Narrative Review
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The Airway Microbiome and Childhood Asthma – What Is the Link?

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Abstract
In this paper we propose to describe the available evidence from the literature on upper airway microbiome and its association with paediatric asthma and allergy. Recent advances in sequencing the bacterial 16S ribosomal RNA (16S rRNA) gene have enabled research into the complex communities of bacteria, known as the microbiome, that exist outside and inside the human body. Although the upper airways have long been recognised to host a microbiome, the lower airways are now known to contain a rich and diverse microbiome. This review first describes the microbiome of the upper and lower airways and then explores associations between the microbiome in the airways and bowel and asthma in children. The characteristics of the microbiome differ between nose and mouth, and between the mouth and bronchus in terms of burden and diversity of bacteria and in the predominant phyla present. There is a small literature which suggests that there are differences in the airway microbiome in early life between children who later have asthma compared to those who do not develop asthma. **Conclusion.** At the time of writing it is not clear whether the microbiome may cause childhood asthma, whether the conditions in the asthmatic airway encourage a different microbiome or whether a third factor confounds the relationship between airway microbiome and childhood asthma.

Key Words: Asthma ▪ Child ▪ Microbiome.

Introduction

Bacterial communities outside and inside the human body are complex, and the introduction of 16S rRNA gene sequencing, which amplifies and sequences the 16S rRNA gene in bacterial communities, has given some insight into the "microbiome" and its association with asthma, cystic fibrosis and broncho pulmonary dysplasia (1-3). This methodology has moved us from a position of very little objective microbiological data to one where we have an abundance of data. The "microbiome" describes microorganisms of all types i.e. commensal (healthy), symbiotic and pathogenic bacteria, viruses and fungi that share the human body (4). The human microbiome is found at different body sites, including those in regular communication with the external environment, i.e. the skin, the respiratory tract and the gastrointestinal tract.

One important leap forward in our understanding from this pioneering research has been the understanding that there is a healthy community of bacteria in the lower airways. Previously the lower respiratory tract was considered sterile since bacteria could not be cultured from sputum and bronchoalveolar lavage samples from healthy individuals using older methodologies. The bacteria which are associated with lower respiratory tract infection, e.g. *S pneumoniae* and *H influenzae*, are part of everyone's healthy commensal upper and lower airway microbiome.

Microbial colonization is multifactorial, and it starts at the time of birth and is influenced by many factors including maternal microbiome, mode of birth, breastfeeding, older siblings, pet exposure,

vaccination, infection and antibiotic exposure in the early life. These early exposures influence the composition of the airway microbiome and provide immunity or increase susceptibility to certain infections (5-7). Studies have shown that there is considerable variation in microbiota between individuals and within individuals at the various sites and over time and this variation extends to geographical diversity which may be influenced by factors such as weather, diet and genetics (8, 9).

Asthma is a chronic inflammatory disease of the airways affecting both adults and children. Asthma is a common condition and has an incidence of 0.67/1000 (95% CI 0.6-0.7) in children aged 0-14 years in the Tuzla canton of the Federation of Bosnia and Herzegovina, with a significantly higher incidence in boys compare to the girls (10). In the UK there are over one million children with asthma (11) and one child is admitted to hospital for an asthma attack every 20 minutes (11). Asthma causation is multifactorial with both genes and environment playing an important role.

One environmental exposure implicated in asthma causation is respiratory tract infection by bacteria and virus, but as with many exposures implicated in asthma causation the relationship between asthma and infection is complex. In some cohorts lower respiratory infection is associated with reduced risk for later asthma (12) but in others the severity and frequency of respiratory tract infections in the early years of life have been linked with increased risk for subsequent development of asthma (5, 6). The 'hygiene hypotheses', described in 1989, proposed that reduced infections (from older siblings) may explain the rise in atopic conditions such as hayfever (13), and over time the underlying mechanism has evolved. A recent expert group have proposed that the "Westernised" lifestyle may mean that the immune system's tolerance of commensal bacteria is replaced by an inflammatory response to bacteria which results in asthma (14).

"Circumstantial evidence" leading to a suspicion that a certain different host bacterial communities ("dysbiosis" is a term which describes microbial imbalance) are important to asthma causation comes from studies which link antenatal maternal antibiotic use, delivery by caesarean section and formula feeding with increased risk for asthma (15-17); these associations support the paradigm that the maternal bacterial community is important to the development of asthma by influencing her offspring's own bacterial community. These associations (15-17) do not prove causation and may arise due to confounding, reverse causation and publication bias. The positive association between early antibiotics and later asthma may also be confounded since "asthmatic" episodes of shortness of breath, cough and wheeze may be diagnosed as "infection" in young children and treated with antibiotic. In addition to the confounding, reverse causation and publication bias, much of the literature linking "early bacterial milieu" to asthma is based on surrogates such as self-reported and retrospectively reported breast feeding. Whilst it is highly plausible that the characteristics of the bacteria in the upper and lower airways are important determinants of asthma, what is required is an objective index of the characteristics of those bacterial communities, e.g. species, burden or diversity

Our aims are to identify and review the literature describing the airway microbiome in children and to explore evidence that links the microbiome to asthma outcomes. The literature search was carried out in August 2019 within the human literature using the keywords microbiome, microbiota, asthma, allergy, pediatric asthma, bacteria, airways in OVID MEDLINE. Further studies were sought from the reference lists of the preliminary search results.

Mechanisms for Microbiome to Cause Asthma

There are several proposed mechanisms which explain the presence of a different microbiome in children with asthma compared to children without asthma (18). These mechanisms are not exclusive:

1. Colonisation with an "abnormal" airway microbiome may cause asthma. Dysbiosis is defined as an imbalance or disruption of the microbial diversity and the presence of a "dysbiotic" com-

munity on the airways may interact with local cells (including epithelial and smooth muscle cells) and cause asthma. The dysbiosis may be in the upper or lower respiratory tract (or both). This model would explain how asthma runs in families (assuming each generation is inoculated with dysbiotic microbiome from their parents) and how asthma may have an onset at any age (assuming dysbiosis can develop at any age). See figure one (scenario A).

2. A primary abnormality of the airway epithelium causes asthma and also provides an environment which leads to dysbiosis (19). This putative airway epithelial cell dysfunction leads to a break in the epithelial barrier which introduces inhaled allergens to the immune system and leading to immune sensitisation (20). In this paradigm the epithelial abnormality would explain the association between asthma and allergy, and here the microbiome present is a result of abnormal airways in asthmatic patients and not a cause of asthma. See figure one (scenario B).
3. Epithelial abnormalities (with associated dysbiosis) in the skin or bowel allow immune sensitisation which in some individuals causes asthma. This paradigm requires an intrinsic pulmonary risk factor to explain why the majority of people who are atopic do not have asthma. See figure one (scenario C).

Differences between Upper and Lower Airway Microbiome

A key question which has not been resolved is whether the upper or lower airway microbiome is more relevant to asthma causation. Since the upper airway (defined as proximal to the vocal cords) is more easily accessed, there are more studies de-

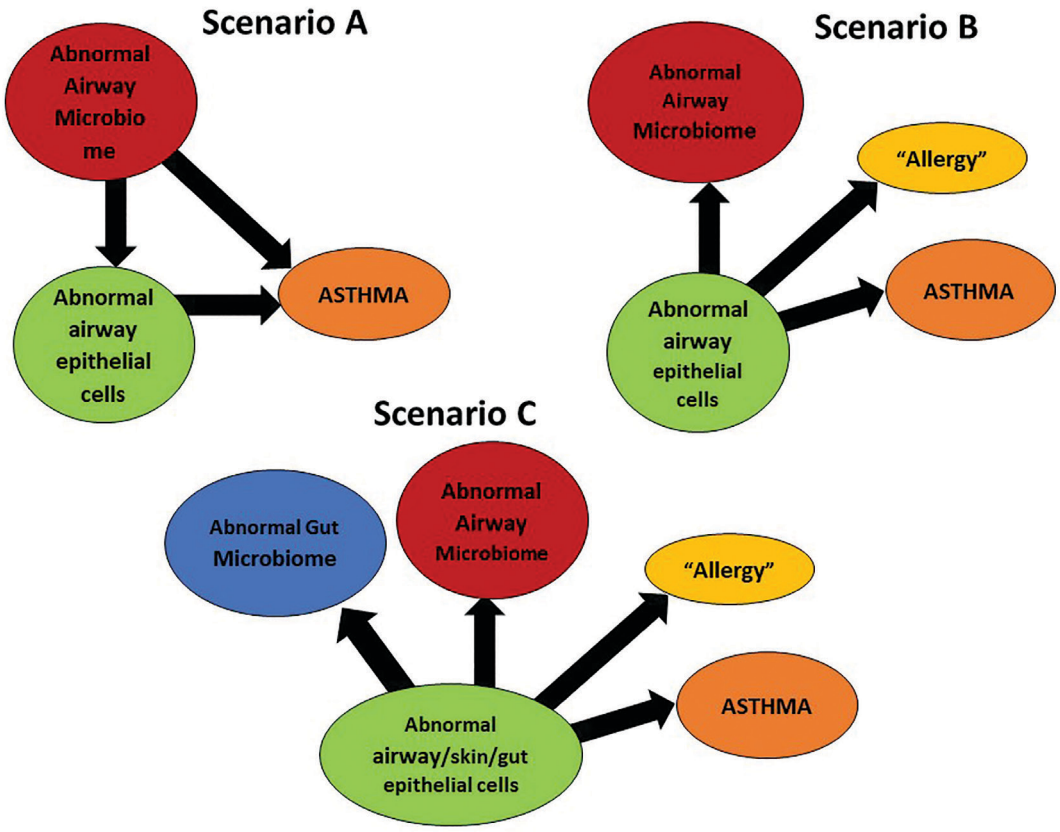

Figure 1. Schematic Diagram Showing Three Different Mechanisms Linking the Airway Microbiome to Childhood Asthma.

scribing associations between the upper respiratory microbiome and asthma outcomes than the lower respiratory microbiome. There are several studies which have compared the microbiome in both upper and lower airways in children with and without respiratory conditions and these demonstrate that the upper airway microbiome is not a reliable index of the lower airway microbiome (3, 21-26). Collectively these studies (3, 21-26) find the greatest difference is between nasal and bronchial microbiome with oro-pharyngeal being intermediate. One group of researchers have suggested using a combination of both nasal and oral microbiota may give a better representation of the lower airway microbiota which could be useful to assess the lower airway without using invasive techniques (21).

These studies make the question whether the upper or lower airway microbiome (at a single time point or over time) is more relevant to asthma causation even more intriguing, since if there are such marked differences between the upper and lower airway microbiome then only one or the other (or neither) is important. The upper airway is the initial point of interaction between inhaled environmental exposures so asthma may develop due to initial interactions in the upper airway, but asthma is a disease of the lower airways and it seems reasonable that mechanisms in the lower airway are more important to asthma causation. In addition, the microbiome not only varies between sites within the human body but also shows variation over time (22). At this point in time we simply do not know the answer to this question and the unified airway concept would argue that the upper and lower airway are essentially the same. What we do know is that there are associations between the upper and lower respiratory microbiome and respiratory outcomes in children. Also there is evidence that the microbiome of the intestine and possibly the skin may be related to asthma or, in the case of the latter, allergy.

Association between the Upper Airway Microbiome and Asthma

Samples from the nose, the nasopharynx and the hypopharynx in early life have been linked to childhood asthma in at least three longitudinal studies. In a prospective cohort study (COPSAC) from Denmark, acute wheezy episodes were significantly associated with presence of bacteria (*Streptococcus, Haemophilus and Moraxella*) in the hypopharyngeal aspirates and this was independent of the significant association with viral infections (23). A follow-up study in the same Danish cohort revealed that children who developed asthma at school age (seven years) had shown an abnormal immune response to these bacteria at 6 months of age with significantly increased levels of IL-5 and IL-10. The authors concluded that inability to clear the pathogenic bacteria from airways in early life can lead to chronic airway inflammation and therefore susceptibility to asthma (24). A similar relationship between the nasopharyngeal microbiome in the first year of life and subsequent development of asthma was observed in an Australian cohort study (25). Table one presents the bacterial phyla associated with later asthma. In a longitudinal analysis of data from a Finnish cohort study (STEPS) there were associations between some species of bacteria (e.g. Moraxella) with an increased risk of respiratory infections later in childhood (26). When the same cohort was followed up, frequency of both upper and lower respiratory tract infections was associated with an increased risk of developing asthma at seven years of age (27). Although there was no attempt to link early life microbiome to later asthma, these two studies suggest that such a link may exist.

In a nested case-control study amongst a cohort of children followed from birth to 18 months of age the authors (28) observed distinct nasal microbiota amongst the cases (rhinitis with and without wheeze) compared to healthy controls. Nasal swabs were gathered from as early as three weeks to three months of age. Authors concluded that nasal microbiome is associated with development of early onset rhinitis and wheeze in infants.

In a cross-sectional case-control study from Korea researchers showed a higher proportion of Firmicutes in upper airway samples from a group with asthma compared to controls and also children with asthma in remission. They also observed

Table 1. Bacterial Phyla Identified in the Upper and Lower Airways which Have Been Linked with Asthma (3, 23, 24, 26, 27, 29-32)

Bacterial phyla in the upper airways associated with childhood asthma	Bacterial phyla in the lower airways associated with childhood asthma
Firmicutes	*Firmicutes*
Actinobacteria	*Actinobacteria*
Proteobacteria	*Proteobacteria*
Bacteroidetes	*Bacteroidetes*
Fusobacteria	*Fusobacteria*
	Tenericutes

higher proportions of *Staphylococcus, Streptococcus, Dolosogranulum* and *Corynebacterium* in the asthma group (31).

Although the relation between bacteria and asthma causation is already complex, a further dimension of complexity may be added by the possibility that viruses may interact by causing reduced bacterial diversity and thereby modify the microbiome-host relationship (33). This interaction between virus-bacteria-microbiome can also play in the other direction a symbiotic relationship, whereby host respiratory bacteria can facilitate binding of Human rhinovirus (HRV) and respiratory syncytial virus (RSV) to the epithelial cells facilitating an inflammatory response, increasing the number of bacterial cell receptors, suppressing the immune system and/ or impacting on the commensal bacteria (34). This was shown in a small longitudinal cohort study of thirty-two infants in Bern, Switzerland (35). Their results showed that symptomatic HRV infections are linked with short term change in bacterial density and diversity and more frequent symptomatic infections have a long-term impact on diversity of the microbiota at the end of first year of life.

Associations between the Lower Airway Microbiome and Asthma

Very few studies have examined the association between lower airway microbiota and asthma. Hilty et al. (3) collected lower airway samples from adults and children with and without asthma and demonstrated how the upper and lower airways have distinct microbiomes and that children with asthma are more likely to have a microbiome rich in members of the Firmicutes phyla such as *Haemophilus* (3). An et al. compared the microbiota in a small sample of children with and without asthma (N=20) and showed an abundance of Proteobacteria amongst those with asthma (N=7) whereas Fusobacteria was the dominant phyla in those without (29). Table one lists the bacterial phyla in the lower respiratory tract which have been associated with asthma.

In another study when researchers analysed both bacterial and fungal populations in samples of the bronchoalveolar lavage, a significant difference was observed in the abundance of bacterial and fungal genera amongst children with and without severe asthma (30). Fungi are also thought to interact with bacteria in the respiratory tract. Pathogenic bacteria such as *P. aeruginosa* and *S. aureus* have been isolated from lungs in Cystic Fibrosis patients along with fungal species of *A. fumigatus* and *C. albicans* (36). The role of the fungi in the airway microbiome and asthma is currently unknown.

Association between the Gastrointestinal Microbiota and Asthma

There is a close relationship between the gastrointestinal (GI) and respiratory tracts, gastro-oesophageal reflux may expose the respiratory tract to upper GI bacteria and fecal-oral transmission may expose the upper airways to lower GI bacteria. The relationship between the gut microbiota

(analysed using fecal samples) and its association with asthma and atopy has been studied in longitudinal cohort studies (37-39). Arrieta et al. have shown that dysbiosis of the gut microbiota (fungal and bacterial) in the first 100 days of life is a risk factor for the development of atopic wheeze in children (37). Lack of abundance of certain species such as *Bifidobacterium, Akkermansia, Faecalibacterium, Roseburia* and a higher abundance of the bacterium *Veillonella* and the fungi *Candida* and *Rhodotorula* was linked with an increased risk of developing asthma in some of these studies. Results from a Danish cohort study revealed that lack of maturation of the gut microbiome in the first year of life contributed to increased risk of developing asthma at age five (39). In the same study maternal asthma although did not affect the microbial colonies of children it did act as an effect modifier increasing their risk of developing asthma and this the researchers associated with inborn immune deviation.

Conclusion

We are currently at the start of a line of enquiry into the role of the microbiome in childhood asthma. When the paediatric and adult data are considered, it is almost beyond reasonable doubt that the microbiome in the upper and lower airway is different in people with asthma compared to their peers who do not have asthma. What remains to be determined is whether a dysbiosis is the cause or the result of asthma. Longitudinal studies are required to determine the true nature of the relationship between the microbiome and asthma. These studies also need to consider:

- The role of viral infection and fungal colonisation in the airways,
- Using a robust objective index of airway function as an outcome alongside the rather subjective "asthma",
- Confounders including genetic factors and antibiotic use and
- There are likely to be different asthma endotypes, only one/some of which are related to the microbiome.

The COPSAC data (24) provide encouragement to this exciting line of research. Almost 40 years ago it was suggested that a chronic eosinophilic inflammatory condition in a derivative of the embryonic foregut (i.e. peptic ulcer disease in the stomach) was caused by infection. This suggestion was thought highly unlikely until *H pylori* was isolated in the stomach mucosa. Is it possible that an infection of a currently "unknown" bacteria might cause a chronic eosinophilic inflammatory condition in another derivative of the embryonic foregut? Let's see.

Conflicts of Interest: The authors declare that they have no conflict of interest.

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