Impact of bacterial infections on airway diseases

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ABSTRACT: Bacterial infections play an important role as aetiologic agents in acute exacerbations of chronic obstructive pulmonary disease (COPD). Modern investigational tools, including bronchoscopy, microbial molecular epidemiology and measurement of specific immunity have established that bacteria cause up to 50% of acute exacerbations in COPD.

Acute exacerbations have enormous economic costs and contribute to morbidity, mortality and impairment in health related quality of life. Chronic bacterial persistence in the lower airways and lower respiratory tract in patients with COPD is not innocuous. It is likely to contribute to persistent airway inflammation and might contribute to acute airway exacerbations or progression of airway obstruction. Further investigation is required in these fertile areas of investigation.

Bacteria also play a role in asthma exacerbations, but their role is less well defined than with patients with COPD. *Mycoplasma pneumoniae* may be associated with asthma chronicity. Chronic airway infection models document that *M. pneumoniae* plays a role in airway remodelling, including angiogenesis, vascular remodelling and airway wall thickening.

Recent studies have identified patients with asthma as an at-risk group for invasive pneumococcal disease. The feasibility and cost effectiveness of a pneumococcal vaccination strategy among persons with asthma deserves careful, immediate attention.

KEYWORDS: Airways disease, asthma, bacterial infections, chronic obstructive pulmonary disease, pneumonia, vaccination

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COPD

Chronic obstructive pulmonary disease (COPD) is the fourth leading cause of chronic morbidity and mortality in the USA and is currently the fourth leading cause of death in the world [1–3]. While mortality related to other leading causes of death (cardiac disease and malignancy) has declined substantially over the last decade, the mortality of COPD has been increasing worldwide [3].

Potential roles of bacterial infection in COPD

The contributions of bacterial infection to the aetiology, pathogenesis and clinical course of COPD have been a source of controversy for several decades. Bacterial infection has been assigned a pre-eminent role in the pathogenesis of COPD; bacterial infections have also been dismissed as a mere epi-phenomenon [4–7].

Bacterial infection might contribute to the pathogenesis and clinical course of COPD in three ways: 1) lower respiratory tract infection during childhood might impair lung growth; 2) bacteria could account for a substantial portion of acute exacerbations of chronic bronchitis, a cause of considerable morbidity and mortality; 3) chronic colonisation of the lower respiratory tract or persistence in respiratory tissues could induce a chronic inflammatory response and thus contribute to the pathogenesis of disease.

Childhood lower respiratory tract infections

Adults who experience childhood lower respiratory tract infection consistently show a lower forced expiratory volume in one second (FEV1) and often lower forced vital capacity (FVC) when compared with others in the cohort who did not experience a childhood infection [8–10]. The extent of decrease in FEV1 is unlikely to cause symptomatic pulmonary disease, but could make the individual susceptible to the effects of additional injurious agents, such as tobacco smoke or exposure to airborne pollutants. While the association between childhood lower respiratory tract infection and impaired lung function in adulthood is well established, it is uncertain that this association reflects a cause–effect relationship. Two possibilities exist. Childhood infection could damage a vulnerable lung undergoing rapid postnatal growth and development. Alternatively, genetic factors may predispose

certain individuals to lower respiratory tract infections as well as a lower FEV1 in childhood. In one instance, infection antedates impaired lung growth while in the other instance impaired growth antedates the respiratory infection.

Bacterial pathogens as aetiologic agents in acute exacerbations of COPD

There is considerable debate regarding the definition of an acute exacerbation of COPD. An acute exacerbation remains a subjective, symptom based, patient reported clinical event. Acute exacerbations are usually defined as a minor increase (or new onset) of two or a major increase (or new onset) of one of the following respiratory symptoms: cough, dyspnoea, sputum production, sputum tenacity, or sputum purulence. The altered symptoms must be present for at least 24 h and must be of greater intensity than normal day-to-day variability.

Bacteria, viruses and atypical pathogens are implicated as aetiologic agents of up to 80% of acute exacerbations [11]. While considerable controversy exists regarding the role of bacteria in acute exacerbations, recent studies that have utilised modern investigational tools including bronchoscopy, microbial molecular epidemiology and measurement of specific immunity, have established that bacteria cause up to 50% of acute exacerbations [12]. Significant bacterial infection was noted in ~50% of patients with acute exacerbations who had bronchoscopic sampling of lower airway secretions [13–15]. Lower concentrations of bacteria were present in the lower airways of 25% of patients who had stable COPD. Bronchial mucosal biopsies obtained from patients intubated for acute exacerbations revealed intracellular nontypable *Haemophilus influenzae* (NTHI) in 87% of patients with exacerbations, compared with 33% of patients who had stable COPD. Intracellular NTHI were not noted in any healthy controls [16]. The increased rate of isolation of pathogenic bacteria from the lower respiratory tract during acute exacerbations compared with stable COPD substantially supports the pathogenic role of bacterial in acute exacerbations.

Early studies found no difference in the isolation rate of bacterial pathogens during exacerbations and stable COPD [17, 18]. These cohort studies did not differentiate among strains of pathogens isolated from sputum over time. A recent longitudinal cohort study demonstrated that the acquisition of a novel bacterial strain was associated with a greater than twofold increase in risk for an exacerbation. This increased risk for exacerbation was seen with acquisition of novel strains of NTHI, *Moraxella catarrhalis* and *Streptococcus pneumoniae*, but not with strains of *Pseudomonas aeruginosa*. This study provides support for bacteria as aetiologic agents in a substantial portion of exacerbations. Additionally, this study suggests that infection with a bacterium that is new to the host is a major mechanism of recurrent acute exacerbations in COPD [19].

Further support for the role of novel bacterial strains in the aetiology of acute exacerbations of COPD comes from studies of specific immune responses following exacerbations. An immune response to the infecting NTHI strain was demonstrated after 61% of exacerbations associated with strains that were new to the patient; an immune response to the infecting NTHI strain was noted in only 21% of exacerbations associated with a pre-existing strain. New bactericidal antibodies that developed after an exacerbation were highly strain specific, showing bactericidal activity for only 12% of heterologous strains isolated from other patients. Development of a strain-specific immune response provides further support for a causal role of bacteria during acute exacerbations [20].

Considerable confusion exists regarding the importance and incidence of *Mycoplasma pneumoniae*, *Chlamydophila* (formerly *Chlamydia*) *pneumoniae* and Legionella species infections in acute exacerbations of COPD. This confusion is related both to the definition of exacerbations used in the studies as well as to methods used to determine the presence of infection with one of these microbes. In studies with rigorous methodology to exclude pneumonia and rigorous diagnostic criteria, including a strict four-fold increase in titre or a positive culture, *M. pneumoniae* or Legionella species are rare in acute exacerbations and the incidence of *C. pneumoniae* is ~5% [21, 22].

The impact of repeated exacerbations on pulmonary function is a matter of intense debate [7, 23]. In a longitudinal study of 101 patients, acute exacerbations of COPD significantly impaired pulmonary functions and increased symptoms with a mean time for recovery of 6 days and 7 days, respectively [24]. However, even by 35 days recovery was incomplete in 25% (by pulmonary function) and 14% (by symptoms) of patients. Thus, even single episodes trigger prolonged disability in a subset of patients. More ominous, though controversial, is mounting evidence that more frequent acute exacerbations of COPD accelerate physiological decline. Early cohort studies that included nonsmokers concluded that chronic bronchitis is unrelated to the development of chronic airflow obstruction [25–27], but more recent data imply that repeated acute exacerbations of COPD are indeed associated with increased decrements in airflow [28, 29]. The number of respiratory infections influences the rate of decline in FEV1 in those who continued smoking in the Lung Health Study. Smokers suffered an additional 7 mL decline in FEV1 for every additional exacerbation [29]. The negative impact of exacerbations on lung function has also been demonstrated in a longitudinal study of patients with emphysema due to α_1-antitrypsin deficiency. Significant correlations between exacerbation frequency and rate of decline in FEV1 were noted [30]. Thus, the influence of infections on the progression of COPD may be more evident in patients with added risk factors for rapid progression of disease, such as active smokers or patients with α_1-antitrypsin deficiency.

The importance of acute exacerbations of COPD are also shown by their economic costs, estimated at $1.2 billion in patients aged >65 yrs and $419 million in patients aged <65 yrs. This burden is greatest in those requiring hospitalisation, who account for the bulk of costs of acute exacerbations [31, 32]. Emergency room visits for acute exacerbations alone predict significant impairment in health-related quality of life, whereas episodes severe enough to warrant hospitalisation result in marked impairment in the next 6 months [33]. Hospitalisations for acute exacerbations of COPD are associated with a high risk of readmission in both the USA and elsewhere [34–37]. Thus, acute exacerbations of COPD result in major symptomatic, physiological and economic burden.

Chronic persistence of bacteria in respiratory tissues

NTHI are present in up to three-quarters of healthy adults. Virtually all patients with chronic bronchitis are colonised with NTHI if serial cultures are performed. Colonisation with NTHI is a dynamic process; new strains are periodically acquired and replace old strains [38]. The adherence of NTHI to the human respiratory tract is a complex interaction of bacterial adhesins and host molecules. NTHI most likely initially encounters mucus in the respiratory tract. Mucus is a complex mixture of debris, cells and secreted molecules including mucins. Mucins bind NTHI and may influence bacterial adhesion to the epithelium. Binding of mucin is mediated by outer membrane proteins [39, 40]. An understanding of the molecular interactions of mucin with NTHI might allow development of strategies to prevent colonisation and infection.

Multiple adhesins expressed by NTHI are involved in adherence to the respiratory epithelium [41]. NTHI express pili that mediate adherence to mucosal cells [42]. Pili are hair-like projections with a distal tip adhesin. Nonpiliated strains of NTHI are also capable of adhering to human epithelial cells, suggesting the presence of nonpilus adhesins [43]. While numerous adhesins have been identified and the ability of the bacterium to modulate expression of adhesins has been defined, the conditions under which specific adhesins are expressed in patients are unclear.

NTHI's niche in the human respiratory tract is not limited to the surface of epithelial cells. A small percentage of adherent NTHI enter epithelial cells *via* an entry process that involves actin filaments and microtubules [44]. Bacteria may penetrate epithelial cells by paracytosis or passage between cells. Bacteria pass through confluent layers of epithelial cells without affecting the permeability or viability of the cells [45]. Intracellular NTHI are protected from the bactericidal activity of antibiotics and antibody-mediated bactericidal activity [46]. It is believed that NTHI preferentially, or perhaps exclusively, enter nonciliated cells within the population [47]. *In vivo* studies also confirm NTHI penetrate the mucosal surface. NTHI are present in macrophage-like cells in the adenoids of children [48], and NTHI are diffusely present in the epithelium, the submucosal of the bronchi, the bronchioles, the interstitium, and the alveolar epithelium in lung explants from patients undergoing lung transplantation [49]. Finally, NTHI may persist in the respiratory tract in biofilms. *H. influenzae* is known to form biofilms *in vitro* and *in vivo* [50]. Bacteria present in the airways in the form of biofilms may yield negative cultures because routine cultures are less sensitive in detecting bacteria in biofilms. *H. influenzae* is known to grow as a biofilm and planktonically under different conditions. NTHI may shift from planktonic growth to biofilms as a strategy of persistence.

Chronic bacterial persistence in the lower airways could perpetuate inflammation and contribute to progression of disease [7]. Cell-free supernatants of NTHI inhibit ciliary beat frequency [51] and induce ciliary dyskinesia and ciliostasis. NTHI stimulates mucin secretion [52] and causes airway epithelial injury *in vitro* [53]. Finally, the presence of bacteria in the lower airways induces the production of inflammatory mediators by the airway epithelium. Different species elicit different degrees of inflammatory reaction. The most intense inflammation is generated by *P. aeruginosa,* followed by *H. influenzae; M. catarrhalis* provokes significantly milder inflammatory response [11, 54]. NTHI and lipooligosaccharide from NTHI increase interlukin (IL)-6, tumour necrosis factor-α and IL-8 production [55].

It is increasingly apparent that chronic persistence of bacteria in the lower airways of patients with COPD is not innocuous. NTHI has the ability to disrupt ciliary motility, induce mucus hypersecretion and damage airway epithelium. NTHI persistence stimulates airway epithelial cells to produce proinflammatory cytokines, especially those that promote neutrophil chemotaxis. The relation of persistent airway inflammation to the symptoms of acute exacerbations and to progression of airway obstruction is a fertile area of investigation (fig. 1).

ASTHMA

The incidence of asthma has increased worldwide over the past decade. Factors that influence the development and severity of asthma include environmental exposures, genetic predisposition, environment/gene interactions, atopy, diet, socio-economic status and infection. Certain bacteria are contributors to some asthma exacerbations, and bacteria may play a complex role in the development of asthma [56].

Exacerbations of asthma

The precise incidence of bacterial infection in asthma exacerbations has not been determined. In most instances, studies have not demonstrated cause and effect relationships. Several pathophysiological mechanisms may account for bacterial involvement in asthma exacerbations. Sinusitis is an important trigger of asthma exacerbations. Sinusitis is extremely common in asthmatic patients, with a prevalence ranging from 53–75%. *S. pneumoniae* and *H. influenzae* account for 76% of positive cultures in sinusitis [57]. *M. catarrhalis* is more commonly isolated from the upper airway of asthmatics than from the upper airway of normal patients. *M. catarrhalis* was present in one-third of normal patients. Altogether, 70% of well asthmatics and 75% of asthmatics experiencing exacerbations had positive cultures for *M. catarrhalis* on pharyngeal swabs. No causal relationships were identified in this study [58]. A recent serologically based prospective study demonstrated a link between atypical infectious organisms and asthma exacerbations [59]. One hundred patients hospitalised with exacerbations of asthma were compared with hospitalised surgical patients with no history of lung disease or history of a upper respiratory tract infection in the month before admission. *M. pneumoniae* was identified more than any other pathogen in the asthmatic group (18 *M. pneumoniae*, 11 influenza A, eight *C. pneumoniae*, six adenovirus, five influenza B, five Legionella species and three *S. pneumoniae*) and in the control group (three *M. pneumoniae*). Interestingly, *M. pneumoniae* was the sole infectious agent in only eight of the 18 patients in which *M. pneumoniae* was identified, illustrating the difficulty in ascertaining a cause–effect relationship of *M. pneumoniae* as the cause of the hospitalisation.

Several studies have evaluated the role of *M. pneumoniae* and *C. pneumoniae* in acute wheezing in children [60, 61]. *M. pneumoniae* was detected in 20% and *C. pneumoniae* in 5–15%
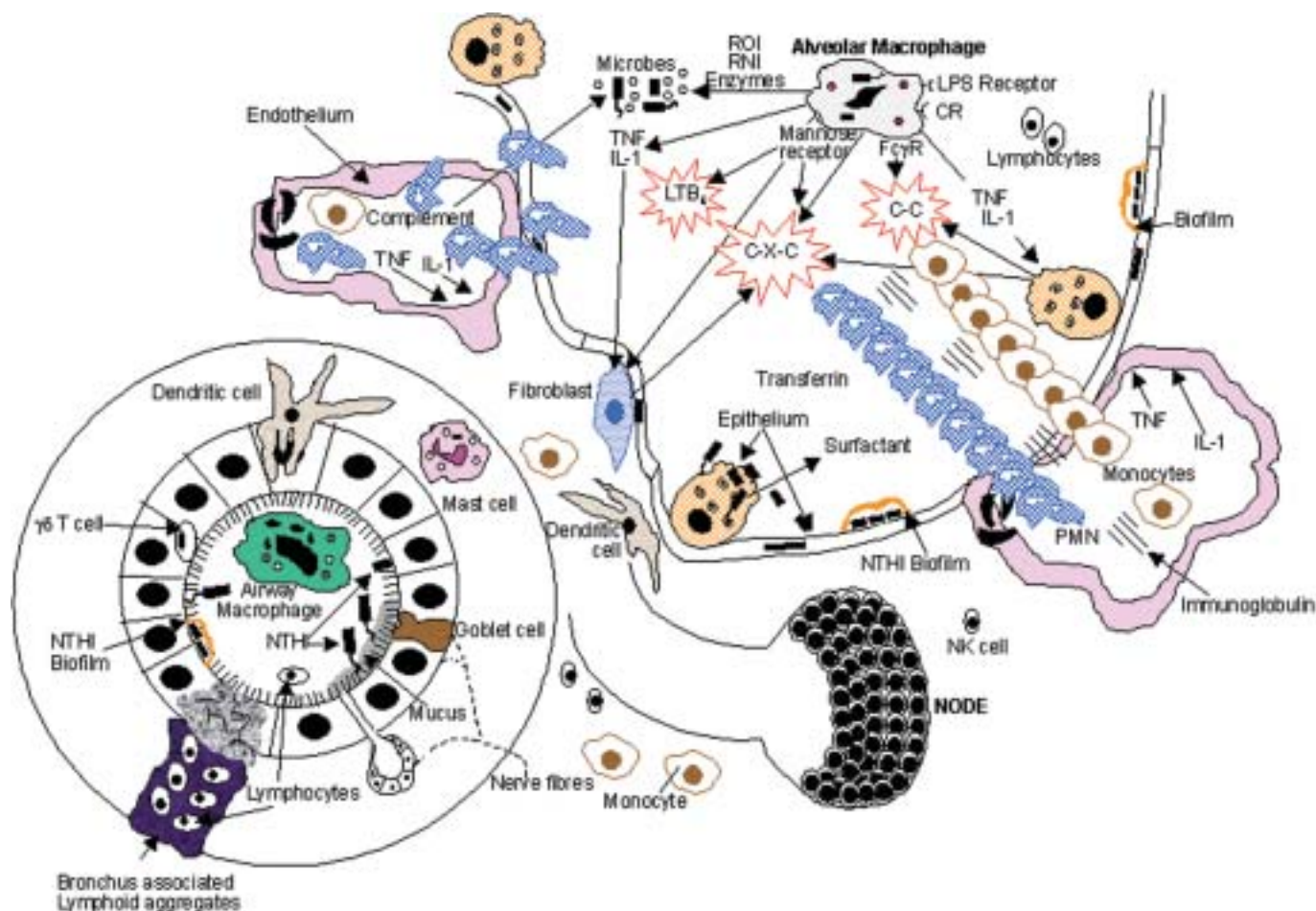
FIGURE 1. Host defences of conducting airways and alveoli. Conducting airways are lined by ciliated epithelium, which moves mucus generated by bronchiolar glands and goblet cells cephalad. Nontypable *Haemophilus influenzae* binds to mucus *via* pili and to respiratory epithelia *via* pili and nonpilus adhesins. Bacteria also penetrate epithelial cells. Bacteria may persist in the respiratory tract in biofilms. Airway macrophages ingest and kill small inocula of most inspired and airborne bacteria. Ingestion of microbes stimulates alveolar macrophages to produce tumour necrosis factor-α (TNF-α), C-C and C-X-C chemokines and leukotriene B4 (LTB$_4$), which mediate inflammatory responses in the airway and lower respiratory tract. IL-1: interleukin 1; LPS: lipopolysaccharide; NK: natural killer; PMN: polymorphonucleates. Modified with permission from: Toews GB. Pulmonary Clearance of Infectious Agents. *In*: Fishman's Pulmonary Diseases and Disorders, 3rd edn, vol 2. Eds: Fishman AF, Elias JA, Fishman JA, *et al.* McGraw Hill, New York, 1997, p. 1893.

of children with wheezing compared with 7.5% and 2.5%, respectively in healthy control children. Acute *M. pneumoniae* infection was confirmed in 50% and *C. pneumoniae* infection in 8.3% of patients experiencing their first wheezing episode. Further studies are needed to confirm the association between bacterial infection and asthma exacerbation and to determine the prevalence of such infection in patients with acute exacerbations of asthma.

Role of bacteria in chronic asthma

M. pneumoniae and *C. pneumoniae* may be associated with asthma chronicity [62]. The role of *M. pneumoniae* and *C. pneumoniae* infections with chronic asthma has been evaluated using PCR, culture and serology to detect *M. pneumoniae*, *C. pneumoniae* and viruses. Altogether, 56% of asthmatic patients had a positive PCR for *M. pneumoniae* (n=25) or *C. pneumoniae* (n=7). Positive results for PCR were found in broncholaveolar lavage (BAL) fluid or biopsy samples. Only one of 11 control

subjects had a positive PCR for *M. pneumoniae*. Cultures for these organisms were negative in all patients. A significantly greater number of tissue mast cells were noted in the group of patients who were PCR positive.

Asthmatic patients infected with *M. pneumoniae* or *C. pneumoniae* may benefit from prolonged treatment with clarithromycin [63]. Treatment with clarithromycin resulted in significant improvement in FEV1, and FVC; the greatest improvement was noted when PCR-positive patients received clarithromycin. While the studies to evaluate the effect of clarithromycin in patients with asthma are randomised, double-blind and placebo controlled, the number of patients enrolled in these studies is small. Further study is needed to assess if the presence of *M. pneumoniae* and *C. pneumoniae* is an epiphenomenon or related to the pathogenesis of asthma. The effects of clarithromycin could be secondary to its antimicrobial effects, its anti-inflammatory effects or to a combination of these two therapeutic effects.

Animal models of *Mycoplasma pneumoniae* infection

Murine models of acute and chronic *M. pneumoniae* respiratory tract infection show many similarities to human disease and allow a complementary investigation of the complex interactions of Mycoplasma infection and the host [64–67]. *M. pneumoniae* infection in chronic mouse models can be detected up to 1.5 yrs after resolution of the pneumonia [64]. Murine lungs exhibit inflammatory changes, alteration in lung histology, antibody production, cytokine release into the BAL fluid and persistent airflow resistance [64]. Bronchial hyperresponsiveness and airway neutrophilia have also been demonstrated during chronic *M. pneumoniae* infection [66]. Chronic airway infection models have also been utilised to characterise the mechanisms underlying angiogenesis, vascular remodelling and airway wall thickening [67]. Such remodelling can be induced in concert with inflammatory responses to Mycoplasma infection. Vascular leak is promoted by vascular endothelial growth factor and is opposed by angiopoietin 1. Both molecules are stimulated by *M. pneumoniae* infection. The role of *M. pneumoniae* infection in the airway remodelling noted in chronic asthmatics deserves further detailed study.

AIRWAYS DISEASE AS A RISK FACTOR FOR INVASIVE PNEUMOCOCCAL DISEASE

Streptococcus pneumoniae is a cause of substantial morbidity and mortality in the USA, particularly among patients with high risk medical illness [68]. Pneumococcal vaccination has been shown to prevent invasive disease from this ubiquitous pathogen, particularly in high risk groups [68, 69]. While chronic obstructive pulmonary diseases (emphysema and chronic bronchitis) have long been included as an at-risk group for whom pneumococcal vaccination is recommended, guidelines for pneumococcal vaccinations specifically excluded subjects with asthma and the guidelines for the management of asthma do not include pneumococcal vaccination as a strategy to prevent infectious complications. A recent nested, case-controlled study documented that asthma is an independent risk factor for invasive pneumococcal disease [70]. Subjects with asthma who had a history of one or more hospitalisations or visits to an emergency department for asthma, or who were given a prescription for a course of corticosteroids as rescue therapy, or a long-term course of oral corticosteroids (120 days or more) or prescriptions for three or more β-agonists medications during the year before the index date, were classified as having high-risk asthma. All other subjects with asthma were classified as having low-risk asthma. The annual incidence of invasive pneumococcal disease was 4.2 episodes per 10,000 subjects with high-risk asthma, and 2.3 episodes per 10,000 subjects with low-risk asthma. Subjects aged ≥65 yrs experience 8.3 episodes per 10,000 subjects as a comparison [71]. This recent study provides strong evidence for the addition of asthma to the list of conditions that increase the risk of invasive pneumococcal disease. The feasibility and cost effectiveness of a pneumococcal vaccination among persons with asthma deserves careful, immediate study.

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