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The role of acute and chronic respiratory colonization and infections in the pathogenesis of COPD

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ABSTRACT

COPD is a major global concern, increasingly so in the context of ageing populations. The role of infections in disease pathogenesis and progression is known to be important, yet the mechanisms involved remain to be fully elucidated. While COPD pathogens such as *Haemophilus influenzae*, *Moraxella catarrhalis* and *Streptococcus pneumoniae* are strongly associated with acute exacerbations of COPD (AECOPD), the clinical relevance of these pathogens in stable COPD patients remains unclear. Immune responses in stable and colonized COPD patients are comparable to those detected in AECOPD, supporting a role for chronic colonization in COPD pathogenesis through perpetuation of deleterious immune responses. Advances in molecular diagnostics and metagenomics now allow the assessment of microbe–COPD interactions with unprecedented personalization and precision, revealing changes in microbiota associated with the COPD disease state. As microbial changes associated with AECOPD, disease severity and therapeutic intervention become apparent, a renewed focus has been placed on the microbiology of COPD and the characterization of the lung microbiome in both its acute and chronic states. Characterization of bacterial, viral and fungal microbiota as part of the lung microbiome has the potential to reveal previously unrecognized prognostic markers of COPD that predict disease outcome or infection susceptibility. Addressing such knowledge gaps will ultimately lead to a more complete understanding of the microbe–host interplay in COPD. This will permit clearer distinctions between acute and chronic infections and more granular patient stratification that will enable better management of these features and of COPD.

Key words: acute exacerbations of chronic obstructive pulmonary disease, chronic obstructive pulmonary disease, colonization, infection, microbiome.

Abbreviations: AECOPD, acute exacerbation of COPD; AFB, acid-fast bacilli; BAL, bronchoalveolar lavage; BODE, Body-mass index, airflow Obstruction, Dyspnea, and Exercise; BOLD, Burden of Obstructive Lung Disease Study; CD, cluster of differentiation; cfu, colony forming units; COPD, chronic obstructive pulmonary disease; CXCL, chemokine (C-X-C motif) ligand; CXCR, CXC chemokine receptor; E1A, adenovirus early region 1A; FEV_1, forced expiratory volume in 1 s; FVC, forced vital capacity; GOLD, Global Initiative for Chronic Obstructive Lung Disease; HIV, human immunodeficiency virus; ICAM-1, intercellular adhesion molecule 1; ICD, International Statistical Classification of Diseases and Related Health Problems; ICS, inhaled corticosteroid; IFN, interferon; Ig, immunoglobulin; IL, interleukin; IPA, invasive pulmonary aspergillosis; LABA, long-acting β2-agonist; LLN, lower limit of normal; LTB4, leukotriene B4; MARCO, macrophage receptor with collagenous structure; MDR, multidrug resistance; MMP, matrix metalloproteinase; MPO, myeloperoxidase; NE, neutrophil elastase; NF-κB, nuclear factor kappa-B; NTHi, non-typeable *Haemophilus influenzae*; NTM, non-tuberculous mycobacteria; PAMP, pathogen-associated molecular pattern; PCR, polymerase chain reaction; PPM, potentially pathogenic microorganism; PRR, pattern recognition receptor; ROS, reactive oxygen species; RSV, respiratory syncytial virus; RV, residual volume; TB, tuberculosis; Th, T-helper; TLR, Toll-like receptor; TNF-α, tumour necrosis factor alpha; YKL-40, Chitinase-3-like protein 1.

INTRODUCTION

The role of microbes and infection in obstructive lung disease was first proposed in the 1950s by the British

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hypothesis. This states that recurrent infection and mucus hypersecretion were the primary causes of progressive airways obstruction in smokers. However, subsequent studies failed to detect significant associations between infection and lung function decline and consequently exposure to noxious particles and gases was advanced as the principal cause of COPD.[1] A wealth of evidence shows that the isolation of bacteria, viruses and fungi in the airways of stable COPD patients is in fact significant and has implications for disease pathogenesis, progression and treatment. This places a renewed emphasis on microorganisms isolated from the COPD lung, driving us to revisit the original British hypothesis.[2] Advances in diagnostic technologies now permit a more accurate detection of specific pathogens in COPD while molecular studies shed light on the immune response elicited by COPD-associated microorganisms. Metagenomic studies have revealed microbial consortia in both healthy and diseased individuals, helping to define pathogenic and beneficial microbes associated with various disease states. This has major implications for our understanding of COPD and its therapy, particularly in the face of antibiotic-mediated lung dysbiosis during therapy. Therefore, both acute and chronic infections are linked to COPD progression and exist as components of overarching lung microbiome architecture, which is modulated during therapeutic intervention. Characterizing this dynamic interaction between microbes, therapy and disease states is now a central focus in COPD research. Furthermore, the emergence of antibiotic resistance in COPD-associated pathogens is also a growing international concern and greater emphasis on antimicrobial stewardship and rational therapies is of increasing importance. Ultimately, the role of microbes and infection in COPD may reveal novel prognostic markers of disease which in turn provide scope for more focused interventions and novel therapeutic approaches.

ACUTE INFECTION IN COPD: VIRAL AND BACTERIAL SUPERINFECTION AND THEIR ROLE IN COPD EXACERBATION

Periodic worsening of respiratory symptoms in patients with COPD is known as acute exacerbations (AECOPD). They can have marked effects on lung function, quality of life and health socio-economic burden. The exact nature and cause of these events can often be difficult to establish clinically, but in general infections are largely blamed, with up to 80% of AECOPD linked to either bacterial or viral pathogens.[3,4] Because a certain subset of COPD patients have frequent exacerbations, the concept of inherent susceptibility to acute infection in COPD subsequently triggering AECOPD events has been developed. Several studies have now demonstrated that COPD patients feature impaired innate immunity, the first line of defence against infection. Alveolar macrophages resident in the airways provide the key initial response to bacteria by both recognizing and removing harmful pathogens through phagocytosis, but in COPD, these functions may be impaired. Recently, Berenson *et al*. investigated Toll-like receptor (TLR) expression and responses in alveolar macrophages collected from exacerbation-prone and exacerbation-free COPD patients.[5] In response to exposure to common COPD-related pathogens including *Moraxella catarrhalis*, *Streptococcus pneumoniae* and *Haemophilus influenzae*, alveolar macrophages from exacerbation-prone patients had diminished TLR2 expression and cytokine responses. Similarly, alveolar macrophages from COPD patients appear to phagocytose *H. influenzae* and *S. pneumonia* poorly compared with those of control patients yet they still release reactive oxygen species (ROS).[6,7] Together, these studies suggest that COPD patients have impaired responses to bacterial colonization and infection which in turn increases susceptibility to AECOPD and promotes an oxidative stress response (Fig. 1).

Defects in innate immunity may also play a role in increased susceptibility to viruses. Recognition of viral infection by the innate immune system is essential for coordinating an effective antiviral response in the airways, yet in patients and mice with COPD, the cascade from recognition to response falters. This involves increases in phosphatidylinositol 3-kinase responses and impaired antiviral stress granule formation including type I interferon (IFN) signalling.[8] In the healthy host, pathogen recognition by TLR3 triggers IFN pathways that then can hinder viral replication and mediate adaptive immune responses. In epithelial cells, constitutive type 1 IFNs are critical for protection.[9,10] In these cells and lung fibroblasts exposed to cigarette smoke, responses are reduced and even stimulation with IFN-β yields a diminished antiviral response.[11] Furthermore, mucociliary clearance, which is key for the removal of virus from the airways, appears to be perturbed in COPD. Cigarette smoke exposure reduces both the number and length of cilia,[12] while goblet cell hyperplasia in COPD leads to more viscous mucus in the airways, further impeding proper ciliary motion.[13] In mouse models, respiratory viruses themselves can induce mucus hypersecretion, contributing to the inability to clear the virus from the airway.[14]

Of the 80% of exacerbations with an infectious cause, approximately half are bacterial in origin while approximately 30% are viral.[4,15] Numerous studies have been performed to classify the most common pathogens implicated in AECOPD using sputum samples to identify bacteria and nasopharyngeal swabs to identify viruses. In a landmark 2002 study, Sethi *et al*. traced longitudinal sputum cultures in 81 moderate-to-severe COPD patients during both times of clinical stability and exacerbation.[16] Interestingly, the acquisition of a new strain of bacteria (discovered through molecular typing) was highly associated with the development of an AECOPD. The relative risk of an exacerbation varied with each new species: 1.69 for *H. influenzae*, 1.77 for *S. pneumoniae* and 2.96 for *M. catarrhalis*. Since then, more sensitive quantitative PCR methods of identifying bacteria relative to standard culture methods have enabled better quantification and typing of bacterial strains during exacerbations. In one study, while species such as *H. influenzae* and *S. pneumonia* were identified in stable states, their relative loads, determined by PCR, were considerably higher during exacerbations (10^8 cfu/mL vs 10^7 cfu/mL for both species).[17] The most prevalent species identified during exacerbation

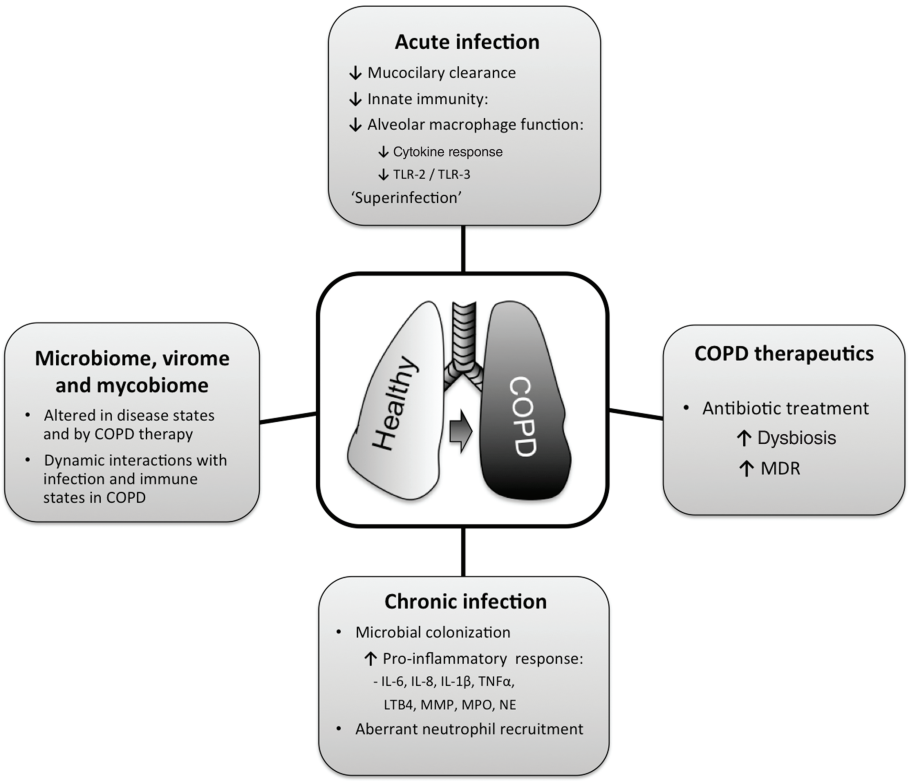

Figure 1 Microbial factors affecting COPD disease pathogenesis and progression. Pathogenic microbes associated with acute and chronic COPD infection influence disease progression. Increasingly, the role of the microbiome and its associated virome and mycobiome is recognized. As microbiome architecture is profoundly altered by COPD therapy, dynamic interaction between microbiology, infection and therapy likely occurs during COPD disease progression. LTB4, leukotriene B4; MDR, multidrug resistance; MMP, matrix metalloproteinase; MPO, myeloperoxidase; NE, neutrophil elastase; TLR, Toll-like receptor.

was *S. pneumoniae*, followed by *H. influenzae*, *M. catarrhalis* and *Legionella pneumophila*. In more severe COPD patients (forced expiratory volume in 1 s (FEV_1) < 50% predicted), *Pseudomonas aeruginosa* was also frequently encountered as a cause of exacerbations (Table 1).[24] *Pseudomonas aeruginosa* infections appear to portend a worse prognosis with higher 30- and 90-day mortality rates compared with exacerbations not associated with this bacterium.[25]

Viruses implicated in the development of AECOPD include respiratory syncytial virus (RSV), rhinovirus, human metapneumovirus, influenza, parainfluenza, adenovirus and coronavirus.[33–35] A meta-analysis of 19 studies (totalling 1728 patients) evaluated the pooled prevalence of respiratory viruses in AECOPD and found that rhinoviruses/enteroviruses were the most commonly encountered virus (16%), followed by RSV (10%) and influenza (8%). Of lesser prevalence were coronaviruses (4%), parainfluenza (3%), human metapneumovirus (3%) and adenovirus (2%).[28] Co-infection with two or more viruses has also been reported,[36] with one study noting that in their cohort,

Table 1 Summary table of COPD disease state and associated microorganisms

	Prevalence (%)			
COPD-associated organisms	AECOPD	Stable COPD	Sample size (*n*)	Reference
Bacteria				
Haemophilus influenzae	26	17–35	410†	17–21
Streptococcus pneumoniae	25	7.5–17	442†	17,22,23
Moraxella catarrhalis	19	2–22	247†	17,22,23
Pseudomonas aeruginosa	13–29	—	332†	24,25
Chlamydophila pneumoniae	24	43	141	26
Non-tuberculous mycobacteria	22	—	73	27
Viruses				
Rhinovirus/enterovirus	16	—	1728	28
Respiratory syncytial virus	10	—	1728	28
Influenza virus	8	—	1728	28
Adenovirus	2	—	1728	28
Fungi				
Aspergillus spp.	—	14	141	29
Pneumocystis jirovecii	—	8–55	137	30–32

†Cumulative sample size is reported for multiple studies; — means not reported.
AECOPD, acute exacerbations of COPD.

only the patients with viral co-infections were severe enough to be admitted to the hospital for their exacerbations.[34]

While simultaneous bacterial and viral pathogens have been detected during acute exacerbations, it remains unclear whether these co-infections carry any greater risk for poor outcomes in comparison to single infections.[37] They indeed may simply represent more severe disease as other studies have detected.[38,39] More compelling is the notion of 'superinfection', where an acute viral infection in COPD may set up the necessary microenvironment in the lung for a subsequent bacterial infection or vice versa. In an experimental rhinovirus infection, where COPD and control patients were nasally inoculated with low doses of rhinovirus, only COPD patients developed significant increases in sputum bacterial load post-viral infection.[40] While sputum viral loads peaked between days 5 and 9 post-inoculation, bacterial loads peaked later around day 15, confirming that in these studies viral infection can promote bacterial superinfection. Subjects who ultimately developed secondary bacterial infections had lower baseline FEV_1 compared with those who remained free from secondary infections. A plausible mechanism was suggested whereby rhinovirus infection induces neutrophil elastase (NE) production, which then degrades the antimicrobial peptides elafin and secretory leukoprotease inhibitor, both of which may be important in protecting subjects from bacterial infections.[40] These data suggest that AECOPD events thought to be bacterially induced may in fact have been preceded by an initial viral infection. Moreover, greater vigilance for superinfections in patients with more severe lung function may be warranted.

CHRONIC INFECTION IN COPD

Role in COPD pathogenesis

Microorganisms are commonly detected in the airways in stable COPD and are considered 'colonizers' in the absence of acute infective symptoms (Table 1). However, the term colonization in this context may be debated, as the microorganisms identified in stable COPD are not necessarily benign. Collective evidence favours the vicious cycle hypothesis whereby an inciting event (e.g. smoking) leads to an impaired innate immune response and results in bacterial colonization.[41] This in turn promotes airway and systemic inflammation leading to COPD progression. As airway inflammation persists despite smoking cessation, the presence of a persistent stimulus—independent of cigarette smoke—is consistent with a role for colonizing bacteria in the pathogenesis of COPD.[42,43]

Recognition of pathogen-associated molecular patterns (PAMPs), by pattern recognition receptors (PRRs) expressed on epithelial and innate immune cells, activates signal transduction pathways including nuclear factor kappa-B (NF-κB), p38 mitogen-activated protein kinases, phosphoinositide-3-kinase and IFN regulatory factors.[44] This results in the production of pro-inflammatory mediators such as cytokines and chemokines. In turn, the recruitment of neutrophils to the airways ensues, a feature characteristic of bacterially colonized COPD patients.[45] Neutrophils are anti-infective and have recently been found to have modulatory functions.[46] In COPD, neutrophils are intrinsically altered with abnormal host defence functions and have unusual chemotactic behaviour and migratory structure.[47] Activated neutrophils cause lung destruction and emphysematous change through the release of oxygen radicals and proteolytic enzymes, including NE that reduces ciliary function, induces epithelial damage and is a potent stimulator of mucin production.[47] The resulting mucus production and impaired mucociliary clearance leads to further bacterial colonization and airway obstruction. Additionally, increased levels of airway inflammatory markers such as IL-6, IL-8, IL-1β, TNF-α, leukotriene B4 (LTB4), matrix metalloproteinase (MMP), myeloperoxidase (MPO) and NE are found in stable COPD patients with bacterial colonization compared with those without bacterial colonization, strongly implicating the colonized state with a deleterious immune response.[45,47] Such persistent inflammation is associated with increased daily symptoms, exacerbations and mortality.[48,49] Defective phagocytosis is also characteristic of COPD particularly that of alveolar- and monocyte-derived macrophages, which in turn likely contributes to chronic bacterial colonization and infective exacerbations.[6] This is clearly evidenced by the observed impaired phagocytosis of *H. influenzae* and *M. catarrhalis* with increasing COPD severity.[50] In addition, alveolar macrophage surface receptors (TLR2, TLR4 and scavenger receptor (MARCO)) that respond to intracellular signalling and phagocytosis are seen to be diminished in both COPD and smokers.[51–53] Chronic bacterial colonization also activates adaptive immune responses, with the development of B-cell lymphoid follicles and production of mucosal IgA and serum IgG antibody including strain-specific IgG antibody against bacterial colonizers, a phenomenon characteristic of the COPD airway.[54] Increases in lymphoid follicles have been noted in the small airways of patients with severe COPD, possibly secondary to chronic airway infection.[55] Thus, impaired innate immune responses likely contribute to microbial airway colonization while the activation of adaptive immunity suggests a state of heightened immune surveillance associated with lung function decline in established COPD.

Bacterial infection and colonization

Potentially pathogenic microorganisms (PPMs) have been isolated in up to 74% of stable COPD patients.[56] The commonest PPMs identified include *H. influenzae*, *M. catarrhalis*, *S. pneumonia*, *P. aeruginosa* and *Chlamydophila pneumoniae* similar to the culprit repertoire in AECOPD and severe asthma.[57] Bacterial colonization is, however, a dynamic process with alterations in pathogen type, load and strain over time. Increases in bacterial load and changes in species are associated with greater lung function decline and airway inflammation.[18,19]

Non-typeable *H. influenzae* (NTHi) is the most common bacteria isolated in both the stable state and during AECOPD. It alone accounts for up to half of all bacteria isolated in the lower airways of stable COPD patients.[17,18,20] The differing cell surface proteins

facilitate bacterial adhesion to the respiratory mucosa including outer membrane proteins P2 and P5 that bind to mucin, and adhesin (HMW1 and HMW2) which adhere to the epithelial and extracellular matrix.[58–60] Lipooligosaccharide, a key component of the NTHi cell wall, and the outer membrane protein P6 are potent immunomodulators for macrophages leading to increased phagocytosis and cytokine secretion, including IL-8 and TNF-α which drive neutrophil recruitment.[60–63] Given the defective phagocytic ability of the COPD alveolar macrophage, a failure of bacterial clearance potentially leads to the state of persistent infection.[6,63] Furthermore, NTHi is uniquely able to survive intracellularly within the alveolar macrophages.[64] Through its secreted proteases, it cleaves IgA and in turn protects against IgA-mediated cytotoxicity.[65] Where persistent, COPD NTHi infection of the lower airway results in increased inflammation with elevated levels of IL-8, IL-1β, MPO, TNF-α and MMP-9 detectable in patient samples.[19,66] Critically, the organisms' immunoevasive ability causes chronic airway inflammation which in turn correlates with poorer health status and lung function.[67,68]

Moraxella catarrhalis, the second most prevalent colonizing organism in stable COPD (seen in 2–22.5%), has been shown in previous studies to be linked to a heightened airway inflammation (IL-8, TNF-α and NE).[18,22,69] In contrast to NTHi,[70] this bacterium is cleared after a relatively short colonization period and the development of immune responses that are protective against the homologous strain.[54]

Streptococcus pneumoniae is another important bacterial organism (7.5–17%) in stable COPD.[22] Its polysaccharide capsule reduces the effectiveness of host defence mechanisms, which normally cause its clearance, leading to its airway persistence and colonization. In turn, this is associated with increased exacerbations, emphysema, lower diffusion capacity and pneumonia.[71]

The role of *P. aeruginosa* in COPD pathogenesis is underscored by its frequent isolation in patients with more severe disease.[67,72] Two types of *Pseudomonas*-related infection are described in the COPD setting: short-term colonization followed by clearance and long-term persistent colonization.[73] Risk factors for *Pseudomonas* isolation in COPD include bronchiectasis, antibiotic exposure, previous hospitalization, steroid use and worse BODE index.[72] The mucoid phenotype is most likely to cause persistent colonization and, amongst *P. aeruginosa*-positive COPD patients, there is a significant association between colonization and disease severity.[73] Despite this, a nested case–control study comparing mortality showed no difference in relation to *Pseudomonas* status in stable COPD; however, in AECOPD, recent work has reaffirmed this organism's association with higher COPD mortality rates.[25,74]

Finally, chronic *C. pneumoniae* infection in COPD has been brought to light by a number of observational studies. Through its induction of a cytokine response (TNF-α, IL-1β, IL-6 and IFN-α), airway inflammation and critical airway remodelling ensue. Prevalence varies widely (0–65%) likely attributable to the lack of samples collected, standardization in applied microbiological methodology and lack of diagnostic testing in routine use.[26,75] Despite this, serum *C. pneumoniae*-specific IgG and IgA antibody levels are found in higher titres in COPD when compared with a control group. Furthermore, a statistically significant association between chronic *C. pneumoniae* infection and COPD has been established.[76] Employing combinations of both PCR and serological testing reveal that chronic *C. pneumoniae* infection is associated with more severe COPD, faster lung function declines and, importantly, exacerbations.[26] These relationships however are not clear cut as a separate study of 110 COPD patients showed no association between *Chlamydia* and either COPD exacerbation frequency or FEV_1.[77] In this latter study, IgG titres were used to define chronic *C. pneumoniae* infection which may explain the observed disparity. Furthermore, it is likely that *Chlamydia* resides in the lower respiratory tract and sampling needs to occur from this site. Standardization and validation of reproducible and robust diagnostic tests to clearly define *C. pneumoniae* infection in COPD are necessary and will likely clarify existing differences observed in our current evidence base.

Tuberculosis and non-tuberculous mycobacteria

Mycobacterial diseases, both non-tuberculous mycobacteria (NTM) and tuberculosis (TB), have long been associated with COPD, whether thought to be related to an inability to clear these chronic pathogens in the setting of structurally damaged lung or potentially the concurrent burden of bronchiectasis increasingly appreciated in COPD.[78] Studies of NTM in COPD cohorts have revealed that roughly one-fifth of COPD patients culture NTM in their sputum.[27,79] Moreover, in 126 COPD patients undergoing lung volume reduction surgery, histological evidence of mycobacterial disease (necrotizing granulomas) occurred in 14 (11%) patients, 8 of whom had acid-fast bacilli (AFB)-positive stains.[80] Conversely, COPD appears to be a major co-morbidity in patients with NTM, with estimates of prevalence ranging from 24% to 79%.[81,82] Not merely an innocent bystander, NTM infections in COPD portend a worse prognosis with more frequent exacerbations and a more rapid decline in FEV_1.[79] However, long-term, prospective studies on the impact of NTM on COPD mortality and antimycobacterial treatment outcomes in COPD have yet to be performed.

The relationship between TB and COPD dates back to 1955 when Anno and Tomashefski first described airflow obstruction and air trapping in patients with TB.[83] Since then, the link between TB and COPD has largely focused on COPD as a sequela of TB infection, a risk that consistently appears to be independent of smoking histories. The proportion of COPD patients found to harbour a history of TB are listed in Table 2, while worldwide estimates of the prevalence of airflow obstruction in cohorts of TB patients are listed in Table 3. Patients with a history of TB carry anywhere from a 1.8 (worldwide estimate) to 8.9 (South Africa) times overall risk of developing COPD.[89,93,97,100,113] In particular, TB remains an important risk factor for COPD in non-smokers, with TB patients carrying an

Table 2 TB as a risk factor for COPD

Author	Year	Country	*n*	Definition of COPD	Proportion of COPD patients with history of TB	Adjusted OR (95% CI) for COPD
Chan-Yeung *et al.*[84]	2007	Hong Kong	289 COPD 289 Controls	FEV_1/FVC <70%	24 (8.3%)	1.52 (0.45–5.19)
Menezes *et al.*[85]	2007	Brazil Chile Mexico Uruguay Venezuela	5571	FEV_1/FVC <70%	Not provided	2.57 (1.69–3.93)
Caballero *et al.*[86]	2008	Colombia	494 COPD 5045 Controls	FEV_1/FVC <70%	62 (12.6%)	2.94 (1.58–5.49)
Lam *et al.*[87]	2010	China	8066	FEV_1/FVC < LLN	167 (32%)†	1.37 (1.13–1.67)†
Lamprecht *et al.*[88]	2011	BOLD (14 countries)	4291 Never smokers	FEV_1/FVC <70%	28 (5.4%)	Females: 1.29 (0.52–3.23) Males: 3.09 (0.60–15.95)
Idolor *et al.*[89]	2011	Philippines	722	FEV_1/FVC <70%	15 (10.6%)	6.31 (2.67–15.0)
Danielsson *et al.*[90]	2012	Sweden	86 COPD 462 Controls	FEV_1/FVC <70%	4 (4.7%)	5.99 (0.82–44)
Govender *et al.*[91]	2011	South Africa	110 COPD 102 Controls	Pulmonologist diagnosis	17 (15%)	7.7–8.1
Lee *et al.*[92]	2011	South Korea	3687	FEV_1/FVC <70% or FEV_1/FVC < LLN	FEV_1/FVC <70%: 82 (27.9%) FEV_1/FVC < LLN: 89 (30.3%)	FEV_1/FVC <70%: 2.56 (1.84–3.56) FEV_1/FVC < LLN: 2.64 (1.97–3.52)
Hooper *et al.*[93]	2012	BOLD (14 countries)	4733	FEV_1/FVC < LLN	Not provided	1.72 (1.19–2.48)
Perez-Padilla *et al.*[94]	2012	Chile Venezuela Brazil Uruguay Mexico	2278 Never smokers	FEV_1 < FVC <70%	Not provided	5.82 (2.22–15.28)
Hwang *et al.*[95]	2014	South Korea	1384	FEV_1 < FVC <70%	44 (29.5%)†	3.12 (2.01–4.67)†
Smith *et al.*[96]	2014	China	317 399 Never smokers	FEV_1 < FVC <70%	Not provided	Females: 2.36 (2.06–2.71) Males: 1.81 (1.40–2.34)
Amaral *et al.*[97]	2015	BOLD (14 countries)	18 644	FEV_1/FVC < LLN	Not provided	2.51 (1.83–3.42)
Chan *et al.*[98]	2015	Taiwan	96 COPD 104 Non-COPD	ICD-9 or ICD-10 code	7 (7.3%)	Not provided
Jaganath *et al.*[99]	2015	Peru	2957	FEV_1 < FVC <70%	Not provided	Females: 7.02 (3.63–13.59) Males: 3.12 (2.02–4.83)
Jo *et al.*[100]	2015	South Korea	9488	FEV_1 < FVC <70%	94 (12.6%)	2.55 (1.86–3.50)
Lee *et al.*[101]	2015	South Korea	3473 Never smokers	FEV_1 < FVC <70%	Not provided	4.5 (2.3–8.7)
Lee *et al.*[102]	2016	South Korea	15 063	FEV_1 < FVC <70%	Non-smokers 81 (20.3%)‡ Smokers 139 (16.9%)‡	Non-smokers: 4.73 (3.63–6.17)

†TB defined as radiographic evidence of prior, inactive TB.

‡GOLD II–IV patients only.

BOLD, Burden of Obstructive Lung Disease Study; CI, confidence interval; FEV_1, forced expiratory volume in 1 s; FVC, forced vital capacity; GOLD, Global Initiative for Chronic Obstructive Lung Disease; ICD, International Statistical Classification of Diseases and Related Health Problems; LLN, lower limit of normal; OR, odds ratio; TB, tuberculosis.

estimated 1.3–5.8 times the risk of developing COPD compared with those without TB.[94,96,101,102] Likewise, COPD patients are at higher risk of developing TB. In Pakistan, for instance, 7.5% of COPD patients were found to have TB-positive sputum cultures.[114] Even in non-endemic regions, for example in Sweden, COPD patients carry a three times higher likelihood of developing active TB, conferring a twofold higher risk of death compared with COPD patients without TB.[115]

Why TB may predispose individuals to COPD, and vice versa, is a matter of some speculation, as outlined in Figure 2. Both conditions share a number of common risk factors, including cigarette smoking and biomass fuel exposure. In addition, the spread of HIV has

Table 3 COPD rates among patients with a history of TB

Author	Year	Country	*n*	Definition of COPD	Prevalence of COPD in patients with history of TB
Snider *et al.*[103]	1971	United States	1403	FEV_1/FVC <70%	589 (42%)
Willcox *et al.*[104]	1989	South Africa	71	FEV_1/FVC <70% and/or RV > 120%	48 (68%)
Plit *et al.*[105]	1998	South Africa	74	FEV_1/FVC <70%	21 (28%)
Ramos *et al.*[106]	2006	Brazil	50	Not provided	12 (24%)
Pasipanodya *et al.*[107]	2007	United States	107	FEV_1/FVC <70%	16 (15%)
Girdler-Brown *et al.*[108]	2008	Lesotho	184	FEV_1/FVC <70%	37 (20.1%)
Baig *et al.*[109]	2010	Pakistan	92	FEV_1/FVC <70%	26 (55.3%)
Rhee *et al.*[110]	2013	South Korea	595	FEV_1/FVC <70%	457 (76.8%)
de la Mora *et al.*[111]	2015	Mexico	70	FEV_1/FVC <70%	24 (34.3%)
Manji *et al.*[112]	2016	Tanzania	501	FEV_1/FVC <70%	210 (42%)

FEV_1, forced expiratory volume in 1 s; FVC, forced vital capacity; RV, residual volume; TB, tuberculosis.

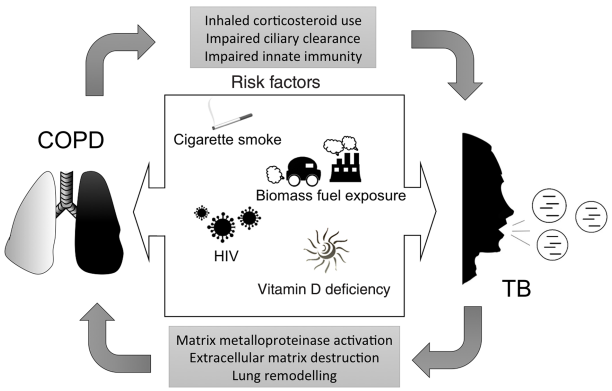

Figure 2 A proposed schematic representation of the interactions between COPD and tuberculosis (TB). While both conditions share a number of risk factors, TB may contribute to the development of COPD through matrix metalloproteinase-mediated lung remodelling. COPD may also contribute to TB infection by impairing immune responses, ciliary function and through exposure to inhaled corticosteroids.

triggered not only a resurgence in TB rates, but also an accelerated form of COPD affecting HIV patients as early as in their 30s and 40s. Recent interest in the role of vitamin D in airway diseases and pulmonary infections has suggested that certain vitamin D-binding protein polymorphisms may also predispose patients to both COPD and TB. Once contracted, TB may set the stage for future COPD by activating MMPs. Destruction of the extracellular matrix and caseous necrosis is critical to the formation of cavitary lesions by which TB can then propagate and aerosolize. At the same time, however, the upregulation of MMPs may also help to trigger emphysema, as multiple studies have shown an increase in MMP-1, -2, -8 and -9 in emphysematous lung tissue. The ensuing lung remodelling may result in significant airway disease. Conversely, COPD itself may promote subsequent TB infection by impairing immune responses and ciliary function, two key defences against pathogenic microbes. Innate immune dysregulation against pathogens such as *H. influenzae* and *S. pneumoniae* have been well documented in COPD; while similar work on TB has yet to be confirmed. The phagocytic disruption observed in COPD may indeed hold validity for TB as well. For instance, cigarette smoke-exposed mice have been shown to demonstrate impaired macrophage responses to TB in comparison to control mice. These injuries may be in part mediated by chronic exposure to inhaled corticosteroids (ICSs).

Fungal infection and colonization

The clinical significance of fungal infections in COPD is poorly understood and likely underestimated. Fungal colonization is observed in severe COPD patients. Whether such colonization is a marker of advanced COPD or contributes to disease progression remains to be studied. *Aspergillus* spp. are frequently isolated from the airways of COPD patients during exacerbations (16.6%) and even during follow-up (14.1%).[29,116] In an observational study, positive *Aspergillus* cultures in COPD patients were associated with increased sputum cell counts.[117] The incidence of AECOPD in the preceding year and concomitant bacterial pathogen isolation were associated with increased *Aspergillus* detection. Positive *Aspergillus* cultures in COPD patients are associated with increased sputum neutrophils suggesting the presence of host immune response in relation to the organism.[117]

Up to 8–15% of COPD patients have hypersensitivity to *Aspergillus*, with associated reduced lung function.[118,119] Whether the use of antifungals may mitigate such declines in pulmonary function remains to be studied. Isolation of *Aspergillus* in the airway may represent temporary passage, benign carriage or be an early sign of invasive pulmonary aspergillosis (IPA), especially with high steroid use for recurrent exacerbations conferring host immunosuppression. Increasing evidence suggests that COPD patients are at risk of IPA with resulting high mortality rates; up to 22% of COPD patients with detection of *Aspergillus* in airway culture had IPA.[120] Innate immune responses play an important role in *Aspergillus* clearance mechanisms. Inhaled *Aspergillus* conidia bind to surface receptors within terminal airways via galactomannan, which enhance uptake by alveolar macrophages and dendritic cells leading to a cascade of immune responses. Recruitment of neutrophils and alveolar macrophages leads to

ingestion, phagolysosomal degradation and killing of the fungal conidia. TLR2- and TLR4-dependent signals further contribute to host recognition and the inflammatory response to both *Aspergillus* conidia and hyphae.[121] Steroids therefore interfere with alveolar macrophage function, thereby increasing the risk of IPA through this and other defective innate immune responses in COPD including functionally abnormal neutrophils and TLRs.[122]

Pneumocystis jirovecii is another opportunistic fungal pathogen of the COPD lung (8–85% of patients).[30–32,123] *Pneumocystis* colonization is described in 36.7% of severe COPD patients (GOLD stage IV) compared with 5.3% of those with milder disease (GOLD stages 0–III). Colonization is associated with severe airflow obstruction independent of smoking.[30,124] Increased systemic inflammation (IL-8, TNF-α and IL-6)[31,124] and proteases (MMP-12) responsible for parenchymal destruction and emphysema in COPD have been found in higher concentrations in COPD patients colonized with *Pneumocystis*. This suggests that the organism is unlikely an innocent bystander and may contribute to disease pathogenesis and progression. An upregulation of chemokine ligands (CXCL9, CXCL10 and CXCL11) of the CXCR3 receptor predominantly expressed on activated T-helper (Th) 1 lymphocytes is also observed and may prime Th1 inflammatory pathways contributing to the progression of COPD in these cases.[32]

Viral infections

Although respiratory viruses are associated with asthma and COPD and cause acute cell damage, release of ROS and activation of NF-κB, their significance in stable COPD is less well understood (Table 1). Adenovirus DNA is detectable in lung tissue of stable COPD patients and when compared with healthy smokers, a 5–40-fold increase in adenovirus early region 1A (E1A) expression is observed in patients with emphysema.[125,126] In animal models, even latent adenovirus infection amplified the inflammatory responses (increased cluster of differentiation (CD) 8 cells, macrophages and neutrophils) and emphysematous destruction,[127] and increased IL-8 and intercellular adhesion molecule 1 (ICAM-1) expressions are also described in human epithelial cell transfection with adenovirus E1A genes.[128,129] RSV is also common in stable COPD with prevalence rates of up to 33%.[130–132] An RNA virus of the Pararmyxoviridae family and a common cause of acute respiratory tract infections, its presence in stable COPD is unsurprisingly linked to higher levels of both airway and systemic inflammation.[130,131]

THE ROLE OF THE MICROBIOME IN COPD PATHOGENESIS AND PROGRESSION

There is an emerging role for the microbiome in both stable and during exacerbations of COPD that has been recently reviewed elsewhere.[133–141]

While most research has focused on the bacteriome, viruses (virome) and fungi (mycobiome) are also important constituents of the microbiome. The respiratory tract contains genetic material from eukaryotic viruses such as anelloviruses, herpesviruses and papillomaviruses, as well as retroviruses that may establish persistent asymptomatic infections and bacteriophages, which dominate the virome.[142–145] In the lung, the virome is highly variable between individuals and is strongly influenced by environmental conditions such as oxygen availability.[144] Unfortunately, in many studies, a large proportion of sequencing reads are not matched to a reference genome and it is unclear whether the identified viruses are commensal or a latent infection. Given these difficulties, it is challenging to directly compare the entire virome in COPD patients and healthy subjects. Nevertheless, COPD patients have increased total viral load, as well as an increased prevalence or abundance of influenza, cytomegalovirus and Epstein–Barr virus.[146–148] Ultimately, the role of acute viral infection including rhinovirus, RSV and influenza, which clearly contribute to inflammation, pathology and exacerbations of COPD,[28,149,150] remains to be fully elucidated. As previously described, latent adenoviral infection is associated with increased inflammation and alveolar destruction[126] while total and influenza-specific viral loads of COPD patients without symptoms of infection correlated with inflammatory cell numbers in the airways.[148] Moreover, human rhinovirus-encoded proteinase 2a has been proposed to elicit a duel Th1/Th2 response in COPD patients through its impact on dendritic cell maturation, thereby driving inflammation, allergy and airway obstruction.[149]

Although fungi are only detectable at low abundance in healthy individuals, several members of the mycobiome are consistently identified in the lower respiratory tract, including *Cladosporium* and *Aspergillus* spp.[120,151] Moreover, the outgrowth of *Aspergillus* can lead to IPA, with COPD as the most common predisposing condition. Colonization with *Pneumocystis* was associated with COPD development and GOLD stage classification.[30,152] *Pneumocystis* spp. are capable of inducing COPD-like disease in animal models both with and without cigarette smoke exposure.[153,154] In the oral cavity, smoking is associated with increased prevalence of *Candida* spp. as a consequence of the ability of cigarette smoke to increase the growth, adherence and immunostimulatory activity of *Candida albicans*.[155,156] Cigarette smoke also increased the content of chitin in the cell wall of *C. albicans*. While it is not clear whether cigarette smoke causes similar changes in fungi that colonize the lung or if fungi in the oral cavity affect the lung through microaspiration, notably, the activity of chitotriosidase and the chitin-binding protein YKL-40 are increased in the BAL and alveolar macrophages of COPD patients.[157,158] Even serum levels of YKL-40 and chitotriosidase are elevated in COPD patients and inversely correlate with lung function, indicating that host responses to fungal colonization may contribute to COPD progression.[159]

The bacterial microbiome, virome and mycobiome are not isolated and act in concert. Indeed, bacteria and phage populations are strongly correlated,[142,144] while eukaryotic viruses and fungi can affect bacterial

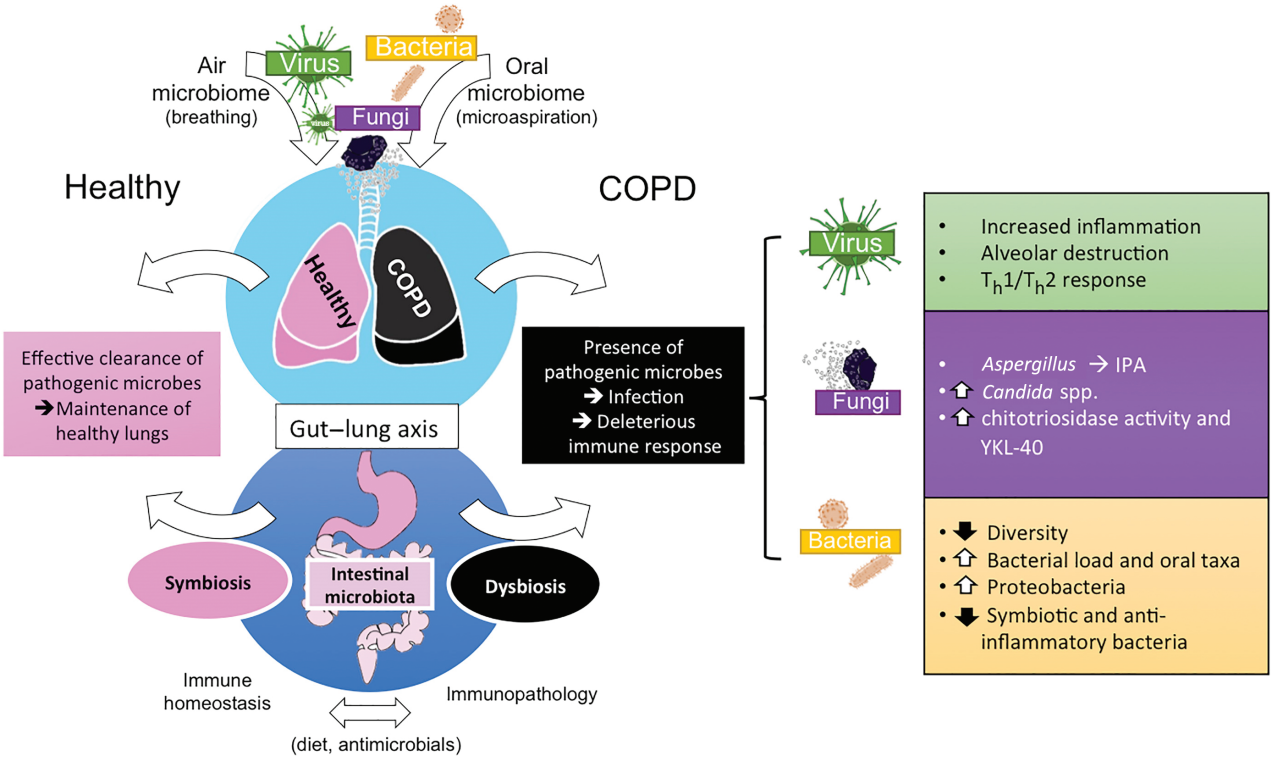

Figure 3 Role of the microbiome in COPD pathogenesis and progression. Bacteria, viruses and fungi enter the lung through breathing and microaspiration of oral microbes. In the healthy lung, homeostasis is achieved through an appropriate immune response and pathogen clearance. In COPD, an increased abundance of pathogenic organisms and oral taxa is seen in association with reduced overall bacterial diversity. Consequently, perpetuation of a deleterious immune response occurs with associated COPD progression. Host–microbe dialogue may also proceed via the gut–lung axis further exacerbating disease symptoms as demonstrated in other chronic respiratory disease states. IPA, invasive pulmonary aspergillosis; Th, T-helper.

growth.[150,151] Furthermore, the gastrointestinal microbiome interacts with the respiratory microbiota and plays a critical role in the development of several chronic respiratory diseases, and likely COPD (Fig. 3).[133]

THE EFFECT OF INFECTION ON COPD DISEASE PATHOGENESIS, PROGRESSION AND THERAPEUTICS

Antibiotic, bronchodilator and steroid use

The prominent role of bacteria in inciting acute COPD exacerbations has placed antibiotics at the forefront of COPD therapy. In 1987, Anthonisen *et al.* reported a 13% increase in treatment success rate with antibiotic therapy compared with placebo, particularly in patients with increased dyspnoea and sputum production and purulence.[160] Since then, antibiotics covering pathogens such as *H. influenzae*, *S. pneumoniae* and *M. catarrhalis* have become mainstays of therapy and numerous trials have confirmed their beneficial effects.[161–163] Nonetheless, the notion that all acute exacerbations must be treated with antibiotics has been called into question, given that not every exacerbation is the result of a bacterial infection. Efforts to better identify the subset of exacerbating COPD patients who may benefit from antibiotics have explored biomarkers such as procalcitonin,[164,165] C-reactive protein[166] and neutrophil CD64 expression,[167] but few of these have yet made it into mainstream clinical decision algorithms.

The pleiotropic effects of antibiotics, particularly those of the macrolide class that also have anti-inflammatory properties, led to their use in the prevention of exacerbations. Azithromycin, for instance, has not only antimicrobial activity against both Gram-positive and -negative pathogens, but also anti-inflammatory and immunomodulatory effects.[168,169] Treatment in stable COPD patients reduced severe exacerbations, and in a population of patients with frequent COPD exacerbations, chronic use of azithromycin resulted in a significantly longer time to first exacerbation and a reduced yearly exacerbation rate.[170,171] Azithromycin was particularly effective in reducing exacerbations requiring both antibiotics and systemic corticosteroids and appeared to be more effective in older patients with milder disease.[172] The long-term effects of chronic azithromycin therapy on the lung microbial community, however, are yet to be determined although initial reports suggest that while the overall bacterial burden remains stable, the alpha diversity of the lung decreases significantly.[173] The implications of these changes are yet to be established.

While these therapies have proven beneficial for COPD patients, it is important to remember that other COPD treatments can have untoward microbial effects. ICSs, for instance, have been shown to increase the risk

of both pneumonia and TB. In initial randomized controlled trials studying ICS and ICS–long-acting β2-agonist (LABA) combination drugs, a higher rate of pneumonia was noted in the ICS-containing treatment groups.[174] Subsequent analyses, however, have specifically implicated fluticasone as the ICS most likely to increase the risk of pneumonia, as budesonide does not appear to confer a significant risk.[175–178] Similarly, the risk for developing TB while on ICS therapy is significant in moderately to highly TB endemic areas. Research in Taiwan and South Korea has demonstrated up to 25 times greater risk of developing TB while on ICS treatment, with a dose-dependent effect.[179] Thus, in endemic areas, clinicians should be vigilant for the development of TB in COPD patients on ICS therapy. In addition to geographical distinctions, infection is not uniform in COPD, with changes in pathogen, strain and load over time.[16,18] Different species trigger distinct inflammatory responses.[67] Furthermore, host factors play an essential role in infection manifestation and outcome. This results in variable response to antibiotic treatments in COPD. Therapy such as long-term macrolides can be beneficial with reductions in exacerbation rates in a selected group of patients with COPD.[180] This effect has been linked to anti-inflammatory properties of macrolides likely explained by their impact on the microbiome, which, in turn, modulates immune tone. Culture-independent molecular techniques have constructed a comprehensive analysis of the microbial community in the lower airway and yet how the complexities in interactions between the host, pathogen and the environment contribute to disease manifestation and progression in COPD is far from understood. Future studies are required to better define the contribution of infection in COPD pathogenesis and intervention studies incorporating antimicrobial and anti-inflammatory approaches in the treatment of COPD. The application of whole-genome metagenomics in this context may uncover mechanisms beyond the scope of culture-based and amplicon sequencing approaches revealing precise genetic and metabolic pathways that intersect key immune responses contributing to disease progression.

COPD in the Asia-Pacific region

The differences in risk factor exposure, ethnogeography and genetic background in Asia result in distinct characteristics of Asian COPD, which is currently overlooked by existing COPD treatment guidelines. Many of the COPD risk factors, including indoor and outdoor air pollution, occupational exposure to dust and fumes, a history of repeated childhood respiratory tract infections, intrauterine growth retardation, pulmonary TB and poor socio-economic status, are observed at higher prevalence in the Asia-Pacific region. Of particular note is the higher prevalence of pulmonary TB among Asian COPD patients.[181] Concomitant NTM, TB and bronchiectasis in COPD have been associated with higher risk of exacerbations, greater declines in pulmonary function and increased risk of mortality.[78,79] Furthermore, Asia is an epicentre of antimicrobial resistance with high prevalence of multidrug resistance (MDR) organisms, which include respiratory pathogens.[182] Consequently, there may be differing responses to antibiotic therapy during bacterial exacerbation of COPD and an array of variable organisms identified during AECOPD in the Asia-Pacific region. Gram-negative bacteria including *P. aeruginosa*, *Klebsiella pneumoniae* and *Acinetobacter* spp. were more common in AECOPD in Asia-Pacific region.[183] The distinction in risk factor exposure as compared with western cohorts, underutilization of spirometry, higher prevalence of TB, bronchiectasis and antibiotic resistance all pose unique challenges for better diagnosis and treatment of COPD particular to the Asia-Pacific region.

Emerging antimicrobial resistance in COPD: implications for disease pathogenesis and progression

The excessive mucus production in COPD patients is a fertile ground for the stimulation of chronic bacterial infection. Pulsed therapies of antibiotics are frequently used to control bacterial multiplication, which consequently promotes the development of antimicrobial resistance. Prolonged use of macrolides for the prophylaxis and treatment of COPD exacerbations has been shown to increase antimicrobial drug resistance in *H. influenzae*.[184] The commonly used drug azithromycin has a long half-life of around 66 h, which can lead to drug concentrations below the minimum inhibitory concentration for an extended period of time.[185] This prolonged exposure to subinhibitory concentrations of the drug may be one of the reasons behind the increased resistance to macrolides. Indeed, a study by Desai *et al*. on COPD patients with *S. pneumoniae* infection showed a correlation between exposure to macrolides and development of resistance.[186] They advised for the use of alternative antibiotics in patients who have been taking macrolides in the previous 6 months.[186] A 15-year longitudinal study conducted by Pettigrew *et al*. concluded that fluoroquinolones represent the best choice for eradicating *H. influenzae* in COPD patients.[184] In their study, none of the bacterial isolates developed resistance to fluoroquinolones.[184] Even though *P. aeruginosa* is a relatively uncommon cause of COPD exacerbations, its incidence is on the rise and the rapid emergence and spread of MDR (defined by resistance to at least three antibiotic classes) is a serious cause for concern. A comparative study conducted in Spain has demonstrated that the carriage of MDR *P. aeruginosa* in COPD patients was associated with increased mortality rates and more recent work in AECOPD reveals a high burden of antibiotic resistance.[25,187] The lung environment found in COPD patients is also favourable to the formation of biofilms, a mechanism by which bacteria, particularly *P. aeruginosa* and *H. influenza*, evade antibiotic killing. In addition to limiting antimicrobial penetration, the biofilm triggers the emergence of relatively quiescent bacteria that become phenotypically drug resistant to most antibiotics, therefore increasing their persistence for prolonged periods of time.[188] Currently, the most effective way of curbing antimicrobial resistance is to

reduce the use of antibiotics by prescribing them only to patients with demonstrated pathological bacterial infection. In this respect, the use of sensitive markers of bacterial infection such as procalcitonin to guide the start of antibiotic therapy was shown to significantly reduce the use of antibiotics in COPD patients.[189] There is also the potential that the new therapies developed for steroid-resistant asthma may also be beneficial in COPD and possibly avoid the infection-inducing effects of ICS.[190]

Utility of mouse models and new translational techniques

The majority of studies described above are of the associations between infections and microbiomes with COPD in humans. These studies are limited by the type of samples that can be collected and typically the cross-sectional nature of study design. Mouse models of cigarette smoke-induced experimental COPD that accurately recapitulate the hallmark features of the human disease have been generated.[191,192] Chronic models that use only inhalational exposures drive the development of airway inflammation and remodelling, mucus hypersecretion, emphysema and changes in lung function similar to those in humans.[191,192] This can be achieved in a relative short time frame of 8 weeks with exposures similar to those of a pack-a-day human smoker.[191] Importantly, these and other models have increased susceptibility to respiratory bacterial and viral infections that are relevant to those in humans described above.[8,9,46] They are being used to assess the mechanisms of the associations between infections, microbiomes and COPD and have recently implicated roles for immune defects, microRNAs, mast cell proteases and many other factors. In combination with parallel ex vivo human sample and primary cell culture analysis, they provide powerful tools for exploring these associations and are valuable for testing new drugs.[7–9,53,57,170] Translatability into humans can be addressed by applying treatments specifically to those patients with alterations in drug targets. Through similar analysis of comparable tissues from mice and humans, using similar techniques such as the interrogation of precision-cut lung slices, important therapeutic questions can be examined in future COPD studies.[193–195]

CONCLUSIONS

Although the role of pathogenic microbes in AECOPD has been extensively investigated, their clinical significance in stable but colonized COPD patients remains less clear. The question of whether colonizing microbes actively contribute to COPD pathogenesis and more importantly progression may be difficult to address with the current evidence base, given technical challenges in detecting these organisms and the subtlety of their association with disease over long periods of time. Nevertheless, immune responses observed in stable and colonized COPD patients reflect the corollary of those seen in AECOPD supporting a role for microbial colonization in COPD pathogenesis most likely through a perpetuation of negative immune responses over time. Initial work has started to uncover changes in the lung microbiome associated with the COPD state and may reveal further variables to be considered in the investigation of microbe–COPD interactions. The parallel use of mouse models and interrogation of human samples ex vivo and in primary cell culture will likely progress mechanistic insights and drug development. Importantly, however, other variables of microbiome composition may confound pathogen-centric investigations of disease–microbe interactions and it is likely that large-scale studies will be required to address the current gaps in our knowledge of bacterial, viral and fungal microbiota and their association with COPD. Precipitous drops in the costs of DNA sequencing, coupled with the technological innovations in metagenomics, may soon bring such technology into the realm of routine diagnostics in COPD. This could allow for refined patient phenotyping and stratification that in turn will allow more focused and personalized therapy based on microbiology. Finally, the impact of antimicrobial therapy and the use of antibiotic prophylaxis on the respiratory microbiome provide further challenges. The extensive remodelling of the microbiome that occurs in response to COPD antibiotic treatment while recognized has unclear long-term consequences for patients.

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