Does lung microbiome play a causal or casual role in asthma?

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Abstract

Asthma is the most common chronic disease in childhood. The pathogenesis of asthma is multifactorial and is thought to include environmental factors interacting with genetics during pregnancy and in the first years of life. In the last decades, a possible role of gut microbiota in allergic disease pathogenesis has been demonstrated. Next generation sequencing techniques have allowed the identification of a distinct microbiome in the healthy lungs. The lung microbiome is characterized by the prevalence of bacteria belonging to the phylum *Bacteroidetes* (mostly *Prevotella* and *Veilonella* spp) in healthy subjects and to the phylum *Proteobacteria* in asthmatics (mostly *Haemophilus*, *Moraxella*, and *Neisseria* spp). In asthma, as well as in other diseases, the lung microbiome composition changes due to a disruption of the delicate balance between immigration and elimination of bacteria. The lung microbiome can interact with the immune system, thus influencing inflammation. Early infections with viruses, such as respiratory syncytial virus, may alter lung microbiome composition favoring the emergence of Proteobacteria, a phylum which is also linked to severity of asthma and bronchial hyperreactivity. Lastly, antibiotics may alter the gut and lung microbiota and potentially disturb the relationship between microbiota and host. Therefore, antibiotics should be prescribed with increasing awareness of their potential harmful effect on the microbiota in young children with and without asthma. The potential effects of probiotics and prebiotics on lung microbiome are unknown.

KEYWORDS
16S ribosomal RNA, lung microbiome, microbiome, microbiota, pediatric asthma

1 | INTRODUCTION

Asthma is one of the most common chronic disease worldwide, affecting almost 300 million people with a continuously increasing prevalence.[1] It is also the main chronic disease in childhood, affecting 10% of children. A great effort is currently being focused on the search for preventive strategies, which seems feasible based on evidence suggesting that the onset of atopy and asthma may be strictly connected to several events occurring in very early stages of life. In particular, exposure to antibiotics during fetal and neonatal period,[2] being born by cesarean section,[3] formula feeding, maternal diet, and the variety of microbes one is exposed to may play a key

Abbreviations: 16S rRNA, 16S ribosomal RNA; BAL, broncho-alveolar lavage; COPD, chronic obstructive pulmonary disease; NGS, next generation sequencing; RSV, respiratory syncytial virus.

TABLE 1 Terminology used in this work

Microbiome	The term refers to bacterial communities associated with the human body identified by molecular methods. While this term is often used interchangeably with microbiota, sometime in a broader sense encompassing also viruses and fungi, in this review the term refers to all the genomes of bacteria found in the human body, analyzed trough the sequencing of 16S rRNA gene.
Microbiota	In the strict sense, the term refers to bacteria associated with the human body studied by traditional culture.
Virome	In the strict sense, the term refers to viruses associated with the human body.

role.[4] The mechanisms proposed by which these factors predispose to asthma are numerous, but what seems be a common feature is their effects on gut microbiota. Indeed, in the last decades many papers have been published on the role of gut dysbiosis in the pathogenesis of allergic diseases. The gut has probably been thoroughly studied because collecting samples is relatively easy compared to other bodily sites.[5] However, researchers have recently demonstrated that specific communities of bacteria are present also in the lungs and that their composition may play a crucial role in the development of asthma, as well as in the development of other respiratory diseases. Studies on the human microbiota have been complicated by a certain degree of confusion in terminology: with the term "microbiota," Authors usually refer to bacteria associated with the human body, while with "microbiome" they refer to the array of genomes of such microbes, as identified by next generation sequencing (NGS) techniques. These terms are often used interchangeably, and sometimes to include all the microorganisms associated to the human body, encompassing also viruses and fungi. As the literature regarding the composition of lung bacterial communities' is based on NGS studies, in this work, as in the great majority of the previous studies on this subject, we will refer to the microbial communities of the lungs as "lung microbiome" (Table 1).[6]

1.1 | Does the lung have its own microbiome?

The theory that healthy lower airways are sterile is one of the dogmas in the history of medicine: however, with the advent of NGS techniques and their application in microbiology, this hypothesis has been reconsidered due to the frequent detection of bacterial communities in healthy lower airways, as well as in other body sites formerly believed to be sterile.[7] As very few of these bacteria, if any, can be isolated in culture, this finding has two important consequences: (1) Traditional culture isolation techniques can no longer be considered the gold standard for bacteria detection and identification. (2) Most body sites probably have their own resident microbial flora. New molecular techniques applied to bacteriology include amplification, sequencing, and analysis of the gene encoding 16S ribosomal RNA (16S rRNA), ie, a small nucleic acid present in multiple copies in bacterial cells and highly conserved among prokaryotes. This gene can be used as a bar-code for definition of phyla and genera in specific databases and allows, therefore, taxonomic investigation of bacterial communities[6] (Figure 1). Hilty et al[8] used NGS for the first time on lower airways samples from human subjects. This study included 24 adults (5 with chronic obstructive pulmonary disease [COPD], 11 with asthma and 8 healthy controls) and 20 children (13 with severe asthma and 7 healthy controls), all in clinical stability.[8] Nasal and pharyngeal swabs were collected from all study subjects, 23 of whom also underwent bronchoscopy with protected brushing and/or bronchoalveolar lavage (BAL). Their results showed that bacteria belonging to the phylum *Bacteroidetes* (*Prevotella* spp in particular) were present, albeit in small amounts, in the airways of healthy subjects. These microorganisms are Gram-negative anaerobic bacteria, that are part of the normal oral and vaginal flora and are not easily isolated by culture. In contrast, subjects with asthma and COPD harbored mainly *Proteobacteria*, including *Haemophilus*, *Moraxella*, and *Neisseria* spp. In addition to showing that sick and healthy individuals have a different microbial flora, this work was the first to report that the bronchial tree of healthy subjects is colonized by bacteria. Further studies confirmed

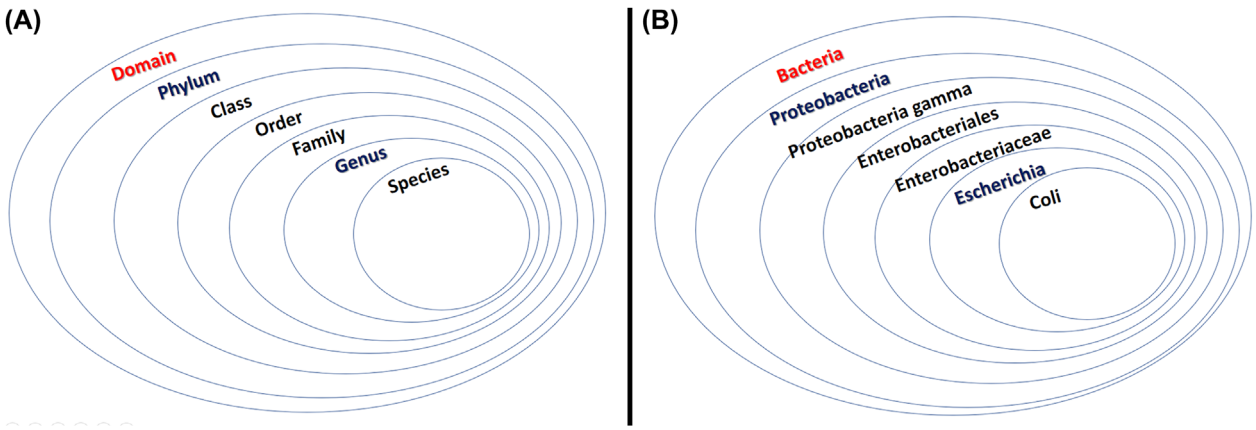

FIGURE 1 A, Bacteria taxonomy; B, Taxonomical classification of *Escherichia coli* as defined the hierarchical organization showed in (A)

this finding and evidenced that phylum *Firmicutes* (especially *Veilonella* spp, but also *Streptococcus* spp) is also highly represented in healthy individuals,[9] showing that the lung microbiome is more dynamic but less varied compared to the gut microbiome. It is now clear that lung microbiome composition depends on the resident flora of the upper airways, which, therefore, represents the main reservoir for lower airways colonization.[9–10] Recently, Dickson et al[11] demonstrated that the oropharynx microbiome is nearly identical to the one of the right upper lung lobe, but differs from that found in the other lobes. This could be explained by the fact that, for anatomical reasons, the right upper lobe is the most susceptible to immigration of bacteria by microaspirations, which are common also in healthy subjects.[12–13] It is therefore possible that bacteria reach the lower airways through repeated microaspirations of oropharyngeal secretions and, to a lesser extent, through diffusion from the mucosa or by direct inhalation.[9] In children, microaspirations also involve secretions from the nasopharynx,[14] that are facilitated by the peculiar anatomy of upper airways and increased production of nasal secretions during childhood.[9,15] Any bias related to the risk of contamination by medical procedures was excluded, demonstrating that the passage of the bronchoscope through the upper airways did not significantly alter results.[11,16] But what happens once the oropharyngeal bacteria reach the lower airways? Dickson et al[17] developed the "adapted island model," which postulates that the composition of lung microbiome is determined by three factors: (1) Migration of bacteria from the upper airways; (2) Elimination of bacteria by mucociliary clearance, cough and host defenses; and (3) Levels of bacterial replication driven by local growth conditions, such as pH, nutrient availability, oxygen tension, and presence, amount, and type of immune cells. In healthy subjects, the lung environment is inhospitable, so that bacterial replication rates are low. Consequently, the microbiome is transient and shaped by continuous migration and purging of bacteria. Respiratory diseases may change local conditions to the point of creating niches that favor persistence and replication of bacteria. Supporting this notion, chronic colonization by bacteria that are difficult to eradicate is frequently observed during severe respiratory ailments in general, especially in advanced stages.[10,17–18] Taken together, these findings demonstrate that the lower respiratory tract does have its own flora that may change according to the individual health status.

1.2 | Lung microbiome and immune response

The role of the lung microbiome in pathogenesis and the natural history of respiratory diseases is incompletely understood. A growing body of evidences suggest that the lung microbiome plays an important role in the development and homeostasis of the immune response. As a consequence, it is conceivable that dysbiosis might facilitate the onset of respiratory diseases[19] and, at the same time, that the immune response affects the composition of pulmonary microbiome.[20] In other words, interactions between the host's immune system and lung microbiome may be crucial for the development of allergy and asthma,[21] as observed for the gut microbiome.

In the mouse model, for example, members of the phylum Bacteroidetes and, in particular, *Prevotella* spp, have been shown to reduce pulmonary inflammation, neutrophil recruitment, and pro-inflammatory cytokine production mediated by Toll-like receptor 2, unlike *Haemophilus influenzae*, which has a proinflammatory influence.[22] Moreover, it has been demonstrated that asymptomatic human adults who exhibit lung microbiome with higher percentage of taxa characteristic of the oral cavity have increased numbers of inflammatory cells in the lower airways mucosa and higher levels of exhaled nitric oxide.[9] Furthermore, in humans, a continuous and diverse exposure to bacteria in the first years of life has been associated with reduced chances of subsequent development of asthma and atopy.[23] It is likely that such exposure occurs mainly at the airways, which represent the widest surface of the human body exposed to the external environment. Recently, a gut-lung axis has been proposed, in analogy to the close relationship between the lung and gut microbiome observed in the mouse model. In mice, the microbial flora resident in the two body sites continuously communicates via blood circulation, allowing reciprocal modulation, brought about by local immune response, and microbiome composition.[20]

1.3 | Lung microbiome and asthma

It is well known that viral infections of the lower airways in the first 3 years of life are associated with increased risk of developing asthma in later years, especially when caused by respiratory syncytial virus (RSV) and rhinovirus.[24] However, only recently the lung microbiome was proposed as having a causal role in the genesis and maintenance of chronic inflammation of the airways. This hypothesis stemmed from the previously cited work by Hilty et al.,[8] performed on asthmatic adults and children, and from yet other studies showing a higher prevalence of phylum *Proteobacteria*, together with a greater diversity in the bacterial composition of the lung microbiome, in both asthmatic children and adults, compared to healthy individuals.[7,8,20] It is interesting to note that, in a prospective study carried out in 2007, 21% of the traditional cultures from hypopharyngeal aspirates from 321 infants, still in good health but at risk of asthma due to asthmatic mothers (cohort of the COPSAC study), were positive for *Moraxella catarrhalis*, *Haemophilus influenzae* (which are members of phylum Proteobacteria) and *Streptococcus pneumoniae*, alone or in combination. The presence of these bacteria was associated with an increased risk of episodic wheezing or asthma at the age of 5.[25] In 2015, the results of the first investigation on the features of the nasopharyngeal microbiome in the first year of life was published.[26] Here, the 16S rRNA sequencing was used in a birth cohort of 234 children at high risk for asthma/allergy (cohort of the Childhood Asthma Study). Nasopharyngeal aspirates were collected at multiple times in the first year of life from these patients, both in healthy conditions or during respiratory infections. The microbiome was found to be mainly composed by the phyla *Proteobacteria*, *Firmicutes*, and *Actinobacteria*, but its percentage composition changed according to the health status: the genera mostly present in patients with respiratory infections were,

again, *Haemophilus*, *Streptococcus*, and *Moraxella spp*; conversely, *Staphylococcus*, *Alloiococcus*, and *Corynebacterium* spp were those most common in health. *Staphylococcus* or *Corynebacterium* were the first genera to appear after birth and, in general, were subsequently replaced by *Moraxella* or *Alloiococcus*, along with the occasional and transient presence of *Moraxella*, *Streptococcus*, and *Haemophilus* during acute viral infections. Follow-up demonstrated that detection of *Streptococcus* within the first 2 months of life is more common in children who will later develop aeroallergen sensitization and it is a predictor of asthma at the age of 5.[26]

Another study examined 33 healthy infants and 99 infants who were assessed during respiratory infection. Analyses, which also included detection of RSV, were performed on nasopharyngeal specimens, pointing out that the microbiome changed during RSV infection, showing an increase in *Proteobacteria* (*Moraxella* and *Haemophilus spp*) and *Streptococcus* and *Corynebacterium* spp.[27] The authors conclude that the well-known higher risk to develop asthma following RSV infection[24] could be associated with the alteration in the composition of the bacterial microbiome induced by RSV. Accordingly, a recent clinical study where the nasopharyngeal microbiome of 106 children with RSV infection and 26 healthy infants was evaluated, further confirmed the higher prevalence of *Streptococcus pneumoniae* and *Haemophilus influenzae* during RSV infection.[28] Moreover, the transcriptome profiles of children with RSV infection and high levels of *Streptococcus pneumoniae* and *Haemophilus influenzae* in the airways were characterized by an increased expression of Toll-like receptor genes as well as by activation of macrophages and neutrophils, which were in turn associated with more serious clinical manifestations.[28,29]

The evidence accrued thus far suggests that an imbalance of the respiratory microbiome favoring higher levels of the phylum *Proteobacteria* and *Streptococcus* spp in the first years of life could contribute to asthma pathogenesis. The dysbiosis could play both a direct effect on the immune response and an indirect effect in modulating concomitant viral infections.[29]

1.4 | Lung microbiome and asthma severity

Lung microbiome may also affect the clinical course of asthma. Dozens of studies have confirmed that lung microbiome in asthmatics under treatment with inhaled steroids and/or rescue bronchodilators shows a higher prevalence of Proteobacteria, along with a greater diversity in bacterial composition compared to healthy subjects.[30] Moreover, Huang et al[31] took protected specimen brushing samples from 65 adult subjects with mild asthma and under suboptimal control (ie, with persistent symptoms on Asthma Control Questionnaire after four weeks of standardized treatment with inhaled fluticasone). They demonstrated that the prevalence of *Proteobacteria* and the diversity of bacterial composition correlate with bronchial hyperreactivity. In the same study, clarithromycin, administered to 16 patients, reduced bronchial hyperreactivity significantly, particularly in subjects with high bacterial diversity prior to treatment. This suggests that the beneficial effect of macrolides observed in some asthmatic patients is a combinatorial result of their intrinsic anti-inflammatory properties, and the antibiotic effect on the lung microbiome.

While the microbiome in subjects with severe asthma is also significantly enriched in Actinobacteria,[32] in patients with severe steroid-resistant asthma *Haemophilus parainfluenzae*, proved to favor steroid resistance in macrophages.[33] New studies on lung microbiome are expected to shed light on many aspects of severe asthma, as well as on non-Th2-mediated asthma, which is typical of adults and is characterized by predominantly neutrophilic inflammation.[34,35]

There is no evidence so far that changes in lung microbiome are involved in asthma exacerbations, but a hypothesis to explain what happens at this level has been proposed. This is called "the dysbiosis-inflammation cycle" model and suggests the existence of a bidirectional interaction between lung microbiome and immune response: according to this model, any inflammatory process (such as the one evoked by a viral infection) would trigger a cascade of events altering the factors regulating the microbiome composition, favoring growth of some bacteria. This would further fuel inflammation and immune responses, thus leading to a vicious circle eventually exacerbating disease. Worsening of the disease, therefore, would not be due to acute infection by a single bacterium emerging from the microbiome, but rather to respiratory dysbiosis causing changes in microbiome composition and dysregulation of inflammation. This would explain why acute bacterial respiratory infections, caused by overgrowth of a single bacterium, respond quickly to antibiotics, while these drugs are ineffective during asthma exacerbations.[17]

1.5 | Future perspectives

Additional studies are warranted to gain further insight on the causal role of bacterial colonization in the development of allergy and asthma. It will be important to analyze the impact of short- and long-term treatment with antibiotics, because antibiotics may alter the gut and lung microbiome, thus interfering with their immunomodulatory action.[36,37] Notably, a few studies have shown some beneficial effects of macrolides on bronchial inflammation,[31] while others suggest that antibiotics may play a causal role in the development of pediatric asthma.[38] Physicians should, therefore, be aware of these potential risks and should prescribe these drugs only when necessary, especially in the case of newborns and infants.[20,29]

Even if the evidence available seems to suggest a causal role of changes in the composition of lung microbiome in asthma pathogenesis, whether such changes are primary or secondary to inflammation has not yet been clarified sufficiently. In this respect, it will be interesting to assess whether diet or probiotics and prebiotics administration may modulate microbiome composition. Some studies have evaluated the effects of these dietary supplements on the gut microbiota and demonstrated some beneficial effects both in treating and preventing asthma.[36] Such data are not yet available for lung microbiome.[36]

On a more general issue, another point to be clarified is whether the bacterial genomes detected by molecular methods in the lower airways belong to resident bacteria or are relics of past infections.

Culture isolation techniques would be the most suitable method to ascertain whether bacteria from respiratory specimens are alive or not, but the available techniques are still unable to reproduce the lung habitat enabling their growth[7]: to this aim, new culture conditions and media need to be developed soon. To advance our knowledge in the near future, data obtained from transcriptional, proteomic and metabolomic analyses will provide gene expression and metabolic data deriving only from living cells.[6]

In any case, even if we were highlighting genes belonging to bacteria no longer present in the airways, this fact still helps interpret the effects of their passage in the airways.[39] Lastly, although most interest is currently directed toward the bacterial microbiome, studies on virome and mycobiome and their interactions with microbiome are also being published.[7,29] The evidence that is emerging, along with what was already known about viruses, will possibly lead to a stronger concept of asthma as a chronic inflammatory condition triggered, exacerbated and maintained by the respiratory microbiome in a broad sense.[39]

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