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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 propionate 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 evolutionarily 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 propionate 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 propionate 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

![Gut Microbiota Diagram](image)

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