Lung Microbiome for Clinicians

New Discoveries about Bugs in Healthy and Diseased Lungs

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Abstract

Microbes are readily cultured from epithelial surfaces of the skin, mouth, and colon. In the last 10 years, culture-independent DNA-based techniques demonstrated that much more complex microbial communities reside on most epithelial surfaces; this includes the lower airways, where bacterial culture had failed to reliably demonstrate resident bacteria. Exposure to a diverse bacterial environment is important for adequate immunological development. The most common microbes found in the lower airways are also found in the upper airways. Increasing abundance of oral characteristic taxa is associated with increased inflammatory cells and exhaled nitric oxide, suggesting that the airway microbiome induces an immunological response in the lung. Furthermore, rhinovirus infection leads to outgrowth of *Haemophilus* in patients with chronic obstructive pulmonary disease, and human immunodeficiency virus–infected subjects have more *Tropheryma whipplei* in the lower airway, suggesting a bidirectional interaction in which the host immune defenses also influence the microbial niche. Quantitative and/or qualitative changes in the lung microbiome may be relevant for disease progression and exacerbations in a number of pulmonary diseases. Future investigations with longitudinal follow-up to understand the dynamics of the lung microbiome may lead to the development of new therapeutic targets.

Keywords: lung; microbiome; antibiotics; immune responses; inflammation

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Dimensionality of the Microbial Kingdom

In relative terms, we are the small creatures living in an enormous and complex ecosystem of microbes. These microbial communities were previously called "microflora," which is now replaced by the term "microbiome" (Table 1). Our bodies have 10 times more bacterial cells than the totality of human cells and 100 times more genetic material than our own genome (1, 2). The human gut alone contains around 1,000 different bacterial species. Millions of microbes populate our bodies and coexist with us peacefully throughout our lives. Indeed, the microbiome is crucial to our health, emphasizing the importance of understanding the role that this "second genome" carries on our epithelial surfaces. By definition, the microbiome includes all microbes in the human body, including bacteria, viruses, and fungi, although, for technical reasons, most initial research growth in this area has focused on bacteria.

Historically, microbiology has relied on culture techniques that study individual species in isolation. For example, Koch's postulates, a cornerstone of defining which pathogens are necessary and sufficient to cause an infectious disease, require *ex vivo* isolation and culture of the responsible microbe (3). Culture dependence has markedly limited a sophisticated understanding of host–microbe interaction because the vast majority of microbial species have never been successfully cultured. Most bacterial growth is dependent on specific microenvironments that have not been reproduced experimentally.

Two major scientific breakthroughs have bolstered our ability to characterize the complex microbial ecosystems that exist on our epithelia. First, with the efforts directed toward sequencing of the human genome during the Human Genome Project, inexpensive next-generation DNA sequencing techniques have been developed (4). When applied to bacterial ribosomal rRNA genes, this technology has enabled culture-independent description of bacterial communities without the need to rely on growing microbes in culture media. Second,

Table 1. Glossary of terms

Microbiota	All the microbes that are found in a particular region or habitat; the term "microflora" is no longer used
Microbiome	The totality of the microbes with their genes that are harbored by the microbiota and the milieu in which they interact
Metagenome	The genetic information of the whole microbiota, usually obtained by whole genome sequencing. This is the functional genetic potential provided by the genomes of many individual organisms
Metatranscriptome	Sum of genetic information in microbial mRNA, usually obtained by mRNA sequencing. Provides insights on what is functionally active in a microbial community
Virome	Collection of all the viruses in an environment
16S ribosomal DNA	A specific DNA gene that is unique to prokaryotic cells
Taxonomy	The science of identifying species and arranging them into a classification
Operational taxonomic unit (OTU)	Specific sequences based on sequence similarity (typically threshold is 97%) to reference genes. This is taken as a proxy for species-level divergence
Taxon	A group of phylogenetically related microbes that belong to the same taxonomic group, such as order, family, or genus
Amplicon	An amplified fragment of DNA from a region of a marker gene (such as 16S rDNA) that is generated by PCR
Sequencing	Technique allowing thousands or millions of DNA sequences to be obtained from a given sample
Richness	Number of different taxa within a single population
α Diversity	How many types of sequences in a sample
β Diversity	How many different types of sequences are shared among samples
Resilience	The capacity of the microbiome to absorb disturbance and reorganize itself while undergoing change, so as to retain essentially the same function, structure, and identity
Dysbiosis	A condition in which the normal structure of the microbiome is disturbed, often through external pressures such as disease states or medication

and equally important, bioinformatic algorithms were developed to organize and interpret the massive data sets spewing forth from the sequencing machines (5). These advances have allowed investigators to formulate novel hypotheses to understand the interaction between the microbiome and the host immune response and pathophysiological phenotypes.

Just as paleontology is able to define the evolutionary relationships between vertebrate fossils by comparing the similarities and differences of bones from different species, phylogenetic taxonomy of all living things is achieved by quantitatively comparing the similarities and differences in 16S ribosomal RNA (rRNA). Because all free-living organisms have ribosomes and ribosomal RNA, this approach is comprehensive and has gained general acceptance in the science of evolutionary biology (6). There are invariant regions of the ribosomal RNA used for priming the sequencing reaction and variable regions useful for discriminating among species. Multiplexing provides up to tens of thousands of sequence reads per sample at a relatively low cost (currently well below $100 per sample) (7). The improvement in sequencing techniques and lower cost now allow genome-wide sequencing to evaluate microbial DNA (metagenomics) or RNA (metatranscriptomics). With the use of these "meta-omic" approaches we can characterize genes, transcripts, and eventually proteins and metabolites from thousands of microbes. This new field of microbial epigenetics has now allowed the analysis of biochemical function and systems-level microbial interactions that are relevant for understanding the role of our nonhuman inhabitants.

The Human Microbiome Project was added to the National Institutes of Health Roadmap for Medical Research in 2007 and has invested more than $200 million aiming to characterize the microbial communities found at several different sites on the human body, including nasal passages, oral cavities, skin, gastrointestinal tract, and urogenital tract, and to analyze the role of these microbes in human health and disease. Thus far, the gastrointestinal tract has been the most intensively studied organ in microbiome research, where the role of the gut microbiota in shaping the mucosal immune system is being defined (8, 9). In the gut mucosa, specific microbiota have been linked to obesity (10–12), coronary artery disease (13–15), *Clostridium difficile* colitis (16, 17), type 2 diabetes (18), and inflammatory bowel disease (19, 20). These studies provide evidence for the relevance of the microbial environment to human health and suggest new, potentially therapeutic venues (21). The lung was not included as one of the sites for the Human Microbiome Project, but has received increasing interest in the last 5 years, with the National Heart, Lung, and Blood Institute sponsoring a Lung HIV Microbiome Project among other specific projects.

An Old Dogma to Challenge

For decades few have dared to challenge the preconception of sterility of the lung. It has been accepted that the sole presence of bacteria in the lower airways was a pathological phenomenon. Furthermore, although we have appreciated that the vast majority of the human cavities are inhabited by microbes, we continued to believe that the airways below the vocal cords were sterile. Data produced by culture techniques have shown frequent isolation of oral microorganisms in samples from the lower airways (22, 23). However, because techniques used to sample the lower airways require exposure to the upper airways, contamination was commonly blamed for those results (24–26). It has been observed that microaspiration was a frequent event in normal subjects (27–29). Other factors that had limited culture-dependent techniques include the low culturable bacterial burden, difficulties growing fastidious bacteria such as anaerobes, and inability to describe complex microbial communities.

More recently, the use of culture-independent techniques that assay microbial nucleic acids and antigens has allowed identification of potential pathogens in culture-negative respiratory specimens (30, 31). Furthermore, the lower airways of normal individuals harbor low levels of oral bacteria such as *Prevotella* species and *Veillonella* species (32, 33). These findings and other experimental models (34) have now challenged the dogma that the lower airways are normally sterile. However, using 16S rDNA PCR techniques, current lung microbiome data from the healthy lung may only reflect bacterial DNA fragments and not viable live bacteria (32, 33, 35–40). Although more data are needed to solve the viability issue, there is growing evidence that, similar to other epithelial surfaces, the airway microbiota exerts an effect on the immunological homeostasis of the lung mucosa in normal individuals (33).

Culture data in subjects with acute lung infections and chronic airway colonization secondary to chronic obstructive lung disease (COPD) and cystic fibrosis (CF) have shown that the most common source of microbes in the lower airway is the upper airway. Through microaspiration, there is seeding of oral microbes into the lower airways. Microaspiration occurs in normal individuals (27) and its prevalence is higher in several lung diseases, including COPD, asthma, obstructive sleep apnea, CF, and lung infections due to atypical (such as nontuberculous mycobacteria) and typical microorganisms (28, 41–45). This can occur through reduced coordination of breathing with swallowing followed by gastroesophageal reflux (28, 29, 41, 42). Furthermore, there is impairment of clearance of microorganisms from the lower airways in COPD and CF (46, 47). However, evaluation of the lung microbiome in normal subjects demonstrates that some specific bacteria have higher relative abundance in the lung than would be expected if they originated from the upper airways (35, 39). Furthermore, environmental exposures, frequent antibiotic and/or antiinflammatory use, or diet might exert a selection pressure on the lower airway microbiome (48).

What Is the Role of the Airway Microbiota?

Microbes exert an important physiological function in shaping the immune response of the airway mucosa (Figure 1). Studies done in European cohorts have shown that exposure to diverse microbes during childhood (such as growing up on a farm) is protective against asthma and allergies (49–51). This hypothesis is coincident with data from gut microbiota research, where early exposure to certain bacteria is needed for immune maturation in early life (8, 9). Under this view, commonly referred to as the "hygiene hypothesis," restricted microbial exposure leads to inadequate "priming" of the immune system during maturation, resulting in helper T type 1 (Th1)–Th2 cell subset imbalances, regulatory T cell deficiency, and innate immune abnormalities. Changes in diet, improved sanitary conditions, and increased use of antibiotics may limit the exposure to environmental microbes and be responsible for the increase in autoimmune diseases observed more recently (49, 52–57). In a mouse model, inhalation of an innocuous strain of *Escherichia coli* could reprogram dendritic cells and macrophages in the lungs, resulting in protection against allergic responses (58). This model suggests that direct exposure of the airways to bacteria is sufficient to elicit a protective effect.

However, the immune priming of the lung mucosa through microbial environmental exposure may not be a local event. There is increasing recognition of the presence of cross-talk between the gut and lung mucosa. Children whose stomach is colonized with *Helicobacter pylori* are 40–60% less likely to develop asthma than children who are not carriers (59, 60). The presence of *H. pylori* in the stomach is associated with decreased intestinal microbiome diversity, which is also associated with increased risk for allergic rhinitis and peripheral blood eosinophilia (61). Lessons from animal models show that disruption of the gastrointestinal microbiota may lead to abnormal immune responses (62–66).

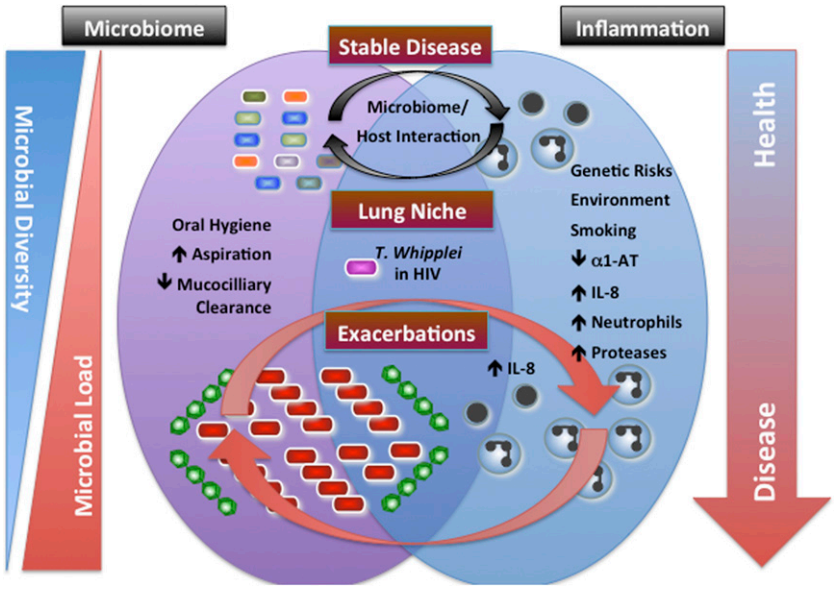

Figure 1. Conceptual model of the interaction between the lung microbiome and host immune response.

Ultimately, both the gut and lung mucosa may function as a single aerodigestive system and share the physiological function of immune surveillance and shaping the host response. Alternatively, changes in gut microbiome may be a marker of dysbiosis in other mucosas such as the upper airways (oral) and lower airways and explain a direct microbiome–respiratory immune mucosa interaction. Several lines of investigation are focused on trying to dissect the nature of this "cross-talk" between the gut and lung mucosa.

Lessons Learned from the Study of Airway Microbiota in Lung Diseases

Defining the contribution of the lung microbiome to various disease states is challenged by "carryover" as well as disease-induced changes in lung structure, cellular composition, and immune function that affect the microbial colonization of the airways. Although it is difficult to adjust for all possible confounders in human studies, there are several lessons to be learned from studies performed in various airway diseases.

In childhood asthma, two observations about the microbial exposure early in life highlight the importance of the microbiome in the development of the immune system. First, childhood exposure to a diverse microbial environment, either by farm habitation or pet exposure, is a protective factor reducing the risk of asthma (49, 67). Second, the acquisition of an airway microbiota enriched with pathogenic microorganisms (such as *Streptococcus pneumoniae*, *Moraxella catarrhalis*, and *Haemophilus influenzae*) in infancy increases susceptibility to asthma (68). Importantly, although the proinflammatory role of pathogenic bacteria such as *S. pneumoniae*, *M. catarrhalis*, and *H. influenzae* is well defined, less is known about what determines a "healthy" microbial exposure. The risk-reducing bacteria responsible for the "hygiene hypothesis" are poorly defined.

In adults with asthma, the airway microbiota has a high bacterial burden (69). In a cohort of asthmatic subjects with clinically stable but suboptimally controlled asthma (symptoms of respiratory tract infection or asthma exacerbation within 6 wk was an exclusion criteria), bronchial hyperresponsiveness was associated with increased bacterial burden and microbial diversity (number of different taxa in a sample) in airway brushing samples. Subjects with asthma also have an increase in proteobacteria in their sputum compared with healthy individuals (70). These data suggest that perturbations of the commensal microbial community might directly or indirectly influence the clinical phenotype in asthma and highlights the potential "pathogenic" role of commensal bacteria.

In patients with COPD, studies of the lung microbiome may help understand airway inflammation, hyperresponsiveness, exacerbations, and alveolar wall apoptosis. However, it is still unclear whether there is a unique lung microbiome in early-stage COPD (36, 40, 71). At a more advanced stage (GOLD 4) increased bacterial colonization and recurrent infections are associated with increased risk of exacerbations and accelerated loss of lung function (72). Furthermore, in advanced COPD, there is reduced bacterial diversity as compared with healthy individuals or those with milder cases of COPD (36). The apparent contradiction between the higher microbial diversity seen in asthma versus the lower diversity in COPD may be related to differences in the treatment that these subjects receive or reflect a predisposition of the lung microbiome in COPD to have a pathogen that dominates the microbial environment. Importantly, α diversity is a summary statistic of a single population and provides a partial snapshot of how complex a microbiome is without considering the specific taxa involved in the microbial community. To clarify this, it will be important to study early stages of COPD without the effects of antiinflammatory and antibiotic drugs on microbial diversity. In end-stage COPD, the microbiome of lung explants differs from healthy lung (40). Studies that evaluated the impact of smoking on the airway microbiome have found differences in the upper airway microbiome, with lower relative abundance of *Porphyromonas*, *Neisseria*, and *Gemella* and higher relative abundance of *Megasphaera* species, *Streptococcus*, *Veillonella*, *Atopobium* species, and *Actinomyces* (39, 73). Despite these differences in the upper airway microbiome, the lower airway microbiome of healthy smokers does not seem to differ from that of nonsmokers (33, 39), suggesting that other factors related to the environment or the host might be responsible for changes in the lung microbiome observed in COPD. The use of inhaled corticosteroids and bronchodilators may account for some of these differences in lung microbiome composition (48). It is unclear to what extent a dysbiotic lung microbiome is the cause or the consequence of changes in host immune response. A provocative finding is that there are regional variations of airway microbiota within a single individual (36).

Although chronic colonization of airways might be relevant for the host immune response, the presence and frequency of exacerbations have been associated with accelerated decline of FEV_1 and disease progression in several lung diseases. In COPD, exacerbations occur after infection with a new bacterial strain or change in bacterial load (74), and this dysbiotic microbiome has been associated with increased inflammation. In severe COPD exacerbations requiring mechanical ventilation, there is a diverse bacterial community suggesting a polymicrobial cause (75). This highlights the potential for ecological interaction of bacterial strains during exacerbations. The core of this bacterial community may be composed of previously unrecognized lung pathogens such as oropharyngeal and gut-associated bacterial species. Molyneaux and colleagues reported that infection with rhinovirus in patients with COPD was associated with increased bacterial load and change in microbiota composition (71). This highlights how different components of the microbiome other than bacteria (i.e., viruses and fungi) might be fundamental to the understanding of the intermicrobial relationships that may shape the airway microbiome. In this study, rhinovirus infection led to a change in the abundances of many pathogenic and nonpathogenic bacteria with a reduction of Streptococcaceae, Veillonellaceae, and Prevotellaceae and an increase in *Haemophilus* species and Neisseriaceae on Day 15. Interestingly, these changes in microbiota would not have been noticed on the basis of culture techniques and were associated with increased lung inflammation as measured by inflammatory cells and neutrophil elastase in the sputum. This is in line with other investigations suggesting that enrichment of the lung microbiome with oral microbes may be a contributing factor to the persistence of inflammatory cells (33).

In patients with bronchiectasis, poor mucus clearance is associated with persistent

bacterial colonization, airway obstruction, inflammation, and progressive tissue destruction, a picture complicated by frequent development of acute exacerbations (76). Although *H. influenzae*, *Pseudomonas aeruginosa*, and *S. pneumoniae* are the most frequently isolated pathogens by culture (77), new studies using culture-independent methods have shown a much more complex and diverse polymicrobial community. Tunney and colleagues compared culture-dependent and culture-independent techniques in sputum samples from patients with clinically stable bronchiectasis, detecting aerobic genera including *Achromobacter*, *Stenotrophomonas*, and *Streptococcus*, and anaerobes including *Prevotella*, *Veillonella*, and *Actinomyces* (78). Sequencing observed a much more diverse airway microbiome with airway microbial communities clustering into two groups: one composed of a relatively small number of taxa that dominate the environment (usually *Haemophilus*, *Pseudomonas*, and *Streptococcus*), and another with less commonly found taxa present in low abundance and higher diversity. ALlthough the agreement between the presence and absence of bacteria detected by culture and sequencing was relatively good for some aerobic bacteria, it was extremely poor for several other aerobic bacteria (*Haemophilus*, *Staphylococcus*, and *Streptococcus*) and anaerobic genera (such as *Prevotella* and *Veillonella*). Samples obtained during exacerbations had an increase in anaerobic microbes relative to aerobic microbes, suggesting that this compositional change may be an important factor contributing to the onset of exacerbations missed by culture techniques (78).

In CF, disease progression has been characterized by chronic infection with *Staphylococcus aureus*, *Burkholderia cepacia*, and *Pseudomonas aeruginosa* (79). With the use of sequencing, oral anaerobes, such as *Prevotella*, *Veillonella*, and *Rothia*, are also commonly recognized in airway microbiota (80, 81). Moreover, one other characteristic of the microbiome in the sputum of patients with CF is an increased ratio of Firmicutes to Bacteroidetes and lower microbial diversity when compared with the sputum of control subjects (82). Longitudinally, reduction in bacterial diversity is associated with disease progression and colonization with pathogens (83). The low bacterial diversity found in CF has been associated with higher inflammation, more advanced disease stage, and worse prognosis (83, 84). Dietary manipulations could restore microbial diversity in the airways in CF (85). Furthermore, low microbiota diversity also precedes the development of an exacerbation (86). An interesting parallel to the observed decreased microbial diversity associated with worse lung disease in CF is the observation that limited gut microbial diversity is associated with advanced inflammatory bowel disease (87, 88). It is likely that dynamic changes of airway microbiota occur over time, where a change from a "healthy" well-balanced polymicrobial microbiome to an "unhealthy" restricted airway microbiota renders the airway increasingly susceptible to a dominant pathogen, for example, *Pseudomonas aeruginosa*, and consequent lung injury. A confounder frequently cited is that intensive antibiotic therapy is common with more advanced disease and may be a primary driver of decreasing diversity in patients with CF. However, evaluation of the dynamic changes in airway microbiota in CF may be an important example of the relevance of understanding the resilience of the polymicrobial ecosystem as a whole that is only possible through culture-independent techniques.

Iwai and colleagues compared the oral and airway microbiome in HIV-infected patients treated with antimicrobials for acute pneumonia (89). The lungs exhibited a significantly higher relative abundance of multiple members of the Proteobacteria, including several known pathogens such as *Klebsiella pneumoniae* and *Pseudomonas* species, which may contribute to the high prevalence of recurrent pneumonia in HIV-infected patients.

Study of the lung microbiome in immunodeficiency states suggests that airway microbiota characteristics are determined by the host immune response as well. In subjects with immunodeficiency due to HIV and no obvious lung disease, the lung microbiome is enriched with *Tropheryma whipplei* as compared with control subjects (38). When longitudinal follow-up was performed, the relative abundance of *T. whipplei* decreased with antiretroviral therapy. Importantly, the increased relative abundance of this taxon in the lung as compared with paired upper airway samples suggests that the true niche of *T. whipplei* is the lung. Using whole-genome shotgun sequencing, the genome of *T. whipplei* found in the lung differed from reference genes obtained from patients with Whipple disease. Differences in the genome of *T. whipplei* from the lung were characterized by reduced genome size, lack of mobile DNA elements, and changes in genes encoding membrane proteins, suggesting that this organism evolved to adapt to a distinct lung environment. This is consistent with the presence of different selective pressures n various epithelial cell surfaces, thereby resulting in a distinct microbiota pattern.

In interstitial lung diseases such as idiopathic pulmonary fibrosis and sarcoidosis, there are few data about the composition of the airway microbiome (90). The common use of immunosuppressing drugs in the setting of chronic inflammation due to the disease process likely represents a major confounder in the study of the lung microbiome in these diseases.

In lung transplantation, there is increased bacterial load with enrichment with betaproteobacteria, especially *Burkholderia*, lower microbial diversity, and the presence of fungal species (91, 92). Major confounders in the study of the lung microbiome in transplantation are diversity of disease, heterogeneity of lung damage, and frequent antibiotic and steroid use (92). It is of increasing interest to determine whether changes in airway microbiota are associated with lung graft functionality, susceptibility to infections, or the development of bronchiolitis obliterans syndrome.

Lung Microbiome: What Can We Expect from Future Investigations?

Although none of the described culture-independent techniques are currently available from a clinical laboratory to guide individual patient care, this is an area of logarithmic growth where one can expect that new findings will become relevant to clinical care. We still need to understand what constitutes a "healthy" airway microbiome. By analogy with what it is known about the role of the gut microbiome in shaping the gut immune system, a healthy airway microbiome may be relevant to "prime" the innate immune system and produce optimal helper T subset cell maturation. This may allow

development of therapies attempting to manipulate the bacterial community for prophylaxis before an invasive procedure (e.g., intubation) or with the goal of preserving lung function in disease states. Potential therapeutic options could include the use of probiotics (living bacteria intended to benefit health), prebiotics (food ingredients that confer specific changes in the microbiome and lead to beneficial effects in the host), or antibiotics. Therapies attempting to modify the composition of the airway microbiota range in scale from eliminating individual strains of a single species (e.g., with antibacterial conjugate vaccines) to replacing the entire community with a new intact airway microbiota (similar to the use of fecal transplantation in cases of *C. difficile* colitis). Similar to the rationale for using probiotics in diet, a provocative concept would be whether a healthy airway microbiome could be nurtured with the use of certain microbial species or microbial nutrients that would restore or promote microbial diversity in the airways. Conversely, other commonly used inhaled medications, such as steroids, may shape the lung microbiome by inducing antiinflammatory changes in the host (48). A clinically relevant special case is the use of azithromycin in chronic airway diseases such as CF, bronchiectasis, COPD, and refractory asthma (93, 94). Analysis of the lung microbiome may decipher whether the beneficial effects of azithromycin in the prevention of exacerbations are antiinflammatory or antibiotic in nature. Furthermore, longitudinal studies are needed to better understand how changes in microbial composition and microbiota structure determine disease progression and exacerbations. Identification of these dynamic changes may be useful as both a biomarker and a potential therapeutic target.

Also, it is expected that nonbacterial microbes (viruses and fungi), mostly neglected in current lung microbiome studies because of technical difficulties, will receive further research interest. Viruses play a major role in chronic inflammatory diseases of the lung such as asthma, COPD, and CF, although few studies have been performed to evaluate the airway virome (95, 96). Improved understanding of the nonbacterial composition of the microbiome will be essential for completing our understanding the interactions of the microbial community in its entirety in the settings of health and disease. To this end, whole-genome sequencing (shotgun metagenomics) is a more promising approach. Using metagenomics to evaluate regional differences in the virome in the lung of advanced CF, it has been suggested that the apical lobes of patients with stable CF may house a unique virome characterized by extremely low richness and the predominance of eukaryotic viruses, such as herpesvirus, anellovirus, and papillomavirus (95). Interestingly, on the basis of these data, the most affected areas do not seem to have bacteriophages. The study of the lung virome may also be important to understand the role of bacteriophage communities (viruses that infect and replicate within bacteria, and that may serve as a reservoir for antibiotic resistance genes) in the response to antibiotic treatment. Similarly, specific components of the microbiome may interfere with the metabolism of inhaled drugs. As an example, members of the Comamonadaceae family have been shown to possess steroid-responsive degradation pathways (97). Characterization of the airway microbiome in various disease states could be used to assess the impact of antimicrobial therapy and ensure new rational treatment strategies devised to affect not only known pathogens but also colonizing bacteria that might be relevant to determine the microbiome structure. Reductions in the diversity of the airway microbiota may be a marker of increased risk for outgrowth of certain bacteria associated with disease exacerbations and severity, such as in CF and COPD. This could represent an early biomarker that may lead to the use of novel clinical interventions.

In summary, sequencing enables more comprehensive characterization of airway microbial community composition and is required to detect the presence of less abundant and potentially more difficult-to-culture microbes. The use of culture-independent techniques to study the lung microbiome has challenged our previous belief that the healthy lung was sterile and provides new insight into an important role of the microbiome for the mucosal immune maturation and response. We have shown that the lung microbiome in asymptomatic healthy subjects is characterized by low bacterial burden and is frequently enriched with taxa commonly found in the upper airways (33). The degree of exposure to upper airway microbes may be associated with increased inflammatory markers (33). Evaluation of the lung microbiome in various disease states has identified some changes in microbial diversity and/or enrichment with specific pathogenic or nonpathogenic bacteria. Therefore, it is plausible that specific components of the lung microbiome may prime the immune system to have a beneficial or detrimental immune response. Rather than aggressively prescribing broad-spectrum antibiotics, evaluation of the airway microbiota and its immune interactions may allow better-targeted antimicrobials and probiotic therapies intended to regulate pathogen activity and enhance the efficacy of immune mechanisms. ■

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