



The role of the microbiome in childhood asthma

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It is now well established that the healthy bronchial tree contains a microbiome distinct from that of the upper respiratory tract and that the lung microbiome may be dysregulated in individuals with a chronic respiratory disease, such as asthma. In addition, after birth, gut microbes interact with the host tissue, especially with the lymphatic tissue, thereby guaranteeing efficient immune activation. This review focuses on the available literature on the relationships between the gut microbiome, immune function and asthma in childhood, as well as the therapeutic strategies aimed at acting on the modulation of the microbiome.

First draft submitted: 28 August 2017; Accepted for publication: 30 August 2017; Published online: 14 November 2017

Keywords: asthma • childhood • microbiome

Not long ago, the airways were thought to be germ free. This notion was based on the ineffectiveness of standard microbial culture techniques to reproduce the lung habitat. On the other hand, it is now well established that the healthy bronchial tree contains a characteristic microbiome distinct from that of the upper respiratory tract. Importantly, the lung microbiome may be dysregulated in individuals with a chronic respiratory disease. Furthermore, after birth, gut microbes interact with the host tissue, especially with the lymphatic tissue, and this fact is crucial for guaranteeing efficient immune activation and developing the lymphoid structures. In this review, we will discuss the available literature regarding the relationships between the gut microbiome, development of immune function and predisposition to allergic sensitization and asthma in childhood.

The infant gut microbiome & immune system development

The gut microbiome is an important factor for human health [1] due to its role in the immunity development and behavior of the host [2,3]. Host genetic profile has been related to differences in microbiome composition, thereby suggesting that host genetics can shape the gut microbiome of the host. However, cause–effect relationships behind these links are not yet clear. A survey of the possible mechanisms allowing host genetics to shape microbiota composition in the gut has demonstrated the major role of metabolic functions and the immune system [4]. A considerable impact of other factors, such as diet, may overbalance the effects of host genetic background. Further studies are needed to provide a better understanding of the relations between host genetic profile and gut microbiome composition [4].

The initial colonization of the human gut microbiome begins *in utero*. Culture-based and culture-independent studies have suggested that the fetus is not sterile, since bacteria of the genera *Enterococcus*, *Streptococcus*, *Staphylococcus* and *Propionibacterium* have been isolated in the umbilical cord [5] and meconium samples [6,7] as well as DNA of *Bifidobacterium* and *Lactobacillus* from human placenta [8]. Birth mode (vaginal delivery versus cesarean section; CS) may affect gut microbiome composition in the first 6 months of life [9,10]. Notably, Dominguez-Bello *et al.* [10] found that vaginally delivered infants acquired bacterial communities from the mother's vaginal microbiome dominated by *Lactobacillus* and *Prevotella*, whereas CS infants harbored bacterial communities similar to those found on the skin surface, dominated by *Staphylococcus*, *Corynebacterium* and *Propionibacterium* spp. Despite some studies

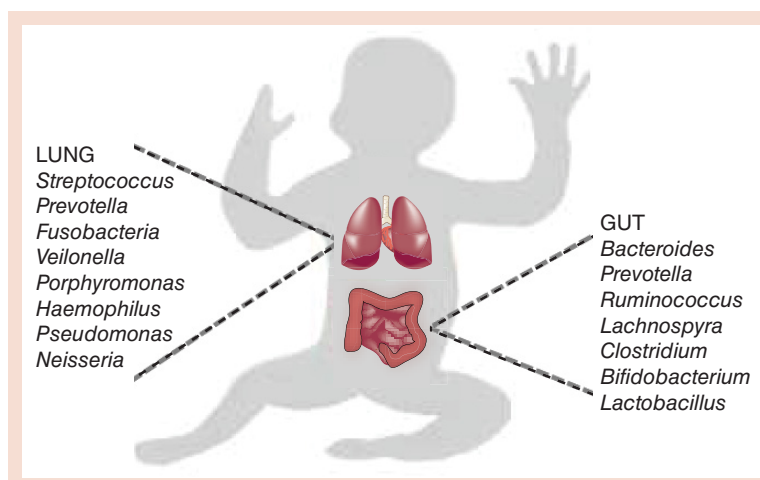


Figure 1. The major bacterial populations in the lung and gut microbiome of the healthy children.

reporting an increased risk of asthma [11], obesity [12], celiac disease [13] and Type 1 diabetes [13] in children born via CS compared with vaginally delivered babies, however, the causality of this relation remains to be demonstrated.

Nutrition also has a major role in the shaping of gut microbiome composition during the first year of life. Backhed *et al.* [14] reported that exclusively breast-fed infants at 4 months had increased levels of *Lactobacilli* and *Bifidobacteria*, while 4-month-old formula-fed infants had elevated levels of *Clostridium difficile*, *Granulicatella adiacens*, *Citrobacter* spp., *Enterobacter cloacae* and *Bilophila wadsworthia*. The chemical composition of breast milk influences the gut microbiome through supplying unique oligosaccharides that are selectively utilized by *Bifidobacterium* spp. [15] and are not digested by the newborn [16].

With the progression of complementary feeding, the gut microbial composition changes and total diversity increases, probably because of the increased fiber and protein intake [17]. The complete transition to family food is characterized by an increased abundance of *Bacteroidaceae*, and a further reduction in *Bifidobacterium* [18,14], and by the third year of life, the microbiome of the infant resembles that of a stable adult microbiome (Figure 1) [19].

Antibiotic treatment is also recognized as one of the most important postnatal factors modulating the microbiome in early life. While assessing the effects of antibiotic exposure on the intestinal microbiome from birth over 2 years of life in a cohort of 43 healthy urban US infants, Bokulich *et al.* [20] found that antibiotic exposures alter bacterial diversity and delay microbiome maturation. In addition, comparing Finnish children aged 2–36 months with and without antibiotic treatments Yassour *et al.* [21] showed that antibiotic exposures affect intestinal microbiome stability and diversity.

By the age of 3 years, both the gut microbiome [19] and the major components of the immune system [22] reached their structural and functional maturity. In this temporal window, a balanced interaction between the host's microbiome and the immune system is critical to protect the child from both infectious and inflammatory diseases [23]. Some studies have reported that in early life, an aberrant gut microbiome, so-called dysbiosis, may contribute to food sensitivities, allergic reactions, asthma, diabetes [24], and later in life, to several intestinal inflammatory diseases [25] as well as metabolic disorders [26].

During pregnancy, type 2 T helper (TH2) cells response is dominant in the fetus to avoid rejection. In the neonatal period, the immune system is also shifted toward a TH2 response, which protects against helminth and parasite infections but may promote allergic reactions [27], and is biased against TH1 phenotype to avoid dangerous proinflammatory responses [28], allowing the colonization of the infant's gut by different microbial species. On the other hand, because of this blunted Th1 response, the neonates are more susceptible to infectious diseases. Later on, the gut microbiome gradually drives the transition of the immune system from TH2 toward TH1 phenotype with IgA secretion and inhibition of IgE synthesis [29,30], reducing the risk of allergy and atopy later in life. According to the 'hygiene hypothesis' [31], which suggests that the absence of a robust microbiome may cause a lack of immune tolerance [32], early exposure to specific microorganisms in infancy promotes immune system development and may confer protection against allergic and autoimmune diseases.

The lung microbiome

In 2010, Hilty *et al.* [33] using culture-independent techniques applied both to the bronchoalveolar lavage and to the bronchial brushings in healthy volunteers and patients with asthma, first demonstrated that the healthy bronchial tree contains a characteristic microbiome distinct from that of the upper respiratory tract, and suggested that this microbiome is altered in the asthmatic airways. Further studies confirmed that in subjects with lung diseases, such as chronic obstructive pulmonary disease [33,34], and cystic fibrosis [35], the composition of the lung microbiome is distinct from that of the healthy lung microbiome.

Although it has been estimated that healthy adults breathing >7000 l of air each day inhale each hour between 1500 and 14,000 organisms for a surface area of approximately 75 m² [36], the bacterial biomass in the healthy lung is low and greatly varying when compared with the skin and the gut. Bacterial loads range from 4.5 log copy numbers per milliliter [37] to 8.25 log copy numbers per ml [38] and from 10 to 100 bacterial cells per 1000 human cells in the bronchoalveolar lavage fluid and in lung tissue samples [34], respectively. In healthy subjects, the total bacterial burden of the lung mainly depends on the balance of microbial immigration and elimination [39] with relatively little amount from the reproduction rates [40] because the lung is in general a low-nutrient environment for microbes compared with that of the GI tract.

The primary source of microbial immigration is the upper airways through subclinical microaspiration of secretions, which commonly occurs in healthy adults during sleep [41]. Additionally, microbial immigration can arise from both the inhalation of atmospheric air containing bacterial cells [36] and the direct dispersion of reproducing microbial colonies. Based on the evidence of similar composition of the oral and lung microbiome [40], it is likely that microaspiration is the dominant way of microbial immigration in the lungs. In healthy subjects, elimination of microbes from the lungs is a continuous process that relies on three factors: mucociliary clearance, coughing and immune pathways, including epithelial cells, T cells, antibody-producing B cells, dendritic cell and macrophages [39]. Nevertheless, the lower airways of healthy people are constantly contaminated by bacteria without evidence of infection, which develop when defense mechanisms are impaired [38,42].

The most prevalent phyla in the airways are *Bacteroidetes* and *Firmicutes*, and to a lesser amount *Proteobacteria* and *Actinobacteria* (Figure 1) [43]. Despite considerable regional variation in environmental factors such as oxygen tension, pH, blood perfusion, alveolar ventilation and temperature [44], regional differences of the microbiome within the lungs of healthy subjects are modest and the respiratory tract harbors a homogeneous microbiota that decreases in biomass from the upper to lower tract [43].

Relationship between the lung microbiome & childhood asthma

The hypothesis that bronchial infection might underlie the asthmatic airways was first sustained in 1991 by epidemiologic studies reporting an association between the development of bronchitis or pneumonia during community outbreaks of *Chlamydomphila pneumoniae* and adult-onset asthma [45]. Since then, several studies have investigated airway microbiome in children or adults with asthma, but few studies involved patients in early infancy (0–12 months). The popular study of Bisgaard *et al.* [46] (COPSAC birth cohort) detected a higher rate of wheeze at 5 years of age by means of conventional culture methods in the hypopharynx among children colonized with *Streptococcus pneumoniae*, *Moraxella catarrhalis* and/or *Haemophilus influenzae* at 1 month of life.

In a more recent study, Kloefer *et al.* [47] demonstrated that in children, a coincident identification of *M. catarrhalis* or *S. pneumoniae* was associated with a greater severity of respiratory tract illnesses, including asthma exacerbations attributed to rhinovirus infection. Most of the studies detected microbial agents either by using PCR approaches or by cultured methods, reflecting only a small fraction of existing taxa. The implementation of new culture-independent molecular techniques (mainly detection and sequence analysis of the conserved gene for bacterial 16S ribosomal RNA) has allowed to better characterize the entire microbial diversity in the airways of subjects with asthma as compared with those of subjects without asthma.

According to the study by Hilty *et al.* [33], pathogenic *Proteobacteria*, particularly *Haemophilus* spp., were much more frequent in the bronchi of adults and children with asthma than in controls. Conversely, *Bacteroidetes*, particularly *Prevotella* spp., were more common in control subjects than in patients. *Staphylococcus* was found to be present in the airways of children with difficult asthma. Accordingly, Huang *et al.* [48] confirmed that *Proteobacteria* are more prevalent in the lower airways of asthmatic patients compared with those of healthy control subjects. A bias of both these studies [33,48] could be the regular use of inhaled corticosteroids in asthmatic patients. However, the small study by Marri *et al.* [49] in patients with mild asthma, not regularly taking inhaled corticosteroids, similarly reported a greater prevalence of *Proteobacteria* compared with healthy control subjects. By contrast, *Firmicutes* and

Actinobacteria were found more frequently in samples from nonasthmatic subjects [49]. In a recent study, Teo *et al.* [50] first assessing longitudinally the nasopharyngeal microbiome by means of culture-independent techniques during the first year of life in the prospective cohort Childhood Asthma Study site in Perth, Western Australia, showed that *Streptococcus* colonization at approximately 2 months of age was significantly associated with chronic wheeze at 5 years of age.

The causal relation between airways microbiome and the development of respiratory diseases, such as bronchial asthma is still unclear. An explanation of this relationship might be offered by the 'hygiene hypotheses'. In 1989, Strachan [31] followed >17,000 British children born in 1958 and found an inverse relationship between hay fever and the number of older siblings in the household hypothesizing that allergic diseases could be prevented by early-life infections. Ten years later, Martinez *et al.* [51] suggested that more than any single or specific infection, the global 'microbial burden' to which the infant was exposed in early life could be more important for the protection against asthma and allergies. Since then, several studies have suggested an association between family size, day-care attendance and pet home exposure on the risk of asthma development. Number of siblings [52], exposure to pets in particular dogs [53], and day-care attendance [52] are inversely related to the prevalence of asthma and allergies. Interestingly, Kramer *et al.* [54] in a large cross-sectional survey in school children of East Germany reported that only children entering daycare in the first year of life were at significantly lower risk of allergies than children attending daycare after their second year.

More recent studies have assessed the microbial composition of house dust through measurement of surrogates, such as lipopolysaccharide (or endotoxin), and muramic acid for Gram-negative bacteria and for Gram-positive bacteria, respectively [55]. To support the hypothesis that bacterial exposure in early life may play a key role in the development of childhood asthma, Maier *et al.* [56] evaluated the effects of environmental exposures to the microbial community of household dust on asthma risk. This study first demonstrated that the dominant bacterial populations in household dust are significantly influenced by the presence of domestic animals and day-care attendance [56].

In addition to the indoor bacterial microbiome, a growing number of studies have explored in many countries worldwide the prevalence of asthma and allergies among children living on farms. The first studies published in this issue, enrolling children of rural communities in Switzerland, Austria and southern Germany, showed a protective farm effect on asthma, allergic rhinitis and atopic sensitization [57–59]. The ALEX study [60] arising from the three previous studies confirmed their findings suggesting that the exposure to livestock and consumption of raw milk in the first year of life had significantly stronger protective effects than later exposures.

Similar results arose from the PARSIFAL study [61], which compared the prevalence of asthma and allergic diseases between farm children, children in anthroposophic families (recruited from Steiner schools), and reference children in Austria, Germany, the Netherlands, Sweden and Switzerland. Growing up on a farm with an anthroposophic lifestyle was found to have a protective effect against both asthma and allergic diseases in childhood. More recently, Amish populations, which maintain traditional farming practices without using mechanized technologies, showed a better protection against risk of asthma and allergies in childhood [62]. Furthermore, a high prevalence of asthma and allergic sensitization has been reported in Hutterites, an ethnoreligious group with similar lifestyles and genetic backgrounds as the Amish, but adopting modern farming technologies [62]. Interestingly, the Gabriela study [63] not only confirmed that children living on a farm were at significantly reduced risk of asthma, hay fever, atopic dermatitis and atopic sensitization compared with nonfarm children but also identified cows, straw and farm milk and fodder storage rooms and manure as specific exposure for asthma and atopic dermatitis, respectively. A meta-analysis of epidemiological studies confirmed that the exposure to dairy farming and unprocessed cow's milk may contribute to the reduction of risk of asthma in children [64]. Ege *et al.* [65] demonstrated that increased diversity of microbial communities present in dust obtained from the homes of children living in a farm was inversely related to asthma as compared with that obtained from the homes of children of nonfarmers.

Exposure to home dust and the bacterial content of house dust has also been assessed in urban indoor environments. Recently, in a high-risk birth cohort of urban and low-income children (Urban Environment and Childhood Asthma, URECA) in inner-city areas of the USA, Lynch *et al.* [66] reported that a reduced content of *Firmicutes* and *Bacteroidetes* in house dust was associated with atopy and atopic wheeze at 3 years of life. Moreover, exposure to high levels of cockroach, mouse and cat allergens in the first year of life was most common among children without atopy or wheeze, suggesting that concomitant exposure to high levels of allergens and bacteria in early life might confer protection against asthma and allergy development.

Importantly, the specific period of life during which the child is exposed to a farm environment is of clinical relevance. Early-life exposure to farming is relevant for allergy protection, suggesting effects during immune

Table 1. List of the principal microorganisms in the respiratory tract or in the gut microbiome that may increase or decrease the risk for asthma.

Asthma risk		Asthma protection	
Phylum	Genus/familie	Phylum	Genus/familie
Proteobacteria	<i>Haemophilus</i>	Bacteroidetes	<i>Prevotella</i>
Actinobacteria	Cellulomonadaceae	Proteobacteria	<i>Helicobacter</i>
Firmicutes	<i>Mycoplasma</i>	Firmicutes	<i>Lachnospira</i>
Chlamydiae	<i>Chlamydophila</i>	Firmicutes	<i>Veillonella</i>
Proteobacteria	Sphingomonadaceae	Actinobacteria	<i>Faecalibacterium</i>
Proteobacteria	Oxalobacteraceae	Actinobacteria	<i>Rothia</i>
Proteobacteria	Comamonadaceae	Firmicutes	<i>Bifidobacterium</i>
Proteobacteria	Nitrosomonadaceae	Firmicutes	<i>Eubacteria</i>
Proteobacteria	Pseudomonadaceae	Firmicutes	<i>Lactobacillus</i>
Proteobacteria	<i>Moraxella</i>		
Firmicutes	<i>Streptococcus</i>		
Firmicutes	<i>Staphylococcus</i>		
Firmicutes	<i>Alloicoccus</i>		
Actinobacteria	<i>Corynebacterium</i>		
Firmicutes	<i>Clostridium</i>		
Fungi	<i>Rhodotorula candida</i>		

maturation potentially through specific regulatory mechanisms. The PASTURE study [67] including a prospective birth cohort from rural areas of five European countries (Austria, Finland, France, Germany and Switzerland) showed that neonates born to mothers exposed to animal sheds during pregnancy had higher numbers of Treg cells with higher suppressive capacity in cord blood compared with newborns to nonexposed mothers. This fact potentially contributes to a protective effect for the development of allergic diseases subsequently in the life.

Some studies focused on overall diversity of the gut microbiome with contrasting findings. A microbial dysbiosis in the intestine of neonates including the reduction in *Escherichia coli* and *Bifidobacteria* content and increase in *Clostridia* was found to precede asthma development [68]. Abrahamsson *et al.* [69] reported that children with asthma have a reduced intestinal bacterial diversity in the first month after birth compared with children without asthma. Thus, early bacterial diversification in the gut seems to be important perhaps indirectly to prevent asthma development later in life. In contrast, Bisgaard [70] did not find any correlation between increase in intestinal bacterial diversity and reduced asthma risk. Importantly, Arrieta *et al.* [71] showed that infants at risk of asthma exhibited transient gut microbial dysbiosis during the first 100 days of life. Furthermore, the relative abundance of the bacterial genera *Lachnospira*, *Veillonella*, *Faecalibacterium* and *Rothia* was significantly decreased in children at risk of asthma.

Several studies have also utilized dietary interventions in mice to alter the intestinal microbiota and examined subsequent effects on allergic airway inflammation. Thorburn *et al.* [72] showed that mice fed on a high-fiber diet had alterations in the gut microbiome and had suppressed allergic airways disease due to enhancement of Treg cells. These findings were replicated in a recent work by Trompette *et al.* [73], in which a high-fiber diet in mice led to an increase of *Bacteroidaceae* and *Bifidobacteriaceae*, and suppressed allergic airway inflammation following house-dust mite exposure. Taken together, these findings suggest the existence of a gut–lung axis probably operating through the influence of the gut microbiome on the lung immune system [74]. Interestingly, a recent study by Fujimura *et al.* [75] showed that neonatal gut microbiome dysbiosis has the potential to promote CD4⁺ T-cell dysfunction, which is associated with childhood atopy. Table 1 summarizes the principal microorganisms in the respiratory tract or in the gut microbiome that may increase or decrease the risk for asthma.

Therapeutic perspectives

Therapeutic strategies aimed to act on the modulation of the microbiome to prevent asthma and allergic diseases include several different approaches. Oral extracts of associations of killed pathogenic bacteria have been used to avoid respiratory tract infections in Europe [76]. Recently, experimental evidence in mice suggests that bacterial lysates can reduce airway inflammation acting by an increase in Treg cells both in the gut and in the airways [76].

The most promising agent is the bacterial lysate (OM-85 BV), which seems to be able to prevent wheezing episodes in children [77], but so far the evidence for a beneficial effect of bacterial lysates in clinical trials is weak.

Alternative strategies to modulate the gut microbiome might include oral supplementation such as prebiotics and probiotics. Prebiotics are nondigestible oligosaccharides of the human milk (human milk oligosaccharides) which arrive intact in the gut where they exert an immunomodulatory effect stimulating the growth of commensal bacteria [78], such as *Bifidobacteria* and *Lactobacilli*. Some beneficial effects such as the reduction of atopic dermatitis and infections have been observed in both experimental studies and clinical trials with human milk oligosaccharides [79,80], but results were not consistent in all studies.

Probiotics are defined as specific live microbial species that may exert a positive effect on the health of the host. The mechanism of action of probiotics is still poorly understood, even if the most plausible seems to be the regulation of the integrity of the epithelial barrier. Over the last years, the use of probiotics, mainly *Bifidobacteria* and *Lactobacilli*, is becoming increasingly used in children [81]. Although there is some evidence for efficacy of probiotics in the treatment of infectious diarrhea in young children [82], their efficacy for allergic diseases and asthma [83] remains to be clearly established because human studies have reported conflicting findings.

The microbiome composition can also be modulated through dietary interventions [84].

In western countries, high fat and low fruit and vegetable content in diets is correlated with an increase in asthma risk [85] and in obesity prevalence [86]. High consumption of fish, fruit and vegetables as in Mediterranean countries seems to be protective against asthma in childhood [87]. In experimental studies, a high-fiber diet in pregnant mice led to suppression of allergic disease in their offspring by potentiating the function of Treg cells probably due to an increase in short chain fatty acids produced by *Bacteroidaceae* [73]. It has been also shown that a high-fiber diet supports the growth of this family of bacteria [73]. Accordingly, diet might affect immune responses by acting on the gut microbiome, and may represent an approach to prevent asthma. However, a systematic review and meta-analysis on probiotic supplementation during pregnancy or infancy for the prevention of asthma and wheeze found no evidence to support a protective association between perinatal use of probiotics and doctor-diagnosed asthma or childhood wheeze [88].

Conclusion

A healthy microbiome is essential to guarantee the host's well being. Accordingly, a body of evidence supports the notion that early-life dysbiosis might play a role in asthma and atopic disease development. It is of note that even transient changes in the gut microbiome maturation in children may induce long-lasting sequelae for immune system maturation and host health.

Future perspective

Interestingly, a novel approach for the prevention of allergic asthma could be the supplementation with probiotics given to the mother during pregnancy or to the infant in early life when the immune system response is still maturing. However, extended follow-up of existing trials, as well as further clinical and basic research are needed to define the value of probiotics in the prevention of asthma in children.

Financial & competing interests disclosure

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

No writing assistance was utilized in the production of this manuscript.

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Executive summary

The gut microbiome

- The gut microbiome is an important factor for human health, because of its role in the immunity development and behavior of the host.

The lung microbiome

- The airways are not germ free and there is evidence that healthy bronchial tree contains a characteristic microbiome.
- The primary source of microbiome is the subclinical microaspiration of secretions, which commonly occurs in healthy subjects during sleep. The microbial immigration can arise also from the inhalation of atmospheric air containing bacterial cells.

Lung microbiome & childhood asthma

- In individuals with respiratory diseases, such as bronchial asthma, the composition of lung microbiome is distinct from that of the healthy individuals.
- The causal relation between airways microbiome and the development of asthma is still unclear.

Therapeutic strategies

- The modulation of the microbiome to prevent asthma and allergic diseases include several different approaches. Though it has been found that bacterial lysates can reduce airway inflammation acting by an increase in Treg cells both in the gut and in the airways, so far the evidence for a beneficial effect of bacterial lysates in clinical trials is weak.
- Alternative strategies to modulate the gut microbiome consist in dietary interventions, including oral supplementation by means of prebiotics and probiotics.

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