The Airway Microbiome and Disease

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Although traditionally thought to be sterile, accumulating evidence now supports the concept that our airways harbor a microbiome. Thus far, studies have focused upon characterizing the bacterial constituents of the airway microbiome in both healthy and diseased lungs, but what perhaps provides the greatest impetus for the exploration of the airway microbiome is that different bacterial phyla appear to dominate diseased as compared with healthy lungs. As yet, there is very limited evidence supporting a functional role for the airway microbiome, but continued research in this direction is likely to provide such evidence, particularly considering the progress that has been made in understanding host-microbe mutualism in the intestinal tract. In this review, we highlight the major advances that have been made discovering and describing the airway microbiome, discuss the experimental evidence that supports a functional role for the microbiome in health and disease, and propose how this emerging field is going to impact clinical practice.

Abbreviations: CF = cystic fibrosis; IBD = inflammatory bowel disease; rRNA = ribosomal RNA

The human microbiome refers to all the microbial communities that inhabit various surfaces of the human body. Bacteria form an important part of the human microbiome, and, indeed, it is well established that the human intestinal tract harbors a bacterial flora whose constituents are an order of magnitude greater in number than the cells in our body. These bacteria reside in a state of mutualism with the host, and serve many functions such as metabolizing dietary material into forms usable by the host and providing maturation signals for hematopoietic and nonhematopoietic cells of the immune system.\(^1\)

Dysbiosis of the host-microbe interaction can underlie inflammatory diseases such as inflammatory bowel disease (IBD),\(^2\) or even obesity.\(^3\) In recent years, analysis of bacterial 16S ribosomal DNA, the gene encoding the 16S ribosomal RNA (rRNA), has revealed that the intestinal tract harbors 500 to 1,000 different species of bacteria.\(^4\) 16S rRNA is a component of the 30S subunit of the bacterial ribosome and gene sequences encoding it contain hypervariable regions, which are conserved within bacterial species. Accordingly, with the advances in sequencing technologies, the analysis of 16S ribosomal DNA has enabled the detection and identification of several bacterial species, including many that cannot be cultured using standard microbiologic protocols. Thus, one reason underlying the expectation that the airways were sterile was that the bacteria present there could not be cultured. It is now clear, based upon work from a number of research groups worldwide, that there are bacteria resident in the airways and, additionally, the bacterial load and proportions of phyla vary under healthy or diseased conditions.

In a landmark article by Hilty and colleagues,\(^5\) the airway microbiome was shown to be different between healthy individuals and those with asthma or COPD. Indeed, the Proteobacteria phylum was overrepresented in the lungs of patients with allergies and those with COPD, while the phylum Bacteroidetes was marginalized. It is interesting to note that similar changes were evident in both asthmatic and COPD airways, which raises the question of whether the change in microbiome constituents drives disease or whether inflammation per se creates a niche for the outgrowth of certain bacteria. In a recent article by Mallia et al.,\(^6\) the authors showed that 60% of individuals with
moderate COPD developed bacterial infections following rhinovirus infection. Although no culturable bacteria were detected in these individuals prior to the rhinovirus infection, it remains possible that levels of bacteria were below the limits of detection using standard culturing protocols. It is plausible that inflammation allows the outgrowth of pathogenic bacteria, perhaps by creating a nutrient-rich niche suitable for their growth. Future studies that use molecular techniques to dissect whether these secondary infections are de novo or, in fact, represent outgrowth of the endogenous airway microbiota will provide a significant step forward in our understanding of the dynamics and implications of the airway microbiome.

THE AIRWAY MICROBIOME IN HEALTH AND DISEASE

The origin of the airway microbiome is yet to be delineated. Endogenous flora from the intestines and the oropharynx, and/or environmental microbes could provide the initial seeding source, or, most likely, a continued source of bacteria (Fig 1). A study by Charlson et al.7 suggested that the bacterial species detected in the lower respiratory tract of healthy individuals likely originated from microaspiration and reflected the microbiome detected in the upper respiratory tract. Certainly, it is critical that techniques are used to avoid possible contamination of lower respiratory tract samples by upper respiratory tract carryover; further work is required to definitively test whether contaminations from upper respiratory tract samples confound all conclusions made on the lower respiratory tract. It is notable that a recent study by Marri et al.8 assessed the microbiota in induced sputum of patients with mild asthma and of healthy individuals. The authors showed a clear increase in Proteobacteria in the former group as compared with healthy individuals, in line with prior studies focusing on the lower respiratory tract of patients with more severe asthma.5 Given the ease of sample collection, these results could have implications for monitoring asthma pathogenesis and management, and suggest that the microbiome of the whole respiratory tract might be consistent; however, additional studies directly comparing different anatomic compartments of the lung are required before further conclusions can be made. In any case, the observation that the constituents of the airway microbiome change depending upon the health status of an individual (eg, having asthma or COPD) suggests there is a degree of microbe-host cross talk in the airways, and particularly encourages further analysis of its functional consequences.

Figure 1. The lung microbiome in health and disease. A, Although the origins of the lung microbiome are unknown, direct exposure to environmental microbes as well as endogenous flora from the intestines and oropharynx could serve as the initial or continued inoculums. B, During homeostasis, the lung microbiome shows diversity in its composition. C, In contrast, in the diseased state, this diversity is reduced, particularly due to the outgrowth of the Proteobacteria phylum. Moreover, increases in the proportion of certain species within the different phyla have been reported. For example, an increase in the proportion of Streptococcus within the phylum Firmicutes is observed in both asthma and COPD.
Established microbial communities in the intestine exist in a dynamic state and vary in response to changes in nutrition and habitat. Future work in the respiratory field is needed to dissect whether the airway microbiome is similarly dynamic and influenced by local changes in energy sources and habitat. Nevertheless, one clear parallel between the intestine and the lung microbiomes is that a greater bacterial diversity is associated with a healthy homeostasis (Fig 1).5,9,10

An important contribution to the field came from Erb-Downward and colleagues,10 who assessed the lung microbiome in a small cohort of never-smokers, healthy smokers, and smokers with COPD. First, the authors found a similar number of bacterial 16S rRNA copies in BAL samples among all their subjects, arguing that rather than changes in the magnitude of the bacterial load, bacterial diversity might be a key differentiating factor. Indeed, in the two patients with the most severe COPD, there was evidence of reduced bacterial diversity as compared with the healthy or milder cases of COPD. This work clearly needs to be validated, but it does align with what might be expected, considering that lack of bacterial diversity in the intestinal tract is associated with an increased incidence of IBD.11,13 The key finding from Erb-Downward and colleagues10 was that the microbiota is different in distinct regions of the airways. Due to the small sample size, it is premature to draw strong conclusions; however, bearing in mind that diseases such as COPD can have localized foci of inflammation or tissue damage, one can speculate that a certain microbiota might associate with that site. Cause or effect still needs to be delineated. More recently, Sze and colleagues13 adopted a similar approach and assessed the microbiome in lung tissue of nonsmokers, healthy smokers, patients with severe COPD, and patients with cystic fibrosis (CF). Similar to Erb-Downward’s group, these authors showed that the bacterial load in the lung was comparable between healthy tissue and COPD lungs, but was significantly higher in CF lungs. Again, aligning with prior work, the authors showed that the constituents of the microbiota in healthy lungs and lungs with severe COPD were distinct.

A recent study compared the microbiome detectible in sputum samples from healthy individuals and patients with CF.3 A strikingly enhanced ratio of Firmicutes to Bacteroidetes was evident in the sputum of patients with CF as compared with healthy control subjects, and β-diversity cluster analysis of both phylum- and family-level sequence classifications revealed a clear dissimilarity between the sequences found in patients with CF and healthy individuals. Importantly, the authors added further weight to their study by associating their findings with clinical parameters. Indeed, high diversity of the bacterial flora was positively associated with reduced clinical parameters.

Although thus far there is limited evidence within the context of lung transplantation, the characteristics of the airway microbiome are likely to have major effects upon lung graft functionality. Possible underlying genetic predisposition or environmental exposures coupled with immunosuppression could lead to the outgrowth or colonization of transplanted lungs with a pathogenic microbiome. One could speculate that such a microbiome could underlie the development of rejection, bronchiolitis obliterans syndrome, or certain infections. A first step toward characterizing the airway microbiome of transplant recipients was made by Charlson and colleagues.14 In their study, they reported that the lower respiratory tract of subjects who had undergone lung transplantation had markedly higher loads of bacteria and, notably, certain fungal species were also detectible. The diversity of the microbiota was also lower in the transplant recipients, which, as mentioned earlier, has been described in COPD and is a characteristic of the intestinal flora in IBD.

A Functional Role for the Airway Microbiome?

In spite of a growing bulk of literature describing the airway microbiome in different disease settings, the evidence for its function is still limited. Studies using axenic (germ-free) mice have the potential to address these questions, since these mice are completely sterile. Thus, in theory, future studies could selectively reconstitute the intestinal or airway microbiome and dissect their relative roles.

Some of the first studies to address the importance of the microbiota in allergic airway disease were performed by Noverr et al.15,16 Their model used antibiotic therapy for the disruption of the intestinal microbiota. Although this did not eliminate the entire host microbiota, it was sufficient to cause the development of allergic airway response independent of the antigen used or the genetic background of the host mice. Consistent with their findings, in a more stringently defined model using germ-free mice, it has been shown that the complete absence of a microbiome results in pulmonary immune responses that are biased toward allergic T helper cells type 2-driven inflammation.17 In the absence of a microbiome, the pulmonary environment contained dendritic cells and macrophages with dysregulated activation states; moreover, the germ-free mice harbored fewer alveolar macrophages, indicative of a role for the microbiome in both maturing and possibly recruiting or differentiating certain cell types (Fig 2).

The dysregulated lung environment in the absence of a microbiome is not limited to effects upon allergic responses, with antiviral immunity against influenza virus being similarly impaired.18 Indeed, the influenza
infection of germ-free mice or mice that received extensive antibiotic treatment leads to an impaired clearance of the virus and a reduced expansion of influenza-specific, cytotoxic T cells.

Olszak and colleagues recently showed that there is a developmental window in neonatal mice when exposure to microbes is critical for appropriate immune maturation. Using germ-free mice, the authors showed that in the absence of microbial exposure, immune-cell development was dysregulated, such that there was an accumulation of invariant natural killer T cells in the lung. The consequence of this cellular accumulation was exaggerated allergic airway inflammation following allergen inhalation. One particularly important finding from this study was that germ-free mice could only be protected against the exaggerated inflammation when they were recolonized with a microbiome during the neonatal period, not when recolonized as adults. Taken together, these studies provide experimental data supporting a functionally positive effect of microbial exposure, particularly during the neonatal period.

Forsythe et al and Karimi et al have shown that in adult mice harboring a normal microflora, oral administration of a specific bacterial strain, *Lactobacillus reuteri*, also attenuated airway hyperresponsiveness. Specifically, the bacterial treatment resulted in a systemic increase in regulatory T cells, which can ameliorate the allergic airway response.

Another study showed that inhalation of an innocuous strain of *Escherichia coli* could reprogram dendritic cells and macrophages in the lungs of normal mice, resulting in long-term protection against allergic responses. This exposure specifically impaired allergic responses, while leaving classic, innate responses against bacterial products such as lipopolysaccharide untouched. In this case, direct exposure to bacteria in the airways appeared to be sufficient for eliciting a protective effect (Fig 2). However, one could equally speculate that colonization of mice with a microbiome from a diseased lung might have the opposite effect upon immune-cell maturation and inflammation. Indeed, a recent in vitro study has shown that certain bacteria associated with patients with asthma...
or COPD (pathogenic Haemophilus species and Moraxella species) intrinsically led to enhanced release of IL-23, IL12p70, and IL-10 by human monocyte-derived dendritic cells, as compared with bacteria associated with healthy lungs (commensal Prevotella species). Thus, within a niche in the lung, discrete interactions between certain bacterial species and the resident immune cells could potentially elicit or maintain local inflammation underlying the progression of chronic lung diseases.

**What Can We Learn From Other Tissues?**

There is a wealth of information available describing host-microbe interactions in the intestine. As mentioned earlier, species diversity is clearly linked to health, while limited diversity is associated with IBD.\(^{11,12}\) Moreover, certain bacterial species have been linked with the differentiation of inflammatory or regulatory T cells in the intestine.\(^{24}\) Thus, placing the respiratory literature into the context of what is known for the intestine, there already are many parallels. This does raise the point that perhaps the phenomenon of the airway microbiome is simply a flow-on effect of what is occurring in the intestine. However, given that there are clear changes in the respiratory microbiome in diseases largely localized to the airways, such as asthma, the argument for solely an intestinal basis for the changes in the lung microbiome is fairly weak. Furthermore, in a recent study by Naik et al.\(^{25}\) the authors elegantly showed that the intestinal and skin microbiomes were distinct, both in their constituents and in their functionality; specifically, direct exposure of the skin to endogenous skin bacteria was crucial for maturation of cells and appropriate immune responses against a skin infection. Such a phenomenon remains to be tested within the context of the lung, and such studies will no doubt be key to our understanding of the importance and implications of the lung microbiome.

**Implications for Clinical Practice**

As the outgrowth of certain bacteria is associated with disease severity in CF and COPD,\(^{9,10,11}\) this could be used as an indicator for timely clinical intervention and successful disease management. Moreover, the presence of specific bacterial markers such as nucleic acids, proteins, or metabolites could be more sensitive than routine diagnostics and may also precede clinical manifestation of disease. But the ultimate goal would encompass harnessing the understanding of the microbial ecology of the airways to maintain a health-promoting microbiome and resist colonization by pathogenic bacteria. To this end, it is crucial to define what constitutes a healthy microbiome in the airways. Studies in preclinical models will help in understanding whether dysbiosis is a cause or consequence of lung disease. Current strategies for the treatment of CF and certain exacerbations of COPD and asthma involve the use of antibiotics; given the work coming to light on possible beneficial effects of the lung microbiome, it might be a future consideration as to whether a healthy microbiome could be nurtured by selective clearance of pathogenic species by antibiotics.

Another approach could be the administration of beneficial bacteria (probiotics) to compete with the pathogenic bacteria and directly or indirectly restore diversity of the airway microbial communities (Fig 2). Indeed, preclinical studies involving probiotics in allergic airway inflammation have been promising.\(^{20,26}\) However, clinical studies using probiotics have not shown significant protection against the incidence of asthma.\(^{27,28}\) It is important to bear in mind that these interventions involved oral administration of the probiotics. If interactions between bacterial communities and immune cells occur distinctly within the lungs, inhaled probiotics could prove to be a more effective approach. The exact formulation of such inhaled probiotics will become clearer as our understanding of the airway microbiome evolves. Interestingly, Madan et al.\(^{29}\) have linked the development of the respiratory microbiome in infants with CF to diet and intestinal microbiota. Their study suggests that dietary manipulations alone could suffice to restore microbial diversity in the airways.

**Conclusion**

On the basis of multiple studies from different international laboratories, the existence of the airway microbiome is now firmly established. Clearly, the field is still in its infancy, and key questions remain to be answered—most notably, what is its function and how can we use it to prevent or treat chronic lung diseases? Is a “pathogenic” microbiome the basis for exacerbations of asthma and COPD? Does it provide the seed for secondary infections? Is there a link between the intestinal microbiota and the lung? As the airway microbiome gains wider acceptance, and as experimental systems develop to allow these questions to be addressed, this could be a field that helps unravel the basis of chronic lung diseases.

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**References**


