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Past, Present, and Future Research on the Lung Microbiome in Inflammatory Airway Disease

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COPD, asthma, and cystic fibrosis (CF) are obstructive lung diseases with distinct pathophysiologies and clinical phenotypes. In this paper, we highlight recent advances in our understanding of relationships between clinical phenotypes, host inflammatory response, and lung microbiota in these diseases. Although COPD, asthma, and CF largely have distinct lung microbiota and inflammatory profiles, certain commonalities exist. In all three of these lung diseases, and in healthy persons, anaerobic taxa that are typically associated with oral microbiota (eg, *Prevotella* species, *Veillonella* species) are present in the airways and associated with increased host inflammatory response. Similarly, across all three diseases, members of the Proteobacteria phylum are associated with more advanced disease. Finally, we highlight challenges in translating these findings into advances in clinical care, including continued knowledge gaps regarding the causal relationships between host inflammatory response, lung microbiota, medication effects, and clinical phenotypes. CHEST 2019; 156(2):376-382

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Despite the range of ecosystems in which microbes live,[1-3] the healthy human lung was thought to be an environment that microbes do not colonize.[4,5] Studies by multiple groups demonstrate that the lower airways are not sterile in the strictest sense.[6-9] Although the debate is still ongoing as to whether this identified lung microbiota is indeed a resident community,[10] microbes (whether resident or transient) within the healthy lungs interact with host immune response and can exacerbate or protect against disease. In the setting of existing inflammatory lung disease, lung microbiota and host-microbiota interactions are also related to disease course; however, these relationships are largely specific to the underlying lung disease. In this paper, we will review three inflammatory airway diseases with distinct pathophysiologies: COPD, asthma, and cystic fibrosis (CF). For each of these three diseases, we will provide a brief overview of some of the recent advances and current challenges on the relationships between lung microbiota, host immune response, and disease phenotype. We will highlight commonalities and disparate findings between these three distinct inflammatory airway diseases, and finally we will relate these findings to

ABBREVIATIONS: CF = cystic fibrosis; ICS = inhaled corticosteroid; NP = nasopharyngeal

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future research directions in lung host-microbial interactions.

COPD

Over the last 10 to 15 years, studies from multiple laboratories have helped highlight the key correlations between the lung microbiota and host response at all stages of COPD.[6,7,9,11-19] For tissue studies, few differences in the microbiota can be identified between smokers and nonsmokers or between mild and moderate COPD.[9,18] Similarly, studies on the BAL bacterial community have identified few differences between smokers, nonsmokers, and early COPD.[7,11] Although Segal et al[11] did not find a difference in the microbial community between smokers, nonsmokers, and early COPD, they did identify a strong relationship between specific taxa (eg, *Veillonella* species, *Prevotella* species) and BAL lymphocyte counts, BAL neutrophil counts, and exhaled nitric oxide.[11] In a separate study, these same taxa also are strongly associated with a Th17 lung inflammation phenotype.[12] Additionally, an epithelial cell-derived signature of IL-17A inflammation is associated with increased airway obstruction and decreased corticosteroid responsiveness among smokers with or without COPD.[19] Because of the increased host inflammation that is associated with *Veillonella* species and *Prevotella* species, it is possible that these taxa could be used to help classify individuals at higher risk of progressing to later stages of COPD.

Lung tissue and bronchial brushing studies comparing the microbiota between later stages of COPD and control samples (either nonsmokers or smokers) have consistently identified broad bacterial community differences.[6,9,13] A consistent finding across studies is an increase of the Proteobacteria phylum and a decrease in the Bacteroidetes phylum in late-stage COPD vs control subjects.[6,13] For the Proteobacteria phylum, *Haemophilus* species were identified in both studies, whereas for the Bacteroidetes phylum, the strongest associated genus was *Prevotella* species.[6,13] In the lung tissue study, *Haemophilus* species relative abundance was positively associated with a number of terminal bronchioles and with an increased volume fraction of neutrophils.[13] Members of the Proteobacteria phylum also have been known to shift in relative abundance at the onset of COPD exacerbations[14-17] and are impacted differently, as are other members of the lung microbiota, by type of treatment for exacerbation (eg, oral corticosteroids vs antibiotics or both).[14,17] Whether inhaled corticosteroids (ICSs) alter lung microbiota composition in COPD is not established, but there is growing evidence of such in adults with asthma.[46,48] Collectively, the observed associations between Proteobacteria and COPD severity and exacerbations confirm culture-based evidence of the importance of specific Proteobacteria in COPD (eg, *Haemophilus* species, *Moraxella* species). However, a more holistic view of microbial community interactions during either stable or exacerbating COPD has provided new hypothesis-generating insights into the participatory role of other microbiota, including non-Proteobacteria, in COPD. Considering not only Proteobacteria but also multiple other genera (eg, *Prevotella* species) could add additional accuracy to disease progression risk stratification. A recent study of exacerbations in COPD and asthma, that identified three biological clusters based on sputum microbiota (Proteobacteria and non-Proteobacteria) and inflammatory mediator profiles, is a promising early example of such an effort.[20]

Although the characterization of the microbiota in COPD has been extensively studied, there are still some crucial questions that need answering. First, small sample numbers are an issue for several of the studies mentioned, particularly earlier investigations. More recent studies of microbiota dynamics in COPD exacerbation have involved larger subject numbers. As an example, most of these previous studies group COPD together as a single disease entity. However, COPD is a heterogenous disease and many different phenotypes exist, a facet increasingly recognized among smokers with mild or no airflow obstruction.[21-23] Some questions that still need to be studied include whether there are microbiota differences between those with functional small airways disease vs emphysema[22] or by distribution of emphysema (eg, panlobular, centrilobular), and between those with more vs less chronic symptoms which impact quality of life and exacerbation risk.[23] Further studies in well-characterized patient cohorts will lead to a better understanding of how the microbiome contributes to the pathogenesis of COPD and its heterogeneous phenotypes.

Research on the microbiota and COPD has arrived at a critical juncture. There is no denying that the observations coming from this line of research indicate that lung communities may be involved in the progression of COPD and that microbes identified in the lung are associated with host inflammation.[6,7,9,11-17] Although there is still a long way to go before the microbiota can be used in a clinical setting, focusing on studies that characterize the microbiota in different phenotypes of COPD and how different taxa interact

with the host inflammation that is associated with worsening COPD stage could lead to the identification of useful microbiota-based biomarkers.

Asthma

The role of microbiota in asthma pathogenesis has, until recently, focused on the early life period and interactions between microbiota (gut and nasopharynx), the maturation of immune responses during infancy, and subsequent risk for asthma during childhood. Earlier epidemiologic studies conducted in Europe and the United States were seminal in highlighting links between differences in environmental microbial exposures and the prevalence of allergic diseases including asthma.[24-26] Numerous studies, using either culture-based or molecular approaches to identify members of the gut microbiota, subsequently described differences in colonization patterns associated with asthma development in children.[27-30]

Work using ex vivo approaches or animal models have aimed to understand mechanisms through which an altered composition of gut microbiota modulate innate and adaptive immune responses resulting in allergic inflammation.[30-33] Findings have implicated a role for various gene functions encoded in gut bacteria and related metabolic products. These include bacterial production of short-chain fatty acids, which may attenuate the ability of bone marrow-derived dendritic cells to initiate allergic responses.[33] Additional metabolic products of interest include the essential amino acid tryptophan[34] and histamine produced by certain bacteria found elevated in the intestinal tract of some subjects with asthma.[35]

The nasopharyngeal (NP) microbiome has garnered increased attention recently because the nasopharynx is easy to access, and also is the most proximal site of interaction between respiratory epithelia and the environment. Severe viral respiratory illnesses are a strong risk factor for asthma development.[36] Studies from large pediatric cohorts have reported links between an altered NP microbiota and frequency of, or symptom severity in, viral illnesses that subsequently associate with increased asthma risk.[37,38] Although methods vary between studies, observations have been consistent in the finding that increased NP relative abundance of particular genera (eg, *Moraxella* species, *Haemophilus* species, *Streptococcus* species) is associated with asthma or more severe respiratory illnesses in children.[37,39-41] Notably, in both the gut or NP microbiomes of children, differences associated with asthma risk have been strongest in early infancy (< 3 months of age),[30,37] highlighting the important dynamics of microbiota-immune interactions in early life.

In contrast with the focus on asthma susceptibility in children, studies of the respiratory microbiome in adults with asthma have identified significant relationships between microbiota patterns and features of chronic asthma, including disease severity and other phenotypic features.[42-46] Asthma's heterogeneity in adults is well recognized, with many phenotypes described by combinations of clinical and inflammatory characteristics.[47] One consistent observation across studies of more severe asthma is airway enrichment in members of the Proteobacteria phylum, which represent many potentially pathogenic respiratory bacteria such as members of the *Haemophilus*, *Moraxella*, and *Neisseria* genera.[6,42,44,45,48] To an unclear extent, these findings may reflect in part long-term treatment with ICSs, which are immunomodulatory and may therefore change the microbial pressure by the host. However, even with ICS use in the background, clinical features that distinguish severe asthma phenotypes may associate with different patterns of airway microbiota composition. These features include stability of asthma control, obesity-associated severe asthma, and airway inflammation patterns.[44,46,49]

Observations that airway inflammation patterns associate with differences in airway microbiota composition suggest the likelihood of bidirectional, microbiota-host interactions that drive or at least modulate asthma phenotype. For example, stratification of patients by some biomarker of type 2 inflammation (eg, blood or sputum eosinophils, fraction of exhaled nitric oxide) is helpful and clinically informative, given the options now available for type 2-directed biologic therapies.[50] Similar stratification of adult study subjects with asthma by type 2 inflammation status (eg, type 2 high/low, eosinophilic/noneosinophilic) has revealed correlative links between differences in airway microbiota composition and the presence or absence of type 2-driven inflammation. Among subjects with severe asthma, those with a neutrophilic sputum inflammatory phenotype[49] or a Th17-driven bronchial epithelial gene expression profile demonstrate a significantly different airway microbiota (enriched in Proteobacteria)[44,49,51] from those with predominantly eosinophilic sputum inflammation. Additionally, these observations may not be limited to severe asthma. A study on adults with mild, well-controlled asthma (not on chronic ICS therapy) identified similar stratification of differences in airway

microbiota composition based on the presence or absence of type 2-driven epithelial cell responses, with patients with type 2 high asthma demonstrating significantly lower airway bacterial burden.[46]

To summarize this section, studies over the last decade have revealed novel associations between differential patterns of respiratory microbiota composition and risk for childhood asthma or phenotypic features of persistent asthma in adults. These links are bolstered by evidence of specific associations with airway immune response patterns, particularly among adults with asthma, whether categorized by pattern of sputum cellular inflammation or epithelial cell gene expression. That there has been some consistency across studies to date in observations of a distinguishing bacterial microbiome signal in those with low type 2 or less/absent eosinophilic airway inflammation invites speculation that one or more subgroups of patients with type 2 low harbor airway microbial dysbiosis as a potential treatable trait.

CF

Over the past decade, studies of the CF lung microbiome have expanded our understanding of the relationships between CF lung microbiota, inflammation, and lung disease. Alterations in lung microbiota and inflammatory markers in CF begin as early as infancy. Lung microbiota, as measured in BAL fluid, differs in infants with CF compared with healthy infant control subjects.[52] Studies of BAL fluid CF animal models[53] and infants with CF[54] demonstrate that inflammation can occur in the absence of culturable bacterial infection, and is associated with structural lung disease.[54] Culture independent studies have demonstrated that the CF lung microbiome is highly diverse in infancy and the first years of life[55,56] and dominated by oral microbiota-associated taxa (eg, *Prevotella* species, *Veillonella* species, *Streptococcus* species, *Fusobacterium* species) that are often not identified on routine bacterial culture.[57] A recent study of BAL samples from young children with CF identified positive associations between these oral microbiota-associated taxa, bacterial burden, and increased host inflammation in young children with CF.[56] These findings emphasize that host-microbial interactions begin early in life in CF and are related to initiation of CF lung disease. These studies also specifically suggest a role for oral microbiota-associated taxa in the host-microbiota interactions leading to early CF lung disease, and are consistent with studies associating *Prevotella* species and *Veillonella* species with lung inflammation in healthy adults.[11,12]

Throughout later childhood and early adulthood, the diversity of CF lung microbiota decreases, as the relative abundance of traditional CF pathogens (many of which are members of the Proteobacteria phylum [eg, *Pseudomonas* species, *Burkholderia* species, *Achromobacter* species]) increases. Similar to findings in COPD and asthma, relative abundances of members of the Proteobacteria phylum in CF are positively associated with levels of inflammation[56,58,59] and advanced lung disease stage.[60]

Although these general trends in CF lung microbiota in association with age and disease stage have been reproducible across multiple studies, other associations between lung microbiota and clinical outcomes differ by disease phenotype. For example, increased relative abundance of anaerobic genera has been identified in association with CF pulmonary exacerbation[61]; however, this relationship is present only at early and intermediate disease stages, and not at advanced lung disease. In addition to disease stage, other phenotypes relevant for stratification of analyses of CF lung microbiota include disease aggressiveness (ie, rate of lung function decline in relation to age),[59,62] dominant taxa,[63] and chronic antibiotic use.[64,65] The interactions and causal relationships between CF lung microbiota, host inflammatory response, antibiotic (and other medication) use, and clinical outcomes in CF, however, remain largely unclear.

Despite these existing knowledge gaps in the drivers of CF lung disease, advances in our understanding of CF lung microbiota, and relevant disease phenotypes, have suggested potential applications wherein knowledge of the lung microbiome may help advance clinical practice. For example, the potential utility of CF respiratory microbiota as a biomarker of treatment response was illustrated in a study of lung function response to treatment with inhaled aztreonam.[64] In this study, patients with higher relative abundances of certain taxa (*Staphylococcus* species, *Prevotella* species, and *Fusobacterium* species) were less likely to have improvement in lung function with inhaled aztreonam treatment.[64] Other studies have identified changes in CF respiratory microbiota after initiation of treatment with ivacaftor, suggesting the potential use of respiratory microbiota as an outcome measure for response to cystic fibrosis transmembrane conductance regulator modulators.[65,66] In the longer-term future, the CF lung microbiome has potential utility as a prognostic

marker for disease course and/or as a target for intervention to prevent advancement of lung disease.

Conclusions

From this review, relationships between the microbiota and host vary by the lung disease being studied; however, many taxa are common to lungs in health and disease. Across these three inflammatory airway diseases with distinct pathophysiology, certain common host-microbial interactions have been identified. For example, members of the Proteobacteria phylum have consistently been identified as enriched in individuals with COPD, asthma, and CF vs healthy control subjects, and have been associated with more advanced disease. Across the three inflammatory airway diseases (and healthy control subjects), the significance of certain anaerobic taxa (eg, *Prevotella* species, *Veillonella* species) is less clear because these taxa are found in states of health, or milder disease phenotypes, but also can be associated with host inflammatory response. Additionally, in asthma and CF, there is rationale to suggest that early life interventions on the microbiota could modulate disease course; however, this has not yet been tested.

Moving forward, there also is common ground to be found in advancing research on host-microbial interactions in COPD, asthma, and CF. Although there are similarities that have been reported across different cohorts for all three diseases, there is a general lack of studies that investigate the reproducibility or repeatability of the currently published findings (eg, in pediatric studies of asthma). Reproducibility or repeatability studies would help illustrate the potential utility of deploying similar research methodologies in different study populations. Doing so would provide useful guidance into how to use microbiota features as a phenotype or risk stratification tool. One such approach could be the use of meta-analyses of existing data. However, pursuing this approach may be limited by the differences in methodologies and nonuniform provision of data and necessary metadata across studies. Additionally, there is the growing need to determine how the clinical heterogeneity observed in COPD, asthma, and CF inform our understanding of host-microbiota relationships within these diseases, which might change existing paradigms about their pathogenesis. Some of these limitations may be solved with the use of more longitudinal studies. The use of longitudinal studies also may provide opportunities to identify noninvasive microbiota-based biomarkers that could have a large impact on disease prevention and diagnosis. This paper focused specifically on the bacterial portion of the microbiota, and this does not mean that viral or fungal lung community members do not play a role in these diseases. Collectively, the lung microbiotas in COPD, asthma, and CF are different from individuals who do not have these diseases. However, knowledge gaps exist regarding the interactions between lung microbiota, host inflammatory response, and medication effects (eg, steroids, antibiotics), and how these complex interactions shape the pathogenesis of lung disease in COPD, asthma, and CF. Ongoing research in these areas will determine how much of the lung microbiota research in chronic airway diseases can be translated into the clinic.

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