

# Microbial dysbiosis in allergic lower airway disease (asthma)

## Abstract

Changed configuration of respiratory tract microbiota (microbial dysbiosis) has also been demonstrated in individuals affected from various allergic airway diseases, primarily asthma. Consistently, gut microbiota alterations via the gut-lung axis or the presence of certain pathogenic bacteria in the lungs during early life have found to an elevated risk for allergic asthma. It is, however, not identified whether microbiota dysbiosis is the to be related reason or simply a result of the related epithelial barrier disturbance in these diseases. More excitingly, potential role of the effect of lung microbiota changes (dysbiosis) in shaping asthma phenotypes was investigated by different researchers. In patients with severe asthma, a more diverse lung dysbiosis is observed. Gammaproteobacteria, mostly the Enterobacteriaceae and Pseudomonadaceae species were found to be elevated in severe asthmatics compared to non-severe asthmatics and healthy individuals. It is still not known that dysbiosis could contribute to the various specifics of asthma, e.g. chronic airway obstruction, frequent attacks, steroid resistance and/or severity of the disease. Also, could primary prevention and/or treatment of lower allergic airway disorders be achieved by using probiotics?

**Keywords:** microbiota, dysbiosis, asthma, Proteobacteria, gut-lung axis, healthy microbiota, microbial dysbiosis, bronchoalveolar lavage, lung microbiota, gammaglobulins, comamonadaceae, sphingomonadaceae, oxalobacteraceae, lung microbiota, actinomycetaceae, enterobacteriaceae

## Introduction

Documentation of occupant bacterial groups existing in human-derived samples using bacterial 16s ribosomal RNA gene sequencing, a culture-independent technique, has changed the microbiology arena. This method, succeeded afterward by metagenomic sequencing, has been tried into fecal specimens, which has yielded to the identification of 100 trillion microorganisms consisted of more than 1.000 separate bacterial species in the gastrointestinal system.<sup>1</sup>

### The gut-lung connection for lung microbiota development

Gut microbiota are significant in affecting the host immune system during infancy and might have an influence on the lung microbiota growth and its function via a 'gut-lung axis'.<sup>2</sup> The gut-lung axis forms innate and acquired immune responses in healthy individuals as well as in patients with lower allergic airway diseases.<sup>2</sup> Gut microbiota alterations through the gut-lung axis or the presence of certain pathogenic bacteria in the lungs during early life have found to be related to an elevated risk for allergic asthma.

In this mini-review, firstly healthy microbiota, then microbial dysbiosis in lower airway diseases of humans will be told. Importance of microbiota diversity in allergic lower airway diseases and some possible mechanisms of probiotic actions in the lower airway microbial dysbiosis are also briefly described. The potential use of probiotics to manage dysbiosis of chronic inflammatory allergic lower pulmonary tract diseases e.g. asthma is discussed though reviewing a few studies as well.

### Healthy microbiota in lower airways

Identification of lower airway microbiota from an analysis of bronchial brushings from healthy adult subjects indicated that the lower airways of the lungs were not sterile, as previously thought. Bacterial communities in the healthy lung isolated from bronchoalveolar lavage (BAL) fluid overlapped those found in the mouth, but the nasal microbiome, which is different from the oral microbiome, did not contribute to the lung microbiota in healthy individuals. The lower airway microbiome resembles that of the oropharynx, which has led to the concept that microbial migration from the mouth represents a major source of the healthy lung microbiota.<sup>3</sup> As mentioned above, the gut microbiome might also have an important influence on the lung microbiome, and the potential lines of communication between the two microbiota are currently not clear.<sup>2</sup>

The use of 16s rRNA gene sequencing methods for identification of lung microbiota has revealed presence of the Proteobacteria (mostly *Haemophilus*, *Moraxella* and *Neisseria* spp.), Firmicutes, Actinobacteria, Fusobacterium and Bacteroidetes (mostly *Prevotella* and *Veillonella* spp.) as the most prevalent phyla in the lower airways of healthy subjects.<sup>4-7</sup> Studies at the genus level confirmed the predominant presence of *Pseudomonas*, *Streptococcus*, *Prevotella*, *Fusobacteria*, *Veillonella*, *Haemophilus* as well as *Neisseria*.<sup>6</sup>

### Basic action mechanisms of healthy microbiota on lower airways

The healthy and/or probiotic bacteria in lower airways cooperate with the epithelial barrier and immune cells by means of pattern recognition receptors, for instance Toll-like receptor (TLR)s, upon

which triggered they can provoke or revoke numerous immune responses.<sup>8</sup> In different types of chronic inflammatory diseases in pulmonary tract, development of microbial dysbiosis in lower airways and disturbed membrane permeability as well as the epithelial barrier dysfunction has also been demonstrated.<sup>9-10</sup>

### Lung microbiota development in lower airways and its affecting factors

In the first 2 weeks of postnatal period, the bacterial capacity in the lungs augmented, and the bacterial phyla shifted from a preponderance of Gammaproteobacteria and Firmicutes toward Bacteroidetes.<sup>10-11</sup> The bacteria present in the lower airways including Bacteroidetes (Prevotella, Veillonella) and Firmicutes (Streptococcus spp.), which are likely to be inhaled and are then able to colonize the bronchi of healthy subjects.<sup>12</sup> Later in life, different environmental factors including breast-feeding, lifestyle, various therapeutic use and some factors favoring microaspiration of gastrointestinal and upper airway secretions into the airways, such as ciliary damage, cough reflex, and gastroesophageal reflux, could contribute to lung microbiota dysbiosis, which is characterized by an increase in bacterial communities, e.g. Proteobacteria, Firmicutes and Actinobacteria in lower respiratory system.<sup>13</sup> Moreover, early viral infections in life, e.g. respiratory syncytial virus (RSV), might modify pulmonary microbiota conformation preferring the appearance of Proteobacteria, associated with severe asthma and bronchial hyperresponsiveness.<sup>14</sup>

Microbial imbalance (dysbiosis) of the gut microbiota has now been well-known to be associated with various disorders, including inflammatory bowel disease, rheumatoid arthritis, depression, and obesity. Consistently, changed configuration of lower respiratory tract microbiota (microbial dysbiosis) has also been demonstrated in individuals affected from various allergic airway diseases such as asthma. It is, however, not identified whether microbiota dysbiosis is the reason or simply a result of the related epithelial barrier disturbance in these diseases. Also, currently one of the unanswered questions is whether changes in the lung microbiota described in asthmatic patients can drive the mechanisms underlying different asthma clinical phenotypes or not.<sup>15</sup>

### Development of microbiota and microbial dysbiosis in lower airways of humans

Neonatal bacterial community dysbiosis has been associated with the development of atopy and recurrent wheeze in childhood, indicating that changes in very early-life gut microbiome composition and microbial dysfunction might underlie infantile and childhood asthma.<sup>16-17</sup> Fecal samples gathered at 3 months of age in infants, who had atopy and wheezing, demonstrated transient gut microbial dysbiosis with a decrease in the bacterial genera Lachnospira, Veillonella, Faecalibacterium and Rothia. Consistently, what was later demonstrated that the comparatively large quantity of the bacterial genera including Lachnospira, Veillonella, Faecalibacterium and Rothia declined considerably the risk of asthma in these children.<sup>16</sup>

**Development of microbial dysbiosis in lower airways of animals:** In germ-free mice without microbiota, administration of gut microbiota from normal neonatal mice protected against the development of allergic airway inflammation. Similar to humans mentioned earlier, administration of the 4 bacterial taxa (Lachnospira, Veillonella, Faecalibacterium and Rothia) into germ-free mice improved airway inflammation in the mature offspring, supporting a pivotal role of these bacterial taxa to inhibit development of allergic bronchitis/asthma.<sup>16</sup>

### Microbial dysbiosis in lower allergic airway diseases and its effect on asthma phenotypes

Lung microbiota is dysregulated (dysbiotic) in asthmatic patients, with changes in community composition and specifically an expansion of Proteobacteria dominated by *Haemophilus* species.<sup>1</sup> Relative abundance of Proteobacteria members of the Comamonadaceae, Sphingomonadaceae and Oxalobacteraceae was found to be highly associated with bronchial hyperresponsiveness.<sup>7</sup> In a study, potential role of the lung microbiome in shaping asthma phenotypes was investigated by different authors such as Chung<sup>18</sup> and Zhang<sup>19</sup>. There was an important positive correlation was found between sputum eosinophils and plenty of Actinomycetaceae. And those patients with a longer period of asthma had large quantity of Gammaproteobacteria e.g. *Pseudomonas*, *Moraxella* and *Haemophilus*.<sup>21</sup> In some reported studies, there has been controversy about raised Proteobacteria density in mild to moderately severe asthma, led by *Haemophilus* and sometimes by *Klebsiella*, *Neisseria* and *Moraxella* species.<sup>4</sup> Another research from severe asthma patients showed that *Bacteroides* and *Fusobacteria* were decreased in both non-severe and severe asthma groups, but Firmicutes was augmented in severe asthmatics when compared to controls, specifically by a boost in *Streptococci*.<sup>19,20</sup>

### Microbial dysbiosis in severe/ poorly controlled/ steroid resistant asthma

In patients with severe asthma, a more diverse lung dysbiosis is observed. Gammaproteobacteria, mostly the Enterobacteriaceae and *Pseudomonadaceae* species were elevated in severe asthmatics compared to non-severe asthmatics.<sup>7</sup> When patients having severe asthma compared with healthy individuals or patients with mild-to-moderate asthma, *Tropheryma whipplei* (phylum Actinobacteria) was found to be amplified.<sup>15</sup> In addition to *Haemophilus influenzae*, an increase in *Tropheryma whipplei* could be secondary to the use of corticosteroid therapy in poorly controlled asthma.<sup>15</sup>

In a study of patients defined as corticosteroid resistant based on lack of response of FEV1 to 1 week of prednisolone therapy, an expansion of *Haemophilus parainfluenzae* was found in BAL fluid.<sup>22</sup> In the study by Goleva<sup>22</sup> on BAL microbiome, corticosteroid-resistant patients showed an overexpression of *Haemophilus parainfluenzae*, which was demonstrated to have the capacity to directly induce steroid resistance.<sup>22</sup>

### Microbial dysbiosis in asthmatic patients taking or not taking inhaled corticosteroids

Receiving oral corticosteroid therapy was associated with the relative large quantity of *Pseudomonas*, *Rickettsia*, *Prevotella*, *Lactobacillus*, and *Streptococcus* species; indeed, there was a diminished relatively large quantity of *Prevotella* (Bacteroidetes) and a raise in *Pseudomonas* species dependent on high steroid utilization.<sup>23</sup> Greater prevalence of Proteobacteria (e.g., *Haemophilus* and *Neisseria* species) were seen in asthmatic patients taking inhaled corticosteroids (ICSs), compared with healthy control subjects.<sup>4,7</sup> In contrast, another study,<sup>24</sup> showed that Proteobacteria were more frequent in asthmatic patients not taking ICSs compared with control subjects.<sup>24</sup>

### Microbial dysbiosis in neutrophilic asthma

Neutrophilic asthma showed reduced bacterial diversity and high prevalence of *Haemophilus influenzae*. Enrichment of *Haemophilus influenzae* has been found in induced sputum specimens from individuals with poorly controlled severe neutrophilic asthma.<sup>15</sup> Dominant species were *Moraxella catarrhalis* or elements of the

Haemophilus or Streptococcus genera associated with higher sputum neutrophil counts.<sup>25</sup>

### Microbial dysbiosis in eosinophilic asthma

A positive association between large quantity of Actinomycetaceae and Enterobacteriaceae with eosinophilic asthma was defined.<sup>26</sup> High prevalence of Haemophilus influenza; Tropheryma whipplei was identified in patients with eosinophilic asthma.<sup>15</sup> Interestingly, in asthma, the patients having the lowest levels of eosinophils were detected to have a changed bacterial spectrum, with more Neisseria, Bacteroides, Rothia species and less Sphingomonas, Halomonas, and Aeribacillus species.<sup>26</sup>

### Treatment of microbial dysbiosis in lower allergic airway diseases

Different research studies have utilized murine models of allergic lower airway diseases to explore the probiotic effects. In germ-free and asthmatic murine models, importance of normal microbiota and benefits of probiotics to restore dysbiosis have been shown.<sup>27-29</sup> Intragastric and even intranasal administration of Lactobacillus paracasei NCC2461 was demonstrated to able to modulate allergic airway inflammation in mice.<sup>30</sup>

In human, there have been several studies studying probiotic effects on allergic lower airway diseases for the last decade. In a recent systematic review assessing these data, 11 studies with a total of 910 children meeting eligibility criteria were re-analyzed. The data showed that the children having fewer asthma episodes was considerably elevated in the probiotics group than in the control one; the decrease in IL-4 and the increase in interferon- $\gamma$  levels were also noteworthy following the probiotic treatment. However, there was no statistical significance in asthma control, symptom scores, and the quantity of symptom-free days, FEV1 and PEF parameters in spirometry evaluation.<sup>31</sup>

This meta-analysis and other studies could not verify or exclude the helpful effects of probiotic use in asthmatic children.<sup>32</sup> Further well-designed randomized controlled trials with bigger sample sizes ought to be done to assess the probiotic effects on allergic lower airway diseases in near future.

### Conclusion

What is currently unidentified? Here are, a couple of them: Firstly, whether microbial symbiosis does contribute to the various specifics of severe asthma, e.g. chronic airway obstruction, exacerbations and steroid resistance or not, it is currently not known. This question should be delineated by using experimental and marine modeling approaches.<sup>18</sup> Secondly, whether primary prevention and/or treatment of lower allergic airway disorders e.g. asthma can be achieved with probiotics through aiming to improve the lung microbiota symbiosis.

### Acknowledgements

None.

### Conflict of interest

The author declares that there is no conflict of interest.

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