



Emerging evidence of the role of gut microbiota in the development of allergic diseases

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Purpose of review

The purpose is to review recent studies examining the role of gut microbiota in allergic diseases and asthma.

Recent findings

Work in experimental models gives further evidence that a disturbed gut microbiota influences the propensity to develop allergic manifestations, and that changing the gut microbiota by dietary means (high fiber/acetate or prebiotics) in pregnancy may reduce the risk of allergic airways disease and food allergy in the offspring, respectively. The gut microbiome in established allergic disease and prior to disease onset has also been assessed in clinical trials. One study demonstrated a strong association between high abundance of *Faecalibacterium prausnitzii* and decreased levels of butyrate and propionate, and established eczema. Lower relative abundance of *Ruminococcaceae* appears to be implicated in food sensitization and to precede the development of atopic eczema. Decreased relative abundance of *Lachnospira*, *Veillonella*, *Faecalibacterium*, and *Rothia* in early infancy was reported to be associated with increased asthma risk. Inoculation of germ-free mice with these genera decreased airway inflammation in their offspring thereby proposing a causal role of bacteria in preventing allergic airways disease.

Summary

Gut microbiome research is an actively developing field. Although candidate bacterial taxa have been reported it still remains unclear which bacteria (or other microbes), in which numbers and combinations, and when during the gut colonization process may prevent allergic diseases and asthma. There is still a call for standardized approaches that will enable direct comparison of different studies.

Keywords

allergic disease, asthma, biodiversity, gut microbiome, homeostasis, immunity

INTRODUCTION

Every human is a habitat for a unique set of micro-organisms (microbiota) [1] and the most abundant and most studied microbiota is found in the gut. The number of bacteria in the human gut exceeds the number of somatic cells by a factor of 10, thus complementing the human genome with at least 100-fold more genes [2]. Despite the fact that the microbiome (microbiota-derived genes) has conserved evolutionary roles, it is also plastic and its composition changes with host age, diet, and environmental exposures [3^{***}]. Normally, the indigenous microbiota and the host have a symbiotic relationship, whereas altered gut microbiota has been associated with immune-mediated diseases, including allergic diseases and asthma [4^{*}].

The first intestinal colonizers are derived from the mother before and during delivery [5,6^{*}] and

there is emerging evidence of an early-life ‘critical window’, when the effects of gut microbial dysbiosis are most influential in immune development [7]. In humans, a plethora of early-life exposures, including delivery mode, perinatal treatment with antimicrobials, infant feeding, and the general environment have the capacity to influence the microbiome composition and disease risk (Fig. 1) [4^{*},8^{**}]. Vaginally delivered infants are primarily colonized by a microbiome enriched for *Escherichia*

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KEY POINTS

- Experimental models show that gut microbiota are critical for normal immune ontogeny and regulation, and suggest that modulation of the gut microbiota may influence susceptibility to allergic diseases and asthma.
- Epidemiological studies and clinical trials report an aberrant gut microbiota to be associated with allergic manifestations and asthma.
- Although candidate bacterial taxa have been reported, it still remains undecided which bacteria (or other microbes) may prevent allergic diseases and asthma.

coli/Shigella, *Bacteroides*, and *Bifidobacterium* [3²²], whereas cesarean-section born infants, who appear to be at higher risk of allergic diseases and asthma [9²³], are typically colonized with skin bacteria, for example, *Staphylococcus* and *Corynebacterium* and have delayed colonization with *Bacteroides* [3²²,10]. Recently, Dominguez-Bello *et al.* [11²⁴] reported that the oral, skin, and anal microbiota of cesarean-section born infants could be partly restored by transfer of maternal vaginal microbes at birth. Undoubtedly, pioneer species influence ecosystem conditions, but whether the restoration with vaginal microbes may have a long-term influence beyond the first month of life remains to be demonstrated.

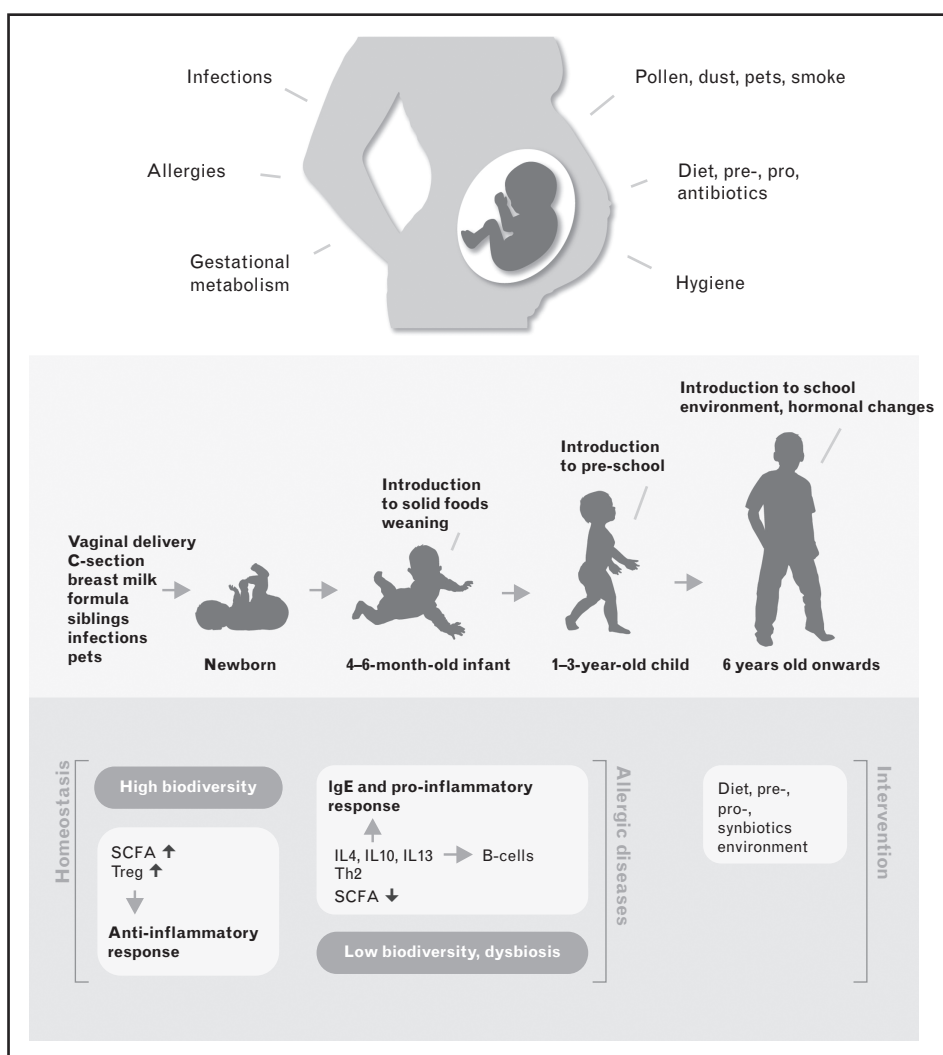


FIGURE 1. Multifactorial cross-talk between the environment and the host. High biodiversity may increase SCFA production; induce regulatory pathways (Treg) and an anti-inflammatory response. Low biodiversity has been associated with reduced SCFA production and a T helper 2 weighted and proinflammatory response. Interventions to favorably influence the intestinal environment include dietary strategies and pre-, pro- and synbiotics. A potential target to restore biodiversity could be through modifications of the environment. SCFA, short-chain fatty acid; Th2, T helper 2.

The step-wise colonization of the gut leads to a more diverse bacterial profile and initiates priming of the immune system, driving tolerance toward nonharmful commensals and dietary antigens, and proper reactions toward invading pathogens (Fig. 1) [4[¶]]. Throughout life there is a constant interaction between external stimuli and the host's immune system at the mucosal surfaces and the gut microbiota is a key player in maintaining homeostasis [12]. Still, it remains unclear which bacteria (or other microbes), in which numbers and combinations, and when, during the whole colonization process, will induce a balanced immune response and protect from the development of immune-mediated diseases. Work in both animal models and in the human setting is aiming to decipher this process and here, we review recent studies examining the role of gut microbiota in allergic diseases and asthma, with an emphasis on human studies.

Animal studies: recent findings

Animal models have contributed greatly to the understanding of the role of gut microbiota in the development of immune-mediated diseases, reviewed by [13[¶]]. Vital *et al.* [14] investigated the development of murine allergic asthma in association with both the gut microbiome and age; young (7–9 weeks) and old mice (>10 months) were sensitized with house dust mite or a vehicle. Changes in microbial community structure with both pulmonary allergy development and age were observed and allergic mice also had higher serum IL-17A compared with controls. As the locally induced allergic airway response was associated with changes in gut microbial composition, this suggests a putative bidirectional gut–lung communication [14]. The authors propose that signals from the inflamed lung may drive changes in the intestinal microbial community structure that further increase inflammation in the lung. Another recent study [15^{¶¶}] found that inoculation of germ-free mice with *Lachnospira*, *Veillonella*, *Faecalibacterium*, and *Rothia* reduced airway inflammation in their offspring, thereby suggesting a role of these taxa in asthma prevention.

Studies on gut microbiota and allergic manifestations

Eczema (or atopic dermatitis) has reached 'epidemic' proportions and is highly associated with a modern lifestyle [16]. Although aberrant interactions between gut microbes and the intestinal immune system have been implicated in this skin disease, the nature of dysfunction remains unclear. In a study by Song *et al.* [17[¶]], the gut microbiome from

132 subjects (from infants to adults), including 90 patients with established eczema, was analyzed. Here, enrichment with species of *Faecalibacterium prausnitzii* was strongly associated with eczema, as were decreased levels of butyrate and propionate, short-chain fatty acids (SCFA) with known anti-inflammatory effects [18,19]. The authors propose that high abundance of *F. prausnitzii* leads to reduced numbers of high butyrate and propionate producers, including those related to the strain A2–165, which ultimately induces aberrant T helper 2 type immune responses to allergens in the skin [17[¶]].

Recent studies have also explored the early-life gut microbiome and the risk of allergies later in childhood. We prospectively examined the gut microbiome in 20 mother–infant pairs in relation to onset of IgE-associated eczema over the first 2.5 years in predisposed children (because of maternal atopy) [20[¶]]. The relative abundance of *Ruminococcaceae* was lower at 1 week of age in infants that developed IgE-associated eczema compared with infants that had no allergic manifestations. As toll-like receptor (TLR)-mediated innate immune response pathways are believed to be vital for the induction of regulatory pathways that suppress exaggerated immune responses in allergic disease, we related the gut microbiome to innate immune responses following activation with specific microbial ligands for TLR2 and TLR4. At this time point, the relative abundance of *Ruminococcus* was inversely associated with TLR2-induced IL-6 and TNF α . There was also an inverse association between Proteobacteria and TLR4-induced inflammatory responses at 1 week and 1 month of age [20[¶]]. Another study that looked into the early-life 'window' used fecal calprotectin as a biomarker of intestinal inflammation and studied its association to the development of allergic disease. High fecal calprotectin at 2 months predicted asthma and eczema by the age of 6 years and was linked to low abundance of fecal *E. coli*. The authors suggest that impaired IL-10 activation because of lack of colonization with *E. coli* could be an explanation for the intestinal inflammation and subsequent risk of asthma and eczema [21]. Collectively, these studies give further evidence that low abundance of potentially immunomodulatory bacteria in infancy are associated with aberrant immune responses and increased risk of allergic diseases.

Two studies from the Canadian Healthy Infant Longitudinal Development study investigators also addressed the question how early gut colonization may contribute to the development of allergic diseases. In their first study [22[¶]], low gut microbiota richness and an elevated *Enterobacteriaceae/Bacteroidaceae* ratio in early infancy were associated with

subsequent development of food sensitization, which could be consistent with another recent study [23] that found food-sensitized children to have reduced numbers of Bacteroidetes bacteria. In the second study [15^{***}] from the Canadian Healthy Infant Longitudinal Development study, infants at risk of asthma exhibited transient gut microbial dysbiosis, with decreased relative abundance of the genera *Lachnospira*, *Veillonella*, *Faecalibacterium*, and *Rothia*. As discussed above, when germ-free mice were inoculated with these four bacterial taxa there was reduced lung inflammation in their offspring, thus possibly demonstrating causation (rather than correlation) between certain bacterial taxa and susceptibility to asthma [15^{***}].

Interventions and reversibility

Dysregulation of the symbiotic host–microbe relationship appears to be a fundamental cause of inflammation and disease development which has led to intense interest in developing prevention and treatment strategies by influencing the gut microbiota [4^{*}].

Probiotics

The WHO and Food and Agriculture Organization of the United Nations define probiotics as ‘live micro-organisms which, when administered in adequate amounts confer a health benefit to the host’ [24]. Recent meta-analyses demonstrate a preventive effect of probiotics on eczema but not on other allergic outcomes [25,26]. The 2015 guidelines from the World Allergy Organization now declare that ‘there is a likely net benefit from using probiotics resulting primarily from prevention of eczema’ in infants at high risk of allergic disease [27], but in concordance with other expert organizations no advice on specific probiotic regimens is given because of the low-quality evidence. Timing, dose, and specific properties of probiotic strains have been suggested as critical factors. There appears to be less benefit of probiotics for treatment of allergic conditions; however, in a large-scale questionnaire survey of Japanese high school students ($n = 21\,802$, response rate 89.3%) regular intake of lactic acid bacteria was associated with the remission of eczema [28]. A review on the current understanding of probiotics for allergy prevention and treatment can be found elsewhere [29^{*}].

Diet, prebiotics, and synbiotics

Diet impacts the gut microbiota and dietary habits have the potential to affect overall intestinal health.

It is well accepted that the composition of the gut microbiota differs depending on the intake of fat, sugar, and fiber. A high-fat/low-fiber diet is accompanied by an increase in the Firmicutes/Bacteroidetes ratio, whereas a high-fiber diet leads to the opposite pattern and increased concentration of SCFA [30]. Thorburn *et al.* [31^{***}] showed that a high-fiber diet influences the gut microbiota composition and increases acetate concentrations in mice. High-fiber or acetate feeding also suppressed experimental allergic airways disease by increasing T-regulatory cell numbers and function. Of note, feeding pregnant mice a high-fiber/acetate diet suppressed the expression of genes linked to both human asthma and experimental allergic airways disease in the offspring. This suggests that influencing the gut microbiota by dietary means, also in pregnancy, may prevent asthma.

Another approach to influence the gut microbiota is by feeding prebiotics. Prebiotics are nutritional supplements, typically nondigestible fiber compounds, designed to promote the growth and functions of bacteria. Prebiotics have the potential to modify immune development in early life [4^{*}] and enrichment of a diet with fibers and oligosaccharides may increase the pool of SCFA in the gut, which in turn beneficially influences immunity and metabolism [32^{***}]. Bouchaud with colleagues [33] used wild-type Balb/c mice to investigate if an early-life prebiotic intervention could prevent allergy. Food supplementation with a galacto-oligosaccharides/inulin mixture during pregnancy and lactation increased bacterial load in both mothers and pups, with higher proportions of *Lactobacillus* and *Clostridium leptum* in pups. As the pups of prebiotic-treated mothers were protected against food allergy and had stronger intestinal permeability and dampened T helper 2 responses [33], this gives further support for the theory that influencing the maternal diet in pregnancy and lactation may prevent allergy development in the offspring. In the human setting there is less evidence however. In the most recent meta-analysis of synbiotics for treatment and primary prevention of eczema, the scoring atopic dermatitis index was: -6.56 (95% confidence interval, -11.43 to -1.68 ; $P = 0.008$) following 8 weeks of synbiotic treatment (six studies, 369 children) [34]. For primary prevention, the pooled relative risk ratio of eczema following synbiotic treatment versus placebo was 0.44 (95% confidence interval, 0.11 to 1.83 ; $P = 0.26$) (two studies, 1320 children). The authors concluded that the results support the use of synbiotics for eczema treatment in children (particularly if the synbiotic contains a mix of bacterial strains, and in children >1 year of age) [34]. As commented by Tang and Lodge [35], the results are



FIGURE 2. Major steps to consider while conducting a microbiota study include the collection and storage of samples, choice of DNA extraction and amplification methods, selection of primers, sequence data analysis for classification and identification of microbial groups, bioinformatics tools, and statistical analysis. Findings should be published and data made publicly available to enable future comparisons between studies. Adapted from [36].

limited by few studies and significant heterogeneity. Thus, despite the encouraging findings in relation to eczema treatment, there is currently insufficient evidence to recommend synbiotics for treatment and prevention of eczema [35].

METHODOLOGIES TO ASSESS THE GUT MICROBIOTA

The possibility to use culture-independent tools to identify different microbes has drastically changed the view of microbial ecology. Application of recently developed specific, sensitive and culture-free methods enables the investigation of complex populations of microbes and their role in health and disease [4[■]]. Human microbiome research is developing rapidly and advances in the field rely on well-executed, controlled and comparable studies (Fig. 2); for a comprehensive review see [36]. Microbiome data are commonly described in terms of relative abundance, richness, and evenness of bacterial taxa within a sample. α -diversity is a compound measure where the Shannon index, which empathizes richness and the Simpson index, which is more weighted toward evenness are frequently used [37]. β -diversity estimates the bacterial diversity between the samples and can be estimated using Bray–Curtis, weighted and unweighted UniFrac or the Jaccard index [36].

It should be stressed that the choice of sequencing platform, the sequencing depth, and how data are preprocessed (e.g. if subsampling is applied) will affect these measures and may have an impact on the final result [38,39]. Collectively, there are many aspects that influence the results and there is a need to develop methods and strategies that allow us to effectively combine and compare results from different studies (Fig. 2) [4[■],36].

CONCLUSIONS

Even though the microbiota research field is growing, there is still a relatively small number of studies that has assessed the gut microbiome in allergic diseases and asthma using new systems biology methods. Ongoing studies are anticipated to deepen

our knowledge of the functional capacities of the gut microbiota and the role of microbes in health and disease. To further our understanding, collaborators from multiple fields, for example, medicine, molecular biology, bioinformatics, and statistics is key to achieve high quality, reproducible, and meaningful results. Ultimately, this knowledge may then underlie new guidelines for disease prevention and treatment by influencing the gut microbiota [4[■]].

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Conflicts of interest

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