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REVIEW

Lung microbiology and exacerbations in COPD

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Abstract: Chronic obstructive pulmonary disease (COPD) is the most common chronic respiratory condition in adults and is characterized by progressive airflow limitation that is not fully reversible. The main etiological agents linked with COPD are cigarette smoking and biomass exposure but respiratory infection is believed to play a major role in the pathogenesis of both stable COPD and in acute exacerbations. Acute exacerbations are associated with more rapid decline in lung function and impaired quality of life and are the major causes of morbidity and mortality in COPD. Preventing exacerbations is a major therapeutic goal but currently available treatments for exacerbations are not very effective. Historically, bacteria were considered the main infective cause of exacerbations but with the development of new diagnostic techniques, respiratory viruses are also frequently detected in COPD exacerbations. This article aims to provide a state-of-the art review of current knowledge regarding the role of infection in COPD, highlight the areas of ongoing debate and controversy, and outline emerging technologies and therapies that will influence future diagnostic and therapeutic pathways in COPD.

Keywords: COPD, exacerbations, bacteria, viruses

Introduction

Chronic obstructive pulmonary disease (COPD) is a chronic progressive disease which is characterized by an inexorable decline in respiratory function, exercise capacity, and health status. This chronic clinical course of COPD is punctuated by periods of increased symptoms that are termed "acute exacerbations". Acute exacerbations are significant events in the course of the disease as they accelerate disease progression, impair quality of life, and are the major contributor to morbidity and mortality in COPD. In addition they are the major cause of excess health care costs as they often result in unscheduled health care visits, treatment costs, and hospitalizations. Therefore, preventing exacerbations is a major therapeutic goal that has not been achieved with currently available treatments. The major causes of exacerbations are respiratory infections with both viruses and bacteria. Although a role for infection in both stable COPD and COPD exacerbations has long been established, controversy remains regarding a number of issues including the effect of bacterial infection in stable COPD, the relative contributions of viruses and bacteria in COPD exacerbations, mechanisms of susceptibility to infection, and the role of antibiotics.

Microbial flora in the normal lung and in COPD patients

In order to investigate the role of infection in COPD, an understanding of the normal microbial flora in healthy individuals is required. Most of the surfaces of the

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upper respiratory tract (including nasal and oral passages, nasopharynx, and oropharynx) are colonized by bacteria in healthy individuals. These organisms constitute the normal flora of the respiratory tract and rarely cause disease. The nose is commonly colonized with predominantly S*taphylococcus epidermidis* and C*ornyebacteria* but S*taphylococcus aureus* can also be present. The nasopharynx is predominantly colonized with non-hemolytic and alpha-hemolytic *Streptococci* and *Neisseria* species with occasional carriage of *Streptococcus pneumonia* and *Haemophilus influenzae*.[1] Determining the prevalence of bacteria in the lower respiratory tract has proved problematic due to the difficulty of obtaining lower respiratory tract samples uncontaminated by upper airway secretions. Bronchoscopy with protected brush specimens is the best method of avoiding upper airway contamination. A report by Rosell et al pooled the results of studies using bronchoscopy in healthy subjects and both stable and exacerbated COPD.[2] In 70 healthy participants, potentially pathogenic microorganisms (PPMs) were cultured in only 6% of subjects and bacterial loads were low. Two subsequent studies not included in this analysis reported that PPMs were present in 10% of subjects undergoing diagnostic bronchoscopy in whom no pathology was found,[3] and in 3% of a group of healthy smokers and ex-smokers.[4] Therefore based on these findings, conventional wisdom has been that the lower respiratory tract in healthy individuals is sterile. However it has been estimated that over 70% of the bacterial species present on mucosal surfaces cannot be cultured using currently available standard culture techniques. Several recent studies have used novel molecular approaches for bacterial detection rather than standard culture techniques and have challenged the idea that the lower respiratory tract is predominantly sterile. These studies will be discussed in further detail in a later section.

In contrast to healthy individuals, bacteria can often be cultured from lower airway secretions in COPD patients. The pooled analysis by Rosell et al reported that PPMs are present at a concentration of 10^2 cfu/mL or greater in 29% of stable COPD patients[2] and Sethi et al detected bacteria in 34.6% of patients.[4] No bacteria were present in patients with mild disease (GOLD criteria), but 24% of moderate patients, 45% of severe patients, and 33% of very severe patients had bacteria detected. This relationship between lung function impairment and bacteria has been reported in some studies[5] but other studies have found no relationship.[3,4,6–8] Studies using sputum to detect bacterial infection have generally found higher rates of bacterial isolation than the bronchoscopy studies with bacteria found in up to half of stable COPD patients.[9–11] This is likely due to higher rates of contamination with upper respiratory organisms in sputum.

The most common organisms cultured in COPD from both sputum and bronchoscopic samples are consistently *Haemophilus influenza*, *Streptococcus pneumonia*, and *Moraxella catarrhalis*. However the bacterial flora in COPD varies with disease severity as Gram negative organisms such as *Pseudomonas aeruginosa* are more commonly detected in patients with more severe airflow obstruction in both stable COPD[12,13] and in exacerbations.[14–16] Other organisms detected less frequently include *Haemophilus parainfluenzae* and *Staphylococcus aureus* but the significance of these organisms is debated (Table 1).

Respiratory microbiome

Our current understanding of the role of bacteria in the pathogenesis and progression of obstructive airways disease is predominantly based upon classical microbial culture techniques. However, these suffer from a number of limitations, are very labor intensive, and using standard

Table 1 The Respiratory Microbiome in Health, in stable COPD and exacerbated COPD

Healthy individuals	Stable COPD (mild to moderate)	Stable COPD (moderate to severe)	Exacerbated COPD
Staphylococcus epidermidis[1]	*Haemophilus influenza*[12,13]	*Haemophilus influenza*[12,13,19]	*Moraxella catarrhalis*[69]
Cornyebacteria[1]	*Streptococcus pneumonia*[12,13]	*Streptococcus pneumonia*[12,13]	*Streptococcus pneumonia*[69]
Staphylococcus aureus[1]	*Moraxella catarrhalis*[12,13]	*Moraxella catarrhalis*[12,13]	*Haemophilus influenza*[70]
Non-hemolytic streptococci[1]	*Haemophilus parainfluenzae*[73]	*Pseudomonas aeruginosa*[12,13,23]	*Pseudomonas aeruginosa*[14,16]
Alpha-hemolytic streptococci[1,19]	*Staphylococcus aureus*[70]	*Haemophilus parainfluenzae*[73]	*Staphylococcus aureus*[70,71]
Neisseria spp.[1]		*Staphylococcus aureus*[70]	*Haemophilus parainfluenzae*[73]
Streptococcus pneumonia (occasional)[1,19]			
Haemophilus influenza (occasional)[1,19]			
Prevotella spp.[19]			
Fusobacteria[19]			
Veillonella[19]			

Abbreviation: COPD, cheronic obstructive pulmonary disease.

conditions can culture only 30% of bacteria.[17] It is therefore understandable that historically the lungs have been considered sterile, despite their continuity with the upper airways, proximity to the gastrointestinal tract, and continuous exposure to the environment. Over the past decade molecular culture independent techniques, initially developed in the ecology field, have identified bacteria previously not amenable to culture.[18] These techniques when combined with advances in sequencing technologies have produced a powerful tool for investigating the role of bacteria in health and disease and have recently begun to shed more light on the role of bacteria in COPD.

Molecular tools rely on genomic evolutionary relationships between bacteria and use similarities in housekeeping genes, such as the highly conserved 16S rRNA gene, to assign phylogeny. These techniques can not only be used to rapidly identify individual bacterial species but also to build up a picture of the complete microbial community in an environment (the microbiome), offering a more comprehensive analysis than classical culture-based techniques.

Hilty et al employed these techniques to clearly demonstrate the presence of a wide variety of bacteria in the airways of healthy non-smoking subjects, establishing the existence of a respiratory microbiome and challenging the dogma of lung sterility.[19] Their study also suggested the healthy respiratory microbiome differs from that associated with a number of respiratory diseases including COPD.

COPD is linked inherently to smoking and we must therefore first look at the effects of smoking on the respiratory microbiome. Interestingly, three studies have now found no overall difference in total bacterial levels between lower airways samples from smokers, non-smokers, and COPD subjects.[19–21] However Charlson et al have demonstrated changes in bacterial communities in the upper respiratory tract of smokers, with significant loss of bacterial diversity when compared to the oro- and naso-pharynx of non-smokers.[22] Whether this represents outgrowth of an organism from a healthy community leading to loss of diversity remains unclear, but loss of microbial diversity has been associated with an increased incidence of disease in other systems.[23]

Although the number of studies examining the lower airways microbiome in COPD is limited, it is already clear that there is a significant overlap between the bacteria seen in COPD and healthy individuals.[19,20,24] This has led some authors to postulate the existence of a core respiratory microbiome made up of *Pseudomonas, Streptococcus, Prevotella, Fusobacteria, Haemophilus*, and *Veillonella* species whose relative abundance differs between health and disease.[25] Certainly, in the initial study by Hilty et al, *Haemophilus* species were most strongly associated with the presence of COPD,[19] while the dominance of *Pseudomonas* species and associated lower diversity of the microbiome was observed in subjects with moderate or severe COPD but not in mild disease.[20] However, a recent study by Sze et al did not observe any reduction in microbial diversity in lung tissue from patients with very severe COPD (GOLD stage IV). Although they do report evidence of a unique bacterial community structure in this group with a significantly greater abundance of Firmicutes compared to smoking and non-smoking controls.[21]

Using explanted lungs, Erb-Downward and colleagues were able to characterize the microbial communities at different locations without the need for bronchoscopy, thereby avoiding any potential upper airways contamination. They demonstrated bacterial communities similar to those seen in bronchoalveolar lavage (BAL), and interestingly demonstrated significant micro-anatomical differences in community composition within the lungs. Highlighting the power of technology, the segmental bronchus of the left upper lobe bronchus in one subject was dominated by *Haemophilus*, while in the distal bronchus of the left upper lobe bronchus, *Stentrophomonas* dominated the community.[20]

Molecular microbiology has currently raised more questions than it has answered in the respiratory field. These techniques have clearly demonstrated the presence of PPMs in the lungs of healthy smokers and COPD subjects and have begun to identify differences between the bacterial communities in these groups and between severe and mild disease. However, it remains unclear what impact this could have on our understanding of the disease pathogenesis and progression. How does the microbiome change before, during, and after an exacerbation? What effect do viral infections have on the bacterial communities? Further studies are clearly needed to answer these questions but with these tools and the evolving field of metatranscriptomics, we have the opportunity to study the role of bacteria in obstructive lung disease more comprehensively than ever before.

Significance of bacterial infection in stable COPD

The presence of bacteria in the lower respiratory tract in stable COPD patients is usually termed "colonization" rather than "infection", implying that the bacteria present have no or minimal pathological significance. However, studies have now established that there may be relationships between the presence of bacteria and both airways inflammation and

adverse clinical outcomes in COPD patients. Therefore the term "colonization" may be misleading and the presence of bacteria in COPD patients may not be as benign as previously thought.

Increased levels of cellular and soluble inflammatory markers in the airways including neutrophils, CXCL8, leukotriene B_4 (LTB_4), myeloperoxidase (MPO), matrix metalloproteinase (MMP), tumor necrosis factor-α (TNF-α), IL-1β, IL-6, IL-12, and neutrophil elastase (NE) have been reported in stable COPD patients with bacterial infection.[4,6,8,11,26–29] In addition two studies have reported a quantitative relationship between bacterial load and sputum inflammatory markers.[6,9] Bacterial infection is also associated with adverse clinical outcomes with an increased frequency of exacerbations,[9,11,30] impaired health status,[26,29] and systemic inflammation.[26,29] One study did not find an association between bacterial infection and exacerbation frequency.[30] Therefore these studies would appear to provide evidence that bacterial infection, even in patients who are clinically stable, causes airways and systemic inflammation, increases exacerbations, and impairs health status. However, as these studies were cross-sectional they could not determine the direction of the association between bacterial colonization and the outcomes measured. Bacterial infection may cause increased airways inflammation but reverse causation may be equally plausible, ie, patients with greater airways inflammation may be more susceptible to developing bacterial infection. Parameswaran et al attempted to resolve this issue this by studying the effect of the acquisition of a new strain of *Moraxella catarrhalis* on sputum inflammatory markers.[31] Sputum samples collected after acquisition of *Moraxella catarrhalis* had higher levels of TNF-α, CXCL-8, and NE compared to pre-acquisition samples, supporting the hypothesis that bacteria induce airways inflammation. However, inflammatory markers in subjects who did and did not develop infection were not compared prior to acquisition so the hypothesis that airways inflammation predisposes to bacterial infection is still possible.

This study also highlighted the fact that bacterial colonization/infection is not a static phenomenon. This was further demonstrated by Wilkinson et al who studied 30 stable COPD patients over 1 year and collected sputum at the start and end of this time period.[10] Fifty three percent of subjects had bacteria present in sputum at the start of the study. After 1 year half of these had the same bacterial species but in the other 50% of subjects a different bacterial species was present. There was a significant increase in the mean bacterial load over the year and this correlated with a reduction in the forced expiratory volume in 1 second (FEV_1), as did acquisition of a new bacterial species. A study of 40 COPD patients over an 8-month period used molecular typing to identify the strain of sputum organisms. Fifty-three percent of patients had PPMs in sputum at both time points but only 15% had the same organism, whereas 38% had acquired a new organism.[27]

Therefore, both the presence and type of bacteria in the airways in stable COPD are not static phenomena and may be affected by factors such as disease progression, exacerbations, and treatments including antibiotics and inhaled corticosteroids. Conversely the presence of bacteria can influence disease progression and exacerbations in COPD patients. The relationships between clinical outcomes and bacteria in COPD are undoubtedly complex and further studies to investigate these should be long-term longitudinal studies using strain typing in addition to culture. If a causal relationship between the presence of bacteria and adverse outcomes in stable COPD patients is conclusively demonstrated, then intervention studies with antibiotics in stable COPD may be warranted.

Mechanisms of susceptibility to bacterial infection in COPD

In healthy individuals, bacteria are constantly inhaled but infection does not develop as a result of sophisticated host defenses. These include mechanical factors such as tight epithelial lining cells and mucociliary clearance, antimicrobial peptides (including pentraxin-3, lysozyme, lactoferrin, defensins, elafin, secretory leukoprotease inhibitors [SLPI], and cathelicidin), local immune responses such as secretory IgA, resident phagocytes such as airway macrophages, and acquired immune responses.[32] The presence of bacterial infection in the lower airways suggests that host pulmonary defenses are impaired and a number of abnormalities in pulmonary immune mechanisms have been reported in COPD. Cigarette smoking has a number of effects on the mechanical barriers in the lung that may favor the development of infection. Smoking reduces ciliary beat frequency, induces squamous metaplasia resulting in reduced numbers of ciliated cells, and increases goblet cells and submucosal mucous glands.[33] This is likely to result in excess production and impaired clearance of mucous from the airways, which favors bacterial growth. Impaired mucociliary clearance[34] and ciliary abnormalities have been reported in smokers and COPD[35] and therefore these may be factors contributing to bacterial infection in COPD. There is also evidence that cigarette smoke damages the epithelial junctions leading to increased epithelial permeability.[36]

Another mechanism that has been extensively investigated is impaired function of airway immune cells such as neutrophils and alveolar macrophages in COPD. These cells play a key role in removing microorganisms present in the airways by phagocytosis and initiating immune responses. Absolute numbers of macrophages and neutrophils in the airways are actually increased in smokers and COPD patients, and therefore, research has focused on whether macrophage function in COPD is impaired. A number of studies have reported that macrophage phagocytosis of microorganisms is impaired in COPD[37–42] and a number of mechanisms of impaired function of macrophages in COPD have been investigated. Reduced levels of the toll-like receptor TLR2 have been reported in macrophages[43] and neutrophils[44] from COPD patients. MARCO (macrophage receptor with collagenous structure) is a class A scavenger receptor expressed on macrophages that mediates binding and uptake of Gram-positive and Gram-negative bacteria.[45] Cigarette smoking reduces the expression of MARCO on macrophages resulting in impaired responses to infection in vitro[46] and in vivo in mice.[47] Interest in macrophage function has increased recently with studies that have shown that macrophage function can be restored with pharmaceutical agents,[42,47–49] raising the possibility that improving the phagocytic capacity of macrophages is a potential therapeutic option in COPD.

Antimicrobial peptides (AMPs) are soluble molecules present in the airway surface fluid that constitute an important first line of defense against both bacterial and viral pathogens. AMPS include surfactant proteins (SP), defensins, elafin, cathelicidin, SLPI, and lysozyme. Studies of AMPs in COPD have had varying results with both increased[50] and decreased[51,52] human β-defensin, increased α-defensin,[53] and increased SLPI and elafin[52] reported. Therefore, it remains unclear whether reduced levels of AMPs contribute to impaired immune responses in COPD.

α1-Antitrypsin (AAT) is a member of the serine protease inhibitor (*SERPIN*) supergene family and AAT deficiency is associated with the development of early-onset COPD. The key function of AAT is the inhibition of proteases such as neutrophil elastase, and excess protease activity is considered the key mechanism underlying the development of COPD in AAT deficiency. New evidence has emerged that, in addition to anti-protease activity, AAT possesses anti-inflammatory, immunomodulatory, and both antibacterial[54] and antiviral properties.[55] Therefore, it is possible that AAT deficiency contributes to susceptibility to infection in COPD. AAT deficient patients experience frequent exacerbations[56] but there is no evidence that infections are more frequent than non-AAT deficient patients. AAT augmentation reduces exacerbations and this will be discussed further in the "Novel therapies" section.

Animal models of bacterial infection in COPD

Since there are no existing human models of experimental bacterial infection in COPD, there has been considerable interest in the use of animal models to investigate the role of bacterial infection in COPD. Animal models use three main approaches to establishing a COPD phenotype: inhalation of noxious stimuli (most commonly cigarette smoke exposure), instillation of tissue-degrading proteinases such as elastase, and genetic manipulation.[57,58] Gaschler et al administered cigarette smoke to mice for 8 weeks and subsequently challenged intranasally with nontypeable *H. influenzae* (NTHI). This led to increased pulmonary inflammation, upregulation of inflammatory mediator expression but decreased bacterial burden compared to control mice.[59] Drannik et al similarly showed that cigarette smoke altered the response to infection with *Pseudomonas aeruginosa* with a worsening of clinical score, increased inflammation, and increased expression of pro-inflammatory cytokines.[60] Although cigarette smoke-induced models have the advantage of using the primary disease-causing agent, even with prolonged exposure, only mild pulmonary abnormalities are seen in mice, equivalent to human GOLD stage 1 or 2 disease.[58,61] Therefore, studies using such models may be of less relevance to the study of bacterial infection which occurs more frequently in patients with more severe disease. Instillation of proteinases such as porcine pancreatic elastase produces more severe emphysematous changes which mimic those seen in advanced disease and therefore other studies have chosen to use these techniques to model bacterial COPD exacerbations.

Pang et al used a mouse model of elastase-induced emphysema to show that pulmonary clearance of NTHI was impaired.[62] They also showed that intercellular adhesion molecule 1 (ICAM-1) was downregulated in the airway epithelium of elastase-treated mice and suggested that this may provide an underlying mechanism for the impaired bacterial clearance observed. However, notably, they did not show any differences in localized lung inflammation between the two groups and therefore, whether delayed bacterial clearance leads to clinically relevant exacerbation of disease in this model is unclear. Ganesan et al used a more complex model system of combined lipopolysaccharide

and elastase administration to mimic airway inflammation and emphysema in mice.[63] When challenged with NTHI, elastase/LPS mice showed delayed bacterial clearance with an increase in neutrophilic inflammation and prolonged mucus secretion assessed by mucin gene expression and periodic acid schiff (PAS) staining on lung histology. Furthermore, ex vivo macrophages taken from elastase/LPS mice showed deficient phagocytosis and this was demonstrated to be due to decreased expression of scavenger receptor A. This receptor has previously been shown to be important in bacterial clearance in mice[64] and genetic mutations are also associated with increased risk of developing COPD in humans.[65] However, the direct applicability of these findings to mechanisms leading to susceptibility to bacterial infections in patients with COPD requires further characterization.

Developing animal models of chronic bacterial infection has been difficult due to rapid clearance of bacterial agents by the rodent immune system, typically within 1–2 days of administration.[57] Wang and colleagues used a hamster model of elastase-induced emphysema and administered *H. influenzae* encased in agar beads.[66] This led to prolonged survival and persistence of bacteria for up to 3 weeks in emphysematous airways. Such models may be invaluable in the future to aid the study of bacterial colonization in COPD and its relevance to exacerbations and disease pathogenesis. Furthermore, studies using molecular techniques are also ongoing to assess the bacterial flora in the lower respiratory tract of COPD mouse models. It remains to be seen whether this will correlate with the emerging evidence that bacterial communities are disordered in human patients with COPD.[19,20]

In summary, animal models have provided some useful insights into the mechanisms of host defense against bacterial infection in COPD. However, COPD is a complex, multisystem disorder that is incompletely understood in humans and therefore, the direct relevance of animal studies to clinical disease remains unclear. Future studies should aim to further evaluate which aspects of human bacterial COPD exacerbations existing models accurately mimic, as well as translating findings from existing animal work into clinical disease studies.

Bacteria and COPD exacerbations

Perhaps equally controversial to the role of bacteria in the pathogenesis of stable COPD is the contribution of bacteria to COPD exacerbations.[67] Bacteria are often detected in COPD exacerbations but the high isolation rates of bacteria in stable COPD means the presence of bacteria does not prove a causative role. As bacteria are often present in patients who are clinically stable, studies have attempted to determine whether bacterial infection does in fact induce exacerbations and the potential mechanisms underlying this.

New acquisition of bacteria

Detection rates of bacterial infection in samples collected during exacerbations are high, but as bacteria are often present in stable COPD, this does not prove that the organism is newly acquired and caused the exacerbation. There are surprisingly few studies directly comparing infection rates in stable and exacerbated patients. Two studies from the East London COPD cohort reported infection rates of 48.2% and 43% in sputum in stable patients compared to 69.6% and 76% in exacerbated patients[68,69] ($P = 0.009$ for the study by Hurst et al,[69] P-value not provided for the study by Wilkinson et al[68]). The pooled analysis by Rosell et al detected bacterial infection in 29% of protected brush specimens from stable COPD patients and 54% of exacerbated patients ($P < 0.001$).[2] However, Papi et al reported a non-significant higher incidence of bacterial detection in exacerbated patients (54.7% vs 37.5% when stable, $P = 0.08$)[70] and Bafadhel et al detected bacterial infection in 28% of stable patients and 35% of exacerbations (P-value not provided).[71] Therefore, not all studies have conclusively demonstrated that infection rates are higher in exacerbated patients.

Sethi et al analyzed changes in bacteria in 81 patients over a 56-month period using both culture and molecular typing methods.[72] Isolation of a bacterial pathogen by culture was associated with a significant increase in the incidence of exacerbations. An exacerbation was present in 23.6% of visits at which pathogens were isolated from sputum compared with 18% of visits at which no pathogens were isolated from sputum ($P < 0.001$). An analysis of individual bacterial species showed that isolation of *M. catarrhalis* and *S. pneumoniae* was associated with a significant increase in the frequency of exacerbations whereas *H. influenzae, P. aeruginosa,* and Gram-negative bacilli were not. However, as can be seen from these data, the majority of visits at which a pathogen was detected were not associated with an exacerbation. Therefore, acquisition of bacterial infection in a COPD patient does not automatically result in an exacerbation and it is likely that there are both host and pathogen factors that determine the outcome of bacterial infection. Further studies are needed to identify the key factors that determine the outcome of bacterial infection in COPD.

Increased bacterial load

If acquisition of a new organism does not occur in all exacerbations, another possible mechanism of exacerbation is an increase in the load of a pre-existing organism present in stable COPD patients. Two studies from the East London cohort reported a significant increase in bacteria load at exacerbation compared to the stable state.[68,69] Conversely, Sethi et al reported no significant difference between concentrations in sputum during stable disease and exacerbation for *H. influenzae*, *H. haemolyticus*, and *M. catarrhalis* and actually found an inverse relationship between exacerbation occurrence and bacterial concentrations in sputum for *S. pneumoniae* and *H. parainfluenzae*.[73] When paired comparisons of concentrations of the same strain of pathogens isolated from the same patient during exacerbations and stable visits was performed, only *H. influenzae* was present at a higher concentration in exacerbation samples. Therefore, whether increase in bacterial load is a major mechanism of exacerbation in COPD remains unclear.

New bacterial strain

Detection of bacteria by culture does not distinguish between different strains of the same organism. Acquisition of a new strain of a bacterium has been investigated as another mechanism of exacerbation. In the study by Sethi et al described previously, isolation of a new bacterial strain was associated with a significant increase in the frequency of exacerbation. Thirty-three percent of visits at which new strains were isolated were associated with exacerbations, compared with 15.4% of visits at which no new strains were isolated ($P < 0.001$).[72] The relative risk of an exacerbation in association with the isolation of a new strain of *H. influenzae* was 1.69 ($P < 0.001$), whereas there was no association between the isolation of *H. influenzae* by culture and an exacerbation. The relative risk of an exacerbation in association with the isolation of a new strain was 2.96 for *M. catarrhalis* and 1.77 for *S. pneumoniae* so strain analysis strengthened the association seen with culture alone. New strains of *P. aeruginosa* were not associated with exacerbations. Further evaluation of this study reported that bacteria identified as variant *H. influenzae* were subsequently characterized as *Haemophilus haemolyticus*.[74] Re-assessment of the results accounting for the previously misinterpreted *H. haemolyticus* showed that new strain acquisition of *H. influenzae* was associated with a four-fold increase in the incidence of COPD exacerbation. Additional follow-up of these patients subsequently also reported a significant association between acquisition of *P. aeruginosa* and exacerbation contrary to what was previously reported[13] and a study of *M. catarrhalis* determined that acquisition of a new strain is associated with 10% of exacerbations.[75] In another publication from this group, the clinical and inflammatory responses associated with acquisition of a new strain of *H. influenzae*, *S. pneumoniae, M. catarrhalis*, and *P. aeruginosa* were compared with exacerbations in which a pre-existing strain of one of these four species was present, exacerbations in which *S. aureus* or Gram-negative bacilli other than *P. aeruginosa* were isolated and pathogen-negative exacerbations.[76] Of the 150 exacerbations, 26% were new strain, 10% were pre-existing strain, 20% were other pathogen, and 44% were pathogen-negative exacerbations. New strain exacerbations had the largest increase in airway inflammation with significantly greater changes in TNF-α and NE when compared with pathogen-negative and other pathogen exacerbations and serum CRP levels were significantly higher with new strain exacerbations in comparison to each of the other three groups. New strain exacerbations were also associated with greater clinical symptom scores.

Chin et al studied the pro-inflammatory effects of different strains of *H. influenzae* and demonstrated that strains isolated during exacerbations caused more airway neutrophil recruitment in a mouse model of bacterial infection and more inflammatory pathway activation in primary human airway epithelial cells cultures when compared with bacterial strains isolated from stable patients.[77] Therefore, these data suggest that acquisition of a new bacterial strain is likely to be an important mechanism linking bacterial infection and COPD exacerbations, and that the risk of exacerbation differs with different *H. influenzae* strains.

Thus, the role of bacteria in COPD exacerbations is complex and the previously held concept of a newly acquired bacterial infection causing an exacerbation based on studies using sputum culture is likely to prove simplistic. New paradigms have emerged from studies using more sophisticated molecular techniques such as strain typing, and as these become more widely available, our understanding of the role of bacteria is likely to evolve further.

Virus infection in COPD

Historically, bacterial infections have been considered the predominant infectious causative agents of COPD exacerbations and this is reflected in the widespread use of antibiotics in their treatment. Epidemiological data reporting a greater frequency of exacerbations in the winter months,[78] and frequent coryzal symptoms preceding exacerbations suggest that exacerbations may also be associated with respiratory virus infections.[79] Studies investigating the role of viruses

in COPD exacerbations carried out in the 1970s and 1980s detected viruses in only ~10%–20% of exacerbations,[80,81] casting doubt on the role of virus infection. However, the diagnostic methods used in these studies had low sensitivity, especially for the detection of rhinoviruses, which are the most common cause of viral upper respiratory tract infections. Following the development of PCR-based techniques for the detection of respiratory viruses in clinical samples, the role of viruses in a number of clinical syndromes including COPD exacerbations has been re-evaluated. More recent studies using PCR have detected the presence of a virus in 47%–56% of exacerbations.[70,82–85] In most studies, picornaviruses (predominantly rhinoviruses) were the most frequently detected viruses, followed by influenza, parainfluenza, respiratory syncytial virus (RSV), and adenoviruses.[86] Therefore, these reports suggest that up to half of COPD exacerbations are associated with respiratory virus infection. However, the role of virus infection in COPD exacerbations continues to be debated for a number of reasons and some authorities have questioned whether virus infections can cause exacerbations in their own right or whether they simply predispose to secondary bacterial infection. PCR-based diagnostic techniques are able to detect very small amounts of viral RNA or DNA and therefore do not definitively prove the presence of live virus or prove a causative relationship. As is the case with bacteria, viruses can be detected in stable COPD but this has been studied to a much lesser extent and detection rates have varied from 0%[83,87] to 19%.[82] Few studies have investigated both viral and bacterial infection in the same exacerbations and detection rates have varied from 6%–25%.[70,85] However, it is also possible that the role of viral infections in COPD exacerbations has been underestimated, as patients are evaluated at the time of presentation which often occurs considerably later than the onset of exacerbation and viruses may no longer be detectable by this time point. As rapid diagnostic methods and antiviral agents become available, the relationship between virus infections and COPD will no longer be of just academic interest but will have potential therapeutic implications and therefore warrants further study.

Experimental rhinovirus infection in COPD

A novel tool for investigating relationships between virus infection and COPD exacerbations is experimental rhinovirus infection. Experimental infection studies have been previously conducted in asthmatics and yielded important insights into the mechanisms linking virus infection to exacerbations in asthma.[88,89] Our group has recently reported the first experimental rhinovirus infection study in COPD patients.[90] COPD patients and non-obstructed control subjects were infected with rhinovirus followed by sequential measurement of symptoms, lung function, inflammatory markers, and virus load. Rhinovirus infection induced symptomatic colds that were followed by the typical features of a COPD exacerbation, ie, lower respiratory symptoms, increased airflow limitation, and airways inflammation. Virus was detected in airway samples following inoculation but prior to the onset of symptoms and viral clearance was followed by symptom resolution and return of inflammatory markers to baseline levels. Virus load correlated strongly with inflammatory markers and the rhinovirus was grown from airway samples, confirming the presence of live virus. Therefore, this study is the first to directly link respiratory virus infection to symptoms, airflow obstruction, and airways inflammation in COPD patients so providing novel evidence that rhinovirus infection causes COPD exacerbations.

Virus infection and stable COPD

The majority of studies that have investigated virus infection in both exacerbated and stable patients have detected viruses at a greater frequency during acute exacerbations compared to stable state. However, there is some evidence that RSV is detected more frequently in stable patients with one study reporting a frequency of around 25% in the stable state.[79] A further study found RSV in 30% of sputum samples from stable patients, with detection being related to greater airway inflammation and to a faster decline in lung function.[91] However, other studies have not reported high rates of RSV infection in stable COPD patients.[70,82,92] A study comparing virus loads between infants with acute respiratory infections and adult COPD patients found that virus loads were 2000-fold higher in the infants, suggesting low-grade virus infection in COPD.[93] The disparity between these findings is likely to be due to a combination of factors including differing sensitivity of PCR techniques used, differences in severity of COPD patients included, or differences in populations studied.[94] Latent infection by adenovirus has also been proposed to be involved in the pathogenesis of COPD. Lung tissue from COPD patients has been demonstrated to carry more group C adenoviral DNA than matched non-obstructed smokers.[95] Latent adenoviral infection in combination with cigarette smoke exposure in a guinea pig model caused an increase in lung volumes, airspace volume, and reduced surface to volume ratio compared to smoke exposure alone.[96] Additionally, adenovirus detection has

been shown to be similar in exacerbated and stable COPD patients.[97] Some authors have postulated that the presence of RSV and adenovirus in stable COPD may contribute to the pathogenesis of the disease as there are some common pathologic features between respiratory viral infection and COPD including a predominance of CD8+ T lymphocytes. However, this remains a largely unproven hypothesis.

Relationships between exacerbation etiology and clinical and inflammatory parameters in COPD

COPD exacerbations are associated with systemic and pulmonary inflammation and increased levels of inflammatory mediators and cells have been measured in airway samples including tumor necrosis factor-alpha (TNF-α),[98] IL-8,[98,99] IL-6,[100] leukotriene B4,[101] and neutrophils,[70,99,102,103] lymphocytes,[99,103] and eosinophils.[70,99,103] Few studies have examined the relationships between the clinical and inflammatory parameters and specific pathogens. It has been reported that virus-positive exacerbations are associated with more symptoms, greater falls in lung function,[79,104,105] higher sputum levels of IL-6,[104,105] and IP-10,[106] however, these studies did not perform bacterial culture. Conversely, others have reported that only exacerbations associated with purulent sputum (presumed bacterial) are associated with airways inflammation and greater falls in lung function[68,101,107,108] and that bacterial load correlates with levels of inflammatory markers,[108] but did not perform virological analysis. Studies that directly compared clinical and inflammatory parameters in bacteria and virus-associated exacerbations have had contrasting results. Two studies reported that the presence of bacteria but not viruses was associated with airways inflammatory markers such as neutrophils, IL-8, and TNF-α.[69,109] Papi et al found no differences in clinical outcomes between bacteria- and virus-associated exacerbations. Both were associated with increased neutrophils and neutrophil elastase but only virus-associated exacerbations had significantly increased sputum eosinophils.[70] However, Bafadhel et al reported bacterial exacerbations were associated with increased sputum total cell count, neutrophils and eosinophils, and CXCL-8, TNF-α, IL-1β, CCL5, CCL17, CXCL11, CCL3, and CCL13, whereas virus-associated exacerbations had greater falls in lung function and increased CCL5, CXCL10, and CXCL11.[71] Sethi et al compared the inflammatory responses to different bacteria species in COPD exacerbations and reported that *H. influenzae* and *M. catarrhalis* were associated with inflammatory markers in sputum whereas the inflammatory profile of *H. parainfluenzae*-associated exacerbations was no different from pathogen-negative exacerbations.[108] Therefore, no clear pattern of clinical features or inflammatory markers has emerged that is specific to either bacterial or viral infection in COPD exacerbations. It is possible that the inflammatory pathways activated are the same irrespective of exacerbation etiology; however, studies are needed with intensive sampling and advanced diagnostic methods for both bacteria and viruses to investigate this further.

Susceptibility to virus infection in COPD

Cigarette smoking increases susceptibility to infection with respiratory viruses, but it is not established whether COPD is associated with increased risk of virus infection. In vitro studies have shown that cigarette smoke impairs the release of IFN-β and IFN-α.[110] BAL cells from COPD patients infected ex vivo with rhinovirus, demonstrated deficient induction of IFN-β with similar trends for deficient induction of IFNs-α and -λ, associated with deficiency of the interferon-stimulated gene CXCL10.[90] Similar findings have been reported in a mouse model where persistence of rhinovirus, increased airways inflammation, and deficient induction of IFNs-α, β, and -γ were reported in COPD mice compared to controls.[111] However, in vitro rhinovirus infection of epithelial cells from COPD patients resulted in higher virus load and increased inflammatory mediators, but no differences in interferon production compared to cells from control subjects.[112] Rhinoviruses bind to cells via intercellular adhesion molecule-1 (ICAM-1, major group rhinoviruses) or members of the low-density lipoprotein receptor family (minor group rhinoviruses). ICAM-1 is upregulated on the bronchial epithelium of patients with COPD,[112,113] and therefore, it is possible that increased ICAM-1 levels may permit greater virus binding and increased viral entry into epithelial cells in COPD patients. Further studies investigating mechanisms of virus infection in COPD are required and this may lead to potential future therapies for virus-induced exacerbations.

Treatment of infection in COPD exacerbations

The controversy surrounding the role of bacteria in COPD is mirrored in the debate regarding the efficacy of antibiotics in COPD exacerbations. The current GOLD guidelines acknowledge this ongoing controversy and the poor quality of the clinical trials on antibiotics. This is reflected in the fact that there is no Grade A evidence on which the guidelines based their recommendations.[114] However, antibiotic use in COPD exacerbations is widespread with >80% of patients in secondary care and 50% of patients

managed in primary care treated with antibiotics.[117,118] Therefore, antibiotic use in COPD may be excessive and contribute to antimicrobial resistance.

Antibiotics in COPD exacerbations

In the GOLD guidelines, the evidence for antibiotic use in COPD exacerbations is classed as Category B which is defined as "few randomized trials exist, they are small in size, they were undertaken in a population that differs from the target population of the recommendation, or the results are somewhat inconsistent." There are numerous studies of antibiotics in COPD exacerbations but the majority of these are head-to-head comparisons of different antibiotics and there are few placebo-controlled trials. The placebo-controlled trials that are available are affected by a number of methodological issues including small numbers of patients, different outcome measures assessed at different time points, inclusion of patients without COPD, varying severities of exacerbation, and different antibiotics. A Cochrane review in 2006 identified 11 placebo-controlled trials of antibiotics in COPD including a total of 917 patients. Antibiotic use was associated with significant reductions in mortality, treatment failure, and sputum purulence, but no improvement in arterial blood gas parameters or peak expiratory flow.[119] However, there was considerable heterogeneity across the trials and a number of issues that cast doubt on how applicable these findings are to modern management of COPD exacerbations. The review included trials carried out over a wide time period (1965–2001) with only four studies post-1990 and as the definition of COPD has changed markedly, no uniform COPD classifications such as the GOLD stages could be determined from the studies. Studies included a mixture of exacerbation severities ranging from patients treated in the community to patients requiring mechanical ventilation, corticosteroid use was poorly documented, a range of different antibiotics was used, and outcome measures such as quality of life measures not recorded. Therefore, it is debatable whether it is valid to extrapolate these findings to modern day practice. A more recent study included hospitalized COPD patients, all of which were treated with oral corticosteroids and randomized to receive doxycycline or placebo.[120] The primary end point was clinical response on day 30 and the study found no difference between groups in this outcome measure. Doxycycline was shown to be superior to placebo in a number of secondary outcomes. On day 10, clinical success was achieved in 80% of patients in the doxycycline group and 69% in the placebo group (OR: 1.9; 95% CI: 1.1–3.2; $P = 0.03$) in the intention-to-treat analysis but this significant difference was lost in the per-protocol population. Open-label antibiotic treatment for lack of efficacy, day 10 symptom scores, and positive bacteriological cultures were higher in the placebo group. However, treatment failure and changes in lung function were not significantly different between groups.

Therefore, despite the widespread use of antibiotics in COPD exacerbations, the evidence for a beneficial effect is not compelling. Studies have attempted to identify factors that can identify patients who will benefit significantly from antibiotics therapy. Anthonisen et al studied 173 COPD patients with 362 exacerbations for 3.5 years and treated with antibiotics or placebo in a randomized, double-blind, crossover fashion.[121] Exacerbations were classified into three groups: type 1 exacerbations comprised worsening dyspnea, increased sputum volume, and purulence, type 2 exacerbations were any two of these symptoms, and type 3 was any one of these symptoms with evidence of fever or an upper respiratory tract infection. Outcome was defined as success, no resolution, or deterioration, all assessed at 21 days. Patients with a type I exacerbation receiving antibiotics had a higher success rate compared to placebo (62.9% vs 43%, $P = 0.01$) but no benefit was seen for patients with type II (70% vs 60%) and type III (74% vs 70%) exacerbations. However in the study by Daniels et al the treatment effect did not differ significantly between the Anthonisen groups.[120] A meta-analysis of ten trials including 1557 patients by Puhan et al reported that the effects of treatment varied according to the severity of exacerbations. Antibiotics did not significantly reduce treatment failure in mild to moderate exacerbations treated as outpatients but did so in severe exacerbations requiring hospital admission.[122] These findings were confirmed by Quon et al and together these studies indicate that the beneficial effect of antibiotics may be restricted to more severe exacerbations.[123] Sputum purulence has also been proposed as a marker of bacterial infection[124–128] and there is evidence that withholding antibiotics from patients without purulent sputum is safe,[124,129] but these studies were not randomized.

The use of procalcitonin (PCT) as a marker of bacterial infection has been studied in a number of clinical syndromes including COPD exacerbations. Procalcitonin levels in the serum are low in the healthy state and become elevated in response to bacterial infections, but this response is attenuated by cytokines released in response to viral infection. Christ-Crain et al evaluated the use of procalcitonin to guide antibiotic therapy in respiratory tract infections including patients with COPD exacerbations.[130] Patients were assigned to either a standard therapy arm where antibiotics were prescribed according to clinical judgment, or a procalcitonin-guided arm in which antibiotics were given only to patients with raised

PCT levels. Eighty-seven percent of the COPD patients in the standard therapy group received antibiotics compared to 38% in the procalcitonin group ($P = 0.0001$) and there were no differences in outcomes between the groups. This group then carried out a similar study in COPD exacerbations only and reported that antibiotic prescribing was reduced by 32% in the procalcitonin group compared to the standard therapy group.[131] There were no differences in outcomes between the two study arms and importantly, antibiotic prescribing was not different up to 6 months after the index exacerbation. There was no relationship between PCT levels and sputum purulence, Anthonisen type, or sputum cultures.

Therefore, despite the widespread use of antibiotics in COPD exacerbations, the evidence for this usage is surprisingly weak. Antibiotic use has a number of adverse effects both in terms of direct side effects for patients and effects on antimicrobial resistance. With increasing use of self-management plans for COPD patients[132] and increasing numbers of elderly patients with COPD, the prescribing of antibiotics and the associated adverse effects are likely to increase with current management strategies. The validation of biomarkers and identification of clinical characteristics that identify exacerbations likely to be bacterial are urgently needed to guide rational antibiotic prescribing in COPD.

Antibiotics in stable COPD

Recognition of the potential role of bacteria in amplifying airways inflammation in stable COPD has led to interest in treating bacterial infection not only in exacerbations but also in stable patients. This approach is not new as trials of prophylactic antibiotics in chronic bronchitis were first carried out in the 1960s. A meta-analysis of nine such trials showed some reduction in exacerbation time and a small but non-significant effect on exacerbation frequency.[133] The lack of clear efficacy and concerns around the development of antibiotic resistance meant that this approach to antibiotic use in COPD was not pursued until more recently. Long-term macrolide antibiotics have been used in a number of chronic respiratory diseases including cystic fibrosis, asthma, and obliterative bronchiolitis. It is unclear whether the clinical benefits of macrolides are due to their antimicrobial effects as anti-inflammatory and immunomodulatory effects have also been described in vitro. In view of their beneficial effect in pulmonary diseases, their effect in COPD has been examined. Seemungal et al assessed the effects of erythromycin administered over 1 year in COPD and reported a significant reduction in exacerbations in the treatment group.[134] No effect on airway or systemic inflammatory markers was seen, suggesting that exacerbations were reduced through an antibacterial mechanism. A trial of the macrolide antibiotic azithromycin 250 mg once daily for 1 year in patients with COPD reported a reduction in exacerbations and improvement in quality of life measures in the treatment arm.[135] However, azithromycin was associated with a slight increase in hearing impairment and a doubling of macrolide resistance in respiratory pathogens isolated from nasopharyngeal swabs. An association between exposure to macrolides and macrolide resistance in pneumococci in COPD has also been reported,[136] and therefore, caution may be required before embarking on long-term antibiotic treatment in large populations of COPD patients.

The risk of development of antibiotic resistance is reduced if either short courses or pulsed courses of antibiotics are used. A short course (5 days) of moxifloxacin in stable COPD patients with bacterial infection demonstrated eradication rates of 75% compared to 30% with placebo ($P = 0.01$). However, infection rates were similar at 8 weeks and there was no difference in exacerbation rates over a 5-month follow-up period indicating that the effect of the antibiotic was short-lived.[137] A randomized controlled trial of the fluoroquinolone antibiotic moxifloxacin to patients with COPD as a pulsed dose for 5 days every 8 weeks reported reduced exacerbations in the per-protocol analysis, but no significant effect was noted on exacerbations in the intention-to-treat analysis.[138] A subgroup analysis of the per-protocol group showed that patients with mucopurulent or purulent sputum at baseline had a 45% reduction in the occurrence of exacerbations. There was no evidence to suggest development of significant bacterial resistance during the trial.

Therefore, the role of antibiotics in stable COPD remains to be clearly defined and the benefits weighed against the potential risks of increased microbial resistance and other adverse effects.

Novel treatments for COPD exacerbations

In view of the evidence implicating respiratory viruses in COPD exacerbations, anti-viral agents may have potential as treatment in COPD exacerbations. Drugs have been developed that are active against rhinoviruses including the capsid-binding agent pleconaril.[139] Despite demonstrating clinical benefit in reducing the severity of symptoms, pleconaril was not approved as a treatment for the common cold. Pleconaril has also been investigated as a treatment for asthma exacerbations but the results have not been reported yet.[140] If antiviral agents demonstrate benefit in asthma, then trials in COPD may be warranted. The observation that AAT

has antimicrobial activity suggests administration of AAT as a potential novel therapeutic approach for COPD.[55] In AAT-deficient COPD patients, AAT augmentation therapy is associated with reductions in exacerbations[141,142] and in cystic fibrosis, inhaled AAT reduces *Pseudomonas* ssp. load.[54] However it remains to be determined whether administration of AAT is beneficial in non-AAT deficient COPD.

Conclusion

In conclusion, infection with both bacteria and viruses plays a major role in both stable COPD and acute exacerbations. It is clear that infection can have multiple outcomes including resolution, chronic infection, and exacerbation, and complex interactions between host and pathogen factors are likely to determine the outcome. Previous concepts of "colonization" in stable patients are no longer valid with the evidence that even in stable patients, the presence of bacteria is associated with adverse outcomes. New diagnostic methods that can detect different strains of the same bacterial species and reveal the presence of bacteria not detected by culture methods are likely to further advance our understanding of the role of bacteria in COPD. Finally, the development of anti-viral agents and the validation of biomarkers that can identify specific infectious agents will lead to the use of pathogen-directed therapy in exacerbations.

Disclosure

The authors report no conflicts of interest in this work.

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