

Is There a Role for Probiotics in the Prevention or Treatment of Food Allergy?

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Abstract A balanced gut microbiota is crucial for the development of healthy immunoregulation and gut barrier function to allow brisk immune responses to pathogens and systemic hyporesponsiveness to harmless antigens such as food. Although the first allergic disease to manifest itself, atopic eczema, is not equivalent to food allergy, pre- and postnatal administration of specific probiotic strains has emerged as a promising tool for the prevention of this condition, with potential implications for food allergy development. For food allergy proper, however, we lack markers and risk factors and mechanisms, i.e., targets for preventive measures. The focus here is therefore on the treatment. Indeed, the potential of specific probiotic strains to alleviate food allergy resides in their ability to modify antigens, repair gut barrier functions, balance altered microbiota, and restore local and systemic immune regulation. In patients with multiple food allergies, induction of oral tolerance by specific probiotics continues to attract research interest.

Keywords Food allergy · Gut barrier · Microbiota · Oral tolerance · Probiotics · Regulatory T cells · Prevention · Treatment

Introduction

Food allergy, like other allergic conditions, is an immune-mediated disease characterized by dysregulated immune

responses to innocuous antigens encountered by the body. The majority of food-allergic reactions are provoked by relatively few foods including cow's milk, hen's eggs, peanuts, tree nuts, fish and shellfish [1]. The overall prevalence rates of challenge-proven allergy to any food have been estimated to range from 1 % to 10.8 % [2]. The prevalence of food allergy is age-dependent, affecting particularly infants and young children, among whom milk and egg allergies dominate [3].

There is a substantial body of evidence suggesting that decreased environmental exposure to microbes is associated with an increased prevalence of allergic disease. In the industrialized world environmental changes reducing microbial contact early in childhood have occurred parallel with the growing epidemic of atopic eczema, food allergy, allergic rhinoconjunctivitis and asthma, but also with disorders such as inflammatory bowel disease, type 1 diabetes and obesity [4–7]. The development of these conditions implies a failure to generate and maintain a balanced host-microbe interaction promoting a tolerogenic milieu for harmless antigens in the gut mucosae. An imbalance in the compositional configuration of the gut microbiota, dysbiosis, alters the host-microbiota homeostasis, which is requisite for the development and function of immune cells in the gut-associated lymphoid tissue. The importance of this reciprocal regulation of the microbiota and immune system culminates in early infancy, when the balance between homeostasis and inflammation programs later disease risk.

While the normal response to food antigens is the generation of antigen-specific regulatory T (Treg) cells, in patients with food allergy the dominant immune response is a Th2-skewed T-cell response and, in the case of IgE-mediated allergy, the production of food-specific IgE antibodies from B-cell-derived plasma cells. The symptoms may vary according to age: in early childhood, gut and skin involvement is common, whereas respiratory symptoms and the oral allergy (or pollen-food-related) syndrome are more frequent in older children and adults [3, 8].

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Food Allergy: The Focus is in the Gut and in Infancy

According to Hippocrates (460–377 B.C.), all diseases begin in the gut, and the mucosal surface of the gastrointestinal tract indeed acts as an important organ of host defense. In addition to its principal physiological function, digestion and absorption of nutrients, a protective interface is created between the internal environment and the constant challenge from antigens such as food and microorganisms in the external environment.

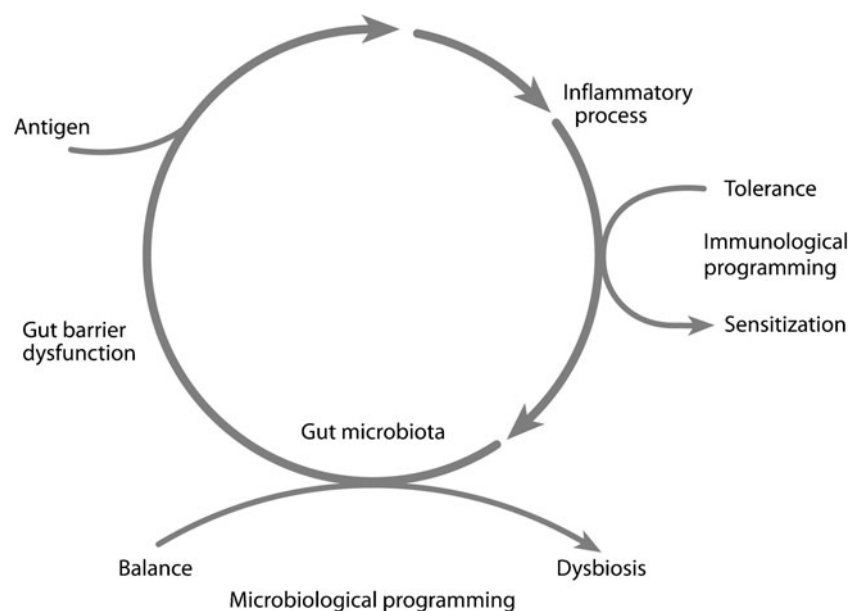
The unique immune system in question comprises an important element in the total immunologic capacity of the host constituting two arms: immune exclusion performed by the innate immune system and immune regulation pertaining to a specific systemic hyporesponsiveness, oral tolerance, induced by prior oral encounter with the antigen [9]. Mucosal surfaces in the intestinal tract are also continuously exposed to a massive microbial load, both detrimental and beneficial. The key element in health is the equilibrium between tolerance and immunity. A critical balance needs to be generated and maintained between protective mucosal immunity, including a vigorous immune response to pathogenic antigens, and systemic hyporesponsiveness specific to ubiquitous antigens such as food and beneficial commensal microorganisms. The key mechanisms by which the commensal microbiota induces Treg differentiation involve transforming growth factor- β (TGF- β). However, the microbiota have an impact not only on the development of Tregs, but also on their functional regulation [10].

Many immunoregulatory aberrations such as inadequate production of antiinflammatory cytokine TGF- β favor sensitization instead of tolerance induction and prevail in early infancy. Moreover, during a variable period after birth, the mucosal immune system is functionally immature [11]. The

binding of antigens to the immature gut microvillus membrane is excessive compared to the mature mucosa and the uptake of intact macromolecules is increased. Furthermore, the immature enterocyte preferably responds to antigen challenges with proinflammatory cytokines. In the context of inflammation, again, a greater amount of antigens may traverse the mucosal barrier and the routes of transport may be altered [12]. During the ensuing mucosal dysfunction caused by immaturity, infection or hypersensitivity reaction, the normal pattern of antigen handling is impaired [13], which may in turn further evoke aberrant immune responses and lead to a vicious circle of sensitization (Fig. 1). These data would imply that an allergic response to dietary antigens is caused by failure of the gut-associated lymphoid tissue to establish or maintain oral tolerance to these antigens; immaturity of the gut barrier may lead to aberrant antigen transfer and immune responses and thus explain the predisposition to food allergy development in infancy. At a later age, inflammatory bowel disease is characterized by analogous dysregulated antigen handling in the gut, and in particular failure in immunological tolerance to microbial exposures [9], and conversely, disruption in the microbiota may lead to clinically significant immune dysfunction [14].

To date, diseases as diverse as allergy, inflammatory bowel disease, diabetes and metabolic syndrome are either causally or consequently linked to dysbiosis. Characteristic of all of these, however, is a rising trend in prevalence in Westernized countries [15–17]. The normal diverse gut microbiota contributes to host health by promoting the development and function of the immune system and gut barrier. In return, the microbiota strains benefit from the specific environment and interface with the host in terms of available nutrients. The host-microbe interaction is thus mutually advantageous, and the

Fig. 1 During mucosal dysfunction, caused, e.g., by immaturity or hypersensitivity reaction, antigen handling is impaired. This may, in turn, evoke aberrant immune responses and lead to a vicious circle of sensitization. A balanced gut microbiota counterregulates immune dysfunction and strengthens impaired gut barrier function



importance of this reciprocal relationship, the way microbiota and host immune system co-evolve, culminates in clinical states associated with immaturity or inflammation in the gut barrier function.

Gut Microbiota

Exposure of the Fetus to Bacteria

Conventionally, the human fetus has been considered sterile and microbial colonization has been thought to begin during the delivery process. However, this dogma has recently been challenged by reports that bacteria are found in the umbilical cord blood and placenta, suggesting that term fetuses are by no means sterile and that a prenatal mother-to-child microbiota transfer may exist [18–20, 21•]. Contact with the complex bacterial load of the extrauterine world during pregnancy and following birth is initiated by changes in the mother's intestinal microbiota during pregnancy and alterations in the intestinal barrier allowing transfer of bacteria from the intestine to breast milk, for example [22, 23]. Current knowledge suggests that the exposure of the fetus to microbes takes place through bacteria residing in the amniotic fluid, very likely derived from the placenta and cord blood and reported to be present in the meconium of cesarean-born infants [24]. Under normal circumstances, microbes from the maternal vaginal and colonic microbiota provide the largest and most diverse initial inoculum of important bacteria to the newborn infant.

Colonization of the Infant Gut Following Delivery

Following delivery, the compositional development of the gut microbiota takes place in a stepwise pattern, with sequential periods of specific microbial strains appearing and disappearing in the breastfed infant. The initial stage of colonization during the first days of life is characterized by facultative anaerobic bacteria (e.g., *Staphylococcus*, *Streptococcus*, *Enterococcus* and *Enterobacteriaceae* spp.), which are rapidly outnumbered by anaerobes due to high levels of *Bifidobacterium* and, as recently reported, also *Bacteroides* [25]. These are followed by members of the Firmicutes. In general, vaginally delivered and breast-fed infants have been demonstrated to form a relatively simple microbiota dominated by *Bifidobacterium* species during the first weeks of life, and breast-fed infants harbor a fecal microbiota containing twice as many *Bifidobacterium* cells when compared to formula-fed infants. On the other hand, formula-fed neonates are likely to harbor a more diverse microbiota, including *Enterobacteriaceae*, *Bacteroides* and *Enterococcus* [26, 27]. After formula feeding, *Atopobium* has been found in significant numbers in the infant feces, and the concentrations of bifidobacterial species have been reported to

be decreased while at the same time *Bacteroides* populations increase [28, 29]. After weaning, the diversity of the microbiota rapidly increases, and several studies suggest that the microbiota become adult-like already by 2–3 years of age [4, 30].

Although the differences between the impact of formula feeding and breastfeeding on intestinal microbiota development have decreased, there still appear to be small divergencies suggesting that breastfed infants have a relatively simple microbiota dominated by several bifidobacterial species while formula-fed infants harbor a more diverse population with a wider variety of *Bacteroides*, *Enterococcus* and *Enterobacteriaceae*. On another level, the microbiota profile of breastfed infants is usually dominated by *Actinobacteria* (mainly genus *Bifidobacterium*) and various genera of Firmicutes, while formula-fed infants have an increased abundance of species with larger amounts of clostridia and *Bacteroides* [25, 31].

Breast Milk Modulates Gut Colonization

Early colonization is modulated by breast milk, which contains factors promoting the growth of bifidobacteria and, according to recent research reports, also a variety of bacteria such as staphylococci, bifidobacteria and lactic acid bacteria, which support healthy immune maturation [32, 33]. It has recently been reported that the human milk microbiome changes over lactation [23]. In colostrum, *Weissella*, *Leuconostoc*, *Staphylococcus*, *Streptococcus* and *Lactococcus* appear to be predominant, while in later milk samples, oral cavity bacteria including *Veillonella*, *Leptotrichi* and *Prevotella* increase. It has also been observed that milk from obese mothers contains a different and less diverse bacterial community compared with milk from normal-weight mothers. In a similar manner, milk from mothers who have undergone elective cesarean delivery contain a different bacterial community than do milk samples from individuals giving birth by vaginal delivery [23].

Taken together, breast-milk and infant feces have specific identical bacterial strains, which suggests that breastfeeding contributes to the bacterial transfer from the mother to the infant [23, 34]. Consequently, healthy term breast-fed infants whose gut microbiota are dominated by bifidobacteria may be considered the model of healthy early colonization and microbial contact.

Is There a Target for Probiotics in Food Allergy?

Mechanisms of Food Allergy: How Much Do We Know?

Absorption and processing of ingested allergen, immune responses and target organ hyperreactivity are regarded as determining elements in the expression of food allergic disorders.

Tolerance is defined as any mechanism by which a potentially injurious immune response is prevented, suppressed or shifted to a non-injurious class of immune response [35]. The systemic hyporesponsiveness to dietary antigens is induced in mesenteric lymph nodes as a result of inactivation of antigen-specific T-cells when antigen-loaded dendritic cells (DCs) seed antigens from the intestinal mucosa [36]. Thus, the phenomenon is both an antigen-specific and an active process. A subset of DCs mediate the selective induction of Treg cells in response to an antigen encountered in the gastrointestinal mucosa. CD103+ DCs are migratory and traffic to the mesenteric lymph nodes, where, under homeostatic conditions, gut intraluminal antigens are presented by CD103+DCs [37]. These DCs also induce the development of Treg cells through mechanisms dependent on factors such as TGF- β and retinoic acid [38]. Anti-inflammatory cytokine TGF- β is necessary for the induction of tolerance through the gastrointestinal mucosa, whereas the data about the role of IL-10 in oral tolerance are more controversial [39–42].

The power of the intestinal microbiota in oral tolerance induction to the IgE response was documented in germ-free mice [43]. In contrast to control mice, germ-free animals maintained a T helper 2-type immune response to orally administered ovalbumin. This could be corrected by the reconstitution of the microbiota at the neonatal stage, but not by any reconstitution implemented at a later age. These findings point to a decisive role of the gut microbiota early in life.

Changes in gut microbiota composition have been documented in patients with food allergy [44], and these may precede the development of allergic disease [45, 46]. A strong inflammatory response may be mounted to microbiota bacteria, leading to perpetuation of the inflammation and gut barrier dysfunction. Intestinal permeability can be secondarily increased as a result of inflammation in the intestinal mucosa induced by viruses, bacteria or dietary antigens [47]. A greater amount of antigens may thus traverse the mucosal barrier, and the routes of transport may be altered. These data would imply that an allergic response to dietary antigens is caused by failure of the gut-associated lymphoid tissue to establish or maintain oral tolerance to these antigens.

The gut microbiota *per se* does not, however, represent the only or even primary treatment target in patients with food allergy. Recent characterization of the immunomodulatory potential of specific strains of the gut microbiota, probiotics, beyond their effect on the composition of the microbiota, has led to novel applications, e.g., in allergic diseases. The potential targets of the probiotic effect in food allergy are presented in Table 1.

Food Allergy: Treatment Targets

Inflammation, irrespective of the etiology or trigger, is frequently accompanied by an imbalance in the intestinal microbiota [48]. Inflammation and infection are again frequently accompanied by an imbalance in the intestinal microbiota. Inflammation then aggravates gut barrier dysfunction, allowing aberrant antigen uptake and immune regulation. The necessary initial step is thus management of the inflammatory response (Fig. 1) induced by the offending food antigen.

The key elements in the current approach to food allergy are:

- Identification of the offending antigen (diagnosis)
- Elimination of the offending antigen (for an individually determined period of time)
- Reintroduction of the antigen and tolerance induction

Probiotic Effects in the Gut and Beyond

Probiotics, live microorganisms that, when administered in adequate amounts, confer a health benefit on the host [49], currently represent one of the best-characterized immunomodulatory compounds for the allergic host. They may aid in degradation/ structural modification of enteral antigens, normalization of the properties of aberrant indigenous microbiota and of gut barrier functions, regulation of the secretion of proinflammatory mediators and direction of the development of the immune system during the critical period of life when the risk of allergization is heightened.

Table 1 Potential targets of probiotic effect in food allergy

Target of effect	Studies	Comments
Prevention	Ref. 65–83	A moderate risk reduction for eczema especially in studies with pre- and postnatal administration of probiotics. No direct evidence of effect on food allergy
Treatment	Ref. 99, 100**	Lactobacillus casei CRL431 and Bifidobacterium lactis Bb-12 – no effect on cow's milk allergy. Lactobacillus rhamnosus GG – hastened tolerance acquisition in cow's milk allergy
Tolerance induction	Ref. 101–104	Attenuation of barrier dysfunction and proinflammatory response

The modern view on probiotic therapy is based on specific effects of clearly defined strains or strain combinations.

Selection of Probiotics

Hitherto, specific probiotics have been selected and characterized based on their *in vitro* properties, for example, tolerance to gastric acid, adhesion to the intestinal mucus or epithelial cells, and competitive exclusion of target pathogens [50–52]. Recent work on intestinal microbiota aberrancies and on the genomic properties of probiotics and probiotic combinations has revealed new possibilities for future selection criteria involving both the health benefits and the safety of probiotics, extending the idea of specific strains and strain combinations to specific microbiota compositions that would be safe and applicable even during the early life of the infant.

Genomic research corroborates the conception that different species, and even strains of the same species, show clinically important differences in these characteristics [53, 54]. Thus, the probiotic potential of specific strains differs, and their properties cannot be extrapolated from other, even closely related strains. Unfortunately, information published thus far frequently lacks accurate information on strain and strain combination identity and characteristics. A good example of such deficits may be deposits missing from international culture collections and deposit numbers, which should be reported along with the mode of administration and the preclinical documentation, together with the selection criteria. We have extended this concern also to the food matrix used to deliver the probiotic to the study subject, providing experimental evidence that the impact of *Lactobacillus rhamnosus* GG (ATCC 53103) on antigen transport depends on the quality of protein, unhydrolyzed vs. hydrolyzed [55], in the diet. We have also drawn attention to the importance of quality control of current probiotics in preserving their original properties [56, 57], and these need to be further investigated.

The use of probiotics is currently extending from research to recommendations [58, 59], but rigorous scientific effort is still required to validate specific strains with anti-allergic potential for preventive and therapeutic applications. The production of functional probiotic foods presupposes stability of strain features in the final product. This requirement is emphasized in treating young infants with impaired gut barrier function, aberrant gut microbiota and sensitization to dietary substances. Questions of probiotic efficacy have arisen because of the European Union health claim regulation, which requires efficacy to be demonstrated in healthy normal consumers [60, 61]. This requirement calls for new types of efficacy studies, but for clinical practice, studies on patients or studies on prevention are still required in the same manner as hitherto. Human intervention studies and subsequent meta-analyses will continue to provide evidence-based recommendations on probiotic

use. Such recommendations have been given by an American expert group, and the use of probiotics in necrotizing enterocolitis, childhood diarrhea, inflammatory bowel disease, irritable bowel syndrome and *Clostridium difficile* diarrhea has been reviewed. Recent positive experiences with bacteriotherapy (fecal microbiome transplant) may also open new therapeutic possibilities, and a positive recommendation has been made for use of microbiota transplant in severely resistant *C. difficile* diarrhea [62].

Recent advances in microbiota and probiotic research are likely to further alter future selection criteria. More specific data will also be obtained by treatment in *Clostridium difficile* colitis by fecal transplant and alleviation of obesity by fecal transplants from lean individuals [63, 64]. These reports suggest that future selection criteria will involve more specific knowledge of probiotic combinations and their use in patients with severe microbiota-associated challenges.

Clinical Evidence of Probiotics in Food Allergy

Several papers have been published on the preventive effect of probiotics on the development of atopic eczema, mainly in infants of families with a history of allergic disease. Although atopic eczema is not tantamount to food allergy, moderate to severe eczema is often associated with food allergies, and results from eczema studies may thus have implications for food allergy development.

Timing, duration, and the strain or strain combination seem to be major factors in determining the beneficial effects of probiotics. In most of studies [65–74], but not in all [75], where probiotics have been administered both pre- and postnatally, a significant reduction in the cumulative incidence of eczema and/or IgE-associated eczema up to 7 years of age has been demonstrated. Huurre and associates [76] reported a reduced risk of IgE sensitization in probiotic-treated infants in a subgroup of sensitized mothers, though no effect on eczema was seen.

In one recent study, maternal administration of probiotics during pregnancy and breast-feeding reduced the risk of eczema during the first 2 years of life [77]. A meta-analysis, again, concluded that maternal probiotic supplementation during pregnancy and lactation significantly reduced the risk of eczema in children by 2–7 years of age with the use of lactobacilli but not with other probiotic species and strains [78]. Another meta-analysis [79] including probiotic prevention studies found that probiotic treatment reduced the risk of eczema [RR=0.79 (95 % CI=0.71–0.88)] regardless of the time of the probiotic use (pre- or postnatal) or the subject receiving the probiotics (mother or infant).

In most studies using only postnatal probiotic administration, no effect on eczema has been demonstrated [80–82], though a controversial result has also been obtained [83].

Probiotic treatment with LGG, *Bifidobacterium lactis* Bb12, or *B. breve* M-16 V for up to 8 weeks showed diminished eczema symptoms in children compared to placebo treatment [84, 85]. Likewise in several other probiotic treatment studies improvement of clinical symptoms has been demonstrated [86–89]. However, a number of studies have not confirmed the beneficial effects of probiotics in the treatment of atopic eczema [90–95]. According to several systematic reviews and meta-analyses, the evidence for a beneficial effect of probiotics in the treatment of atopic eczema is inconclusive [96–98]; this probably reflects the great heterogeneity in populations and probiotic strains used in the studies.

Studies investigating the effect of probiotics on challenge-confirmed food allergy are scant. In one randomized double-blind placebo-controlled study of infants with challenge-proven cow's milk allergy, administration of *L. casei* CRL431 and *B. lactis* Bb12 for 12 months did not affect the acquisition of tolerance to cow's milk [99]. Berni Canani and associates [100•] prospectively evaluated the effect of five different dietary treatment strategies on the rate of acquisition of tolerance in infants with cow's milk allergy, confirmed by the double-blind placebo-controlled food challenge. The groups compared received one of the following treatments: extensively hydrolyzed casein formula (EHCF), EHCF + *Lactobacillus rhamnosus* GG (LGG), hydrolyzed rice formula, soy formula or amino acid-based formula. After the treatment period of 12 months, the proportion of children acquiring tolerance to cow's milk protein was significantly higher in the groups receiving EHCF (43.6 %) or EHCF + LGG (78.9 %) than in the other groups, this implying the ability of LGG to enhance tolerance development in cow's milk-allergic children. This is in accordance with the results from studies showing that LGG is able to modulate immune functions through different pathways including enterocytes, monocytes, mast cells, DCs and Tregs [100–104].

Conclusions

There is strong evidence to support the fundamental impact of the gut microbiota in the development and functional regulation of the immune system. At an early age, both processes are vulnerable; the stable adult-type microbial community develops concomitant with the consolidation of the immune responder type. There is, moreover, reciprocal regulation of these systems: the compositional development of the gut microbiota stimulates and shapes the maturation of immune cells, and immune regulation supports the development of a healthy microbiota. Differences in the neonatal gut microbiota were shown to precede the expression of atopy, suggesting that the composition of the gut microbiota is crucial for the maturation of the human immune system to a non-allergic mode. Promising results have been reported regarding perinatal

administration of specific probiotic strains in reducing the risk of atopic eczema. As atopic eczema in infancy is accompanied by food allergy, repercussions on their incidence are expected, but not proven. Nevertheless, probiotics counterregulate food allergy, not only by providing maturational signals for the gut-associated lymphoid tissue and balancing the generation of pro- and anti-inflammatory cytokines, but also by their capacity to reduce the dietary antigen load by degrading and modifying macromolecules. Probiotics reverse the increased intestinal permeability characteristic of children with atopic eczema and food allergy, and enhance gut-specific IgA responses, frequently defective in children with food allergy. Promotion of gut barrier functions by probiotics also includes normalization of the gut microecology, alterations in which have been demonstrated in allergic subjects. Recent studies indicate that certain probiotics alleviate allergic inflammation both locally and systemically, and may aid in tolerance induction. The heterogeneity of results in probiotic studies in general may be explained by different study protocols, especially in relation to the probiotic strains used and the study populations. More data are needed regarding specific probiotic strains and their combinations, as well as the optimal food matrix and mode of treatment.

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Compliance with Ethics Guidelines

Conflict of Interest Merja Nermes, Seppo Salminen and Erika Isolauri declare that they have no conflict of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

References

Papers of particular interest, published recently, have been highlighted as:

- Of importance
- Of major importance

1. Sampson HA. Update on food allergy. *J Allergy Clin Immunol.* 2004;113:805–19.
2. Rona RJ, Keil T, Summers C, Gislason D, Zuidemeer, Sodergren E, et al. The prevalence of food allergy: a meta-analysis. *J Allergy Clin Immunol.* 2007;120:638–46.
3. Dupont C. Food allergy: recent advances in pathophysiology and diagnosis. *Ann Nutr Metab.* 2011;59(suppl):8–18.
4. Nylund L, Satokari R, Nikkilä J, Rajilic-Stojanovic M, Kalliomäki M, Isolauri E, et al. Microarray analysis reveals marked intestinal microbiota aberrancy in infants having eczema compared to healthy

- children in at-risk atopic disease. *BMC Microbiol.* 2013 Jan;23:12. doi:10.1186/1471-2180-13-12.
5. Hersoug LG, Linneberg A. The link between the epidemics of obesity and allergic diseases: does obesity induce decreased immune tolerance? *Allergy.* 2007;62:1205–13.
 6. Mai XM, Becker AB, Sellers EA, Liem JJ, Kozyskyj AL. The relationship of breastfeeding, overweight and asthma in preadolescents. *J Allergy Clin Immunol.* 2007;120:551–6.
 7. Kero J, Gissler M, Hemminki E, Isolauri E. Could the Th1 and Th2 diseases coexist? Evaluation of asthma incidence in children with celiac disease, type 1 diabetes or rheumatoid arthritis. A register study. *J Allergy Clin Immunol.* 2001;108:781–3.
 8. Sicherer SH. Determinants of systemic manifestation of food allergy. *J Allergy Clin Immunol.* 2000;106:S251–7.
 9. Duerkop BA, Vaishnava S, Hooper LV. Immune responses to the microbiota at the intestinal mucosal surface. *Immunity.* 2009;31:368–76.
 10. Kamada N, Nunez G. Role of the gut microbiota in the development and function of the lymphoid cells. *J Immunol.* 2013;190:1389–95.
 11. Renz H, Brandtzaeg P, Homef M. The impact of perinatal immune development on mucosal homeostasis and chronic inflammation. *Nat Rev Immunol.* 2011;12:9–23.
 12. Cani PD, Possemiers S, Van de Wiele T, Guiot Y, Everard A, Rottier O, et al. Changes in gut microbiota control inflammation in obese mice through a mechanisms involving GLP-2-driven improvement of gut permeability. *Gut.* 2009;58:1091–103.
 13. Peng HJ, Turner MW, Strobel S. Failure to induce oral tolerance to protein antigens in neonatal mice can be corrected by transfer of adult spleen cells. *Ped Res.* 1989;26:486–90.
 14. Isolauri E. Development of healthy gut microbiota early in life. *J Paediatric Child Health.* 2012;48 Suppl 3:1–6.
 15. Gupta R, Sheikh A, Strachan DP, Anderson HR. Time trends in allergic disorders in the UK. *Thorax.* 2007;62:91–6.
 16. Henderson P, Wilson DC. The rising incidence of paediatric-onset inflammatory bowel disease. *Arch Dis Child.* 2012;97:585–6.
 17. Salihu HM, Bonnema SM, Alio AP. Obesity: what is an elderly population growing into? *Maturitas.* 2009;63:7–12.
 18. Satokari R, Grönroos T, Laitinen K, Salminen S, Isolauri E. Bifidobacterium and Lactobacillus DNA in the human placenta. *Lett Appl Microbiol.* 2009;48:8–12.
 19. Jiménez E, Fernández L, Marín ML, Martín R, Odriozola JM, Nueno-Palop C, et al. Isolation of commensal bacteria from umbilical cord blood of healthy neonates born by caesarean section. *Curr Microbiol.* 2005;51:270–4.
 20. Perez PF, Doré J, Leclerc M, Levenez F, Benyacoub J, Serrant P, et al. Bacterial imprinting of the neonatal immune system: lessons from maternal cells? *Pediatrics.* 2007;119:e724–32.
 21. Rautava S, Collado MC, Salminen S, Isolauri E. Probiotics modulate host-microbe interaction in the placenta and fetal gut: a randomized double-blind placebo-controlled trial. *Neonatology.* 2012;102:178–84. *The study showed that maternal supplementation of Bifidobacterium lactis and B. lactis in combination with Lactobacillus rhamnosus GG for 14 days before elective caesarean section modulated the expression of Toll-like receptor-related genes in the placenta and in the fetal gut.*
 22. Koren O, Goodrich JK, Cullender TC, Spor A, Laitinen K, Bäckhed HK, et al. Host remodeling of the gut microbiome and metabolic changes during pregnancy. *Cell.* 2012;150:470–80.
 23. Cabrera-Rubio R, Collado MC, Laitinen K, Salminen S, Isolauri E, Mira A. The human milk microbiome changes over lactation and is shaped by maternal weight and mode of delivery. *Am J Clin Nutr.* 2012;96:544–51.
 24. Jiménez E, Marín ML, Martín R, Odriozola JM, Olivares M, Xaus J, et al. Is meconium from healthy newborns actually sterile? *Res Microbiol.* 2008;59:187–93.
 25. Jost T, Lacroix C, Braegger CP, Chassard C. New insights in gut microbiota establishment in healthy breastfed neonates. *PLoSOne.* 2012;7:e44595. doi:10.1371/journal.pone.0044595.
 26. Favier CF, de Vos WM, Akkermans AD. Development of bacterial and bifidobacterial communities in feces of newborn babies. *Anaerobe.* 2003;9:219–29.
 27. Roger LC, Costabile A, Holland DT, Hoyles L, McCartney AL. Examination of faecal Bifidobacterium populations in breast- and formula-fed infants during the first 18 months of life. *Microbiology.* 2010;156:3329–41.
 28. Bezirzoglou E, Tsiotsias A, Welling GW. Microbiota profile in feces of breast- and formula-fed newborns by using fluorescence in situ hybridization (FISH). *Anaerobe.* 2011;17:478–82.
 29. Roger LC, McCartney AL. Longitudinal investigation of the faecal microbiota of healthy full-term infants using fluorescence in situ hybridization and denaturing gradient gel electrophoresis. *Microbiology.* 2010;156:3317–28.
 30. Palmer C, Bik EM, DiGiulio DB, Relman DA, Brown BO. Development of the human infant intestinal microbiota. *PLoS Biol.* 2007;5:e177.
 31. Azad MB, Konya T, Maughan H, Guttman DS, Field CJ, Chari RS, et al. Gut microbiota of healthy Canadian infants: profiles by mode of delivery and infant diet at 4 months. *CMAJ.* 2013 Mar 19;185(5):385–94.
 32. Gueimonde M, Laitinen K, Salminen S, Isolauri E. Breast milk: a source of bifidobacteria for infant gut development and maturation? *Neonatology.* 2007;92:64–6.
 33. Jost T, Lacroix C, Braegger C, Chassard C. Assessment of bacterial diversity in breast milk using culture-dependent and culture-independent approaches. *Br J Nutr.* 2013 Mar 14;110. [Epub ahead of print].
 34. Martín V, Maldonado-Barragán A, Moles L, Rodríguez-Baños M, Campo RD, Fernández L, et al. Sharing of bacterial strains between breast milk and infant feces. *J Hum Lact.* 2012 Feb;28(1):36–44.
 35. Weiner HL. Oral tolerance: immune mechanisms and the generation of Th3-type TGF-beta-secreting regulatory cells. *Microbes Infect.* 2001;3:947–54.
 36. Chen W. Dendritic cells and (CD4+)CD25+ T regulatory cells: crosstalk between two professionals in immunity versus tolerance. *Front Biosci.* 2006;11:1360–70.
 37. Schultz O, Jaensson E, Persson EK, Liu X, Worbs T, Agace WW, et al. Intestinal CD103+, but not CX3CR1+, antigen sampling cells migrate in lymph and serve classical dendritic cell functions. *J Exp Med.* 2009;206:3101–14.
 38. Coombes JL, Siddiqui KR, Arancibia-Carcamo CV, Hall J, Sun CM, Belkaid Y, et al. A functionally specialized population of mucosal CD103+ DCs induces Foxp3+ regulatory T cells via a TGF-beta and retinoic acid-dependent mechanism. *J Exp Med.* 2007;204:1757–64.
 39. Mucida D, Kutchukhidze N, Erazo A, Russo M, Lafaille JJ, Curotto de Lafaille MA. Oral tolerance in the absence of naturally occurring Tregs. *J Clin Invest.* 2005;115:1923–33.
 40. Zhou Y, Kawasaki H, Hsu SC, Lee RT, Yao X, Plunkett B, et al. Oral tolerance to food induced systemic anaphylaxis mediated by the C-type lectin SIGNR1. *Nat Med.* 2010;16:1128–33.
 41. Cassani B, Villablanca EJ, Quintana FJ, Love PE, Lacy-Hulbert A, Blanker WS, et al. Gut-tropic T cells that express integrin alpha4beta7 and CCR9 are required for induction of oral immune tolerance in mice. *Gastroenterology.* 2011;141:2109–18.
 42. Verhasselt V, Milcent V, Cazareth J, Kanda A, Fleury S, Dombrowicz D, et al. Breast milk-mediated transfer of an antigen induces tolerance and protection from allergic asthma. *Nat Med.* 2008;14:170–5.
 43. Sudo N, Sawamura S, Tanaka K, et al. 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.
44. Thompson-Chagoyan OC, Fallani M, Maldonado J, Vieites JM, Khanna S, Edwards C, et al. Faecal microbiota and short-chain fatty acid levels in faeces from infants with cow's milk protein allergy. *Int Arch Allergy Immunol.* 2011;156:325–32.
 45. 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.
 46. 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.
 47. Rautava S, Ruuskanen O, Ouwehand A, Salminen S, Isolauri E. The hygiene hypothesis of atopic disease- an extended version. *J Pediatr Gastroenterol Nutr.* 2004;38:378–88.
 48. Winter SE, Lopez CA, Bäumlér AJ. The dynamics of gut-associated microbial communities during inflammation. *EMBO Rep.* 2013;14:319–27.
 49. FAO/WHO. Guidelines for the evaluation of probiotics in food. Food and Agricultural Organization of the United Nations and World Health Organization. Working Group Report 2002.
 50. Tuomola E, Crittenden R, Playne M, Isolauri E, Salminen S. Quality assurance criteria for probiotic bacteria. *Am J Clin Nutr.* 2001;73:393–8.
 51. Ouwehand AC, Salminen S, Tolkkio S, Roberts P, Ovaska J, Salminen E. Resected human colonic tissue: new model for characterizing adhesion of lactic acid bacteria. *Clin Diagn Lab Immunol.* 2002;9:184–6.
 52. Collado MC, Meriluoto J, Salminen S. Measurement of aggregation properties between probiotics and pathogens: in vitro evaluation of different methods. *J Microbiol Methods.* 2007;71:71–4.
 53. Kankainen M, Paulin L, Tynkkynen S, von Ossowski I, Reunanen J, Partanen P, et al. Comparative genomic analysis of *Lactobacillus rhamnosus* GG reveals pili containing a human mucus-binding protein. *Proc Natl Acad Sci U S A.* 2009;106:17193–8.
 54. Azcarate-Peril MA, Altermann E, Goh YJ, Tallon R, Sanozy-Dawes RB, Pfeiler EA, et al. Analysis of the genome sequence of *Lactobacillus gasseri* ATCC 33323 reveals the molecular basis of an autochthonous intestinal organism. *Appl Environ Microbiol.* 2008;74:4610–25.
 55. Pessi T, Sutas Y, Martinen A, Isolauri E. Probiotics reinforce mucosal degradation of antigens in rats: implications for therapeutic use of probiotics. *J Nutr.* 1998;128:2313–8.
 56. Elo S, Saxelin M, Salminen S. Attachment of *Lactobacillus casei* strain GG to human colon carcinoma cell line Caco-2: comparison with other dairy strains. *Lett Appl Microbiol.* 1991;13:154–6.
 57. Grześkowiak Ł, Isolauri E, Salminen S, Gueimonde M. Manufacturing process influences properties of probiotic bacteria. *Br J Nutr.* 2011 Mar;105(6):887–94.
 58. Koletzko S, Niggeman B, Arato A, Dias JA, Heuschkel R, Husby S, et al. Diagnostic Approach and management of cow's milk protein allergy in infants and children: ESGPHAN GI committee practical guidelines. *J Pediatr Gastroenterol Nutr.* 2012;55:221–9.
 59. Guarino A, Albano F, Ashkenazi S, Gendrel D, Hoekstra JH, Shamir R. European Society for Paediatric Gastroenterology, Hepatology, and Nutrition/European Society for Paediatric Infectious Diseases: evidence-based guidelines for the management of acute gastroenteritis in children in Europe: executive summary. *J Pediatr Gastroenterol Nutr.* 2008;46:619–21.
 60. van Loveren H, Sanz Y, Salminen S. Health claims in Europe: probiotics and prebiotics as case examples. *Annu Rev Fo Sci Technol.* 2012;3:247–61.
 61. Salminen S, van Loveren H. Probiotics and prebiotics: health claim substantiation. *Microbiol Ecol Health Dis.* 2012;23:18568. doi:10.3402/mehd.v23i0.18568.
 62. Floch MH, Walker WA, Madsen K, Sanders ME, Macfarlane GT, Flint HJ, et al. Recommendations for probiotic use-2011 update. *J Clin Gastroenterol.* 2011 Nov;45(Suppl):S168–71.
 63. van Nood E, Vrieze A, Nieuwdorp M, Fuentes S, Zoetendal EG, de Vos WM, et al. Duodenal infusion of donor feces for recurrent *Clostridium difficile*. *N Engl J Med.* 2013;368:407–15.
 64. Kassam Z, Lee CH, Yuan Y, Hunt RH. Fecal microbiota transplantation for *Clostridium difficile* infection: systematic review and meta-analysis. *Am J Gastroenterol.* 2013;108:500–8.
 65. 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.
 66. Kalliomäki M, Salminen, 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.
 67. Kalliomäki 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.
 68. Kukkonen K, Savilahti E, Haahtela T, Juntunen-Backman K, Korpela R, Poussa T, et al. Probiotics and prebiotic galactooligosaccharides in the prevention of allergic diseases: a randomized, double-blind, placebo-controlled trial. *J Allergy Clin Immunol.* 2007;119:192–8.
 69. Abrahamsson TR, Jakobsson T, Böttcher MF, Fredrikson M, Jenmalm MC, Björkstén 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.
 70. Wickens K, Black P, Stanley TY, 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.
 71. 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 caesarean-delivered children but not in the total cohort. *J Allergy Clin Immunol.* 2009;123:335–41.
 72. Niers L, Martin R, Rijkers G, Sengers F, Timmerman H, van Uden NN, et al. The effects of selected probiotic strains on the development of eczema (the PandA study). *Allergy.* 2009;64:1349–58.
 73. Dotterud CK, Storro O, Johnsen R, Oien T. Probiotics in pregnant women to prevent allergic disease: a randomised, double blind trial. *Br J Dermatol.* 2010;163:616–23.
 74. Kim JY, Kwon JH, Ahn SH, Lee SL, Han YS, Choi Y, et al. Effect of probiotic mix (*Bifidobacterium bifidum*, *Bifidobacterium lactis*, *Lactobacillus acidophilus*) in the primary prevention of eczema: a double-blind, placebo-controlled trial. *Pediatr Allergy Immunol.* 2010;21(2Pt2):e386–93.
 75. Kopp MV, Hennemuth L, Heinzmann A, Urbanek R. Randomized, placebo-controlled trial of probiotics for primary prevention: no clinical effects of *Lactobacillus* GG supplementation. *Pediatrics.* 2008;121:e850–6.
 76. 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.
 77. 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.
 78. 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.
 79. 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.
80. Soh SE, Aw M, Gerez I, Chon 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.
 81. Rautava S, Arvilommi H, Isolauri E. Specific probiotics in enhancing maturation of IgA responses in formula-fed infants. *Pediatr Res*. 2006;60:221–4.
 82. 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.
 83. West CE, Hammarstrom ML, Hernell O. Probiotics during weaning reduce the incidence of eczema. *Pediatr Allergy Immunol*. 2009;20:430–7.
 84. Majamaa H, Isolauri E. Probiotics: a novel approach in the management of food allergy. *J Allergy Clin Immunol*. 1997;99:179–85.
 85. Isolauri E, Arvola T, Sutas Y, Moilanen E, Salminen S. Probiotics in the management of atopic eczema. *Clin Exp Allergy*. 2000;30:1604–10.
 86. Rosenfeldt V, Benfeldt E, Nielsen SD, Michaelsen KF, Jeppesen DL, Valerius NH, et al. Effect of probiotic *Lactobacillus* strains in children with atopic dermatitis. *J Allergy Clin Immunol*. 2003;111:389–95.
 87. Woo SI, Kim JY, Lee YJ, Kim NS, Hahn YS. Effect of *Lactobacillus sakei* supplementation in children with atopic eczema-dermatitis syndrome. *Ann Allergy Asthma Immunol*. 2010;104:343–8.
 88. Yesilova Y, Calka O, Akdeniz N, Berktaş M. Effect of probiotics on the treatment of children with atopic dermatitis. *Ann Dermatol*. 2012;24:189–93.
 89. Wu KG, Li TH, Peng HJ. *Lactobacillus salivarius* plus fructo-oligosaccharide is superior to fructo-oligosaccharide alone for treating children with moderate to severe atopic dermatitis: a double-blind, randomized clinical trial of efficacy and safety. *Br J Dermatol*. 2012;166:129–36.
 90. Viljanen M, Savilahti E, Haahtela T, Juntunen-Backman K, Korpela R, Poussa 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.
 91. 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.
 92. Brouwer ML, Wolt-Plompen SA, Dubois AE, van der Heide S, Jansen DF, Hoijer MA, et al. No effects of probiotics on atopic dermatitis in infancy: a randomized placebo-controlled trial. *Clin Exp Allergy*. 2006;36:899–906.
 93. Folster-Holst R, Muller F, Schnopp N, Abeck D, Kreiselmaier I, Lenz T. Prospective, randomized controlled trial on *Lactobacillus rhamnosus* in infants with moderate to severe atopic dermatitis. *Br J Dermatol*. 2006;155:1256–61.
 94. Sistik D, Kelly R, Wickens K, Stanley T, Fitzharris P, Crane J. Is the effect of probiotics on atopic dermatitis confined to food sensitized children? 2006;36:629–33.
 95. Gruber C, Wendt M, Sulser C, Lau S, Kulig M, Wahn U, et al. Randomized, placebo-controlled trial of *Lactobacillus rhamnosus* GG as treatment of atopic dermatitis in infancy. *Allergy*. 2007;62:1270–6.
 96. Boyle RJ, Bath-Hextall FJ, Leonardi-Bee J, Murrell DF, Tang ML. Probiotics for treating eczema. *Cochrane Database Syst Rev*. 2008;4, CD006135.
 97. 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;121:116–121e11.
 98. Tang ML, Lahtinen SJ, Boyle RJ. Probiotics and prebiotics: clinical effects in allergic disease. *Curr Opin Pediatr*. 2010;22:626–34.
 99. Hol J, van Leer EH, Elink Schuurman BE, de Ruiter LF, Samsom JN, Hop W, et al. The acquisition of tolerance towards cow's milk through probiotic supplementation: a randomized controlled trial. *J Allergy Clin Immunol*. 2008;121:1448–54.
 100. •• Berni Canani R, Nocerino R, Terrin G, Frediani T, Lucarelli S, Cosenza L, et al. Formula selection for management of children with cow milk allergy influences the rate of acquisition of tolerance: a prospective multicenter study. *J Pediatr*. 2013. doi:10.1016/j.jpeds.2013.03.008. [Epub ahead of print]. *Provided data that Lactobacillus rhamnosus GG enhances tolerance acquisition in extensively hydrolysed casein formula in infants with cow's milk allergy*.
 101. Pan SJ, Kuo CH, Lam KP, Chu YT, Wang WL, Hung CH. Probiotics and allergy in infants – an update review. *Pediatr Allergy Immunol*. 2010;21:e659–66.
 102. Ghadimi D, Fölster-Holst R, de Vrese M, Winkler P, Heller KJ, Schrezenmeier J. Effects of probiotic bacteria and their genomic DNA on TH1/TH2-cytokine production by peripheral blood mononuclear cells (PBMCs) of healthy and allergic subjects. *Immunobiology*. 2008;213:677–92.
 103. Donato KA, Gareau MG, Wang YJ, Sherman PM. *Lactobacillus rhamnosus* GG attenuates interferon- γ and tumor-necrosis factor- α -induced barrier dysfunction and pro-inflammatory signalling. *Microbiology*. 2010;156:3288–97.
 104. Mileti E, Matteoli G, Iliev D, Rescigno M. Comparison of the immunomodulatory properties of three probiotics strains of *Lactobacilli* using complex culture systems: prediction for in vivo efficacy. *PLoS One*. 2009;4:e7056.