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The gut microbiota and its role in the development of allergic disease: a wider perspective

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Abstract

The gut microbiota are critical in the homeostasis of multiple interconnected host metabolic and immune networks. If early microbial colonisation is delayed, the gut associated lymphoid tissues (GALT) fail to develop, leading to persistent immune dysregulation in mice. Microbial colonisation has also been proposed as a major driver for the normal age-related maturation of both Th1 and T regulatory (Treg) pathways that appear important in suppressing early propensity for Th2 allergic responses. There is emerging evidence that resident symbionts induce tolerogenic gut-associated Treg cells and dendritic cells that ensure the preferential growth of symbionts; keeping pathogenic strains in check and constraining proinflammatory Th1, Th2 and Th17 clones. Some effects of symbionts are mediated by short-chain fatty acids, which play a critical role in mucosal integrity, local and systemic metabolic function and stimulate the regulatory immune responses. The homeostatic IL-10/TGF-β dominated tolerogenic response within the GALT also signals the production of secretory IgA, which have a regulating role in mucosal integrity. Contrary to the "sterile womb" paradigm, recent studies suggest that maternal microbial transfer to the offspring begins during pregnancy, providing a pioneer microbiome. It is likely that appropriate microbial stimulation both pre- and postnatally are required for optimal Th1 and Treg development to avoid the pathophysiological processes leading to allergy. Disturbed gut colonisation patterns have been associated with allergic disease, but whether microbial variation is the cause or effect of these diseases is still under investigation. We are far from understanding what constitutes a "healthy gut microbiome" that promotes tolerance. This remains a major limitation and might explain some of the inconsistency in human intervention studies with prebiotics and probiotics. Multidisciplinary integrative approaches with researchers working in networks, using harmonised outcomes and methodologies are needed to advance our understanding in this field.

Introduction

The epidemic rise in allergic disease over the last decades has coincided with progressive westernisation (increased hygiene, smaller family sizes, dietary change and excessive antibiotic use). As one of the leading candidates in the allergy epidemic, there has been longstanding interest in the critical role of microbials for normal immune development and regulation. To explain this rise, Strachan introduced the hygiene hypothesis suggesting that microbial exposures in childhood are critical for normal immune development (1). This hypothesis was later revised by the "gut microbial deprivation hypothesis", proposing that the observed changes in early intestinal colonisation patterns over the last decades in Western countries have resulted in failure to induce and maintain tolerance (2).

The infant gut microbiota and its corresponding genes (the microbiome) undergo dynamic changes during development, resulting in an adult-like microbiome at about 3 years of age (3). This process is influenced by genetic, epigenetic and environmental factors such as country of origin, delivery mode, antibiotics and breastfeeding (4). Even if there is large variation in acquisition and colonisation of bacterial species in infancy, it was recently shown in different populations that infant microbiomes have lower species richness that adults and many bifidobacteria (3). The adult human gut harbours up to 100 trillion bacteria and outnumber human cells tenfold, and the microbiome has been estimated to contain 150-fold more genes that the host genome (5). The use of new molecular biology techniques using the conserved 16S rRNA gene for phylogenetic analyses that can detect also unculturable bacteria have advanced our understanding of the gut microbiome, both in health and disease (6). This has furthered our understanding of the role of microbials also in relation to allergic disease. Although the "Developmental Origins of Health and Disease (DoHAD) hypothesis (7) was originally studied in the context of cardiovascular and metabolic disease (7, 8) there is emerging

evidence that early gut microbiota establishment during critical periods of development has the potential to influence the risk of developing environmentally influenced disease, including allergic disease. Studies have reported that infants born by caesarean section (CS) are at greater risk for developing asthma (9-11) and atopy (9). A contributing factor for this increased risk might be through effects on intestinal colonisation patterns as recent studies using culture independent methods have shown lower abundance of *Bacteroides* (12-15) and lower diversity within the Bacteroidetes phylum (13) in CS-delivered infants. Lower overall diversity (16-20) and diversity within Bacteroidetes (16) in early infancy have also been observed to precede development of allergic manifestations in clinical studies, lending further support for this concept.

Below, we discuss the gut microbiota and its intricate relationship with immune and metabolic networks from an evolutionary perspective, the role of perinatal programming by the microbiota and strategies to improve gut microbial patterns for allergy prevention.

An ancient and intimate relationship - the immune system and the microbiome

The microbiota play an integral part in the homeostasis of multiple interconnected host metabolic and immune networks (21). This reflects the blended co-evolution of these systems together with the myriad of microbes that colonise cutaneous and mucosal surfaces of all multicellular organisms. It may be more fitting to consider the 'holobiont' and its 'hologenome' (sum of genetic material of the microbiota and its host) as the 'unit of selection' in evolutionary change (22). With around 99% of the hologenome comprising genes of microbial origin, it could be argued that microbial DNA has had a dominant role in human evolution (21). Viewed in this way it is also obvious that our hologenome is a dynamic and changing entity, with great potential for changes in genomic composition both within an individual and between

generations, according to environmental conditions. It is also not hard to understand how dynamic changes in the microbiota can alter immune maturation and metabolic function (23, 24).

Microbes have shaped evolution of the immune system, and still do. The innate immune system that provides the main defence pathways in invertebrates has been remarkably successful. It has been highly conserved across evolution, and shows striking homology with the toll-like receptors (TLR) and other microbial pattern recognition receptors (PRR) in humans (21). There are also ancient links between the immune and sensory systems, with common receptors for both pheromonal communication and pathogen recognition in invertebrates (25), suggesting other dimensions to host-microbial interactions, even in invertebrates. In fact, there is emerging evidence that commensal microbiota may drive behaviour and speciation. In Drosophila, fascinating studies reveal that changes in the gut microbiome, either by changing the diet or by using antibiotics, will alter mating preferences (26). This could hold true in mammals as well (27), revealing much more sophisticated interactions between the microbiome and the evolution of its host than previously suspected.

The appearance of early precursors of the adaptive immune system, around 500 million years ago in some non-jawed marine vertebrates, has been partly attributed to the mobile microbial DNA elements 'infecting' invaded germ-line cells of our early marine ancestors creatures. These transposons (28, 29) provided the early RAG homologs that evolved into the genes for V(D)J rearrangement, providing the basis for B- and T cell diversity (30). Since then, microbial exposure has provided a dominant evolutionary force in the refinement of these pathways. It has been considered that more complex, larger, and slower growing organisms with gastrointestinal tracts require more complex, more efficient immune systems. However, it is

equally if not more likely, that the main evolutionary advantages of the adaptive immune system do not lie in defence, but in the capacity for much more sophisticated relationships with microbes, for our mutual benefit (22). A key advantage of the adaptive immune system is that it allows symbiotic relationships with the microbial world, selectively promoting beneficial microbes for metabolic and physiological gain.

The gut microbiota and immune development

These evolutionary perspectives provide an important backdrop for understanding the critical role of the microbiome in normal immune development and regulation; again for mutual benefit of the commensal symbiotic microbiota and their host. Both the innate and the adaptive immune system are dependent on early colonisation for optimal development (24). Experimental mouse models have shown that the cellular immune networks of the gut associated lymphoid tissues (GALT) fail to develop if colonisation is delayed beyond a critical window, leading to persistent immune dysregulation and associated disease (31). Even though the human correlate of such a window is unknown, microbial colonisation has also been proposed as a major driving factor for the normal age-related maturation of both Th1 (32, 33) and T regulatory (Treg) pathways (34) during early childhood, that appear important in suppressing early propensity for Th2 allergic responses (35).

More recently it has become clear that resident symbionts induce tolerogenic gut-associated Treg cells and dendritic cells (DC), which in turn ensure the preferential growth of symbionts (36, 37). During early colonisation, both symbionts and more pathogenic strains enter the gastrointestinal tract, and can be found within the normal microbiota of healthy children. Robust immune regulatory systems favour the proliferation of symbionts and ensure that pathogenic strains are kept in check (36). These well-balanced regulatory responses also constrain

proinflammatory Th1, Th2 and Th17 clones, and curb the risk of chronic inflammatory diseases, such as allergies and autoimmune diseases. Some of these pro-regulatory effects of symbionts are mediated by bacterial produced molecules, e.g. butyrate and proprionate from highly oxygen-sensitive anaerobes belonging to the clostridial clusters IV, XIVa and XVIII (38-40) and polysaccharide A (PSA) from *Bacteroides fragilis (B. fragilis)* (41, 42). Animal models demonstrate the central role of PSA in correcting the immune dysregulation of germ-free mice and promoting both the cellular and physical maturation of the developing immune system, providing a molecular basis for host-bacterial symbiosis (41, 42). In experimental models of colitis, PSA is the necessary factor for the symbiont-mediated suppression of inflammation seen with *B. fragilis*. This appears to be the result of induction of IL-10-producing CD4+ T cells by DCs that have captured PSA at the epithelial surface (42).

The resulting homeostatic IL-10/TGF-β dominated tolerogenic response within the GALT also signals the production of secretory IgA (SIgA) by B cells. Furthermore, commensal derived TLR signals stimulate SIgA production via induction of APRIL and BAFF, CD40L related cytokines, from gut epithelial cells and DC (43). In humans, this T cell independent isotype switching preferentially induces production of SIgA2, which is more resistant to bacterial proteases than SIgA1 (44). SIgA provides an important first-line effector mechanism regulating mucosal integrity (45). In addition to its neutralizing capacity, SIgA preserves homeostasis and maintains the tight junctions that protect the epithelial barrier against inflammation (46). It is increasingly evident that symbiotic bacteria are important triggers for inducing mucosal immune system maturation and SIgA production (47-49). Proper development of the SIgA system appears to be a critical element in the establishment of ‘mutualism’ which reduces intestinal pro-inflammatory signalling and bacterial epitope expression, allowing survival of the symbionts while reducing host damage from an inflammatory response (50). Symbionts

such as *Lactobacillus* and *Bifidobacterium* are now known to actively enhance SIgA driven immune exclusion of more pathogenic bacteria (51). SIgA-coating of symbiotic bacteria increases local adhesion, reinforces the tight junctions and enhances production of polymeric immunoglobulin receptor and immunomodulatory thymic stromal lymphopoietin (51). Thus, selectively favouring biofilm formation of non-pathogenic bacteria preserves this ancient host-symbiont relationship, while excluding pathogenic bacteria from the epithelial surface and promoting health of both the symbiont and the host. This suggests more complex mutually beneficial inter-relationships between symbionts and local IgA production than previously recognized (52, 53).

The IgA system is re-emerging as an important pathway in the pathogenesis of food allergy. Retarded development of IgA-producing cells or insufficient SIgA-dependent function at the intestinal surface barrier appears to contribute substantially to an individual's threshold for food allergy (45, 54). In new studies using genome-scale DNA methylation profiling in purified CD4+ T cells from infants who developed IgE mediated food allergy at 12-months, we found that many of the differentially methylated sites (distributed across 128 genes), were implicated in the intestinal IgA production pathways, including (*TNFRSF17*, *TGFB3* and *CD80*) (Martino DJ, personal communication). This is consistent with earlier reports of minor dysregulations of both innate and adaptive immunity (especially low levels of IgA) in children with multiple food allergies (55) and previous association between low serum (56-58) and secretory IgA (47, 59, 60) and risk of childhood allergy. As a therapeutic target in allergic disease, human studies have shown that administration of a prebiotic and probiotic combination (perinatally for 6 months in infants) increased levels of faecal IgA and reduced the risk of allergy, including food allergy (61).

Many bacterial metabolites are an important communication tool between the host immune system and the commensal microbiota (Fig. 1), to establish a broad basis for mutualism (48). Of these, the short-chain fatty acids (SCFAs) acetic-, propionic- and butyric acids, are among the most abundant and play a critical role in mucosal integrity, local and systemic metabolic function and stimulates the regulatory immune responses (38-40, 62). Again, these concepts also point to how the changing modern landscape can impact both immune and metabolic homeostasis through its potentially profound effects on our microbiota. Intestinal dysbiosis is increasingly implicated in not only the epidemic rise in allergic disease, but also the parallel rise of a broad range of other immune and metabolic diseases (63).

Microbial exposure and prenatal programming

Many years since the hygiene hypothesis was first proposed (1), reduced diversity of early microbial exposure is still a dominant explanation for the altered patterns of T cells induction that appears to underlie the allergy epidemic. The developing neonatal immune system depends critically on diverse environmental exposures to mature normally. Attenuated development of both regulatory and/or Th1 responses in 'cleaner' environments remain likely contenders in the persistence of allergy-inducing Th2 responses (24, 64, 65).

Most studies investigating the early immunomodulatory mechanisms have focussed on postnatal microbial exposure (66-68). However, it is becoming increasingly clear that the maternal microbial environment during pregnancy is also important in early immune programming (24, 69-71). Experimental murine models demonstrate that maternal treatment with lipopolysaccharide (72, 73) or commensals such as *Acinetobacter lwoffii* (74) and *Lactobacillus rhamnosus* (75) during gestation attenuate allergic sensitisation and airway inflammation in the offspring. Epidemiological studies also indicate that maternal farm

environment exposure during pregnancy protects against allergic sensitisation and disease, whereas exposures during infancy alone have weaker or no effect at all (76, 77). Continued postnatal microbial exposure is also critical for immune maturation and a likely factor for optimal allergy protection (76).

Epigenetic modifications of genes involved in immunomodulatory processes may constitute a viable molecular mechanism through which microbial exposures exert lasting effects on immune function during critical time windows of developmental plasticity (24). The main processes modulating DNA accessibility to establish epigenetic memory occur via posttranslational histone modifications and methylation of DNA CpG dinucleotides (78). These conformational changes produce heritable changes in gene expression and cell phenotype, which are passed on to daughter cells during mitosis. DNA methylation, associated with transcriptional repression, is more rigid than histone modifications, with DNA methyltransferases conferring covalent methyl modifications to evolutionary conserved regulatory gene elements, CpG islands (79). The methylation pattern is thus preserved with high fidelity through cell divisions, assuring preservation of cellular inheritance (79). Many aspects of immune development are under epigenetic control, namely Th1, Th2 and Th17 differentiation (79-82), and human T regulatory cell commitment, which requires demethylation of the *FOXP3* promoter (79, 81-83). Interestingly, the immunoregulatory effects of the gut microbiota derived butyrate and proprionate may be mediated via their inhibitory effects on histone deacetylases, enhancing histone H3 acetylation in the promoter and conserved non-coding sequence regions of the Foxp3 locus, thus enhancing extrathymic induction of Treg cells (38, 39).

During pregnancy, there is a close immunological interaction between the mother and her offspring (84, 85), providing enormous opportunities for the maternal microbial environment to influence the immune development of her offspring (24, 70, 71, 86). Several prenatal environmental exposures can alter gene expression via epigenetic mechanisms. This provides the host with the capacity for physiological adaptations to the anticipated postnatal environment. On the other hand, these early changes in developmental trajectory can also influence the predisposition to later disease (7, 71). Early adaptive epigenetic changes may be more likely to lead to disease if there is 'mismatch' between the *anticipated* postnatal environment and actual conditions that are encountered (87). Although the DoHAD hypothesis was originally studied in the context of cardiovascular and metabolic disease (7, 8), early programming for subsequent vulnerability is also likely in the context of environmentally influenced immune-mediated diseases (71, 88, 89). At this juncture, it seems most logical to optimise microbial diversity from the early prenatal period, into the postnatal years (71).

Contrary to the 'sterile womb' paradigm, recent studies suggest that maternal microbial transfer to the offspring may begin during pregnancy, providing a pioneer microbiome (86, 90). Microbial DNA can be detected during normal healthy pregnancy in amniotic fluid (91, 92), placental (92) and foetal membranes (93), in umbilical cord blood (94) and meconium (95-97). Some of these studies that combined molecular biology with culture methods, reported viable bacteria in umbilical cord blood (94) and meconium (97) of healthy infants. Furthermore, the meconium microbiota has also been reported to be affected by maternal health status and to influence offspring disease development (95). In murine studies, transmission of labelled bacterial strains from the mother to foetus can be demonstrated during pregnancy (94). Evidence for microbial maternal transmission is becoming increasingly widespread across the animal kingdom (90). This may provide the offspring with important microbes at birth,

imprinting the offspring microbiota in preparation for the much larger inoculum transferred during vaginal delivery (13, 98) and breastfeeding (99), and may have shaped the microbiome composition in animal species over evolutionary time. It has been speculated that "heirloom" microbes received from the mother are uniquely evolved to the offspring's genotype and that vertical as compared with horizontal transmission increases the chance for optimal mutualism (90).

The foetal-maternal interface is characterised by high levels of Th2-like (100, 101) and anti-inflammatory (102) cytokines, as well as enrichment of Treg cells (103), functioning to inhibit maternal Th1-mediated immune reactions to foetal alloantigens (104, 105). This cytokine milieu shapes the T helper differentiation (82, 106), and is reflected in the Th2-skewed patterns of neonatal immune responses (24, 107). The Th2 cytokine locus of murine neonatal CD4+ T cells is poised epigenetically for rapid and robust production of IL-4 and IL-13 (24, 108). In some (85, 107, 109) but not all (32, 35) studies, differential neonatal Th2-skewing has been observed in infants later developing allergy. This may reflect prenatal epigenetic effects mediated through the maternal environment or maternal immune responses -- and may be possible to redress by modulating the maternal microbiome during pregnancy. Then, the subsequent failure of Th2-silencing during postnatal maturation may amplify the risk of Th2-mediated allergic disease (24, 107, 110). It is increasingly likely that appropriate microbial stimulation, both pre- and postnatally, are required for optimal Th1 and Treg development and to avoid the pathophysiological process that lead to allergy (71, 76).

Modulation of the gut microbiota in the context of allergic disease

Probiotics

A common approach to modulate the microbial environment has been to use probiotics, predominantly preparations with single or several strains of lactobacilli and bifidobacteria. Early observations that colonisation with lactobacilli and bifidobacteria was reduced in allergic compared with non-allergic individuals (111, 112) formed the basis for this concept. These studies were then followed by studies using non-cultivation based methods, and some (68, 113, 114), but not all (115), show that early colonisation with lactobacilli and/or bifidobacteria is associated with reduced risk of allergic disease. Probiotics are "live microorganisms which when administered in adequate amounts confer a health benefit on the host" (116). They have been variably shown to exert immunomodulatory effects, mostly in experimental models (117) but also in human intervention studies (118-120); their effects being considered strain dependent. Proposed mechanisms of probiotic immunomodulation include increased production of IgA and IL-10, suppression of TNF, inhibition of antigen-induced T-cell activation and circulating soluble CD4 and TLR4 signalling (117). Direct effects on the mucosal barrier leading to enhanced gut integrity have also been demonstrated in experimental models (121) and in clinical trials (122).

Despite some positive results, there appears to be less benefit of probiotics in the treatment of established allergic disease (123), than in allergy prevention. A likely interpretation is that it is more difficult to shape the allergic phenotype when it is already established. In human intervention studies, maternal probiotic supplementation during pregnancy and/or to infants postnatally has been a logical approach for allergy prevention (71, 86, 124). Collectively these studies suggest a protective effect on eczema and atopic eczema (125-127) and combined pre- and postnatal probiotic supplementation appears most efficacious (128, 129). No consistent

protection from other allergic outcomes has been demonstrated (130), and probiotic use is not yet part of allergy prevention recommendations (128, 129). Long-term preventative effects on development of respiratory allergic disease are not yet fully evaluated, but follow-up data (≥5 years of age) from 7 initiated cohorts report no benefit on allergic rhinitis or asthma (130-137), objective markers of lung function (131, 136, 137) or airway inflammation (131, 136-138), suggesting that the strategies used to date have been insufficient, or alternatively, that the airway microbiome should be the primary target (139) .

In a large Norwegian pregnancy cohort, including 40 614 mother-child pairs, probiotic milk consumption in pregnancy (assessed at 22 weeks gestation) was associated with a reduced relative risk of reported of atopic eczema and allergic rhinoconjunctivitis, but not asthma at three years of age (140). The association between probiotics and rhinoconjunctivitis was stronger if both the pregnant mother and the child (from 6 months of age) had consumed probiotics compared with no consumption, or consumption by either mother or child. Most probiotic prevention studies in pregnancy have been initiated late in the last trimester (71, 129) and we speculate that, given the likely role of the maternal microbiome in pregnancy for both immune and metabolic homeostasis (141), it would be logical to investigate the effects of probiotics (and prebiotics) much earlier in pregnancy, at a time when foetal metabolic and immune responses are initiated (71). This is also consistent with the concepts that interventions to prevent later onset metabolic and inflammatory diseases are best targeted during the "first 1000 days" (from conception through infancy) (89). Another limitation of current strategies could be the *choice* of probiotic. So far, mostly strains of lactobacilli and bifidobacteria have been evaluated in clinical trials. Future research is anticipated to provide insight if "next-generation probiotics", i.e. non-conventional indigenous gut bacteria (142), such as butyrate and proprionate producers and immunomodulatory *Bacteroides* strains, are more powerful.

Prebiotics

Breast milk contains a large variety of complex non-digestible oligosaccharides (143, 144). Some of these acts as decoy receptors to prevent attachment of potential pathogens to the intestinal mucosa, and as they are non-digestible, they can pass through the small intestine and enter the colon where they promote colonisation with bifidobacteria, and also lactobacilli (145). A second more direct immune effect seems to be mediated by the production of SCFAs (146). Collectively, and keeping in mind that the amount of oligosaccharides in bovine milk and infant formulas is much lower and the structures less diverse, this provided a strong foundation to explore the effects of prebiotics (non-digestible, fermentable oligosaccharides) in infancy for allergy prevention (129, 147, 148). However, clinical trials are scarce. In the latest Cochrane review, meta-analysis of four studies (1428 infants, all at high-risk) showed a reduction in eczema, but no other allergic outcomes (149). The prebiotic approach is promising, but because of the paucity of studies, considerably more research is needed to evaluate if the positive effects on gut colonisation and immune function (147, 150) translate into clinically relevant benefits (148) in both high and low risk populations, and include other outcomes than eczema.

Conclusions and perspectives

Onset of allergic disease is the result of complex interactions between genetic, epigenetic, environmental and microbiota-driven factors in early life. New discoveries of microbial DNA in the foeto-placental unit and maternal to foetal transmission of labelled bacterial strains in mice suggest that maternal microbial transfer to the offspring may begin during pregnancy thereby providing a pioneer microbiome. It is possible that this pioneer microbiome may affect infant gut colonisation patterns and subsequent susceptibility to allergic disease. Indeed, recent studies have highlighted the importance of perinatal programming of gut microbiota not only

in the context of allergic disease but also in other inflammatory diseases, indicating that these early patterns have the potential to influence health and disease risk throughout life. However, we are far from understanding what constitutes a healthy gut microbiome that promotes immune tolerance (151) . This remains a major limitation, not the least with regard to intervention strategies targeting the gut microbiota. Multidisciplinary integrative approaches should be performed to elucidate the interactions between the host and the gut microbes in the context of allergic disease, and ultimately, even beyond (89). Physiologic, metabolic, immunologic and even behavioural programming have been linked to the gut microbiota, but have traditionally been studied separately. With the advent of new systems biology methods, we have the possibility to study these systems and their interrelationships using a more holistic approach. To solve these complexities, coordinated approaches with researchers from many groups harmonising their outcomes and methodologies, and working collaboratively in networks are needed (89). This may then form the basis for effective strategies to impact healthy gut colonisation, and promote a functional microbiome also for other outcomes than allergic disease.

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Figure

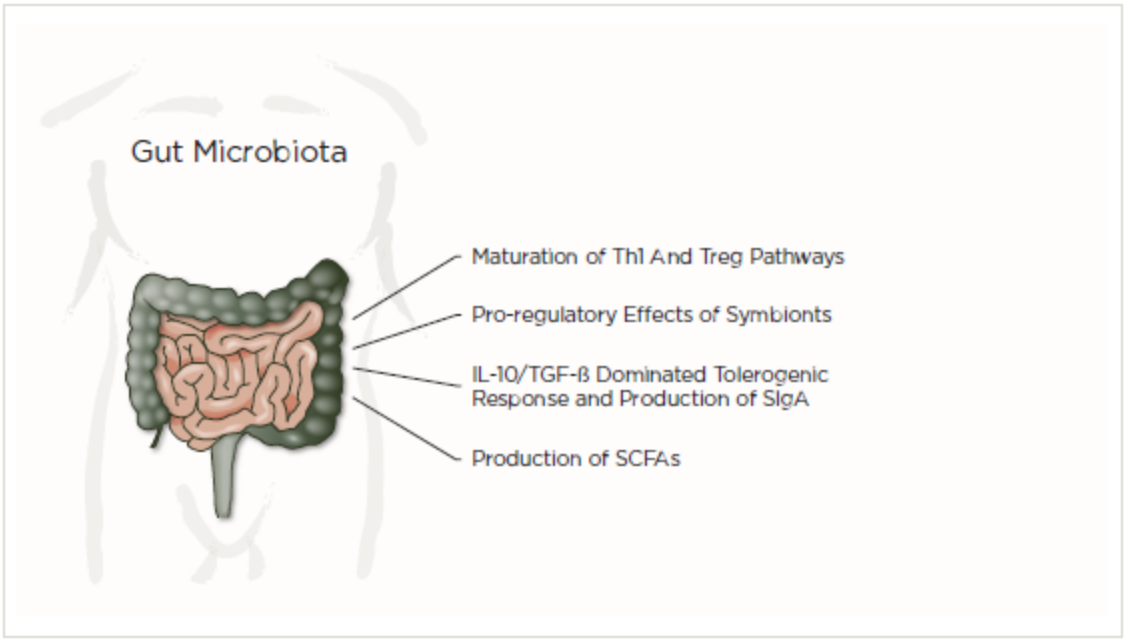

Fig. 1. Gut microbiota are critical in the homeostasis of multiple interconnected immune networks, and mediate their effects via several pathways.

References

1. Strachan DP. Hay fever, hygiene, and household size. Bmj. 1989;299:1259-60.

2. Wold AE. The hygiene hypothesis revised: is the rising frequency of allergy due to changes in the intestinal flora? Allergy. 1998;53(46 Suppl):20-5.

3. Yatsunenko T, Rey FE, Manary MJ, et al. Human gut microbiome viewed across age and geography. Nature. 2012;486:222-7.

4. Adlerberth I, Wold AE. Establishment of the gut microbiota in Western infants. Acta Paediatr. 2009;98:229-38.

5. Qin J, Li R, Raes J, et al. A human gut microbial gene catalogue established by metagenomic sequencing. Nature. 2010;464:59-65.

6. Turnbaugh PJ, Ley RE, Hamady M, et al. The human microbiome project. Nature. 2007;449:804-10.

7. Wadhwa PD, Buss C, Entringer S, Swanson JM. Developmental origins of health and disease: brief history of the approach and current focus on epigenetic mechanisms. Semin Reprod Med. 2009;27:358-68.

8. Barker DJ. The fetal and infant origins of adult disease. Bmj. 1990;301:1111.

9. Kolokotroni O, Middleton N, Gavatha M, et al. Asthma and atopy in children born by caesarean section: effect modification by family history of allergies - a population based cross-sectional study. BMC Pediatr. 2012;12:179.

10. Magnus MC, Håberg SE, Stigum H, et al. Delivery by Cesarean section and early childhood respiratory symptoms and disorders: the Norwegian mother and child cohort study. Am J Epidemiol. 2011;174:1275-85.

11. Thavagnanam S, Fleming J, Bromley A, Shields MD, Cardwell CR. A meta-analysis of the association between Caesarean section and childhood asthma. Clin Exp Allergy. 2008;38:629-33.

12. Azad MB, Konya T, Maughan H, et al. Gut microbiota of healthy Canadian infants: profiles by mode of delivery and infant diet at 4 months. CMAJ : Canadian Medical Association journal = journal de l'Association medicale canadienne. 2013;185:385-94.

13. Jakobsson HE, Abrahamsson TR, Jenmalm MC, et al. Decreased gut microbiota diversity, delayed Bacteroidetes colonisation and reduced Th1 responses in infants delivered by Caesarean section. Gut, epub ahead of print.

14. Penders J, Gerhold K, Stobberingh EE, et al. Establishment of the intestinal microbiota and its role for atopic dermatitis in early childhood. J Allergy Clin Immunol. 2013;132:601-7 e8.

15. Tsuji H, Oozeer R, Matsuda K, et al. Molecular monitoring of the development of intestinal microbiota in Japanese infants. Benef Microbes. 2012;3:113-25.

16. Abrahamsson TR, Jakobsson HE, Andersson AF, et al. Low diversity of the gut microbiota in infants with atopic eczema. J Allergy Clin Immunol. 2012;129:434-40, 40 e1-2.

17. Abrahamsson TR, Jakobsson HE, Andersson AF, et al. Low gut microbiota diversity in early infancy precedes asthma at school age. Clin Exp Allergy. 2013, epub ahead of print.

18. Bisgaard H, Li N, Bonnelykke K, Chawes BL, 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.

19. Ismail IH, Oppedisano F, Joseph SJ, et al. Reduced gut microbial diversity in early life is associated with later development of eczema but not atopy in high-risk infants. Pediatr Allergy Immunol. 2012;23:674-81.

20. Wang M, Karlsson C, Olsson C, et al. Reduced diversity in the early fecal microbiota of infants with atopic eczema. J Allergy Clin Immunol. 2008;121:129-34.

21. McFall-Ngai M, Hadfield MG, Bosch TC, et al. Animals in a bacterial world, a new imperative for the life sciences. Proc Natl Acad Sci U S A. 2013;110:3229-36.

22. Eberl G. A new vision of immunity: homeostasis of the superorganism. Mucosal Immunol. 2010;3:450-60.

23. Nicholson JK, Holmes E, Kinross J, et al. Host-gut microbiota metabolic interactions. Science. 2012;336:1262-7.

24. Renz H, Brandtzaeg P, Hornef M. The impact of perinatal immune development on mucosal homeostasis and chronic inflammation. Nat Rev Immunol. 2012;12:9-23.

25. Benton R, Vannice KS, Vosshall LB. An essential role for a CD36-related receptor in pheromone detection in Drosophila. Nature. 2007;450:289-93.

26. Sharon G, Segal D, Ringo JM, et al. Commensal bacteria play a role in mating preference of Drosophila melanogaster. Proc Natl Acad Sci U S A. 2010;107:20051-6.

27. Theis KR, Venkataraman A, Dycus JA, et al. Symbiotic bacteria appear to mediate hyena social odors. Proc Natl Acad Sci U S A. 2013, epub ahead of print.

28. Rebollo R, Horard B, Hubert B, Vieira C. Jumping genes and epigenetics: Towards new species. Gene. 200;454:1-7.

29. Zeh DW, Zeh JA, Ishida Y. Transposable elements and an epigenetic basis for punctuated equilibria. BioEssays : news and reviews in molecular, cellular and developmental biology. 2009;31:715-26.

30. Fugmann SD. The origins of the Rag genes--from transposition to V(D)J recombination. Seminars in immunology. 2010;22:10-6.

31. 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.

32. Prescott SL, Macaubas C, Smallacombe T, et al. Development of allergen-specific T-cell memory in atopic and normal children. Lancet. 1999;353:196-200.

33. Tulic MK, Hodder M, Forsberg A, et al. Differences in innate immune function between allergic and nonallergic children: new insights into immune ontogeny. J Allergy Clin Immunol. 2011;127:470-8 e1.

34. Tulic MK, Andrews D, Crook ML, et al. Changes in thymic regulatory T-cell maturation from birth to puberty: differences in atopic children. J Allergy Clin Immunol. 2012;129:199-206 e1-4.

35. Prescott SL, Macaubas C, Holt BJ, et al. Transplacental priming of the human immune system to environmental allergens: universal skewing of initial T cell responses toward the Th2 cytokine profile. J Immunol. 1998;160:4730-7.

36. Garn H, Neves JF, Blumberg RS, Renz H. Effect of barrier microbes on organ-based inflammation. J Allergy Clin Immunol. 2013;131:1465-78.

37. Round JL, Mazmanian SK. The gut microbiota shapes intestinal immune responses during health and disease. Nat Rev Immunol. 2009;9:313-23.

38. Arpaia N, Campbell C, Fan X, et al. Metabolites produced by commensal bacteria promote peripheral regulatory T-cell generation. Nature. 2013, epub ahead of print.

39. Furusawa Y, Obata Y, Fukuda S, et al. Commensal microbe-derived butyrate induces the differentiation of colonic regulatory T cells. Nature. 2013, epub ahead of print.

40. Smith PM, Howitt MR, Panikov N, et al. The microbial metabolites, short-chain fatty acids, regulate colonic Treg cell homeostasis. Science. 2013;341:569-73.

41. Mazmanian SK, Liu CH, Tzianabos AO, Kasper DL. An immunomodulatory molecule of symbiotic bacteria directs maturation of the host immune system. Cell. 2005;122:107-18.

42. Mazmanian SK, Round JL, Kasper DL. A microbial symbiosis factor prevents intestinal inflammatory disease. Nature. 2008;453:620-5.

43. He B, Xu W, Santini PA, et al. Intestinal bacteria trigger T cell-independent immunoglobulin A(2) class switching by inducing epithelial-cell secretion of the cytokine APRIL. Immunity. 2007;26:812-26.

44. Cerutti A, Chen K, Chorny A. Immunoglobulin responses at the mucosal interface. Annual review of immunology. 2011;29:273-93.

45. Brandtzaeg P. Food allergy: separating the science from the mythology. Nature reviews Gastroenterology & hepatology. 2010;7:380-400.

46. Mantis NJ, Rol N, Corthesy B. Secretory IgA's complex roles in immunity and mucosal homeostasis in the gut. Mucosal Immunol. 2011;4:603-11.

47. Fagerås M, Tomicic S, Voor T, Björkstén B, Jenmalm MC. Slow salivary secretory IgA maturation may relate to low microbial pressure and allergic symptoms in sensitized children. Pediatr Res. 2011;70:572-7.

48. Geuking MB, McCoy KD, Macpherson AJ. Metabolites from intestinal microbes shape Treg. Cell research. 2013, epub ahead of print.

49. Sjögren YM, Tomicic S, Lundberg A, et al. Influence of early gut microbiota on the maturation of childhood mucosal and systemic immune responses. Clin Exp Allergy. 2009;39:1842-51.

50. Peterson DA, McNulty NP, Guruge JL, Gordon JI. IgA response to symbiotic bacteria as a mediator of gut homeostasis. Cell Host Microbe. 2007;2:328-39.

51. Mathias A, Duc M, Favre L, et al. Potentiation of polarized intestinal Caco-2 cell responsiveness to probiotics complexed with secretory IgA. The Journal of biological chemistry. 2010;285:33906-13.

52. Bollinger RR, Everett ML, Palestrant D, et al. Human secretory immunoglobulin A may contribute to biofilm formation in the gut. Immunology. 2003;109:580-7.

53. Bollinger RR, Everett ML, Wahl SD, et al. Secretory IgA and mucin-mediated biofilm formation by environmental strains of Escherichia coli: role of type 1 pili. Molecular immunology. 2006;43:378-87.

54. Brandtzaeg P. Role of local immunity and breastfeeding in mucosal homeostasis and defence against infections. In: Calder PC, Field CJ, Gill H, editors. Nutrition and Immune Function. vol. 1. Oxon, UK: CABI Publishing; 2002. p. 273–320.

55. Latcham F, Merino F, Lang A, et al. A consistent pattern of minor immunodeficiency and subtle enteropathy in children with multiple food allergy. J Pediatr. 2003;143:39-47.

56. Lundell AC, Hesselmar B, Nordström I, et al. High circulating immunoglobulin A levels in infants are associated with intestinal toxigenic Staphylococcus aureus and a lower frequency of eczema. Clin Exp Allergy. 2009;39:662-70.

57. Taylor B, Norman AP, Orgel HA, et al. Transient IgA deficiency and pathogenesis of infantile atopy. Lancet. 1973;2:111-3.

58. Böttcher MF, Häggström P, Björkstén B, Jenmalm MC. Total and allergen-specific immunoglobulin A levels in saliva in relation to the development of allergy in infants up to 2 years of age. Clin Exp Allergy. 2002;32:1293-8.

59. Kaufman HS, Hobbs JR. Immunoglobulin deficiencies in an atopic population. Lancet. 1970;2:1061-3.

60. Sandin A, Björkstén, Böttcher MF, et al. High salivary secretory IgA antibody levels are associated with less late-onset wheezing in IgE-sensitized infants. Pediatr Allergy Immunol. 2011;22:477-81.

61. Kukkonen K, Kuitunen M, Haahtela T, et al. High intestinal IgA associates with reduced risk of IgE-associated allergic diseases. Pediatr Allergy Immunol. 2010;21(1 Pt 1):67-73.

62. Maslowski KM, Mackay CR. Diet, gut microbiota and immune responses. Nat Immunol. 2011;12:5-9.

63. Greer RL, Morgun A, Shulzhenko N. Bridging immunity and lipid metabolism by gut microbiota. J Allergy Clin Immunol. 2013;132:253-62; quiz 63.

64. Schaub B, Liu J, Hoppler S, et al. Maternal farm exposure modulates neonatal immune mechanisms through regulatory T cells. J Allergy Clin Immunol. 2009;123:774-82 e5.

65. Vuillermin PJ, Ponsonby AL, Saffery R, et al. Microbial exposure, interferon gamma gene demethylation in naive T-cells, and the risk of allergic disease. Allergy. 2009;64:348-53.

66. Böttcher MF, Björkstén B, Gustafson S, Voor T, Jenmalm MC. Endotoxin levels in Estonian and Swedish house dust and atopy in infancy. Clin Exp Allergy. 2003;33:295-300.

67. Kwon HK, Lee CG, So JS, et al. Generation of regulatory dendritic cells and CD4+Foxp3+ T cells by probiotics administration suppresses immune disorders. Proc Natl Acad Sci U S A. 2010 Feb 2;107(5):2159-64.

68. Sjögren YM, Jenmalm MC, Böttcher MF, Björkstén B, Sverremark-Ekström E. Altered early infant gut microbiota in children developing allergy up to 5 years of age. Clin Exp Allergy. 2009;39:518-26.

69. Ege MJ, Mayer M, Normand AC, et al. Exposure to environmental microorganisms and childhood asthma. N Engl J Med. 2011;364:701-9.

70. Jenmalm MC. Childhood immune maturation and allergy development: regulation by maternal immunity and microbial exposure. American journal of reproductive immunology. 2011;66 Suppl 1:75-80.

71. Jenmalm MC, Duchen K. Timing of allergy-preventive and immunomodulatory dietary interventions - are prenatal, perinatal or postnatal strategies optimal? Clin Exp Allergy. 2013;43:273-8.

72. Gerhold K, Avagyan A, Seib C, et al. Prenatal initiation of endotoxin airway exposure prevents subsequent allergen-induced sensitization and airway inflammation in mice. J Allergy Clin Immunol. 2006;118:666-73.

73. Reiprich M, Rudzok S, Schutze N, et al. Inhibition of endotoxin-induced perinatal asthma protection by pollutants in an experimental mouse model. Allergy. 2013;68:481-9.

74. Conrad ML, Ferstl R, Teich R, et al. Maternal TLR signaling is required for prenatal asthma protection by the nonpathogenic microbe Acinetobacter lwoffii F78. The Journal of experimental medicine. 2009;206:2869-77.

75. Blumer N, Sel S, Virna S, et al. Perinatal maternal application of Lactobacillus rhamnosus GG suppresses allergic airway inflammation in mouse offspring. Clin Exp Allergy. 2007;37:348-57.

76. Douwes J, Cheng S, Travier N, et al. Farm exposure in utero may protect against asthma, hay fever and eczema. Eur Respir J. 2008;32:603-11.

77. Ege MJ, Bieli C, Frei R, 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.

78. Jaenisch R, Bird A. Epigenetic regulation of gene expression: how the genome integrates intrinsic and environmental signals. Nature genetics. 2003;33 Suppl:245-54.

79. Suarez-Alvarez B, Rodriguez RM, Fraga MF, Lopez-Larrea C. DNA methylation: a promising landscape for immune system-related diseases. Trends in genetics : TIG. 2012;28:506-14.

80. Janson PC, Winerdal ME, Winqvist O. At the crossroads of T helper lineage commitment-Epigenetics points the way. Biochimica et biophysica acta. 2009;1790:906-19.

81. Janson PC, Linton LB, Bergman EA, et al. Profiling of CD4+ T cells with epigenetic immune lineage analysis. J Immunol. 2011;186:92-102.

82. Vahedi G, A CP, Hand TW, et al. Helper T-cell identity and evolution of differential transcriptomes and epigenomes. Immunological reviews. 2013;252:24-40.

83. Janson PC, Winerdal ME, Marits P, et al. FOXP3 promoter demethylation reveals the committed Treg population in humans. PLoS One. 2008;3:e1612.

84. Jenmalm MC, Björkstén B. Cord blood levels of immunoglobulin G subclass antibodies to food and inhalant allergens in relation to maternal atopy and the development of atopic disease during the first 8 years of life. Clin Exp Allergy. 2000;30:34-40.

85. Sandberg M, Frykman A, Ernerudh J, et al. Cord blood cytokines and chemokines and development of allergic disease. Pediatr Allergy Immunol. 2009;20:519-27.

86. Rautava S, Luoto R, Salminen S, Isolauri E. Microbial contact during pregnancy, intestinal colonization and human disease. Nature reviews Gastroenterology & hepatology. 2012;9:565-76.

87. Godfrey KM, Lillycrop KA, Burdge GC, Gluckman PD, Hanson MA. Epigenetic mechanisms and the mismatch concept of the developmental origins of health and disease. Pediatr Res. 2007;61(5 Pt 2):5R-10R.

88. Martino DJ, Prescott SL. Silent mysteries: epigenetic paradigms could hold the key to conquering the epidemic of allergy and immune disease. Allergy. 2010;65:7-15.

89. Prescott SL. Early-life environmental determinants of allergic diseases and the wider pandemic of inflammatory noncommunicable diseases. J Allergy Clin Immunol. 2013;131:23-30.

90. Funkhouser LJ, Bordenstein SR. Mom knows best: the universality of maternal microbial transmission. PLoS biology. 2013;11:e1001631.

91. Bearfield C, Davenport ES, Sivapathasundaram V, Allaker RP. Possible association between amniotic fluid micro-organism infection and microflora in the mouth. BJOG. 2002;109:527-33.

92. 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.

93. Steel JH, Malatos S, Kennea N, et al. Bacteria and inflammatory cells in fetal membranes do not always cause preterm labor. Pediatr Res. 2005;57:404-11.

94. Jimenez E, Fernandez L, Marin ML, et al. Isolation of commensal bacteria from umbilical cord blood of healthy neonates born by cesarean section. Curr Microbiol. 2005;51:270-4.

95. Gosalbes MJ, Llop S, Valles Y,et al. Meconium microbiota types dominated by lactic acid or enteric bacteria are differentially associated with maternal eczema and respiratory problems in infants. Clin Exp Allergy. 2013;43:198-211.

96. Hu J, Nomura Y, Bashir A, et al. Diversified microbiota of meconium is affected by maternal diabetes status. PLoS One. 2013;8:e78257.

97. Jimenez E, Marin ML, Martin R, et al. Is meconium from healthy newborns actually sterile? Research in microbiology. 2008;159:187-93.

98. Dominguez-Bello MG, Costello EK, Contreras M, et al. Delivery mode shapes the acquisition and structure of the initial microbiota across multiple body habitats in newborns. Proc Natl Acad Sci U S A. 2010;107:11971-5.

99. Cabrera-Rubio R, Collado MC, Laitinen K, et al. 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.

100. Tsuda H, Michimata T, Hayakawa S, et al. A Th2 chemokine, TARC, produced by trophoblasts and endometrial gland cells, regulates the infiltration of CCR4+ T lymphocytes into human decidua at early pregnancy. American journal of reproductive immunology. 2002;4:1-8.

101. Guo PF, Du MR, Wu HX, et al. Thymic stromal lymphopoietin from trophoblasts induces dendritic cell-mediated regulatory TH2 bias in the decidua during early gestation in humans. Blood. 2010;116:2061-9.

102. Svensson J, Jenmalm MC, Matussek A, et al. Macrophages at the fetal-maternal interface express markers of alternative activation and are induced by M-CSF and IL-10. J Immunol. 2011;187:3671-82.

103. Mjösberg J, Berg G, Jenmalm MC, Ernerudh J. FOXP3+ regulatory T cells and T helper 1, T helper 2, and T helper 17 cells in human early pregnancy decidua. Biology of reproduction. 2010;82:698-705.

104. Erlebacher A. Immunology of the maternal-fetal interface. Annual review of immunology. 2013;31:387-411.

105. Boij R, Svensson J, Nilsson-Ekdahl K, et al. Biomarkers of coagulation, inflammation, and angiogenesis are independently associated with preeclampsia. American journal of reproductive immunology. 2012;68:258-70.

106. Hansel TT, Johnston SL, Openshaw PJ. Microbes and mucosal immune responses in asthma. Lancet. 2013;381:861-73.

107. Abrahamsson TR, Sandberg Abelius M, et al. A Th1/Th2-associated chemokine imbalance during infancy in children developing eczema, wheeze and sensitization. Clin Exp Allergy. 2011;41:1729-39.

108. Rose S, Lichtenheld M, Foote MR, Adkins B. Murine neonatal CD4+ cells are poised for rapid Th2 effector-like function. J Immunol. 2007;178:2667-78.

109. Abelius MS, Ernerudh J, Berg G, et al. High cord blood levels of the T-helper 2-associated chemokines CCL17 and CCL22 precede allergy development during the first 6 years of life. Pediatr Res. 2011;70:495-500.

110. Böttcher MF, Jenmalm MC, Björkstén B. Immune responses to birch in young children during their first 7 years of life. Clin Exp Allergy. 2002;32:1690-8.

111. Björkstén B, Naaber P, Sepp E, Mikelsaar M. The intestinal microflora in allergic Estonian and Swedish 2-year-old children. Clin Exp Allergy. 1999;29:342-6.

112. 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.

113. Johansson MA, Sjögren YM, Persson JO, Nilsson C, Sverremark-Ekström E. Early colonization with a group of Lactobacilli decreases the risk for allergy at five years of age despite allergic heredity. PLoS One. 2011;6:e23031.

114. Penders J, Thijs C, Mommers M, et al. Intestinal lactobacilli and the DC-SIGN gene for their recognition by dendritic cells play a role in the aetiology of allergic manifestations. Microbiology. 2010;156(Pt 11):3298-305.

115. van Nimwegen FA, Penders J, Stobberingh EE, 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.

116. Food and Agriculture Organization WHOFW. Report of Joint FAO/WHO expert consultation on evaluation of health and nutritional properties of probiotics in food including powder milk with live lactic acid bacteria. 2001.

117. Prescott SL, Björkstén B. Probiotics for the prevention or treatment of allergic diseases. J Allergy Clin Immunol. 2007;120:255-62.

118. Forsberg A, Abrahamsson TR, Björkstén B, Jenmalm MC. Pre- and post-natal Lactobacillus reuteri supplementation decreases allergen responsiveness in infancy. Clin Exp Allergy. 2013;43:434-42.

119. Prescott SL, Dunstan JA, Hale J, et al. Clinical effects of probiotics are associated with increased interferon-gamma responses in very young children with atopic dermatitis. Clin Exp Allergy. 2005;35:1557-64.

120. West CE, Hernell O, Andersson Y, Sjöstedt M, Hammarström ML. Probiotic effects on T-cell maturation in infants during weaning. Clin Exp Allergy. 2012;42:540-9.

121. Patel RM, Myers LS, Kurundkar AR, et al. Probiotic bacteria induce maturation of intestinal claudin 3 expression and barrier function. Am J Pathol. 2012;180:626-35.

122. Rosenfeldt V, Benfeldt E, Valerius NH, Paerregaard A, Michaelsen KF. Effect of probiotics on gastrointestinal symptoms and small intestinal permeability in children with atopic dermatitis. J Pediatr. 2004;145:612-6.

123. Prescott SL, West CE. Prebiotics and probiotics for treatment of allergic disease. In: UptoDate, Basedow D (Ed), UptoDate, Waltham, MA, 2013. Accessed: 28 Nov 2013.

124. Tang ML, Lahtinen SJ, Boyle RJ. Probiotics and prebiotics: clinical effects in allergic disease. Curr Opin Pediatr. 2010;22:626-34.

125. Doege K, Grajecki D, Zyriax BC, et al. Impact of maternal supplementation with probiotics during pregnancy on atopic eczema in childhood--a meta-analysis. Br J Nutr. 2012;107:1-6.

126. Foolad N, Brezinski EA, Chase EP, Armstrong AW. Effect of nutrient supplementation on atopic dermatitis in children: a systematic review of probiotics, prebiotics, formula, and fatty acids. JAMA dermatology. 2013;149:350-5.

127. Pelucchi C, Chatenoud L, Turati F, et al. Probiotics supplementation during pregnancy or infancy for the prevention of atopic dermatitis: a meta-analysis. Epidemiology. 2012;23:402-14.

128. Fiocchi A, Burks W, Bahna SL, et al. Clinical Use of Probiotics in Pediatric Allergy (CUPPA): A World Allergy Organization Position Paper. The World Allergy Organization journal. 2012;5:148-67.

129. West CE, Prescott, S.L. Prebiotics and probiotics in prevention of allergic disease. In: UptoDate, Basedow D (Ed), UptoDate, Waltham, MA, 2013. Accessed: 28 Nov 2013.

130. Azad MB, Coneys JG, Kozyrskyj AL, et al. Probiotic supplementation during pregnancy or infancy for the prevention of asthma and wheeze: systematic review and meta-analysis. Bmj. 2013;347:f6471.

131. Abrahamsson TR, Jakobsson T, Björkstén B, Oldaeus G, Jenmalm MC. No effect of probiotics on respiratory allergies: a seven-year follow-up of a randomized controlled trial in infancy. Pediatr Allergy Immunol. 2013;24:556-61.

132. Jensen MP, Meldrum S, Taylor AL, Dunstan JA, Prescott SL. Early probiotic supplementation for allergy prevention: Long-term outcomes. J Allergy Clin Immunol. 2012;130:1209-1211.e5

133. 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.

134. Kuitunen M, Kukkonen K, Juntunen-Backman K, et al. Probiotics prevent IgE-associated allergy until age 5 years in cesarean-delivered children but not in the total cohort. J Allergy Clin Immunol. 2009;123:335-41.

135. Loo EX, Llanora GV, Lu Q, et al. Supplementation with Probiotics in the First 6 Months of Life Did Not Protect against Eczema and Allergy in At-Risk Asian Infants: A 5-Year Follow-Up. Int Arch Allergy Immunol. 2013;163:25-8.

136. West CE, Hammarström ML, Hernell O. Probiotics in primary prevention of allergic disease - follow-up at 8-9 years of age. Allergy. 2013;68:1015-20.

137. Wickens K, Stanley TV, Mitchell EA, et al. Early supplementation with Lactobacillus rhamnosus HN001 reduces eczema prevalence to 6 years: does it also reduce atopic sensitization? Clin Exp Allergy. 2013;43:1048-57.

138. Kukkonen AK, Kuitunen M, Savilahti E, et al. Airway inflammation in probiotic-treated children at 5 years. Pediatr Allergy Immunol. 2011;22:249-51.

139. Huang YJ. Asthma microbiome studies and the potential for new therapeutic strategies. Curr Allergy Asthma Rep. 2013;13:453-61.

140. Bertelsen RJ, Brantsaeter AL, Magnus MC, et al. Probiotic milk consumption in pregnancy and infancy and subsequent childhood allergic diseases. J Allergy Clin Immunol. 2013, epub ahead of print.

141. Koren O, Goodrich JK, Cullender TC, et al. Host remodeling of the gut microbiome and metabolic changes during pregnancy. Cell. 2012;150:470-80.

142. Neef A, Sanz Y. Future for probiotic science in functional food and dietary supplement development. Current opinion in clinical nutrition and metabolic care. 2013;16:679-87.

143. Bode L. Human milk oligosaccharides: prebiotics and beyond. Nutr Rev. 2009;67 Suppl 2:S183-91.

144. Bode L. Human milk oligosaccharides: every baby needs a sugar mama. Glycobiology. 2012;22:1147-62.

145. Sela DA, Mills DA. Nursing our microbiota: molecular linkages between bifidobacteria and milk oligosaccharides. Trends in microbiology. 2010;18:298-307.

146. Oozeer R, van Limpt K, Ludwig T, et al. Intestinal microbiology in early life: specific prebiotics can have similar functionalities as human-milk oligosaccharides. Am J Clin Nutr. 2013;98:561S-71S.

147. Lomax AR, Calder PC. Prebiotics, immune function, infection and inflammation: a review of the evidence. Br J Nutr. 2009;101:633-58.

148. West CE. Prebiotics in infancy and childhood; clinical research warranted. Br J Nutr. 2011;106:1628-9.

149. Osborn DA, Sinn JK. Prebiotics in infants for prevention of allergy. Cochrane Database Syst Rev. 2013;3:CD006474.

150. Scholtens PA, Alliet P, Raes M, et al. Fecal secretory immunoglobulin A is increased in healthy infants who receive a formula with short-chain galacto-oligosaccharides and long-chain fructo-oligosaccharides. J Nutr. 2008;138:1141-7.

151. West CE. Gut microbiota and allergic disease: new findings. Curr Opin Clin Nutr Metab Care 2014; 17:261-6.