The Lung Microbiome and Airway Disease

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Abstract

A growing body of literature has demonstrated relationships between the composition of the airway microbiota (mixed-species communities of microbes that exist in the respiratory tract) and critical features of immune response and pulmonary function. These studies provide evidence that airway inflammatory status and capacity for repair are coassociated with specific taxonomic features

of the airway microbiome. Although directionality has yet to be established, the fact that microbes are known drivers of inflammation and tissue damage suggests that in the context of chronic inflammatory airway disease, the composition and, more importantly, the function, of the pulmonary microbiome represent critical factors in defining airway disease outcomes.

Keywords: microbiota; microbiome; airways; asthma; cystic fibrosis

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Microbes represent the oldest and most ubiquitous life form on the planet, and the majority of subsequent life forms have coevolved with microorganisms. Although the diversity of the species within the microbial kingdoms is immense and growing (1), the field of microbiological research has, until recently, been reductionist. Studies have been focused largely on single isolated species, cultured under nutrient-rich conditions that infrequently represent the availability of nutritional resources in microbial ecosystems. Microbes are social organisms and they possess biochemical signaling mechanisms (quorum-sensing systems) that facilitate interspecies and interkingdom interactions, which are known to influence both the burden and the physiology of microbial cells (2). Microbial species exist most commonly, frequently as biofilms, in multispecies communities, sessile communities encased in protective polysaccharides that protect them from environmental insults. Given the clear need to understand microbial

members in a mixed species context, molecular methods have been developed to assess the compositional and functional features of microbial communities.

The most commonly used approach for assessments of community composition involves sequence-based methods in which a biomarker gene (e.g., the 16S or 18S ribosomal RNA [rRNA] gene, or fragments therein), or the interspacer region of the rRNA gene, is amplified by polymerase chain reaction primers. These primers are designed on the basis of regions of the rRNA gene that are highly conserved across the majority of known bacteria (16S rRNA) or fungi (18S rRNA or ribosomal interspacer region) and flank hypervariable regions of sequence within the gene. The resulting amplicon therefore contains genomic fragments from a variety of distinct bacteria or fungi that may be sequenced. The resulting sequence reads are grouped on the basis of a user-defined level of sequence identity (>97% is used typically) into operational taxonomic units or taxa. This approach permits the detection of taxa

within a given microbial kingdom (i.e., bacterial or fungal) and it is semiquantitative in that between-group differences in the relative abundance of these taxa may be assessed.

Although it is important to catalog taxon presence and the relative abundance of organisms within a single microbial kingdom, it has become increasingly apparent that bacterial, fungal, and viral species coexist and play a role in defining the composition and functional attributes of microbial communities (3, 4). Technological advancements in next-generation sequencing platforms have focused on increasing the number of sequence reads generated per sequence run. These advances have permitted a broader assessment (beyond biomarker-based taxon cataloging) of microbial communities and their genomic content. Shot-gun metagenomics, which describes the sequencing of fragmented extracted DNA, permits the assembly of the microbial genomes present in a given sample, in essence capturing the functional

capacity of the microbial community (bacterial, fungal, or viral). A similar approach that is based on extracted RNA (RNASeq/Metatranscriptomics) allows for community-level gene transcriptional profiles to be assessed. The application of mass spectrometry to rigorously collected and appropriately handled samples permits the assessment of a wide array of the proteins (proteomics) or metabolites (metabolomics) produced by the members of microbial communities or their environment. Hence, a growing number of studies in the field now apply a range of these assays in tandem to produce a more comprehensive view of the microbial community composition (microbiota) and, more importantly, the microbiome (communities of microbes and their encoded genomes) function within the human host.

Human Microbiome

More recently, these approaches have been used to study the human microbiome. What has emerged is a portrait of a human superorganism, in which the number of genes encoded by the various species of human-associated microbes dwarfs that of the human host genome. Indeed, a recent update to the integrated gene catalog of functional microbial genes detected in the gut microbiome of 1,257 people from the United States, China, and Europe now comprises 9,879,896 microbial genes (5). Studies are now beginning to establish that this wealth of coevolved microbial pan-genome plays critical roles in regulating the metabolic (6), immune (7), and physiological features (8) of mammalian systems. For example, fermentative microbes in the gut metabolize complex carbohydrates to produce antiinflammatory short-chain fatty acids that also serve as an energy source for intestinal epithelial cells (9). Moreover, microbial communities are not static, but rather, exhibit compositional and functional dynamics in response to a variety of factors, including age (10), mode of delivery at birth (vaginal vs. Caesarian section) (11), host genetics (12), host immune response (13), infections (14), diet (15) (including breastfeeding vs. formula feeding in infancy), diurnal rhythm (16), drugs (including antibiotics) (17), and environmental microbial exposures (18), which should be considered as potential confounding factors in microbiome studies.

Relationships to Diseases of the Lungs and Airways

In healthy subjects, the airways, which represent a large surface area that interacts with the external environment, exhibit a gradient of bacterial burden that is relatively high in the upper airways (19) and substantially diminished in the lower airways (20). The healthy airways also exhibit niche specificity, in that specific sites such as the nares and the oropharynx exhibit distinct distributions of bacterial taxa, with the former enriched for skin-associated *Actinobacteria* and the latter enriched more commonly for members of the *Firmicutes* and *Proteobacteria* (21). In contrast to healthy subjects, patients with airway diseases such as asthma exhibit a significant increase in the burden of bacteria present in the lower airways; moreover, the composition of these lower-airway communities is related to features of the disease (20, 22).

For example, individuals with asthma with the most severe bronchial hyperreactivity exhibit the greatest bacterial burden and diversity in their airways. This is contrary to findings in the gut microbiota, in which chronic inflammatory disorders are associated with bacterial diversity depletion (16), and indicates that, at least for asthma, the nature of microbiota perturbation associated with this chronic inflammatory disease lies in its difference from the healthy microbial state in that specific niche. An independent study of induced sputum from 56 adults, including 26 individuals with severe asthma, indicated that enrichment of specific *Streptococcus* taxa is associated with recent-onset asthma, rhinosinusitis, and sputum eosinophilia (23). Independently, Huang and colleagues demonstrated that the bronchial bacterial community composition in those with severe asthma ($n = 40$) was related to body mass index, sputum total leukocytes, and bronchial biopsy eosinophil numbers, with each of these factors associated with distinct airway bacterial community compositions (22). More specifically, positive associations were observed between biopsy eosinophil numbers and two members of the *Actinobacteria* (*Streptomyces* and *Propionimonas*) in this study (22).

However, correlation does not imply causality, and future studies that assess microbial community function in parallel

with host response and clinical disease features are more likely to identify particular microbial pathways and products associated with specific immunologically and clinically distinct asthma endotypes. What also remains unclear in the field is whether the microbiota differences observed in the airways of individuals with asthma vs. those without asthma are caused by asthma-associated chronic airway inflammation and increased mucus secretion, which lead to the development of a pathogenic community, or by a preceding perturbation that permits a pathogenic community of microbes to establish and drive disease pathology. Hence, understanding the natural history of the disease and the distinct pathways by which subjects converge on disease development will clearly require a multilayered microbial and host assessment of large and well-described birth cohorts.

Temporal bacterial community composition changes in the gastrointestinal tract, particularly in early life are well described (11). However, the community dynamics related to age and pulmonary health status are less well established. In a population of Australian infants ($n = 234$), Teo and colleagues recently demonstrated a temporal evolution of nasopharyngeal bacterial communities over the first year of life (24). The authors also demonstrated that upper-airway bacterial communities dominated by *Haemophilus*, *Streptococcus*, or *Moraxella* were associated with an increased risk of viral acute respiratory infection, lower-airway respiratory involvement, and increased severity. Airway colonization in infancy (<1 yr old) with a *Streptococcus*-dominated community was a risk factor for allergic sensitization at age 2 years and recurrent wheeze (asthma) at age 5–10 years, indicating that early-life airway colonization patterns shape the risk of subsequent airway disease development in childhood, although cause and effect remain to be elucidated.

In a cross-sectional population-based study of participants with cystic fibrosis (CF) ($n = 51$) ranging from 9 months to 72 years of age, with a variety of disease genotypes, Cox and colleagues demonstrated that although relationships existed between the CFTR genotype and community composition, a significant reciprocal relationship between age and lung function was also evident (25). In this patient cohort, loss of lung function in older patients was associated with a phylogenetic contraction within these communities and an expansion of the pulmonary pathogenic genera

including *Burkholderia* and *Pseudomonas*. These observations were confirmed and expanded on in an independent study of adult patients with CF ($n = 6$; age 19–31 yr) whose airways were sampled repeatedly over a 12-year period (26); those who maintained a relatively stable pulmonary status (as assessed by lung function) exhibited stable bacterial community α diversity (the number [richness] and distribution [evenness] of organisms within a community) over this period, whereas those with a progressive decline in pulmonary function exhibited a parallel loss of airway community diversity, indicating that in the case of CF-associated airway disease, an association exists between loss of bacterial diversity and disease progression in this population.

Mechanisms of Host Response and Disease

Defining taxonomic correlates with airway diseases represents a useful approach to identify associated organisms beyond those traditionally implicated. Even more useful is identifying relationships between taxa and markers of host inflammatory response. More recent studies of the airway microbiota have attempted to assess the immune responses associated with microbiota differentials. For example, Iwai and colleagues examined the bacterial communities detected in the bronchoalveolar lavage fluid of 60 Ugandan HIV-infected patients with acute pneumonia (27). Using DNA and RNA extracted in parallel, the authors profiled both the bacterial community composition and, in parallel, the expression of a range of host immune genes (inflammatory cytokines [IL-6, IL-17, tumor necrosis factor- α , IL-4, and IL-13]; a mucin production gene; indoleamine 2,3-dioxygenase, associated with impaired mucosal immunity and increased microbial translocation; and matrix metalloproteinase-9, abundant in lung diseases and involved in the breakdown of extracellular matrix). Patients with a richer and more diverse airway microbiome exhibited lower bacterial burden, enrichment of members of the *Lachnospiraceae* and sulfur-reducing bacteria, and reduced

expression of tumor necrosis factor- α and matrix metalloproteinase-9.

In addition, the study reported that compared with comparable San Franciscan HIV-infected patients with pneumonia, the Ugandan patients' airway microbiota were significantly richer and compositionally distinct, with predicted metagenomes that encoded a multitude of distinct pathogenic pathways including virulence secretion systems, suggesting that the higher rate of mortality in Ugandan HIV-infected patients with pneumonia may be, at least in part, a result of the presence of more diverse and pathogenic lower-airway microbiota. Using 16S rRNA profiling and parallel liquid chromatography mass spectrometry of bronchoalveolar lavage fluid from 39 HIV-infected subjects and 20 HIV-uninfected control subjects, Cribbs and colleagues demonstrated that although variation in overall bacterial community composition was not related to HIV status (discriminatory taxa on the basis of HIV infection status were not assessed), features of the metabolic profile, including enrichment of cysteine, two complex carbohydrates, and 3,5-dibromo-L-tyrosine, were characteristic of HIV-infected patients (28). The study also noted that specific metabolic features, including products of vitamin E, fatty acid, and arginine metabolism, were related to peripheral CD4⁺ cell counts (28).

More recently, using a cohort of 182 HIV-infected patients with pneumonia, Shenoy and colleagues demonstrated the existence of three compositionally distinct airway bacterial microbiota, each predicted to encode metagenomes capable of producing the metabolites detected in paired serum samples from these microbiologically stratified patient groups (29). Each pathogenic microbiota state induced a specific host immune response. These three groups also exhibited differences in mortality outcomes, with the highest-risk group exhibiting increased frequency of ceftriaxone administration, increased presence of culturable *Aspergillus*, and the greatest degree of airway Th2 inflammation. In contrast, the group with the lowest rate of mortality exhibited increased expression of

T-cell immunoglobulin and mucin domain 3 (*TIM-3*) gene expression. *TIM-3*, a glycoprotein expressed by both innate and T cells, is known to down-regulate Th1 proinflammatory activity (30) and to play a key role in the T-cell dysfunction associated with chronic viral infection (31, 32). This latter study suggests that examination of larger study cohorts that permit subgrouping of patients on the basis of the specific type of airway microbial community present are necessary to identify distinct, but repeatable, patterns of pathogenic airway communities that are associated with specific programs of host immune response. It also reinforces the point that assessment of microbial community composition and function in parallel with host immune responses (innate and adaptive), in the context of clinical outcomes, permits the identification of pathogenic airway community states that explain at least partly the observed heterogeneity in patient inflammatory responses and clinical outcomes.

Future Directions

The recent development of ancillary methods to examine microbial community function and the production of bioactive molecules that may mediate the relationship between microbial community members and the human host offers the opportunity to better understand microbiome–host relationships. These developments have precipitated a shift in the field away from primarily correlative and descriptive studies toward an improved mechanistic understanding of how specific pathogenic microbial states in the lower airways modulate host immunity and physiology. It is hoped that these efforts will ultimately lead to improved multivariate biomarker development that includes microbes as key predictors of health status. In addition, these efforts may permit personalized therapies tailored objectively toward the specific microbial dysbiosis and parallel immune dysfunction exhibited by the patient. ■

Author disclosures are available with the text of this article at www.atsjournals.org.

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