Infections in chronic lung diseases 1

The role of the microbiome in exacerbations of chronic lung diseases

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Culture-independent microbiological techniques have shown a previously unappreciated complexity to the bacterial microbiome of the respiratory tract that forces 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 relative growth rates of its members. All these factors change dramatically in chronic lung disease and further during exacerbations. Exacerbations lack the features of bacterial infections, including increased bacterial burden and decreased diversity of microbial communities. We propose that exacerbations are occasions of respiratory tract dysbiosis—a disorder of the respiratory tract microbial ecosystem with negative effects on host biology. Respiratory tract dysbiosis provokes a dysregulated host immune response, which in turn alters growth conditions for microbes in airways, promoting further dysbiosis and perpetuating a cycle of inflammation and disordered microbiota. Differences in the composition of baseline respiratory tract microbiota might help to explain the so-called frequent-exacerbator phenotype observed in several disease states, and might provide novel targets for therapeutic intervention.

Introduction

The natural histories of several chronic lung diseases include exacerbations, which are characterised by abrupt worsening of respiratory symptoms and pulmonary function. Exacerbations cause much of the morbidity, mortality, and expense of chronic lung diseases, and are associated with accelerated disease progression. Exacerbations are also associated with viral exposure and bacterial growth in cultures of respiratory specimens, but the precise relation between resident bacteria, acute infection, and the pathogenesis of exacerbations is controversial.

In the past decade, new culture-independent techniques for microbial identification such as pyrosequencing have shown a previously unappreciated complexity to the bacterial microbiome in the respiratory tract. The lungs and airways, whether in health or in chronic or acute lung disease, harbour diverse communities of microbes that are undetected by conventional culture-based approaches. A new understanding of lung microbiology has called into question long-held beliefs with respect to the pathogenesis of exacerbations of chronic lung disease derived 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, within hours to days, and generally prompt an escalation in therapy. Symptoms might include focal respiratory symptoms, such as cough, increased sputum production, dyspnea, or wheeze, but might also include systemic features such as fever, fatigue, or malaise. The onset of symptoms often precedes worsening of lung function, although in some patients with impaired perception of the severity of dyspnoea, symptoms are only recognised late in the course of an exacerbation. Exacerbations are typically followed by recovery to a newly compromised baseline. Exacerbations are distinct from primary acute lung infections (such as a lobar pneumonia) and from irreversible progression of underlying lung disease (such as progressively obstructive bronchiolitis obliterans).

Modern techniques to study the lung microbiome

Although a comprehensive discussion of modern techniques would exceed the scope of this review, familiarity with basic principles is key to understanding the revelations and difficulties in the field.

Lung microbiome studies have used various molecular techniques to characterise microbial communities in the respiratory tract, but the most commonly used modern method is high-throughput sequencing of the 16S rRNA gene, a small and highly conserved locus in bacterial DNA. A single sequencing run of a respiratory specimen yields thousands of short DNA sequences. The 16S rRNA gene is amplified using universal primers that target the highly conserved variable region V3-V4 of the 16S rRNA gene. PCR products are then sequenced using Illumina or 454 sequencing platforms.

Search strategy and selection criteria

We searched Medline without date or 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”.

The constituents of the respiratory microbiome are 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 a combination of any of these three factors. In healthy people, community membership is primarily determined by regional growth conditions. In advanced lung disease, community membership is primarily determined by regional growth conditions.

Figure 1: Determinants of the respiratory microbiome

The type of respiratory specimen studied and its method of acquisition are important factors to consider in interpretation of the results of lung microbiome studies. Many investigators have used bronchoalveolar lavage (BAL) fluid or protected specimen brushings (PSB). Although passage of a bronchoscope through the upper airways introduces a theoretical risk of contamination of the lung microbiota from pharyngeal microbiota, the anatomical route of bronchoscope insertion (oral or nasal) has no detectable effect on BAL microbiota (appendix). Given the markedly different microbiota in these body sites, the lack of effect of bronchoscope insertion demonstrates the minimal effect of upper respiratory tract contamination on lung microbiota as detected in BAL fluid. Additionally, significant associations have been reported between the microbiota of BAL and PSB specimens and several clinical parameters: severity of airway obstruction; disease prognosis; clinical response to therapeutic intervention; and the identity, number, and behaviour of pulmonary inflammatory cells. Every study using molecular-based culture-independent methods to compare microbiota of healthy people obtained via bronchoscope with that of patients with lung disease has shown substantial differences in the composition of the respective bacterial communities. These observations help to validate the biological and clinical relevance of the lung microbiome 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 used for the analysis of lung microbiota. Although this method introduces a further risk of contamination from upper airway microbiota, features of the microbiota detected in sputum have been significantly associated with patient age, disease severity, airflow inflammation, antibiotic exposure, and response to experimental viral exposure. Thus any noise introduced into sputum specimens from oropharyngeal microbiota does not obscure entirely the meaningful signal from lung microbiota that correlates consistently with other well-established indices of lung health and disease.
microbes (figure 1). Any alteration to the composition of the lung microbiome detected in disease states must be attributable to a combination of these factors. The proportion of the lung microbiome in healthy individuals that comprises resident, reproducing members (subject to regional differences in environmental growth conditions) versus transient microbes (determined only by the rates of immigration and elimination) is an area of investigation. Microbes enter the lungs continuously via inhalation of air (containing $10^4$–$10^6$ bacterial cells per m$^3$ even before reaching the microbe-dense upper airways), microaspiration (frequent even in healthy people), and direct dispersion along mucosal surfaces. The high degree of shared membership in the oral and lung microbiomes, compared with the air, suggests that microaspiration and direct mucosal dispersion contribute more to microbial immigration than inhalation of bacteria. Microbes are removed from the respiratory tract by mucociliary clearance, cough (frequent even in healthy people), and the highly active and diverse antimicrobial mechanisms of innate and adaptive immunity. Local microbial growth conditions in the respiratory tract are heterogeneous. In a single lung, large differences can be found in oxygen tension, pH, blood perfusion, alveolar ventilation, temperature, epithelial cell structure, deposition of inhaled particles and in the number and behaviour of inflammatory cells, all of which have effects on microbial growth rates. The distal alveoli are bathed in pulmonary surfactant, which has bacteriostatic activity against some (but not all) bacterial species, which creates selective pressure on reproducing microbial communities. Thus the steady state of the lung microbiome is in fact 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 substantially reduce the internal surface area of the lungs by as much as 90%. Oesophageal dysfunction and reflux is extremely common (≥70%) in patients with advanced lung disease, and increases the microbial immigration rate and introduces an additional source (gastric) of microbes. Chronic airway diseases such as cystic fibrosis, bronchiectasis, and chronic bronchitis are characterised by impaired mucociliary clearance, which impairs microbial elimination. These diseases are also associated with increased baseline mucus production, which provides a nutrient-rich growth medium for bacteria and pockets of decreased oxygen concentration, and increased temperature. The inflammatory cells in alveoli and airways are both more numerous and active in chronic lung disease than in health, even in the absence of cigarette smoking or exacerbation of disease. Many therapies for chronic lung disease have effects on microbial growth conditions: supplemental oxygen, systemic and inhaled corticosteroids, and systemic and inhaled antibiotics probably all have pleiotropic effects on the influx, efflux, and reproduction rates of lung microbiota. As disease severity 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 local growth conditions and differential reproduction rates in the respiratory tract, shown by the association between disease severity and the identification of persistent bacterial species (colonisers).

In the context of exacerbation of respiratory disease, the topography of the respiratory tract changes further. Hyperventilation accelerates the influx of air-borne microbes and markedly decreases airway temperature. Increased cough accelerates microbial efflux, and the number and activation of inflammatory cells also increases. Byproducts of the host inflammatory response such as inflammatory cytokines, catecholamines, increased temperature, glucose, and free ATP are known growth factors for specific bacterial species, which create selectively favourable growth conditions. Bronchoconstriction alters local oxygen concentrations and pH. Acute mucus production and vascular permeability increase local nutrient supply; airway mucus introduces further gradients of local anoxia and hyperthermia, which selectively favour the growth of specific lung pathogens.

**The microbiome and exacerbations of chronic lung diseases**

**COPD**

Exacerbations of COPD are associated with high mortality, rapid decrease in lung function, and increased health-care costs. Frequency of exacerbations increases with severity of airway obstruction, but many patients experience exacerbations more frequently than would be predicted by disease severity alone (the so-called frequent exacerbator phenotype). Exacerbations are also associated with systemic inflammation, airway inflammation, and increased airway obstruction due to oedema, increased sputum production, and bronchoconstriction.

A relation between COPD exacerbations and respiratory viral infection is inarguable. In case-control and serially sampled cohort studies, viruses have been identified in respiratory specimens in 39–56% of patients with COPD during exacerbations compared with 6–19% of patients at clinical baseline. When patients with COPD were experimentally infected with rhinovirus, they developed the features of COPD exacerbations (cough, sputum production, airway restriction, and inflammation) significantly more than did controls without COPD.

By contrast, the relation between bacteria and COPD exacerbations has for decades been controversial. Although 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 obtained during clinically stable periods.3–7 The culture-determined density of bacteria in sputum is not significantly increased during exacerbation.7 One hypothesis has attributed exacerbations of COPD to the introduction of new strains of pathogenic bacteria to the airways. One longitudinal study of cultured sputum from outpatients with COPD showed that new strains of bacteria were more frequently isolated during exacerbations than during clinical stability (29% vs 13%); however, more than 70% of exacerbations in patients were not associated with the detection of newly identified bacterial strains.7 Because of the limitations of the identification of bacterial strains through specimen culture, and a heterogeneous clinical presentation, the relation between bacteria and COPD exacerbations is unclear.7 The benefit of antibiotic therapy in the treatment of COPD exacerbations is controversial; recent meta-analyses suggest that antibiotics reduce the frequency of treatment failure in inpatients with severe exacerbations, but are of unclear benefit for outpatients with mild or moderate disease.9 A large trial20 showed reduced frequency of exacerbations in patients receiving chronic azithromycin, which sparked renewed interest in the role that bacteria and host inflammation have in the pathogenesis of exacerbations.

Recent culture-independent studies have shown an unappreciated complexity of the respiratory microbiota in COPD exacerbations. The airways of patients with exacerbations in fact harbour dozens or hundreds of phylogenetically distinct bacteria, far more than previously appreciated in culture-based studies. Huang and colleagues8 observed that even in patients who were receiving antibiotics and who were positive for Pseudomonas aeruginosa growth in respiratory cultures, endotracheal aspirate specimens contained hundreds of bacterial species comprising roughly 140 families of bacteria. Subsequent studies have shown comparatively diverse microbial communities in spontaneously expectorated82 and induced89 sputum specimens. In all these studies, the bacterial species identified by culture were also identified by culture-independent methods, but the converse is not always the case: community members identified by culture-independent methods are often unidentified by the use of cultures alone.

Exacerbations of COPD are associated with changes in respiratory microbiota and airway inflammation. Millares and colleagues22 analysed paired sputum specimens from patients with COPD at baseline and during exacerbations, and found that exacerbations were associated with a selective increase in the relative abundance of bacteria typically associated with exacerbations (eg, Haemophilus, Pseudomonas, and Moraxella) despite inconsistent detection in culture. Huang and colleagues86 used paired sputum specimens from patients with COPD at baseline and during exacerbation and found a shift in community composition towards the phylum Proteobacteria. Molyneaux and coworkers87 used a human model of virus-induced COPD exacerbations to systematically compare sputum microbiota in patients at clinical baseline and during exacerbation. Sputum acquired after viral exposure had a substantial shift in bacterial community composition towards the Proteobacteria phylum; this shift was not observed in sputum acquired from healthy controls. None of these three studies showed a decrease in bacterial community diversity, as would be expected in the context of acute infection.26 Importantly, Molyneaux and colleagues found that bacteria in key taxonomic rankings that spiked in abundance during exacerbation were detected in these same patients both before viral exposure and after clinical resolution of the exacerbation.

**Asthma**

Exacerbations cause much of the mortality, morbidity, and health-care expenses of asthma.23,24 Precipitants include allergens, air pollution, and exercise, although, as is the case in COPD, some patients exhibit a frequent-exacerbator phenotype independent of other risk factors.25 Asthma exacerbations are even more strongly associated with viral infections than are the exacerbations of COPD: respiratory viruses (most commonly rhinovirus) are detectable in respiratory specimens from more than 75% of patients with exacerbations,85 and controlled rhinovirus inoculation provokes the airway hyper-responsiveness observed in allergic asthma.86 Bacteria have not traditionally been implicated in asthma exacerbations, because they are infrequently cultured from patients’ sputum, and trials of antibiotics showed no clinical benefit.26 However, recent serology-based and PCR-based approaches have shown a surprising prevalence of Chlamydia pneumoniae and Mycoplasma pneumoniae infections in patients with acute exacerbations.9

Although to date no culture-independent studies have included respiratory specimens obtained from patients with asthma in the context of an exacerbation, several studies have shown that the airways in patients with asthma harbour bacterial microbiota distinct from that of healthy people, suggesting an association between respiratory microbiota, airway inflammation, and susceptibility to exacerbations. In four separate studies analysing respiratory microbiota (with BAL,25,88 bronchial brushings,25,88 and induced sputum25), the microbiota detected in asthmatic airways at baseline was consistently and substantially different from that of healthy controls. All four studies showed a significant increase in community abundance of Proteobacteria, the phylum that contains common Gram-negative respiratory pathogens such as Haemophilus spp, Pseudomonas spp, and Klebsiella spp. Goleva and colleagues26 observed an increase in the concentration of lipopolysaccharide in Proteobacteria-enriched specimens, providing in-vivo...
evidence of the difference in the community composition of microbiota.

These studies have shown associations between the respiratory microbiome and the clinical, physiological, and therapeutic features of asthma. Huang and colleagues showed that airway hyper-responsiveness (assessed by methacholine challenge) was positively associated with bacterial community diversity and community composition (appendix). This association was driven by the increased abundance of members of the phylum Proteobacteria in patients with hyper-responsive airways. The investigators also showed that clinical improvement with clarithromycin was significantly predicted by differences in community diversity in patients’ baseline microbiota (appendix). Independently, Goleva and coworkers noted that differences in baseline microbiota composition were associated with patients’ clinical response to systemic corticosteroids (appendix). In-vitro coculture of macrophages, obtained by BAL, with Haemophilus parainfluenzae (which the authors observed only in corticosteroid-resistant patients) blunted the response of airway macrophages to the effects of corticosteroids, which shows the biological plausibility of a relation between airway microbiota and the hyper-responsiveness that is accentuated during exacerbations.

Cystic fibrosis

Exacerbations of cystic fibrosis are associated with much of the mortality and expense of this diagnosis, and acceleration of decline in lung function. With increasing age, nearly all patients with cystic fibrosis grow specific respiratory pathogens from sputum (most commonly Staphylococcus aureus, P aeruginosa, and H parainfluenzae), both during exacerbations and clinically stable periods. Exacerbations have typically been considered as infectious events and treated primarily with antibiotics, although data supporting this approach are surprisingly sparse.

Two large trials have shown no association between patients’ response to antibiotic therapy during exacerbations of cystic fibrosis and the in-vitro susceptibility of the cultured respiratory pathogen to the antibiotics administered (figure 2A). Furthermore, the clinical course in patients receiving antibiotics during exacerbations is not correlated with changes in bacterial burden in sputum cultures.

In the past decade, the use of culture-independent techniques has revolutionised understanding of the respiratory microbiome in cystic fibrosis at clinical baseline and during exacerbations. Early studies revealed far greater microbial community diversity in sputum specimens than was first appreciated with culture, with viability of many microbes verified both via amplification of transcribed RNA and advanced culture techniques. Studies have since confirmed that the sputum of patients with cystic fibrosis contains diverse bacterial communities, replete both with recognised pathogens and previously unrecognised microbes. Some studies have shown an overall decrease in community diversity with increasing age and disease severity, this loss of diversity is more strongly associated with cumulative antibiotic exposure than disease severity.

Culture-independent techniques have overturned long-held assumptions about the bacterial pathogenesis of exacerbations of cystic fibrosis. Several studies have analysed paired specimens obtained from patients during periods of stability and subsequent exacerbation. All have shown remarkably consistent findings: exacerbations of cystic fibrosis are not associated with increased bacterial density or decreased community diversity (figure 2B and 2C). These results differ from those in patients with bacterial pneumonia, in whom, as expected, the bacterial burden is high, bacterial community diversity is low, and the intensity of the host inflammatory response correlates closely with

Figure 2: Exacerbations of cystic fibrosis lack key features of bacterial infections

(A) The clinical response of patients with cystic fibrosis to antibiotic therapy is not associated with the in-vitro susceptibility of organisms cultured from sputum specimens at the onset of exacerbation, reproduced from Hurley and colleagues. (B) Bacterial density in sputum specimens does not increase either before or at onset of exacerbations, compared with clinical baseline, reproduced from Carmody and colleagues by permission of the American Thoracic Society. (C) Bacterial community diversity in sputum specimens is not lower during exacerbations, reproduced from Stressmann and colleagues.
bacterial burden. These findings strongly challenge the conventional understanding of exacerbations of cystic fibrosis as acute infections of the airways. These same studies have raised the question of whether bacterial community membership changes at the start of an exacerbation, but no consistent pattern of microbial change has emerged. Carmody and colleagues noted that compared with baseline, respiratory microbiota at the time of exacerbation are consistently and significantly increased in abundance of *Gemella* spp, a Gram-positive anaerobe associated with the mouth and upper gastrointestinal tract. How baseline differences in microbiota affect the frequency or features of exacerbations of cystic fibrosis is unknown. Carmody and others showed that dissimilarity between microbiota at baseline and during exacerbation was significantly associated both with high baseline diversity and baseline community domination by *Pseudomonas* spp.

**Non-cystic fibrosis bronchiectasis**

Bronchiectasis that is not attributable to cystic fibrosis can be caused by various predisposing conditions, although most cases are idiopathic. Compared with cystic fibrosis, little is known about the pathophysiology and treatment of the chronic inflammation and exacerbations that characterise its clinical course. New evidence suggests that chronic macrolide therapy decreases the frequency of exacerbations in patients, although as with cystic fibrosis and COPD, whether this is due to antimicrobial effects is unknown.

Several recent studies have used culture-independent analysis to study the microbiome of non-cystic fibrosis bronchiectasis. A study by Tunney and coworkers compared paired sputum and BAL specimens from patients with non-cystic fibrosis bronchiectasis at stability and during exacerbation. They noted no difference in bacterial community diversity at the time of exacerbation before the use of antibiotics, and no increase in aerobic, anaerobic or total bacterial growth by a conventional quantitative culture analysis (ie, number of colony forming units). Bacterial communities both at baseline and exacerbation were largely dominated by Proteobacteria (eg *Haemophilus* spp and *Pseudomonas* spp). Rogers and colleagues noted that the community membership of sputum microbiota at clinical baseline was predictive of the subsequent frequency of exacerbations; importantly, this association was not detected when limited to culture results alone. A study of explanted lung tissue showed similar microbiota in cystic fibrosis and non-cystic fibrosis bronchiectasis, but how generalisable the findings from cystic fibrosis studies are to patients with non-cystic fibrosis bronchiectasis is unknown.

**Idiopathic pulmonary fibrosis (IPF)**

Although the natural history of IPF has traditionally been characterised as one of slowly progressive decline in lung function, the existence of acute exacerbations of IPF has been increasingly recognised as a major cause of mortality in this disease. Exacerbations arise over the course of weeks and are characterised by progressive hypoxaemia, lung infiltrates, the histological presence of diffuse alveolar damage, and the clinical exclusion of infection or heart failure. Mortality is high and no therapies have shown benefit. Many patients with acute exacerbations of IPF have clinical features of infection (fever, cough, and BAL neutrophilia), but no infectious cause has been identified.

Several recent studies have provoked interest in the potential role the bacterial lung microbiome has in the progression of IPF and exacerbations. Richter and others observed a surprisingly high frequency of positive cultures from the BAL specimens in patients with IPF at clinical baseline, and subsequent culture-independent reports have confirmed that in IPF the lungs harbour a distinct bacterial microbiome from that of healthy lungs. Han and coworkers reported a positive association between the presence of specific microbial community members (*Staphylococcus* spp and *Streptococcus* spp) in BAL specimens and disease progression, and investigators of a randomised controlled trial of cotrimoxazole noted reduced mortality in patients with IPF who received antibiotics.

**Key lessons and directions for study**

Despite the presence of airway inflammation in respiratory exacerbations, a consistent finding across disease states is the lack of evidence that exacerbations are attributable to acute bacterial infections of the airways. Of the eight culture-independent studies across COPD, cystic fibrosis and non-cystic fibrosis bronchiectasis that have compared patients’ respiratory specimens obtained at baseline and during exacerbations, all showed no change in the bacterial density or community diversity during exacerbations. This finding differs from studies of bacterial pneumonia, in which (as expected) increased inflammation is strongly associated with increases in bacterial burden and decreases in community diversity. The airway inflammation common to all exacerbations of respiratory disease is surprisingly dissociated from microbial burden and bacterial community domination by one or a few pathogenic species.

Exacerbations differ from acute bacterial infections with respect to several factors (table 1). Acute bacterial infections respond rapidly and consistently to antibiotics, whereas data showing benefit from antibiotics in the treatment of respiratory exacerbations range from minimal to inconsistent to absent. In-vitro tests of the susceptibility of bacteria to antibiotics is clearly of use to predict the response of patients with bacterial lung infections to therapy, but bear no correlation with response to therapy in cystic fibrosis exacerbations. Whereas bacterial lung infections are the most common cause of severe sepsis, exacerbations of respiratory disease rarely (if ever) cause severe sepsis and shock.
These key differences between exacerbations and bacterial infections do not imply that bacteria are uninvolved in exacerbations. Instead, 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 that results in negative effects on host biology. Paired specimen studies have shown substantial changes in the constituents of respiratory bacterial communities during exacerbations, often with large shifts away from Bacteroidetes (the most abundant bacterial phylum in the lungs of healthy people\textsuperscript{[39,40]}) and toward Proteobacteria\textsuperscript{[39,85,86]} and bacteria belonging to other disease-associated taxonomic ranks.\textsuperscript{[87]} The increased frequency of newly isolated strains in cultured sputum specimens from patients with COPD exacerbations compared with baseline (from 13% to 29%)\textsuperscript{[77]} is consistent with the occurrence of respiratory dysbiosis. Altered environmental conditions might disturb the composition of bacterial communities, increasing the relative abundance of species previously undetectable with culture techniques alone and increasing the community’s susceptibility to invasion with new strains.

An analogy can be made between exacerbations of chronic lung disease and exacerbations of inflammatory bowel disease. Both are defined as acute clinical worsening of chronic inflammatory conditions of mucosa-lined luminal organs, associated with dysbiosis and a dysregulated host inflammatory response.\textsuperscript{[81]} These diseases are characterised by loss of the homeostatic balance between resident microbiota and host immunity.\textsuperscript{[82]} Exacerbations in either disease are not caused by an instance of acute infection in which one or a few pathogenic species overtake a body site and directly mediate tissue injury. In both cases, the benefit of antibiotics, if present, comes not from eradication of a target pathogen but instead from selective manipulation of the composition of the microbial community or indirect immunomodulatory effects, which potentially explains the discordance between the sensitivity of bacterial cultures and the clinical response in patients with cystic fibrosis.\textsuperscript{[83,84]} Exacerbations of both chronic lung disease and inflammatory bowel disease are preventable or treatable with macrolides that have antimicrobial and indirect immunomodulatory effects.\textsuperscript{[85,86,87]}

We propose a model for the cycle of host inflammation and respiratory dysbiosis that typifies an exacerbation of respiratory disease (figure 3). An inflammatory trigger (eg, viral infection or allergic exposure) initiates a cascade of host inflammatory responses that acutely alters the microbial growth conditions in the airways. Permeability of the airway wall and mucus production provide a nutrient-dense substrate for bacterial growth.\textsuperscript{[88,92]} Free catecholamines and inflammatory cytokines (eg, tumour necrosis factor α, interleukins 1, 6, and 8) directly promote the growth of specific bacterial species (eg, \textit{P aeruginosa}, \textit{S aureus}, \textit{S pneumoniae}, and \textit{Burkholderia cepacia complex}).\textsuperscript{[93–95]} Inflammatory cells are recruited and activated, killing and clearing bacteria with highly variable effectiveness,\textsuperscript{[96]} creating selection pressure across species. Airway mucus creates local pockets of increased temperature and decreased oxygen tension, favouring the growth of some disease-associated microbes.\textsuperscript{[92,97]} In this model, diverse effects on microbial growth conditions result in disorder and dysregulation of the dynamic homeostasis of the airway microbiome—ie, respiratory dysbiosis. New and abundant community members, some with altered expression of microbial

### Table 1: Comparison of respiratory exacerbations with acute bacterial infections

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<tr>
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<th>Acute bacterial respiratory infections</th>
<th>Exacerbations of respiratory disease</th>
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<tr>
<td>Bacterial density</td>
<td>High (compared with baseline)</td>
<td>Normal</td>
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<tr>
<td>Bacterial community diversity</td>
<td>Low (compared with baseline)</td>
<td>Normal</td>
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<tr>
<td>Bacteria identified in cultures</td>
<td>Usually</td>
<td>Occasionally</td>
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<tr>
<td>Airway inflammation</td>
<td>High, proportionate to microbial burden</td>
<td>High, disproportionate to microbial burden</td>
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<tr>
<td>Sepsis</td>
<td>Common</td>
<td>Never or rare</td>
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<td>Clinical benefit from antibiotics</td>
<td>Rapid, unambiguous</td>
<td>Inconsistent, subtle</td>
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<td>Clinical relevance of in-vitro susceptibility to antibiotics</td>
<td>Critical to clinical response</td>
<td>No relation to clinical response</td>
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**Figure 3: The dysbiosis–inflammation cycle**

An inflammatory trigger initiates airway inflammation, which alters environmental growth conditions of airway microbiota thus creating positive and negative selective pressures. Altered growth conditions result in a disordered microbiome, which provokes further airway inflammation via PAMP and pattern recognition receptor interactions, microbial metabolite signalling to leukocytes and epithelial cells, and other pathways. The result is a self-amplifying cycle of airway inflammation and respiratory dysbiosis. LPS=lipopolysaccharide. LTA=lipoteichoic acid.
virulence proteins or with increased immunogenicity compared with previously abundant members, elicit further airway inflammation through expression of pathogen-associated molecular patterns, and host pattern recognition receptor interactions, including activation of Toll-like receptors. This inflammation further alters bacterial growth conditions in the airway, resulting in a positive-feedback cycle. Tissue injury arises from a combination of direct injury by the newly abundant community members, altered microbial behaviour, and indirect effects of the dysregulated inflammatory response. Homoeostasis is achieved only after the positive-feedback cycle of dysbiosis and inflammation is halted. Although the initial dysbiosis in exacerbations might be secondary to a primary inflammatory trigger, the relation between inflammation and respiratory dysbiosis is probably bidirectional and self-perpetuating, providing a plausible explanation for why the airway inflammation of exacerbations persists long after direct exposure to the inflammatory precipitant.

A distinct but related observation is that the virulence and immunogenicity of a single bacterial species within the respiratory tract is not static, but depends on several host-derived and microbe-derived environmental factors. Published work has shown that the behaviour of a given bacterial strain (as assessed either by gene expression assays of virulence and immunogenicity proteins) is strongly affected by the same environmental factors that differentially affect bacterial growth rates: temperature, concentration of host-derived cytokines and catecholamines, and presence of free glucose. Furthermore, the behaviour and virulence of one strain is affected by the composition of the bacterial community sharing its ecological niche, mediated by direct and indirect microbial interactions. Changes in the metabolism and virulence of microbes (independent of community membership) can modulate inflammatory cascades, as has been proposed for inflammatory bowel disease and the gastrointestinal tract, and modelled with P aeruginosa in Drosophila. Thus the environmental conditions in the airways during inflammation can affect the virulence of individual community members both directly (via the virulence-promoting factors listed above) and indirectly (via the changes in community composition and microbe–microbe interactions in respiratory dysbiosis). This insight is crucial for the future study of bacterial lung disease: the pathogenicity of a 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 inflammatory response of healthy airways to an exposure (eg, viral infection) is measured, proportionate to the insult, and self-limiting. Any disruption to the baseline composition of lung microbiota is muted and short-lived. The key features of exacerbations—sustained respiratory dysbiosis coupled with a prolonged immune response disproportionate to the microbial burden—arise only when dysfunction already exists at baseline on both sides of the respiratory microbiome–immune interface. Even when patients are clinically stable (ie, outside of exacerbations), those with chronic lung disease, when compared with healthy people, have alterations to community membership of respiratory microbiota and activation of airway inflammatory cells (table 2). The composition of respiratory microbiota, established mainly by the balance of immigration and elimination in health, is increasingly determined by regional growth conditions with increasing severity of lung disease (figure 1); during an exacerbation, the effect of regional conditions on bacterial growth becomes all-important. We propose that respiratory exacerbations, with their coupled dysbiosis and airway inflammation, are abrupt, emergent phenomena arising from the chronically disordered but compensated and fragile homoeostasis present in stable chronic lung disease.

An important consequence of understanding the airways as an ecosystem is recognition of the limitations of conventional analytical approaches to modeling its behaviour. Although the conventional model of airway infection suggests linear pathogenesis (a large inoculum of bacteria enters the airways and overwhelms host defences with unrestrained growth and resultant inflammation), in our model, exacerbations instead emerge abruptly from the complex adaptive system of the respiratory microbiome. Ecosystems have been described as prototypical complex adaptive systems, defined by diverse entities interacting with each other in a common space, exhibiting interdependent actions and possessing the capability to adapt to changes in conditions. In linear systems, small changes in conditions result in proportionately small changes in outcomes, whereas in

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<td>Stable</td>
<td>Exacerbation</td>
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<td>Primary ecological determinant of microbiota community composition</td>
<td>Immigration and elimination</td>
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<td>Airway inflammation</td>
<td>Absent or mild</td>
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<td>Proportionate to microbial burden</td>
<td>Increased</td>
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<td>Proportionate to microbial burden</td>
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Table 2: Comparison of respiratory health with stable and exacerbated chronic lung disease states.
complex adaptive systems, outcomes can be abruptly and disproportionately altered by small changes in conditions. Unlike linear systems, complex adaptive systems are not amenable to reductionist models and are instead better modelled via computational methods (eg, agent-based).10,11 These methods might prove essential to understand how respiratory exacerbations arise from the homeoeostasis of clinical stability.9

The respiratory microbiome might partly explain the so-called frequent-exacerbator phenotype in chronic lung disease. In several chronic lung diseases, a subgroup of patients experience exacerbations more frequently than would be predicted by established risk factors.12,13 This frequent-exacerbator phenotype is consistent across years, suggesting a persistent biological predisposition to exacerbations that is unexplained by the current understanding of their pathogenesis. The insights from culture-independent methods have prompted a hypothesis: differences in the composition of baseline respiratory microbiota in terms of species density and diversity might explain the differences observed in the frequency of exacerbations in otherwise clinically similar patients.

Several preliminary observations lend support to this hypothesis. Rogers and colleagues95 showed that in patients with non-cystic fibrosis bronchiectasis, differences in baseline respiratory microbiota were strongly and significantly associated with the number of exacerbations experienced in the subsequent year; importantly, no association was observed between culture-based microbiology results and frequency of exacerbations. Several associations between baseline respiratory microbiota and both airway hyperresponsiveness and the clinical response to therapy have been reported for patients with asthma.22,23 Molyneaux and others96 showed that in patients with COPD after rhinovirus exposure, alterations in the respiratory microbiome were heterogeneous in patients and persistent at 6 weeks. Chronic azithromycin therapy has been shown to decrease the frequency of exacerbations in COPD, cystic fibrosis, and non-cystic fibrosis bronchiectasis,80,101,126 although whether this benefit is attributable to its effects on respiratory microbiota is unknown.97 Two randomised controlled trials100,101 have shown benefit from enteric probiotics in decreasing the frequency of exacerbations of cystic fibrosis. Given the anatomical and ecological continuity of the aerodigestive tract it is possible that this benefit was mediated partly through changes to the respiratory microbiota. In view of the plausibility of this hypothesis and the high morbidity and health-care costs associated with exacerbations of chronic lung disease, an immediate aim of research should be to determine whether the respiratory microbiome can be used to predict when exacerbations of disease are likely to occur and whether its therapeutic manipulation can decrease the frequency of exacerbations.102

Culture-based approaches are inadequate to completely understand the interactions of the host, microbiome, and pathogenesis of exacerbations. Studies of respiratory exacerbations that have used both culture-dependent and culture-independent results have shown markedly different results. Culture-independent techniques can confirm the presence of organisms identified in culture; however, culture-based approaches grossly underestimate the complexity, density, and diversity of respiratory community microbiota. Importantly, culture-independent methods have identified associations between respiratory microbial community features and airway hyperresponsiveness,12 disease prognosis,13 susceptibility to exacerbations105 and the response to experimental viral exposure;14 these associations would have been unappreciated with culture-based methods. Given the intimate relation between the respiratory microbiome and the host, and the potential role of the microbiome in mediation of acute and chronic inflammation, changes in the composition of the respiratory microbiome should be considered as a secondary outcome in all clinical trials targeting a reduction in the frequency of exacerbations in chronic lung disease.

Contributors
RPD did the literature review and wrote the first draft of the article. RPD, FJM, and GBH participated in review and revision of the manuscript and approved the final version.

Declaration of interests
FJM has served on advisory boards relating to COPD topics for Almirall, AstraZeneca, Forest Laboratories, GlaxoSmithKline, MedImmune, Merck, Novartis Pharmaceuticals, Pearl Therapeutics, and United BioSource. He has served on data safety monitoring boards for Novartis and Sanofi. He has consulted for Actelion, Bayer, Boehringer Ingelheim, BoomComm, Comgex, F Hoffmann-La Roche, FB Communications, Forest Laboratories, HLS, Merck/Schering-Plough, Nycomed, Pfizer, Quark, Sanofi, and Teleciris Biotherapeutics. He has served on speakers’ bureaux for Altana/Nycomed, American Lung Association, AstraZeneca, Boehringer Ingelheim, CME Incite, ePocrates, France Foundation, GlaxoSmithKline, Med-Ed, Merck/Schering-Plough, National Association for Continuing Education, Pfizer, Potomac, Vox Medica, and WebMD. His institution has received funds from Boehringer Ingelheim for a clinical trial. He has received royalties from Associates in Medical Marketing and Castle Connolly Medical. He has developed educational materials for the France Foundation, HIT Global, and ePocrates. He has served on steering committees for clinical trials supported by Actelion, Centocor, Forest Laboratories, GlaxoSmithKline, MPex, and Takeda. The other authors declare no competing interests.

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