

Role of the Lung Microbiome in the Pathogenesis of Chronic Obstructive Pulmonary Disease

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Abstract

Objective: The development of culture-independent techniques for microbiological analysis shows that bronchial tree is not sterile in either healthy or chronic obstructive pulmonary disease (COPD) individuals. With the advance of sequencing technologies, lung microbiome has become a new frontier for pulmonary disease research, and such advance has led to better understanding of the lung microbiome in COPD. This review aimed to summarize the recent advances in lung microbiome, its relationships with COPD, and the possible mechanisms that microbiome contributed to COPD pathogenesis.

Data Sources: Literature search was conducted using PubMed to collect all available studies concerning lung microbiome in COPD. The search terms were “microbiome” and “chronic obstructive pulmonary disease”, or “microbiome” and “lung/pulmonary”.

Study Selection: The papers in English about lung microbiome or lung microbiome in COPD were selected, and the type of articles was not limited.

Results: The lung is a complex microbial ecosystem; the microbiome in lung is a collection of viable and nonviable microbiota (bacteria, viruses, and fungi) residing in the bronchial tree and parenchymal tissues, which is important for health. The following types of respiratory samples are often used to detect the lung microbiome: sputum, bronchial aspirate, bronchoalveolar lavage, and bronchial mucosa. Disordered bacterial microbiome is participated in pathogenesis of COPD; there are also dynamic changes in microbiota during COPD exacerbations. Lung microbiome may contribute to the pathogenesis of COPD by manipulating inflammatory and/or immune process.

Conclusions: Normal lung microbiome could be useful for prophylactic or therapeutic management in COPD, and the changes of lung microbiome could also serve as biomarkers for the evaluation of COPD.

Key words: Chronic Obstructive Pulmonary Disease; Lung Microbiome; Pathogenesis

INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is a chronic inflammatory airway disease characterized by persistent airflow limitation, which is a leading cause of morbidity and mortality worldwide (<http://www.goldcopd.org/>). The risk factors of COPD include smoking, indoor use of biomass fuels, occupational exposures, asthma, and early-life infection.

COPD is a multidimensional disease, having complex pathogenesis architecture. Given its heavy economic and social burden, COPD pathogenesis is an active research direction. Inflammation plays an important role in the pathogenesis of COPD, involving many inflammatory cells and mediators. Increased numbers of CD4⁺ and CD8⁺ cells

and macrophages are found in the airway of COPD.^[1,2] Moreover, oxidative stress may amplify the inflammation;^[3] the COPD patients have increased biomarkers of oxidative stress, for example, hydrogen peroxide and 8-isoprostane. Immune response is also related with COPD progression.^[4,5] In addition, protease-antiprotease imbalance in the lungs of COPD patients can break down connective tissue component,

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resulting in emphysema.^[6] Genetic risk factors have been demonstrated to play important roles, and genome-wide association studies identify many genetic variants associated with COPD.^[7-9] Lung microbiome has recently become a new research area for COPD.

This study reviewed the role of lung microbiome in the pathogenesis of COPD. A comprehensive literature search was conducted using PubMed to collect all available studies concerning lung microbiome in COPD. The search terms were “microbiome” and “chronic obstructive pulmonary disease,” or “microbiome” and “lung/pulmonary.”

LUNG MICROBIOME

It has been estimated that more than 70% of the bacterial species on body surfaces cannot be cultured by standard culture techniques,^[10,11] traditional culture-based studies have described the bronchial tree as sterile in healthy individuals,^[12,13] and only low-load colonizing potentially pathogenic microorganisms (PPMs) can be found during diseases. Nowadays, high-throughput sequencing technologies have facilitated the identification of uncultivable microbes in the human body. The development of culture-independent techniques for microbiological analysis (e.g., sequencing of the 16S rRNA gene) shows that bronchial tree is not sterile even in healthy individuals.^[14] The lung is a complex microbial ecosystem, the microbiome is the collection of viable and nonviable microbiota (bacteria, viruses, and fungi) residing in the bronchial tree and parenchymal tissues, which may be important for health, such as colonization resistance, epithelial integrity, and immunoregulation. The composition of lung microbiota varies within the bronchial tree.^[15,16] The alveolar surface is lined with lipid-rich surfactant, which has bacteriostatic effects,^[17] and therefore, the bacterial density in the airways is relatively modest. It is reported that bacterial loads of bronchoalveolar lavage (BAL) range from 4.5 to 8.25 log copies/mL,^[18,19] and lung tissue samples are about 10–100 bacterial cells per 1000 human cells.^[20] To date, most studies focused on bacterial populations, while viral and fungal populations were largely neglected due to technical difficulties.

The composition of the lung microbiome is at least determined by three factors.^[21,22] The first factor is microbial immigration, including microaspiration, inhalation of bacteria, and direct mucosal dispersion. The second is microbial elimination, such as cough, mucociliary clearance, and innate and adaptive host defenses. The third is regional growth conditions (nutrient availability, oxygen tension, temperature, pH, concentration of inflammatory cells, activation of inflammatory cells, local microbial competition, and host epithelial cell interactions). In healthy individuals, the growth conditions are inhospitable for bacterial growth, whereas during diseases, the conditions are suitable for certain bacteria to grow. It is reported that lung microbiota is sex dependent and can be changed by inflammation, antibiotics and corticoids use, etc.^[23]

The pattern of lung microbiome is described as “transient but not resident (TBNR),”^[24] reflecting its complex and dynamic nature. The migration of microbes in the lung is bidirectional and there is no physical barrier in the airway, so the microbiome of the lungs is dynamic and transient. The community composition of bronchial tree is reported to be similar with the upper airways, but the biomass is less, indicating that aspiration from the upper airways may be the source of lung microbiome.^[25-27] Charlson *et al.*^[25] showed that there were similarities in the microbiologic pattern in the oropharynx and bronchial tree, indicating that there is no unique lung microbiome. However, other studies found that certain bacteria were more abundant in the lungs.^[28,29] Further, although bacterial DNA is detectable in the lungs, whether the bacteria are alive or dead is need to be determined. Pezzulo *et al.*^[30] showed that bacteria in the pigs’ lung parenchyma are dead, and bacteria in airways sampled by BAL are a mixture of both alive and dead bacteria. They also demonstrated that dead bacteria in lung might not be lysed and could be detected by quantitative polymerase chain reaction/16S rRNA sequencing, which would result in overestimate of the bacterial load and diversity in the lung.

Many studies are based on sputum and BAL samples, and there are also studies on human lung tissues.^[20] Cabrera-Rubio *et al.*^[31] tested four types of respiratory samples (sputum, bronchial aspirate, BAL, and bronchial mucosa). Sputum and bronchial aspirate samples are upper bronchial tree samples, and BAL and bronchial mucosa are lower bronchial tree samples. Sputum samples showed significantly lower diversity than the other three sample types, while lower bronchial tree samples showed very similar bacterial compositions. Hence, there is a distinct microbiota in the upper and lower compartments, and BAL samples could describe diversity of the bronchial mucosa flora.

The main risk factor for chronic respiratory diseases is tobacco smoke exposure, and smoking alters the composition of the oral microbiome. Whether smoking affects the lung microbiome needs to be determined. Erb-Downward *et al.*^[18] showed that among the healthy smokers, smokers with mild COPD, and nonsmokers, there were no differences in total bacterial load, while the moderate and severe COPD patients lacked bacterial community diversity. Data also suggest that smoking could alter the upper airway microbiome, resulting in enrichment for *Veillonella*.^[28,32]

LUNG MICROBIOME IN CHRONIC OBSTRUCTIVE PULMONARY DISEASE

Disordered bacterial microbiome is believed to participate in the pathogenesis of COPD; it is reported that in about 29% of stable COPD and 54% of acute exacerbation of COPD (AECOPD) patients, PPMs could be detected, and the overgrowth of PPMs and the appearance of *Pseudomonas aeruginosa* in the lower airway were associated with AECOPD.^[12] Lung is not a study site of body in the original

Human Microbiome Project, and lung microbiome research in the context of COPD is still at its early stage, where many reports are inclusive or inconsistent.

Proteobacteria, *Bacteroidetes*, *Actinobacteria*, *Firmicutes*, *Streptococcus*, *Prevotella*, *Moraxella*, *Haemophilus*, *Acinetobacter*, *Fusobacterium*, and *Neisseria* were the most common bacteria, which may account for 60% of the total bacteria in airway.^[31] Rutebemberwa *et al.*^[33] compared abundances of bacterial taxa in normal, COPD, and cystic fibrosis (CF) patients and found different bacterial community composition among the groups and also found that *Novosphingobium* spp was of the highest abundance in advanced COPD patients, which could trigger inflammation. A study analyzed the composition and the gene content of the microbial community in sputum of COPD patients in stability and exacerbation stage, finding that there were no differences in the bronchial microbiome composition. Functional analysis showed that four functional categories showed statistically significant differences with MG-RAST at KEGG level 2, in exacerbation, cell growth and death and transport and catabolism decreased, while cancer and carbohydrate metabolism increased.^[34] However, some researchers showed that the microbiome composition fluctuated with severity of COPD and onset of exacerbation. Huang *et al.*^[35] reported changes in taxonomic composition at the onset of exacerbation, but no changes in community richness, evenness, and diversity. And, Molyneux *et al.*^[36] observed that microbiome composition changed and increase of bacterial abundance in COPD. In another study, the researchers observed a severity-related change of the bronchial microbiome in COPD,^[37] they showed that there was no difference between moderate-to-severe and advanced COPD in microbial community, but patients with moderate-to-severe disease showed greater microbial diversity than patients with advanced disease.

Wang *et al.*^[38] reported dynamic changes in microbiota during COPD exacerbations; 87 patients were recruited and followed up, whose sputum samples were obtained at steady state, during exacerbation, 2 and 6 weeks after recovery. They found that microbiome changed during exacerbations, and there were differences between the two exacerbation phenotypes (bacterial and eosinophilic). In “bacterial phenotype”, *Proteobacteria* was increased, while in “eosinophilic phenotype”, an increase in *Firmicutes* was observed. Lung microbiome dynamics may be a potential biomarker or an intervention target for COPD.

Shotgun metagenomic sequencing enables analysis of the taxonomic composition and functional capacity of the whole microbiome.^[39] Cameron *et al.*^[40] reported metagenomic sequencing of sputum samples from eight COPD patients and ten “healthy” smokers, finding differences in taxonomic composition between the two groups, and microbiome in COPD patients indicated an increased capacity for bacterial growth, such as bacterial cell division, nucleosides and nucleotides, and amino acid, protein, and RNA metabolism. The changes of microbiome composition and

functional capacity were associated with COPD severity (FEV1% of predicted).

There is a research that examined the microbiomes in human lung tissue samples. Sze *et al.*^[20] collected lung tissues from 8 nonsmokers, 8 smokers, 8 COPD (GOLD 4) patients, and 8 CF patients, finding that there was no significant difference in bacterial populations among the nonsmoker, smoker, and GOLD 4 groups, while the CF group had a higher bacterial population than any of the other three groups. Compared with the nonsmoker and smoker controls, the lung bacterial communities of COPD (GOLD 4) group were changed, indicating that a unique bacterial community existed in advanced COPD, while the bacterial diversity in lung tissue from the COPD (GOLD 4) group was the same as the nonsmoker and smoker control groups.

In 2014, Gronseth *et al.*^[41] established a longitudinal study to analyze the microbiota and the host immune system status from protected specimen brushes, small-volume lavage, BAL, and bronchial biopsies in individuals with or without COPD (300 vs. 200). The cohort will be followed for at least 3 years. The results of this large study will be helpful to address the lung microbiome in COPD and its possible mechanism.

INVOLVEMENT OF LUNG MICROBIOME IN CHRONIC OBSTRUCTIVE PULMONARY DISEASE

Most studies to date only demonstrate differences in lung microbial communities between health and COPD; the research of the role of lung microbiome in COPD is rather preliminary. Most studies were correlative and descriptive, and the lung microbiome in COPD pathogenesis and progression remains as uncharted territories.

COPD is characterized by persisting inflammatory/immune response. Inflammation is associated with reduced microbiome complexity.^[42] There is a relationship between the airway microbiota composition and severe lung function decline.^[43] Supraglottic-characteristic flora in lung is associated with lung inflammation (increased BAL neutrophils, lymphocytes, and exhaled nitric oxide [eNO]) in relatively healthy individuals,^[29] and microbiomes play an important role in the development and integrity of the immune system.^[44] Such data suggested that lung microbiome may contribute to the pathogenesis of COPD by manipulating inflammatory and/or immune process.

There are scholars proposing microbiome dysbiosis-inflammation cycle. An inflammatory trigger (e.g., viral infection, allergic exposure, toxic inhalation, and air pollution) initiates airway inflammation, which alters airway microenvironment. Disordered growth conditions result in a disordered lung microbiome, which provokes further airway inflammation.^[22] Dysbiotic microbiome has been associated with increased inflammation. Enrichment of lung microbiome in supraglottic taxa was associated with higher BAL lymphocyte count, BAL neutrophil count, and eNO (biomarker of pulmonary inflammation).^[29] Sputum

CXCL8/interleukin-8 (IL-8) plays a prominent role in COPD,^[45] by recruiting neutrophils, and upregulates airway mucin genes, resulting in mucus production.^[46] It is reported that dysbiosis of the lung microbiota could induce the production of CXCL8/IL-8,^[38] and sputum CXCL8/IL-8 is associated with lung microbiome diversity and community structure. In the emphysema animal model (lipopolysaccharides/elastase-treated mice), the microbiota richness and diversity were decreased, the genera of *Pseudomonas* and *Lactobacillus* were increased, and meanwhile, *Prevotella* decreased.^[47] Drop in bacterial load was associated with attenuated IL-17 production. When mice treated with microbiota-enriched fluid intranasally, IL-17 production was elevated. In mice model, neutralization of IL-17 led to dampened inflammation and reduced disease burden.^[47]

Microbiome plays an essential role in shaping and regulating immune responses and maintaining host immune homeostasis.^[48] Lung microbiome also plays an important role in the homeostasis of lung immune system, and dysbiosis could lead to host immune response. Saeedi *et al.*^[24] described the interaction between lung immune responses and lung microbiome as “Yin Yang model”; there is a dynamic balance between TBNR microbiome of lower airways and immune responses. Changes of host bacterial ecology can have a profound impact on immune subsets, including Th1/Th2 balance and development of Th17, regulatory T-cell, and invariant natural killer T-cells, resulting in disease susceptibility.^[49,50] Lung microbiome enrichment with upper airway taxa could affect immune status. It was reported that enrichment of the lung microbiome with oral taxa resulting by microaspiration could increase expression of inflammatory cytokines and Th-17 lymphocytes and attenuate alveolar macrophage TLR4 response.^[51] Sze *et al.*^[52] researched on control and COPD (GOLD 4) surgically resected lung explants and found that decline in microbial diversity was associated with emphysematous destruction, remodeling of the bronchiolar and alveolar tissue, and further, the lung tissue was infiltrated by CD41 T-cells. Hence, the impairment in lung innate immunity caused by microbial dysbiosis may facilitate the exacerbation of COPD.

The above evidences support the “vicious circle hypothesis”.^[53] The initial factors impair innate immune defenses of lung, leading to a change in the abundance, composition, and diversity of the lung microbiome. The changed lung microbiome would result to maladaptive inflammatory responses, further impairment of lung defenses and further dysbiosis of the lung microbiome, establishing a vicious circle and leading to COPD progression.

CONCLUSIONS

It is recognized that bronchial tree is not sterile in healthy and COPD individuals; the lung microbiome plays a significant role in normal lung function and diseases. The relationships of dysbiosis of lung microbiome and COPD have investigated by many studies, but whether manipulating the bacterial community could treat or prevent COPD has not

been well explored. Carefully designed and well-powered studies are in great need to identify the mechanism of COPD molecular etiology attributable to microbiome.

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Conflicts of interest

There are no conflicts of interest.

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