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The Role of the Microbiome in Exacerbations of Chronic Lung Diseases

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Summary

Culture-independent microbiological techniques have revealed a previously unappreciated complexity to the bacterial microbiome of the respiratory tract, forcing reconsideration of the interactions between host, bacteria and the pathogenesis of exacerbations of chronic lung disease. The composition of the lung microbiome is determined by microbial immigration, elimination, and the relative growth rates of its members; all of these change dramatically in chronic lung disease and further during exacerbations. Exacerbations lack key features of bacterial infections, including increased bacterial burden and decreased community diversity. We propose instead that exacerbations are occasions of respiratory dysbiosis: a disordered respiratory microbial ecosystem with negative effects on host biology. Respiratory dysbiosis provokes a dysregulated host immune response, which in turn alters microbial growth conditions in patient airways, further promoting dysbiosis and perpetuating a coupled cycle of inflammation and disordered microbiota. Differences in baseline respiratory microbiota may help explain the “frequent-exacerbator” phenotype observed across multiple disease states, and may provide novel targets for therapeutic intervention.

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Introduction

The natural histories of numerous chronic lung diseases are punctuated by exacerbations, characterized by abrupt worsening of respiratory symptoms and pulmonary function. Exacerbations are responsible for much of the morbidity, mortality, and expense of chronic lung diseases¹⁻³, and are associated with acceleration of disease progression⁴⁻⁷. Exacerbations have long been associated with viral exposure and bacterial growth from respiratory cultures, but the precise relationships between resident bacteria, acute infection, and exacerbation pathogenesis have been unsettled and controversial^{8, 9}.

In the past decade, novel culture-independent techniques of microbial identification have revealed a previously unappreciated complexity to the bacterial microbiome of the respiratory tract. The lungs and airways, whether in health or afflicted by chronic or acute lung disease, harbor diverse communities of microbes undetected by conventional culture-based approaches¹⁰. This revolution in lung microbiology has called into question long-held beliefs regarding the pathogenesis of exacerbations of chronic lung disease, our understanding of which derives from a half-century of experimentation and observation using culture-based techniques.

What is an Exacerbation?

Exacerbations of chronic lung disease are periods of acute worsening of respiratory symptoms. They arise abruptly, over hours to days, and generally prompt an escalation in medication therapy. Symptoms may include focal respiratory symptoms such as cough, increased sputum production, dyspnea or wheeze, but may include systemic complaints such as fever, fatigue or malaise. Symptom onset often precedes objective worsening in lung function¹¹, though for some patients with impaired perception of dyspnea, symptom onset occurs relatively late in the course of an exacerbation¹². Exacerbations are typically followed by recovery to the patient's prior lung function, though in many cases lung function returns to a newly compromised baseline^{4, 13}. Exacerbations are distinct from primary acute lung infections (such as a lobar bacterial pneumonia) and from irreversible progression of underlying lung disease (as with progressively obstructive bronchiolitis obliterans).

Modern Techniques of Lung Microbiome Study

Though a comprehensive discussion of modern techniques of microbial identification would exceed the scope of this Seminar, a familiarity with basic principles of study is key to understanding the recent revelations and persistent limitations of the field. We provide in references several sources for further reading^{10, 14, 15}.

While early lung microbiome studies employed a variety of molecular techniques to characterize microbial communities in respiratory specimens, the most commonly used modern approach employs high-throughput sequencing of amplicons of the 16S rRNA gene, a small and highly conserved locus of the bacterial genome. A single sequencing run of a respiratory specimen yields thousands of short genome sequences that are then aligned, sorted and classified according to established taxonomic databases. This sequencing-based approach describes communities of microbes in tissue specimens, permitting analysis of

community features such as diversity and relative membership of specific taxonomic groups. Quantitative PCR of the 16S rRNA gene provides an approximation of bacterial burden that correlates grossly with quantitative culture results¹⁶. Sequencing-based approaches thus do not rely upon microbes reproducing in the narrow growth conditions of conventional culture techniques, which fail to identify most human-associated microbes¹⁷.

The type of respiratory specimen studied and its route of acquisition is an important consideration in interpreting lung microbiome studies. Many studies in the field have employed bronchoalveolar lavage (BAL) fluid or protected specimen brushings (PSB). Though passage of a bronchoscope through the upper airways introduces a theoretical risk of contamination via pharyngeal microbiota¹⁸, the anatomic route of bronchoscope insertion (oral or nasal) has no detectable impact on BAL microbiota despite the markedly divergent microbiota present in these body sites^{16, 19, 20} (Figure 1), illustrating the minimal influence of upper respiratory tract contamination on microbiota detected via bronchoscopy. Additionally, as detailed below, significant associations have been reported between the microbiota of BAL and PSB specimens and numerous clinically significant parameters: severity of airway obstruction²¹, airway reactivity²², inhaled medication exposure²³, disease prognosis²⁴, clinical response to therapeutic intervention^{22, 25}, and the identity, number and behavior of pulmonary inflammatory cells^{16, 25, 26}. Every study using molecular-based culture-independent techniques that has compared the microbiota of healthy control subjects obtained via bronchoscope with that of subjects with lung disease has found significant differences in bacterial community composition¹⁰. These observations provide multifaceted validation of the biologic reality and significance of the lung microbiome, as detected in BAL fluid and PSB specimens.

For some lung diseases (cystic fibrosis [CF], bronchiectasis, and chronic obstructive pulmonary disease [COPD]), spontaneously expectorated and induced sputum has been employed for analysis¹⁰. While this introduces additional risk of upper airway contamination²⁷, features of microbiota detected in sputum have been significantly associated with patient age²⁸, disease severity²⁹, airway inflammation^{21, 30}, antibiotic exposure³¹, and response to controlled viral exposure³⁰. Thus any “noise” introduced to sputum specimens by oropharyngeal microbiota does not entirely obscure the meaningful “signal” that correlates consistently with other well-established indices of lung health and disease.

The Microbial Ecology and Topography of the Human Respiratory Tract in Health, Chronic Disease and Exacerbation

A key principle of microbial ecology is the central importance of local environmental conditions in determining the constitution of its resident microbial communities. An oft-cited tenet in the field is that “*Everything is everywhere, but the environment selects.*”³² The growth of a single species, and its relative abundance in its microbial community, is a function of nutrient availability, temperature, pH, oxygen concentration, and innumerable other environmental factors. Thus any inquiry into the respiratory microbiome must begin both with consideration of the unique local microbial growth conditions of the human

respiratory tract, in health and disease, as well as the dynamics of microbial immigration and elimination.

The airways and alveoli are topologically outside of the body and thus continually exposed to the environment. Though the linear distance from nares to alveolus is only half a meter in the average adult, the internal surface area of the lungs is 30 times that of the skin³³. Each day, this enormous terrain is exposed to more than 8000 liters of inhaled air, carried to within 0.1 micrometers of one tenth of the body's total blood volume³⁴. The lungs are arguably the human body's largest and most intimate interface with the outside environment^{33, 35}.

The constitution of the lung microbiome is determined by three factors: microbial immigration, microbial elimination and the relative reproduction rates of its members³⁶ (Figure 2). Any alteration of the lung microbiome detected in disease states must be attributable to some combination of these three factors. The proportion of the lung microbiome in healthy individuals that are resident, reproducing members (subject to regional differences in environmental growth conditions) versus transient microbes (determined solely by rates of immigration and elimination) is a topic of active investigation in the field³⁷. Microbes enter the lungs continually via inhalation of air (which contains 10^4 - 10^6 bacterial cells per cubic meter even before reaching the microbe-dense upper airways³⁸), microaspiration (which is ubiquitous among healthy subjects^{39, 40}), and direct dispersion along mucosal surfaces. The high degree of shared membership between the oral and lung microbiome, compared to the air, suggests that microaspiration and direct mucosal dispersion contribute more to microbial immigration than inhalation of bacteria^{37, 41, 42}. Microbes exit the respiratory tract via mucociliary clearance, cough (which is frequent even among healthy subjects⁴³) and the highly active and diverse antimicrobial mechanisms of innate and adaptive immunity. Local microbial growth conditions within the respiratory tract are heterogeneous. Within a single lung, dramatic regional variation can be found in oxygen tension, pH, relative blood perfusion, relative alveolar ventilation, temperature, epithelial cell structure, deposition of inhaled particles and in the concentration and behavior of inflammatory cells^{36, 44-46}, all of which have demonstrable effects on microbial growth rates. The distal alveoli are bathed in pulmonary surfactant, which has bacteriostatic activity against some (but not all) bacterial strains, further creating selective pressure on reproducing communities⁴⁷. Thus the “steady state” of the lung microbiome is one of constant influx, constant efflux, and spatial heterogeneity in local microbial growth conditions.

Chronic lung disease alters both the topography of the respiratory tract and the dynamics of microbial turnover. Destructive diseases such as emphysema and pulmonary fibrosis dramatically reduce the internal surface area of the lungs by as much as 90%^{48, 49}. Esophageal dysfunction and reflux is extremely common (>70%) among patients with advanced lung disease, increasing the microbial immigration rate and introducing an additional (gastric) microbial source^{50, 51}. Chronic airway diseases such as CF, bronchiectasis and chronic bronchitis are characterized by impaired mucociliary clearance, impairing microbial elimination. The same diseases are associated with increased baseline mucus production, providing both nutrient-rich growth medium as well as pockets of decreased oxygen concentration⁵² and increased temperature⁵³. The inflammatory cells of

the alveolus and airway are both more numerous and more activated in chronic lung disease than in health, even in the absence of cigarette smoking or active exacerbation^{54, 55}. Many therapies for chronic lung disease have profound effects on microbial growth conditions: supplemental oxygen, systemic and inhaled corticosteroids, and systemic and inhaled antibiotics all likely have pleiotropic effects on the influx, efflux and reproduction rates of lung microbiota. As evidenced by the association between disease severity and the identification of persistent bacterial species (“colonizers”) via culture-based testing⁵⁶⁻⁵⁸, as the severity of chronic lung disease worsens, the composition of the respiratory microbiome is determined less by the balance of microbial immigration and elimination (the primary determinant in healthy lungs) and more by regional growth conditions and differential reproduction rates within the respiratory tract (Figure 2).

In the context of a respiratory exacerbation, the topography of the respiratory tract changes further. Hyperventilation accelerates the influx of air-borne microbes and markedly lowers airway temperature⁵⁹. Increased cough accelerates microbial efflux, and the numbers and activation of inflammatory cells increase⁶⁰. Byproducts of the host inflammatory response such as inflammatory cytokines⁶¹⁻⁶³, catecholamines⁶⁴, increased temperature^{53, 65}, glucose and free ATP⁶⁵ are known growth factors for select bacterial species, creating selectively favorable growth conditions. Bronchoconstriction alters regional oxygen concentration and pH. Acute mucus production and vascular permeability increase local nutrient supply; airway mucus also introduces further gradients of local anoxia and hyperthermia, which selectively favor the growth of specific lung pathogens^{52, 53, 66, 67}.

The Microbiome and Exacerbations of Chronic Lung Diseases

Chronic Obstructive Pulmonary Disease

Exacerbations of COPD are associated with high mortality, accelerated loss of lung function and much of the health care costs of the disease⁶⁸. Susceptibility to frequent exacerbations both increases with disease severity and also represents an independent clinical phenotype of the disease that is stable over years⁶⁹. Exacerbations are associated with systemic inflammation⁷⁰, airway inflammation⁶⁰ and worsened obstruction due to airway edema, increased sputum production and bronchoconstriction⁷¹.

A relationship between COPD exacerbations and respiratory viral infection is inarguable. In case-control and serially-sampled cohort studies, respiratory viruses have been identified in respiratory specimens of 39% - 56% of COPD patients at exacerbation compared to 6% - 19% at clinical baseline.^{60, 72, 73}. When COPD subjects are administered a controlled rhinovirus exposure, they develop features of COPD exacerbations (cough, sputum production, airflow limitation and inflammation) significantly more than do non-COPD controls subjects⁷⁴.

In contrast, the relationship between bacteria and COPD exacerbations has been unclear and controversial for decades. While potentially pathogenic bacteria are cultured from respiratory specimens in 51% - 70% of patients during exacerbations, the same organisms are grown from 25 - 48% of specimens acquired during clinically stable baseline periods⁷⁵⁻⁷⁷. The culture-determined density of bacteria in sputum is not significantly

increased during exacerbation⁷⁸. One hypothesis has attributed COPD exacerbations to the introduction of new pathogenic bacterial strains to the airways. In a longitudinal study of cultured sputum in outpatients with COPD, new strains were more frequently isolated during exacerbation compared to clinical stability (29% vs. 13%); however, over 70% of exacerbations were not associated with detection of a newly identified strain⁷⁷. Thus, within the limitations of culture-based identification and a heterogeneous clinical syndrome, the causal relationship between bacteria and COPD exacerbations remains undetermined⁸. Accordingly, the benefit of antibiotic therapy in the treatment of COPD exacerbations remains controversial; recent metaanalyses suggest that antibiotics reduce the frequency of treatment failure in hospitalized patients with severe exacerbations, but are of unclear benefit for outpatients with mild or moderate disease⁷⁹. Recently, a large trial demonstrated reduced frequency of exacerbations in patients receiving chronic azithromycin⁸⁰, sparking renewed interest in the role of bacteria and host inflammation in exacerbation pathogenesis.

Recent culture-independent studies have revealed an unappreciated complexity to the microbiota of COPD exacerbations. Beyond the occasional bacterial species identified in roughly half of specimens via culture, the airways of patients with exacerbations in fact harbor dozens or hundreds of phylogenetically distinct bacterial taxonomic groups. In an early study, Huang et al. observed that even in subjects both receiving antibiotics and with positive *P. aeruginosa* growth from respiratory cultures, endotracheal aspirate specimens contained hundreds of distinct bacterial taxa representing an average of 140 distinct bacterial families. Subsequent studies have found comparably diverse microbial communities in spontaneously expectorated⁸¹ and induced³⁰ sputum specimens. In all reports, the bacteria identified by culture are detected via concurrent culture-independent techniques, but the converse is not the case: prominent culture-independent community members are often not cultured.

Exacerbations of COPD are associated with changes both in respiratory microbiota and associated airway inflammation. Using paired sputum specimens from COPD subjects at baseline and during exacerbations, Millares et al. found that exacerbations were associated with selective increase in relative abundance of taxa classically associated with exacerbations (e.g. *Haemophilus*, *Pseudomonas*, *Moraxella*) despite inconsistent detection via culture⁸¹. Huang et al., using paired sputum specimens of COPD subjects at baseline and during exacerbation, similarly found a shift in community composition towards the Proteobacteria phylum⁸². Molyneaux et al. employed a human model of virus-induced COPD exacerbations to systematically compare sputum microbiota in subjects at baseline and during exacerbation as well as with healthy controls³⁰. Compared to “baseline” sputum and that of post-viral-exposure control subjects, sputum acquired from “exacerbation” COPD subjects contained a significant shift in community composition towards Proteobacteria. None of these three studies observed a decrease in community diversity, as would be expected in the context of acute infection¹⁶. Importantly, Molyneaux et. al. found that key taxonomic groups that spiked in abundance during exacerbation exposure were detected in the same subjects both at baseline before viral exposure as well as after clinical resolution of the exacerbation.

Asthma

Exacerbations are responsible for much of the mortality, morbidity, and expense of asthma^{2, 83}. Precipitants include allergens, air pollution, and exercise, though, as with COPD, some patients exhibit a “frequent-exacerbator” phenotype independent of other risk factors⁸⁴. Asthma exacerbations are even more strongly associated with viral infections than those of COPD: respiratory viruses (most commonly rhinovirus) are detectable in respiratory specimens of more than 75% of patients with asthma exacerbations⁸⁵, and controlled rhinovirus instillation provokes the airway hyperresponsiveness observed in allergic asthma⁸⁶. Bacteria have not traditionally been implicated in asthma exacerbations, as they are infrequently cultured from patient sputum, and early antibiotic trials showed no clinical benefit⁸⁷. Yet recent serology- and PCR-based approaches have revealed a surprising prevalence of *C. pneumoniae* and *M. pneumoniae* infections among patients with acute exacerbations⁹.

Though to date no culture-independent studies have included respiratory specimens acquired from asthmatic subjects in the context of an exacerbation, multiple studies have demonstrated that the airways in asthma harbor a microbiome distinct from that of healthy subjects, suggesting an association between respiratory microbiota, airway inflammation, and susceptibility to exacerbations. In four separate studies analyzing respiratory microbiota (using BAL^{25, 88}, bronchial brushings^{22, 88}, and induced sputum⁸⁹), the microbiota detected in asthmatic airways at baseline has been consistently and significantly distinct from that of control subjects. All four studies demonstrated significantly increased community abundance of Proteobacteria, the bacterial phylum that contains common gram-negative respiratory pathogens such as *Haemophilus* spp., *Pseudomonas* spp., and *Klebsiella* spp.. Goleva et. al. observed an increased concentration of LPS in Proteobacteria-rich specimens²⁵, providing *in vivo* validation of this difference in community membership.

These studies have demonstrated provocative associations between the respiratory microbiome and the clinical, physiological, and therapeutic aspects of asthma. Huang et al. demonstrated that airway hyperresponsiveness (assessed by methacholine challenge) was positively associated both with community diversity and community composition²² (Figure 3A). This association was driven by the increased community abundance of the Proteobacteria phylum among patients with hyperresponsive airways. The authors also demonstrated that clinical improvement to clarithromycin was significantly predicted by differences in patients' baseline microbiota (Figure 3B). Separately, Goleva et al. observed that differences in baseline microbiota were associated with patients' clinical response to systemic corticosteroids²⁵ (Figure 3C). *In vitro* co-culture of BAL-acquired macrophages with *Haemophilus parainfluenzae* (which they observed only in corticosteroid-resistant subjects) blunted response of airway macrophages to corticosteroid effects, demonstrating the biologic plausibility of a relationship between airway microbiota and the hyperresponsiveness that is accentuated during exacerbations.

Cystic Fibrosis

Exacerbations in CF are associated with much of the mortality and expense of the diagnosis, as well as acceleration of lung function decline^{3, 6}. With increasing age, nearly all CF

patients grow select respiratory pathogens from sputum (most commonly *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Haemophilus influenzae*)⁵⁷, both during exacerbations and during clinical stability. Exacerbations have thus classically been considered infectious events and treated primarily with antibiotics, though data supporting this is surprisingly sparse⁹⁰⁻⁹². Provocatively, two large trials have found no detectable association between patients' clinical response to antibiotic therapy during exacerbations and the *in vitro* susceptibility of their cultured organism to their administered antibiotic^{93, 94} (Figure 4A); further, the clinical course of patients receiving antibiotics during exacerbations is unrelated to concurrent change in culture-determined sputum bacterial burden⁹⁵.

In the past decade, use of culture-independent techniques have revolutionized our understanding of the CF microbiome at baseline and during exacerbation. Early studies revealed a far greater microbial diversity in sputum than was appreciated via culture⁹⁶, with viability of many microbes verified both via amplification of transcribed RNA⁹⁷ and advanced culture techniques⁹⁸. Dozens of studies have since verified that the sputum of CF patients contains diverse bacterial communities, replete both with classically-recognized pathogens and previously unrecognized microbes⁹⁹. Several studies have shown an overall decrease in community diversity with age and disease severity^{10, 28, 29}; this loss of diversity is more strongly associated with cumulative antibiotic exposure than disease severity^{29, 31}.

Culture-independent analysis has upended long-held assumptions about the bacterial pathogenesis of CF exacerbations. Multiple studies have analyzed paired specimens acquired from patients during periods of stability and subsequent exacerbation^{29, 100-102}. All have been remarkably consistent in their key findings: CF exacerbations are not associated with increased bacterial density and are not associated with decreased community diversity (Figures 4B, 4C). These results differ starkly from those found in patients with bacterial pneumonia, for which, as expected, bacterial burden is high, community diversity is low, and the intensity of the host inflammatory response correlates tightly with bacterial burden^{16, 103}. These findings, considered with the lack of association between culture-identified pathogens and clinical response summarized above, strongly challenge the conventional understanding of CF exacerbations as acute infections of the airways.

These same studies have asked whether bacterial community membership changes at the time of exacerbation, and no consistent pattern of microbial change has emerged^{31, 97, 98}. Carmody et al. found that compared to baseline, respiratory microbiota at the time of exacerbation contain a consistent and significant increase in abundance of *Gemella* sp., a gram-positive anaerobe associated with the mouth and upper GI tract^{29, 100, 101}. It is unknown how baseline differences in microbiota influence the frequency or character of CF exacerbations; Carmody et. al. observed that dissimilarity between baseline and exacerbation microbiota was significantly associated both with high baseline diversity and baseline community domination by *Pseudomonas* sp.¹⁰¹.

Non-CF Bronchiectasis

Patients with bronchiectasis not attributable to CF comprise a heterogeneous group with a variety of predisposing conditions, though the plurality of cases are idiopathic. Compared to CF, little is known about the pathophysiology and treatment of the chronic inflammation and

episodic exacerbations that characterize its clinical course. New evidence suggests that chronic macrolide therapy decreases the frequency of exacerbations in this population¹⁰⁴, though as with CF and COPD, it is unknown whether this is due to antimicrobial effects.

Several recent studies have employed culture-independent analysis to study the microbiome of non-CF bronchiectasis. A single study by Tunney et al. has directly compared paired sputum specimens of patients with non-CF bronchiectasis at stability and during exacerbation²¹. They observed no difference in community diversity at the time of exacerbation, and by quantitative culture analysis they observed no increase in aerobic, anaerobic or total bacterial growth. Communities both at baseline and exacerbation were largely dominated by Proteobacteria (e.g. *Haemophilus* sp. and *Pseudomonas* sp.). Rogers et al. observed that the composition of sputum microbiota at clinical baseline was predictive of subsequent exacerbation frequency; importantly, this association was not detected when limited to culture-based microbiologic results¹⁰⁵. A study comparing explanted lung tissue found comparable microbiota in CF and non-CF bronchiectasis lungs¹⁰⁶, but it is unknown how generalizable findings of CF studies are to non-CF bronchiectasis patients.

Idiopathic Pulmonary Fibrosis

While the natural history of idiopathic pulmonary fibrosis (IPF) has traditionally been characterized as one of slowly progressive decline in lung function, the clinical phenomenon of acute exacerbations of IPF (AE-IPF) has been increasingly recognized as a major cause of mortality in the disease⁷. Exacerbations arise over the course of weeks and are characterized by progressive hypoxemia, lung infiltrates, the histological presence of diffuse alveolar damage and the clinical exclusion of infection or heart failure. Mortality is high and no therapies are of demonstrated benefit. Many patients with AE-IPF exhibit clinical features of infection (fever, cough, BAL neutrophilia)^{107, 108}, but no infectious etiology has been identified.

Several recent observations have provoked interest in a potential role of the bacterial lung microbiome in the progression of IPF and its exacerbations. Richter et al. observed a surprising frequency of culture-positivity in the BAL fluid of patients with IPF at clinical baseline¹⁰⁹, and subsequent culture-independent reports have confirmed that IPF lungs harbor a distinct microbiome from that of healthy lungs^{24, 110}. Han et al. recently observed a positive association between the presence of specific microbial community members (*Staphylococcus* sp. and *Streptococcus* sp.) in BAL and disease progression²⁴, and a randomized controlled trial of co-trimoxazole found reduced mortality among IPF patients receiving antibiotic therapy¹¹¹.

Key Lessons and Directions for Study

Exacerbations are not acute bacterial infections of the airways but rather instances of dysregulated host immune response coupled with respiratory dysbiosis

Despite the unambiguous presence of airway inflammation in respiratory exacerbations, a startling and consistent finding across disease states has been the conspicuous lack of evidence that exacerbations represent acute bacterial infections of the airways. Of the eight culture-independent studies across three disease states that have compared patients'

respiratory specimens acquired at baseline and during exacerbation, all have found no change in bacterial density or community diversity during exacerbation^{21, 29, 30, 81, 82, 100-102}. This differs starkly from studies of bacterial pneumonia, in which (as expected) increased inflammation is strongly associated with increases in bacterial burden and decreases in community diversity^{36, 103}. The airway inflammation common to all respiratory exacerbations is surprisingly dissociated from microbial load and community domination by one or few pathogenic species.

Exacerbations differ from acute bacterial infections in numerous important respects (Table 1). Acute bacterial infections respond rapidly and consistently to antibiotics, while data showing benefit of antibiotics in treating respiratory exacerbations ranges from subtle to inconsistent to absent^{79, 87, 92}. *In vitro* susceptibility testing is of clear utility in predicting response to therapy in bacterial lung infections¹¹², but bears no correlation with response to therapy in CF exacerbations^{93, 94}. While bacterial lung infections are the most common etiology of severe sepsis¹¹³, respiratory exacerbations rarely (if ever) directly provoke severe sepsis and shock.

These key differences between exacerbations and bacterial infections do not imply that bacteria are uninvolved in exacerbation pathogenesis. Rather, we propose that exacerbations are occasions of respiratory dysbiosis: disorder and dysregulation of the microbial ecosystem of the respiratory tract, coupled with a dysregulated host immune response, resulting in negative effects on host biology. Paired specimen studies have observed significant changes in the constitution of respiratory bacterial communities during exacerbations, often with phylum-level shifts away from Bacteroidetes (the most abundant phylum reported in the lungs of healthy subjects^{10, 16, 88}) and towards members of Proteobacteria^{30, 81, 82} and other disease-associated taxonomic groups¹⁰¹. The increase in frequency of novel strain isolation in sputum observed in COPD exacerbations compared to baseline (from 13% to 29%)⁷⁷ is consistent with the phenomenon of respiratory dysbiosis. Altered environmental conditions provoke a disturbance in bacterial community composition, both increasing relative abundance of previously undetectable species as well as increasing the community's susceptibility to invasion via new strains.

A rich analogy may be drawn between exacerbations of chronic lung disease and exacerbations of inflammatory bowel disease. Both are acute clinical worsening of chronic inflammatory conditions of mucosa-lined luminal organs, associated with profound dysbiosis and dysregulated host inflammatory response¹¹⁴. Both are characterized by an erosion of the homeostatic balance between resident microbiota and host immunity¹¹⁵. Neither is an instance of “acute infection” in any traditional sense, in which one or few pathogenic species overtake a body site and directly mediate tissue injury. In both cases, the benefit of antibiotics, if present, is not via eradication of a targeted pathogen but instead via selective manipulation of microbial community composition or indirect immunomodulatory effects (potentially explaining the discordance between culture sensitivities and clinical response observed in CF^{93, 94}). Both are either prevented or treated by macrolides, which have both antimicrobial and immunomodulatory effects^{80, 104, 116, 117}.

We propose in Figure 5 a model of the cycle of host inflammation and respiratory dysbiosis that typifies a respiratory exacerbation. An inflammatory trigger (e.g. viral infection, allergic exposure, etc.) initiates a cascade of host inflammatory responses that acutely and profoundly alter the microbial growth conditions of the airways. Permeability of the airway wall and mucus production introduce nutrient-dense substrate for bacterial growth^{52, 66}. Free catecholamines and inflammatory cytokines (e.g. TNF- α , IL-1, IL-6, IL-8) directly promote growth of select bacterial species (e.g. *P. aeruginosa*, *S. aureus*, *S. pneumoniae*, *B. cepacia complex*) via interkingdom signaling⁶¹⁻⁶⁵. Inflammatory cells are recruited and activated, killing and clearing bacteria with highly variable effectiveness¹¹⁸, creating a gradient of negative selective pressure across species. Airway mucus creates local pockets of increased temperature and decreased oxygen tension, selectively favoring growth of prominent disease-associated microbes^{52, 53}. Within this model, these diverse effects on microbial growth conditions result in disorder and dysregulation of the dynamic homeostasis of the airway microbiome, i.e. *respiratory dysbiosis*. Newly-prominent community members, some with altered expression of virulence traits and some with enhanced immunogenicity compared to previously prominent members, provoke further airway inflammation via pathogen-associated molecular pattern (PAMP) – pattern recognition receptor (PRR) interactions¹¹⁹, including differential activation of toll-like receptors^{120, 121}. This inflammation further alters growth conditions within the airway, resulting in a self-amplifying feedback loop. Tissue injury arises via a combination of direct injury from newly prominent community members, alteration in microbial behavior, and indirect effects of the dysregulated inflammatory response¹²². Homeostasis is attained only after the feedback loop of dysbiosis and inflammation is severed. While the initial dysbiosis observed in exacerbations may be secondary to a primary inflammatory trigger, the causal relationship between inflammation and dysbiosis is likely bidirectional and self-perpetuating, providing a plausible explanation for why the airway inflammation of exacerbations persists long after direct exposure to the inflammatory precipitant^{13, 30, 68}.

A distinct but related observation in the field is that the virulence and immunogenicity of a single bacterial species within the respiratory tract is not static, but rather contingent on numerous host- and microbe-derived environmental factors. A rich recent literature has demonstrated that the behavior of a given bacterial strain (as assessed either by gene expression or *in vitro* or *in vivo* virulence and immunogenicity) is strongly influenced by the same environmental factors that differentially alter bacterial growth rates: temperature^{53, 65}, concentration of host-derived cytokines and catecholamines^{63, 64} and presence of free glucose⁶⁵. Further, the behavior and virulence of a single strain is dramatically influenced by the composition of the bacterial community sharing its ecologic niche¹²³, mediated by direct and indirect microbe-microbe interactions^{124, 125}. Changes in the metabolism and virulence of microbes (even independent of community membership) can modulate inflammatory cascades, as has been proposed for the gastrointestinal tract and modeled for *P. aeruginosa* in *Drosophila*^{123, 126, 127}. Thus the environmental conditions of the inflamed airways can alter the virulence of individual community members both directly (via the virulence-promoting factors listed above) as well as indirectly (via the alteration of community composition and microbe-microbe interactions observed in respiratory

dysbiosis). This recent insight will be critical for subsequent study of bacterial lung disease: the pathogenicity of a single species cannot be understood outside of its ecological milieu.

The observation that “respiratory exacerbations” do not occur in individuals without chronic lung disease is self-evident but telling. The response of healthy airways to an inflammatory exposure (e.g. viral infection) is measured, proportionate to the insult, and self-limited. Any disruption in baseline lung microbiota is relatively muted and short-lived³⁰. The key phenomena of exacerbations -- sustained respiratory dysbiosis coupled with a prolonged immune response disproportionate to microbial burden -- arise only when dysfunction already exists at baseline on both sides of the microbiome-immune interface. Even when clinically stable (outside of exacerbation states), patients with chronic lung disease, when compared to healthy controls, have alterations both in respiratory microbiota^{10, 128} and in the constitution and activation of airway inflammatory cells^{54, 55, 129} (Table 2). The composition of respiratory microbiota, determined primarily by the balance of immigration and elimination in health, is increasingly determined by regional growth conditions with increasing severity of lung disease (Figure 2); in an exacerbation, the contribution of regional growth conditions abruptly becomes all-important. We propose that the coupled and self-perpetuating dysregulation of dysbiosis and airway inflammation of exacerbations is an abrupt, emergent phenomenon arising from the chronically disordered but compensated, fragile homeostasis present in stable chronic lung disease.

An important consequence of understanding the airways as an ecosystem is recognition of the limitations of conventional analytic approaches in modeling its behavior. While the conventional model of airway infection implies a linear system of pathogenesis (a suitably large bacterial inoculum enters the airways and overwhelms host defenses with unrestrained growth and resultant inflammation), within our modern model, exacerbations instead emerge abruptly from the complex adaptive system of the respiratory ecosystem³⁶. Ecosystems are “prototypical complex adaptive systems”¹³⁰, defined by the presence of diverse entities, interacting with each other within a common space, exhibiting interdependent actions and possessing the capacity to adapt to changes in conditions^{131, 132}. Within linear systems, small changes in conditions result in proportionately small changes in outcomes, whereas in complex adaptive systems outcomes can be abruptly and disproportionately altered by small differences in conditions. Unlike linear systems, complex adaptive systems defy reductionist modeling and are instead better modeled via computational techniques (e.g. agent-based modeling)^{133, 134}. Such techniques may prove essential in understanding how the phenomena of respiratory exacerbations emerge from the homeostasis of clinical stability³⁶.

The respiratory microbiome may partly explain the “frequent-exacerbator” phenotype of chronic lung diseases

In multiple chronic lung diseases, a subgroup of patients experience exacerbations more frequently than predicted by established risk factors^{69, 84}. This “frequent-exacerbator” phenotype is consistent across years, implying a persistent biologic predisposition to exacerbations that is unexplained by our current understanding of their pathogenesis. The revelations of the culture-independent era have prompted a novel hypothesis: differences in

baseline respiratory microbiota may explain the differences observed in exacerbation frequency among otherwise clinically equivalent patients.

Several preliminary observations lend plausibility to this hypothesis. Among patients with non-CF bronchiectasis, Rogers et al. observed that differences in baseline respiratory microbiota were strongly and significantly associated with the number of exacerbations experienced in the subsequent year¹⁰⁵; importantly, no association was observed between culture-based microbiology results and exacerbation frequency. Among patients with asthma, as discussed above, several associations have been reported between baseline respiratory microbiota and both airway hyperresponsiveness and clinical response to therapy^{22, 25}. Molyneaux et al. demonstrated in COPD patients that after rhinovirus exposure, alterations in the respiratory microbiome were both heterogeneous among patients and persistent at six weeks³⁰. Chronic azithromycin therapy has been shown to decrease the frequency of exacerbations in COPD, CF and non-CF bronchiectasis^{80, 104, 116}, though it is unknown whether this benefit is attributable to its unquestionable effects on respiratory microbiota¹³⁵. Two randomized controlled trials have observed a benefit of enteric probiotics in decreasing the frequency of cystic fibrosis exacerbations^{136, 137}; given the anatomic and ecologic continuity of the aerodigestive tract, it is plausible that this benefit was mediated in part via alteration of respiratory microbiota. Given the plausibility of this hypothesis and the high morbidity and expense associated with exacerbations of chronic lung disease, an immediate goal of further study should be to determine both whether the respiratory microbiome can be used to predict exacerbations, and to determine if its therapeutic manipulation can decrease exacerbation frequency¹²⁸.

Culture-based approaches are inadequate to completely understand the interactions of the host, microbiome and disease pathogenesis

Every study of respiratory exacerbations that has employed both culture-dependent and culture-independent results has found marked divergence in their results; while culture-independent techniques faithfully confirm the presence of culture-identified organisms, culture-based approaches grossly understate the complexity, richness, and dynamism of respiratory microbiota. Importantly, culture-independent approaches have identified significant associations between respiratory microbial community features and airway hyperresponsiveness²², disease prognosis²⁴, susceptibility to exacerbations¹⁰⁵, and response to a controlled respiratory viral exposure³⁰; each association would have been unappreciated by culture-based approaches. Given the intimate relationship between the respiratory microbiome and the host, and given the plausible role of the microbiome as mediator of acute and chronic inflammation, changes to the respiratory microbiome should be considered as a secondary outcome of all clinical trials targeting a reduction in exacerbation frequency.

Conclusions

Use of culture-independent techniques of microbial identification has upended our understanding of the role of bacteria in exacerbations of chronic lung disease. Exacerbations are not bacterial infections of the airways, but instead occasions of respiratory dysbiosis:

disorder and dysregulation of the microbial ecosystem that provoke a dysregulated host inflammatory response, which in turn promotes further dysbiosis. Future studies should investigate which features of the respiratory microbiome are associated with subsequent exacerbations and determine whether therapeutic manipulation of the microbiome can decrease exacerbation frequency.

Search Strategy and Selection Criteria

We searched Medline with no date nor language restrictions. Initial search phrases were “exacerbation[All Fields] AND (“microbiota”[MeSH Terms] OR “microbiota”[All Fields] OR “microbiome”[All Fields])” and “DISEASE[All Fields] AND exacerbation[All Fields] AND (“microbiology”[Subheading] OR “microbiology”[All Fields] OR “bacteria”[All Fields] OR “bacteria”[MeSH Terms])” where DISEASE represents “cystic fibrosis,” “COPD,” “bronchiectasis,” “asthma,” or “pulmonary fibrosis.”

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Figure 1. Lack of influence of upper respiratory tract microbiota on BAL microbiota

Though the microbial communities of the mouth and nose differ significantly²⁰ (A), route of bronchoscope insertion (via mouth or via nose) has no appreciable effect on BAL microbiota (B). **A:** Ordination generated from the University of Michigan data collected as part of the NHLBI Lung HIV Microbiome Project^{37, 138}. **B:** Reproduced from Dickson and colleagues¹⁶.

Figure 2. Determinants of the respiratory microbiome

The constitution of the respiratory microbiome is determined by three factors: microbial immigration, microbial elimination and the relative reproduction rates of its members. Any alteration detected in disease states must be attributable to some combination of these three factors. In health, community membership is primarily determined by immigration and elimination; in advanced lung disease, membership is primarily determined by regional growth conditions.

Figure 3. Associations between respiratory microbiota and clinical features of asthma
Among asthmatics, respiratory microbial diversity is positively associated both with bronchial hyperresponsiveness (A) and whether bronchial reactivity improved with clarithromycin therapy (B). Bacterial community composition is significantly different among asthmatics whose airway obstruction improves with systemic corticosteroids (C). **A**, **B**: Reproduced from Huang and colleagues²². **C**: Reproduced from Goleva and colleagues²⁵.

Figure 4. Cystic fibrosis exacerbations lack key features of bacterial infections

A: CF patients' clinical response to antibiotic therapy is not correlated with the *in vitro* susceptibility of organisms cultured from sputum at the onset of exacerbation. **B:** Bacterial density in sputum does not increase prior to or at the onset of exacerbation. **C:** Sputum bacterial community diversity is not lower during exacerbation compared to clinical baseline. **A:** Reproduced from Hurley and colleagues⁹³. **B:** Reproduced from Stressmann and colleagues¹⁰². **C:** Reproduced from Carmody and colleagues¹⁰¹.

Figure 5. The dysbiosis-inflammation cycle

An inflammatory trigger initiates airway inflammation, which alters environmental growth conditions of airway microbiota via positive and negative selective pressures. Disordered growth conditions result in a disordered microbiome, which provokes further airway inflammation via pathogen-associated molecular pattern (PAMP) – pattern recognition receptor (PRR) interactions, microbial metabolite signaling to leukocytes and epithelial cells, and other pathways. This results in a self-amplifying cycle of airway inflammation and respiratory dysbiosis.

Table 1
Comparison of respiratory exacerbations with acute bacterial infections

	Acute bacterial respiratory infections	Respiratory exacerbations
Bacterial density (compared to baseline)	High	Normal
Bacterial community diversity (compared to baseline)	Low	Normal
Culture growth	Usually	Occasionally
Airway inflammation	High Proportionate to microbial burden	High Disproportionate to microbial burden
Sepsis	Common	Never/rare
Clinical benefit of antibiotics	Rapid, unambiguous	Inconsistent, subtle
Relevance of <i>in vitro</i> susceptibility	Critical to clinical response	No relation with clinical response

Table 2
Comparison of respiratory health with stable and exacerbated chronic lung disease

	Health	Chronic Lung Disease (Stable)	Chronic Lung Disease (Exacerbation)
Primary ecological determinant of community composition	Immigration/elimination	Mild: Immigration/elimination Severe: Regional growth conditions	Regional growth conditions
Respiratory dysbiosis	Absent	Moderate, varies with disease severity	Severe
Airway inflammation	Absent/mild Proportionate to microbial burden	Increased Proportionate to microbial burden	Increased Disproportionate to microbial burden