REVIEW ARTICLE

The human microbiota: a new direction in the investigation of thoracic diseases

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ABSTRACT Advancements in next generation sequencing technology have provided means for the comprehensive profiling of the microbial community in the respiratory tract in both physiological and pathological conditions. Recent studies have analyzed the bacterial composition in the respiratory tract of chronic obstructive pulmonary disease (COPD), influenza and tuberculosis patients, and have identified novel targets that may potentially lead to secondary infections. Certain bacteria have also been found to regulate the lung immune system and have unexpected connections with respiratory diseases. Further studies in these areas are necessary to dissect the exact relationship between the dynamics of the microbiota and the health of the respiratory system.

KEY WORDS Human microbiota; chronic obstructive pulmonary disease (COPD); influenza; tuberculosis; thoracic diseases; next generation sequencing

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Introduction

The microbiome is the genome collection of microorganisms in the environmental niche (1). Understanding the human microbiome will hopefully shed light on certain unexplained physiological or pathological phenomenon that was previously tackled by examining the human genome only.

Investigation on microorganisms can be traced back to the seventeenth century via culturing techniques, yet there are quite a number of limitations on the available culture mediums and culturing conditions, and is a qualitative technique only by its nature. More than 70% of the bacterial species on human body surfaces cannot be cultured by current techniques and therefore traditional culture techniques cannot be the single gold standard for microbial investigation. Techniques that aim to identify bacteria based on ribosomal RNA (rRNA) sequences or genomic DNA have gained attention and becoming increasingly popular as a measure to investigate the microbiota present both in normal and diseased lungs (2).

Amongst all, the Human Microbiome Project (HMP) led by the National Institute of Health in the United States is the largest project being conducted (3). The ultimate objective of the HMP is to demonstrate that there are opportunities to improve human health through monitoring and manipulating the human microbiome. The HMP will hopefully lead to paradigm shifts in clinical practice by providing groundwork for determining whether there are associations between microbiome changes and health and providing new means of intervention.

In a study investigating the healthy human lung, it was initially concluded that the healthy lung does not contain a consistent distinct microbiota. Instead, it contains a variety of bacteria largely indistinguishable from that of the upper respiratory flora, demonstrating the high correlation between the microbiota in the upper and lower respiratory tract (4). However, a recent study comparing the microbiota in the lung and oral cavity of smokers and non-smokers has led to a different conclusion. It was observed that certain types of bacteria, e.g., *Enterobacteriaceae* sp. and *Hemophilus*, had significantly higher abundance in the lungs in physiological conditions, contradicting the intuition that these bacteria should be also highly abundant in the oral cavity. Furthermore, the oral cavity microbiota has been shown to greatly vary among smokers and non-smokers (5). This interesting and seemingly contradicting phenomenon raises questions of whether these differences can be correlated with pathological conditions or simply generated because of the differences in experimental design or data interpretation.

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In this review, we report recent advances on the characterization of the microbiota in patients with chronic obstructive pulmonary disease (COPD), influenza and tuberculosis. Significant connections between commensal bacteria and these diseases have been reported, calling for a renewal of our understanding about these classical thoracic diseases.

Chronic obstructive pulmonary disease

COPD is an inflammatory disorder characterized by incomplete reversible airflow obstruction leading to increased mortality and morbidity (6,7).

COPD is divided clinically into emphysema and chronic bronchitis, corresponding to obstruction at the acinar and bronchial levels respectively (8). Epidemiologically, 90% of COPD cases are a result of heavy cigarette smoking, but yet only a minority of smokers will develop COPD (9). The exact mechanism of how smoking may lead to COPD has not been completely understood (10).

The present understanding of the pathogenesis for bronchitis attributes long lasting and repetitive irritation of inhaled substances such as tobacco smoke, dust and silica to be its primary cause. Early clinical features include hyper-secretion of mucus and hypertrophy of sub-mucosal gland, eventually leading to chronic obstruction. The role of infection with other bacteria is believed to be secondary. It is not responsible for the development of bronchitis yet significant in the prognosis of the disease. It is also believed that smoking interferes with biliary action of the respiratory epithelium and leads to direct lung damage, causing even more serious infection. On the contrary, the main cause for emphysema is believed to be protease-antiprotease and oxidant-antioxidant imbalance. Despite that infection occurs commonly in bronchitis; it is only occasionally found in the case of emphysema (9).

Previous classifications of the microbiome based on traditional culture techniques have described the bronchial tree as sterile in healthy subjects (11-13), while low load of colonizing potentially pathogenic microorganisms was found in COPD patients (12,14,15). However, recent studies have indicated that the lungs are in fact not completely sterile even in physiological conditions and further changes in the microbiota may occur in chronic diseases (16).

In COPD patients, a high diversity of bacteria (>100 genera) was observed in the samples collected from various locations in the air tract such as sputum, bronchial aspirate, bronchoalveolar lavage and bronchial mucosa, with *Streptococcus, Prevotella, Moraxella, Haemophilus, Acinetobacter, Fusobacterium,* and *Neisseria* being the most commonly found genera. Other bacteria identified in COPD patients include pathogens such as *Rothia, Tropheryma, Streptococcus, Peptostreptococcus, Leptotrichia, Kingella and Dysgonomonas,* which are known causes of bacteremia and endocarditis (17). Among these samples, sputum showed significantly lower microbiota diversity and lower bronchial tree samples have a distinct bacterial composition as compared to upper ones, demonstrating that sputum and bronchial aspirate may not accurately represent the lower bronchial mucosa flora (17). Moreover, taxa that are differentially abundant in COPD were observed to resemble the oral flora, reinforcing the hypothesis that source of lung microbiome is microaspiration of oral flora (18).

In contrast to an earlier study (19), a more recent and larger study comparing the lung microbiome in moderate and severe COPD patients with normal subjects yielded contradictory results that there is a higher level of microbial diversity in the lung and this discrepancy may be explained by age differences (18). It has also been reported that very severe COPD patients had a greater microbial diversity when compared with less severe patients but a minority of COPD patients exhibited a surprisingly low microbial diversity (20). Last but not least, it was hypothesized that bacterial infection in COPD can to increase risks of exacerbations and accelerate loss of lung function (2).

Influenza

The microbiota in the upper respiratory tract has been shown to have diverse roles in the prognosis of influenza. Efforts have been directed towards studying the bacterial composition that potentially leads to secondary infections, and how commensal bacteria existing in the respiratory tract under physiological conditions may modulate immune responses towards influenza.

The dynamics of the microbiome and secondary infections in influenza

Secondary bacterial pneumonia induced by *Streptococcus* and *Pneumococci* have been suggested to be a major contributor towards the high mortality in the three previous pandemics during 1918, 1957 and 1968 (21). Indeed, a recent study showed that co-infecting mice with influenza A virus (PR8 strain) and *Streptococcus pneumococci* leads to the impairment of macrophage recruitment (22).

Following the outbreak of the pandemic H1N1 (pH1N1) influenza A during 2009, several groups have studied the microbial profiles of influenza patients (23-25). Chaban *et al.* studied the bacterial composition of 65 pH1N1 patients and reported a positive correlation between age and bacterial diversity (25). It was also found that the upper respiratory tract microbiome mainly consisted of *Firmicutes, Proteobacteria* and *Actinobacteria*. These observations are consistent with the results from Leung *et al.* (24), as well as another study focusing on the bacterial composition under physiological conditions (26). Chaban *et al.* also reported that *Proteobacteria*, in particular the *Enterobactericeae*

and *Moraxellaceae* family, was more abundant in influenza patients than normal individuals (25). Interestingly, the study from Leung *et al.* that compared the bacterial composition of pneumonia patients with pH1N1 infection to those without the infection also reported that the amount of *Proteobacteria,* in particular the *Moraxellaceae* family, in their throat swab samples were significantly higher ($P<0.05$) in patients with pH1N1 infection (24). Taken together, these studies imply that the *Proteobacteria* species may be correlated to secondary infection in influenza A.

Indeed, the study from Leung *et al.* demonstrated that the amount of *Pseudomonas* and *Ralstonia* species was significantly increased in patients with pH1N1 infection. Comparative genomic analysis also revealed that the species with increased abundance in pH1N1 were enriched with chemotaxis and flagellar assembly genes, a feature that was absent in species with decreased abundance (24).

Commensal bacteria and its role in the modulation of immune responses in Influenza

Although it has been well established that commensal bacteria play a significant role in the regulation of intestine immune response (27), their role in the respiratory system has remained unclear. To this end, several groups have conducted studies in animal models so as to identify the roles of the microbiota and their corresponding signaling pathways in lung immune response.

Icihnohe *et al.* demonstrated that commensal bacteria promoted the expression of pro-IL-1β and pro-IL-18, which in turn modulate Th1, CTL and IgA immune responses, providing the necessary defense against influenza (28). It was also observed that mice treated with neomycin had diminished immune responses, indicating that neomycin-sensitive bacteria are related to the regulation of the lung immune response. More importantly, their study also showed that treating mice with ampicillin, vancomycin or metronidzaole did not induce changes in the immune responses, demonstrating the specific effects of neomycin. Along the same line, a study by Wu *et al.* revealed that treating mice with neomycin and a probiotic Bifico concurrently reduced lung damage as compared to treating mice with neomycin only (29), further supporting the notion that the existence of the microbiota is crucial in the regulation of the lung immune system.

While the above studies have demonstrated that commensal bacteria is crucial in the proper activation of the immune system, another group has reported that the presence of microbiota in fact also attenuates lung inflammation through the TLR2 signaling pathway. Mice which received *Streptococcus aureus* priming had a longer survival time and less lung injury as compared to mice that were kept in a specific pathogen free environment (30).

Tuberculosis

Tuberculosis, an airborne infectious disease, is well known to be caused by the pathogen *Mycobacterium (M.) tuberculosis* and extensive research has been done on characterizing the *M. tuberculosis* itself (31). Recent efforts have also been diverted towards studying the role of other bacteria in tuberculosis and have reported unexpected findings.

The microbiota and the prognosis of latent tuberculosis infection

It has been estimated that one-third of the world's population is latently infected by *M. tuberculosis* and yet only 10% of the population will ultimately suffer from tuberculosis (32). The true understanding to this phenomenon remains elusive, prompting for more investigation in this direction.

Perry *et al.* have reported an unexpected connection between the presence of *Helicobacter (H.) pylori* in the human body and increased resistance to tuberculosis infection. In a cohort study on patients with latent tuberculosis infection, it was observed that patients that eventually progressed to tuberculosis were about 50% less likely to be *H. pylori* seropositive. A retrospective study on cynomolgus macaques also consistently demonstrated that *H. pylori* seropositivity was associated with a decreased risk of progressing to tuberculosis (33). Although these studies remain observational in nature, this leads to a hypothesis that the presence of *H. pylori* or the status of the microbiota in general may dictate the prognosis of patients with latent tuberculosis infection. Prospective studies utilizing animal models will be required to establish a more definite relationship between these two factors.

Characterization of the microbiota in tuberculosis

Advancements in next generation sequencing technology have also allowed the characterization of the microbiota in tuberculosis patients, complementing the traditional culturing methods to provide a more comprehensive view of possible changes in the bacterial composition. Up to date, two groups have studied the sputum microbiota of tuberculosis patients via 16S RNA pyrosequencing (34,35).

Cui *et al.* reported that tuberculosis patients had an increased diversity in the sputum bacterial composition. In addition, the control group's bacterial composition exhibited a clear clustering pattern, as opposed to a more scattered pattern for tuberculosis patients (34). At the phylum level, both Cui *et al.* and Cheung *et al.* reported that *Firmicutes, Bacteroidetes, Proteobacteria* were the dominant phyla in both tuberculosis patients and healthy control samples, accounting for at least 70% of the sputum microbiota (34,35). These phyla have also

been reported to be dominant in the lung under physiological conditions (10). At the genus level, Cheung et al. reported that *Streptococcus, Neisseria* and *Prevotella* were the most predominant genera, an observation consistent with studies on the sputum microbiota from patients with other lung diseases, but yet differing from the normal lung (10,17,36-38). A few genera belonging to *Firmicutes* were also reported to be more abundant in tuberculosis patients. On the other hand, Cui *et al.* reported a few genera including *Stenotrophomonas and Phenylobacterium* that were unique to tuberculosis patients (34). While changes in the phyla composition were observed by both studies, the results remain somewhat contradictory. These differences may be caused by the dissimilar criteria for patient recruitment and more evidence may be required to draw a definite conclusion in the future.

Discussion

The recent advances made in the relatively young field of lung microbiota characterization have lead to various interesting, and yet contradictory observations, providing the foundation and potential targets for future work on investigating the casual relationship between certain bacteria species and classical thoracic diseases.

While the rapid development of sequencing technologies has opened up possibilities to characterize the microbiota in a novel way, certain technological difficulties still exist and efforts are needed to be made in terms of characterizing its strength and weakness as compared to traditional culturing techniques. Since many studies have reported that traditional culturing techniques are still superior in certain cases (39), it is expected that these two techniques will become complementary to each other and provide a more comprehensive understanding of the microbiota in the respiratory tract.

Our scope of understanding in the change of microbiota of the respiratory tract under pathological conditions is also limited by the fact that lower respiratory tract samples for healthy individuals as a control is scarce, as collecting these samples can be invasive in nature. Current studies on thoracic diseases such as tuberculosis have mainly focused on sputum samples, but further studies are needed to see whether these samples may also accurately represent the microbiota in the lower respiratory tract. We envisage that a better understanding of the respiratory tract microbiota will provide novel insights on the pathogenesis and prognosis of thoracic diseases and open possibilities for interventions based on the modification of microbiota status.

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