Respiratory viral infection: a potential "missing link" in the pathogenesis of COPD

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http://ow.ly/ysSc30mIx77

Cite this article as: Linden D, Guo-Parke H, Coyle PV, *et al*. Respiratory viral infection: a potential "missing link" in the pathogenesis of COPD. *Eur Respir Rev* 2019; 28: 180063 [https://doi.org/10.1183/16000617.0063-2018].

ABSTRACT Chronic obstructive pulmonary disease (COPD) is currently the third most common cause of global mortality. Acute exacerbations of COPD frequently necessitate hospital admission to enable more intensive therapy, incurring significant healthcare costs. COPD exacerbations are also associated with accelerated lung function decline and increased risk of mortality. Until recently, bacterial pathogens were believed to be responsible for the majority of disease exacerbations. However, with the advent of culture-independent molecular diagnostic techniques it is now estimated that viruses are detected during half of all COPD exacerbations and are associated with poorer clinical outcomes. Human rhinovirus, respiratory syncytial virus and influenza are the most commonly detected viruses during exacerbation. The role of persistent viral infection (adenovirus) has also been postulated as a potential pathogenic mechanism in COPD. Viral pathogens may play an important role in driving COPD progression by acting as triggers for exacerbation and subsequent lung function decline whilst the role of chronic viral infection remains a plausible hypothesis that requires further evaluation. There are currently no effective antiviral strategies for patients with COPD. Herein, we focus on the current understanding of the cellular and molecular mechanisms of respiratory viral infection in COPD.

Introduction

Chronic obstructive pulmonary disease (COPD) is a major cause of morbidity and mortality worldwide. Recent estimates indicate that COPD affects one in 10 people globally and is now the third leading cause of death in the USA [1]. There are currently at least 15 million COPD sufferers in the USA [1, 2] and in 2015 the disease was estimated to affect approximately 174 million people globally [3, 4]. Despite the rising prevalence of COPD it is a disease that has received insufficient funding [5]. The clinical course of the disease is associated with a downward spiral of progressive breathlessness punctuated by episodes of acute exacerbation leading to heightened symptom severity and increased risk of mortality [6]. Acute exacerbations of COPD (AECOPD) frequently necessitate hospital admission and are responsible for enormous healthcare costs. In 2005 the European Union (EU) reported that the direct healthcare cost attributable to COPD was €38.6 billion [7] whilst in the USA $50 billion are spent on COPD annually [1]. AECOPD is also associated with accelerated lung function decline and thus implicated in disease

Received: July 07 2018 | Accepted after revision: Nov 19 2018

Provenance: Submitted article, peer reviewed.

progression [6, 8–10]. The growing pressure facing our health services is an important driver to improve the outcome for this disease; however, there are currently no treatments that can meaningfully alter the course of deteriorating lung function or the time to death in COPD. Future research urgently needs to focus on the development of novel therapies. Growing evidence indicates that the majority of disease exacerbations are associated with respiratory viruses and bacteria [11, 12]. Improved understanding of the underlying mechanisms implicated in virus-associated disease exacerbations may help to facilitate the development of potential novel therapies that might serve to reduce exacerbations and hinder disease progression in COPD.

Pathogenesis of airway inflammation in COPD

COPD is characterised by an enhanced inflammatory response of the airway epithelium to inhaled noxious gases and particles, primarily cigarette smoke [13]. Combustion products from biomass fuel, occupational dust and particulate matter from air pollution are important risk factors in non-smokers [3, 13, 14]. There is extensive evidence demonstrating that both protease/anti-protease imbalance and oxidative stress are viable pro-inflammatory mechanisms that contribute to pathogenesis in COPD [15–28]. Only a proportion of smokers develop COPD suggesting that host factors may also be implicated in disease pathogenesis [29, 30]. The neutrophil is a key effector cell that accumulates within the airway mucosa in COPD [31, 32]. Sustained activation of the innate immune response leads to increased episodic neutrophilic inflammation [32, 33]. Dysregulation of adaptive immune mechanisms are also implicated in the chronic inflammation and irreversible "airway remodelling" seen in COPD [13, 34, 35]. T-lymphocytes are believed to play an important role in regulating inflammation in COPD [34]. Resected small airway specimens from subjects with COPD demonstrate an increased number of $CD8^+$ T-lymphocytes and their organisation into lymphoid follicles [13]. The degree of activated immune cell accumulation within the airway mucosa has been shown to correlate strongly with severity of airflow obstruction [13]. $CD8^+$ T-lymphocytes provide adaptive immune defence against viruses; however, they also pose the potential to cause tissue injury through a variety of mechanisms including direct cytotoxic effects, pro-inflammatory signalling and recruitment of other immune cells [34, 36, 37]. Physiologically, $CD8^+$ T-cells normally undergo Fas-induced apoptosis following viral eradication. The finding of abundant $CD8^+$ T-cells and the organisation of B-cells into lymphoid follicles within the airway epithelium in COPD has been suggested to represent an increased "immune surveillance" of the airway mucosa [13]. These findings have fuelled the hypothesis that an additional aetiological factor other than exposure to inhaled noxious agents may be necessary in order to develop COPD. A chronic viral infection could potentially account for this finding.

Viral detection during acute exacerbations of COPD

COPD exacerbations arise in the setting of complex interactions between the host, airway pathogens and environmental pollution [38, 39]. AECOPD leads to lung function decline across the severity spectrum of COPD [6, 8] and patients who suffer from frequent or severe exacerbations experience a more accelerated decline in lung function [6, 40]. Until recently bacteria were believed to be responsible for the majority of exacerbations; however, with the advent of PCR testing the true prevalence of respiratory viruses has been realised [11, 39, 41, 42]. Early studies using serological and viral culture techniques identified viruses in ~10–30% of AECOPD [39, 43]. Following the implementation of PCR it is now estimated that viruses are associated with half of all exacerbations [39, 41, 42, 44]. Rohde *et al.* [45] detected respiratory viruses in 56% of exacerbations using PCR. Respiratory viral infection is associated with poorer clinical outcomes including increased symptom severity and longer duration of hospital stay [41, 44, 46]. A recent systematic review of 19 studies using sputum PCR during AECOPD (n=1972) reported that the most frequently detected viral species are human rhinovirus (HRV) (16.39%), respiratory syncytial virus (RSV) (9.9%) and influenza (7.83%) [11]. Adenovirus (2.07%), coronavirus (4.08%) and human metapneumovirus (2.78%) are detected less frequently [11]. The predominance of HRV, RSV and influenza reflect increased likelihood of infection with these viruses because of co-circulation of multiple genotypes (HRV) and the yearly community wide epidemics of RSV and influenza. This epidemiological data implicates viruses in a significant proportion of COPD exacerbations, however, these findings do not demonstrate causality. Respiratory virus detection in stable COPD has also been reported. McManus *et al.* [41] detected viruses in 12% whilst Rohde *et al.* [45] detected viruses in 19% of stable COPD subjects. PCR may detect minute quantities of viral DNA or RNA and does not confirm the presence of live virus [39]. Bacterial and viral co-infection has also been described in the context of AECOPD leading some to question whether viral pathogens simply contribute to secondary bacterial infection [39, 47]. Based on these findings the role of viruses during COPD exacerbations remains controversial.

Mechanisms of virus-induced COPD exacerbations

The mechanisms of virus-induced COPD exacerbation remain poorly elucidated and clinical data is currently lacking. Respiratory viruses preferentially target airway epithelial cells leading to epithelial cell

sloughing, microvascular dilatation, oedema and immune cell infiltration [48]. There is increased susceptibility to bacterial infection and impaired mucociliary clearance [48, 49]. HRV is the most commonly detected virus during disease exacerbations and the virus that has been studied to the greatest extent in the context of AECOPD. In pursuit of demonstrating causality Mallia and co-workers [50, 51] have conducted human experimental models measuring clinical symptoms, lung function and inflammatory response biomarkers in subjects with COPD following nasally administered rhinovirus inoculum (RV16). Subjects with COPD develop peak cold symptoms at day 10–11 post-inoculation and subsequent exacerbation following 10 to 1000 lower doses of RV16 than those used in subjects with asthma during previous studies [50]. Subjects with COPD experienced forced expiratory volume in 1 s (FEV_1) reduction and increased nasal lavage interleukin (IL)-8 following HRV infection [50]. RV16 viral inoculum has been shown to result in significant increases in sputum neutrophilia and there is correlation between sputum neutrophil elastase, symptom severity and airflow obstruction in subjects with COPD [51]. These findings suggest HRV is a viable inducer of COPD exacerbation [51]. More recently, Footitt *et al.* [52] demonstrated increased indices of oxidative and nitrosative stress and impaired macrophage activity (histone deacetylase, HDAC2) following HRV infection in COPD. The toll-like receptor (TLR)-3 pathway signalling may be implicated in RSV-induced exacerbation [53, 54]. Several studies suggest that viral infection is associated with more frequent and more severe exacerbations [42, 44, 55]. One exacerbation study reported that sputum eosinophilia occurred in the context of viral exacerbation but not bacterial infection [47].

Mechanisms of increased susceptibility to viral infection in COPD

The finding of increased viral load in COPD relative to control subjects following HRV infection suggests that innate antiviral immunity may be deficient [51, 52]. The innate antiviral response following HRV infection relies on interferon signalling, however, this has been shown to be impaired in COPD [51]. Furthermore, the simultaneous insult of cigarette smoke exposure and HRV infection may contribute to increased host susceptibility through inhibiting expression of "interferon simulated genes" and may affect interferon production [56]. A further mechanism of susceptibility to HRV in COPD may be related to increased airway expression of intracellular adhesion molecule (ICAM)-1 in smokers with chronic airflow limitation [57]. McKendry *et al.* [58] recently used an *ex vivo* infection model to demonstrate that $CD8^+$ cells from subjects with COPD have a defective response to H3N2 influenza virus *in vitro*. Programmed cell death protein-1 (PD-1) (also known as CD279) exerts numerous immunosuppressive actions on $CD8^+$ T-cells and appears to be central to this process. In subjects with COPD, PD-1 expression is upregulated in lung $CD8^+$ T-cells compared to unaffected controls following influenza infection [58]. This study also demonstrated reduced T-cell cytotoxicity [58]. These findings indicate that virus-specific mechanisms of susceptibility exist in COPD. Continued cigarette smoke exposure adds insult to injury through impairment of innate immunity. Cigarette smoking is the primary risk factor for COPD and has been shown to impair viral recognition and innate immune responses [59]. Cigarette smoke extract (CSE) pre-treatment of primary bronchial epithelial cells (pBECs) results in a relative immunosuppressive effect with reduced expression of IL-6 and IL-8 in subjects with COPD compared to controls and unaffected smokers [60]. CSE has also been shown to alter host anti-viral immune signalling *via* reduction in viral-mediated interferon (IFN)-β and Retinoic Acid Inducible Gene (RIG)-1 mRNA expression [61]. Dysregulation of adaptive immunity may be an important disease mechanism in COPD and may render patients with COPD more susceptible to acute viral infection. CSE also modulates airway epithelial cell cytokine release in the setting of rhinovirus infection and has been shown to increase the release of CXCL8 [62]. In addition, CSE demonstrates potent inhibition of rhinovirus-induced release of CXCL10 [63]. CXCL10 knockout mice demonstrate reduced ability to control viral infection and impaired T-cell recruitment and activation. Those engaged in the ongoing debate regarding the role for inhaled corticosteroids (ICS) in COPD should consider recent data published by Singanayagam *et al.* [64] showing that fluticasone propionate leads to impairment of both innate and adaptive antiviral immunity and may also contribute to increased mucous production, increased bacterial load and impaired antimicrobial peptide secretion. Furthermore, the finding of herpes simplex virus (HSV)-1 in COPD should alert prescribers. HSV-1 is more commonly detected in those taking higher ICS doses and associated with increased risk of mortality [65].

Chronic viral infection in COPD

In 2004, Hogg *et al.* [13] postulated that the formation of lymphoid follicles and adaptive immune cell infiltration within the airway epithelium in COPD could potentially be explained by "microbial colonisation". A chronic viral infection could account for this finding. Previous attention has focused on persistence of RSV and latent adenovirus infection [44, 66, 67]. However, several epidemiological studies have failed to demonstrate persistence of these pathogens in stable COPD [41, 68, 69]. The adenovirus E1A protein has been shown to be associated with emphysema and appears to upregulate

pro-inflammatory signalling [67, 70, 71]. HSV-1 and Epstein–Barr virus (EBV) may be underestimated in COPD. Human herpes virus infections demonstrate persistence with the ability to cause recurrent infection. In one study our group identified HSV-1 in the sputum of 19% of a cohort of patients admitted to hospital (21 out of 112) [65]. In this study HSV-1 was associated with higher doses of inhaled steroids, worse lung function and an increased risk of mortality [65]. In another study the detection of EBV DNA *via* PCR was significantly increased in the sputum of subjects with COPD but not unaffected smokers [72]. Interestingly, EBV copy numbers were similarly elevated in stable COPD [72]. Polosukhin *et al.* [73] performed immunohistochemistry using lung biopsy specimens demonstrating that small airway inflammation and remodelling is associated with the presence of EBV. Further clinical and mechanistic data is necessary to evaluate the significance of human herpes virus infections in the pathogenesis of COPD.

Rhinovirus infection and COPD

During COPD exacerbation HRV is the most frequently detected respiratory virus [11]. Viral infections may be underestimated because colds, mostly associated with acute viral infection, predict AECOPD even in the absence of detectable virus [74]. HRV is a positive unenveloped single stranded RNA virus that belongs to the Picornaviridae family [75]. Over 100 different HRV serotypes exist [76]. Rhinovirus is perceived as a cause of minor illness; however, it is frequently detected in COPD patients with hypoxic respiratory failure in the absence of other pathogens [76, 77]. HRV infection during childhood is known to predispose to the later development of asthma [76]. There is currently no treatment or vaccine for HRV infection. Human experimental models by Mallia and co-workers [50, 51] demonstrate that HRV is capable of inducing AECOPD. Viral load has been shown to peak at day 5 post inoculation and prolonged HRV shedding has been shown to occur [55, 78]. Re-infection with multiple HRV sub-types can occur and this is more common in subjects with COPD [78]. Unlike other respiratory viruses, such as influenza, HRV infection does not lead to significant airway epithelial damage. HRV infection in subjects with COPD elicits pro-inflammatory cytokines (figure 1) including IL-6, IL-8, tumour necrosis factor (TNF)-α, growth regulated oncogene (GRO)-α, IFN-γ-induced protein 10, RANTES, eotaxin-1, ICAM-1 and neutrophil elastase *via* activation of nuclear factor (NF)-κB [79–82]. HRV also activates epidermal growth factor receptor to release mucin and IL-8 (figure 2) [83]. HRV airway epithelial attachment is mediated *via* ICAM-1 (CD54) in over 60% of serotypes [84]. ICAM-1 is also involved in leukocyte recruitment and activation. ICAM-1 has been shown to be markedly elevated in COPD [57] potentially promoting enhanced HRV binding and entry into the airway epithelial cells [39, 42, 57, 76]. Thus, ICAM-1 has been seen to represent a potential therapeutic target and ICAM-1 inhibition in transgenic mice reduces inflammation, cytokine production and virus load [76]. However, ICAM-1 inhibition also has the potential to impair innate immune capabilities and increase the risk of bacterial infection.

Rhinovirus-induced alterations in lower airway microbiome

A study by Mallia *et al.* [85] reported that following experimental HRV infection in COPD, 60% of subjects demonstrated positive bacterial growth in sputum cultures. Peak sputum viral load precedes peak bacterial load by up to 10 days [85]. The microbiome of subjects with COPD has been shown to differ at baseline from those of normal individuals with lower numbers of proteobacterial species present in COPD [82]. Interestingly, HRV infection induces alterations to the lower airway microbiome in COPD that persist for up to 42 days with a tendency toward pathogenic outgrowths of *Haemophilus influenzae* [82]. This does not occur in unaffected smokers or controls and these subjects demonstrate temporal stability of the microbiome [82]. There is increased bacterial binding and transmigration across well-differentiated airway epithelial cells (16HBE14o) following HRV infection [75]. Viral replication appears to facilitate this process and may be necessary for bacterial transmigration to occur in specific bacterial species such as non-typeable *H. influenzae* (NTHi) [75]. *Pseudomonas aeruginosa* is capable of epithelial transmigration in the absence of viral replication, however, higher rates of cell damage occur in the presence of HRV [75]. These findings suggest that HRV replication supports bacterial transmigration and is relevant to numerous bacterial species that are prevalent in AECOPD [77]. HRV also impairs the airway epithelial barrier by dissociating zona occludens-1 from the tight junction complex [75].

Rhinovirus-induced impairment of innate immune capabilities in COPD

Rhinovirus may lead to impairment of innate immune capabilities increasing susceptibility to bacterial infection in subjects with COPD *via* reduction in sputum antimicrobial peptides including elafin and secretory leukocyte protease inhibitor (SLPI) [85]. Concurrent infection with rhinovirus and NTHi induces synergistic CCL20 production by bronchial epithelial cells that exceeds isolated bacterial or virus infection alone [86]. Airway epithelial cells taken from subjects with COPD demonstrate a pro-inflammatory phenotype at baseline and greater susceptibility to rhinovirus infection [80]. Subsequent increase in the viral load may enhance type 3 IFN responses [80]. These cells also demonstrate increased susceptibility to

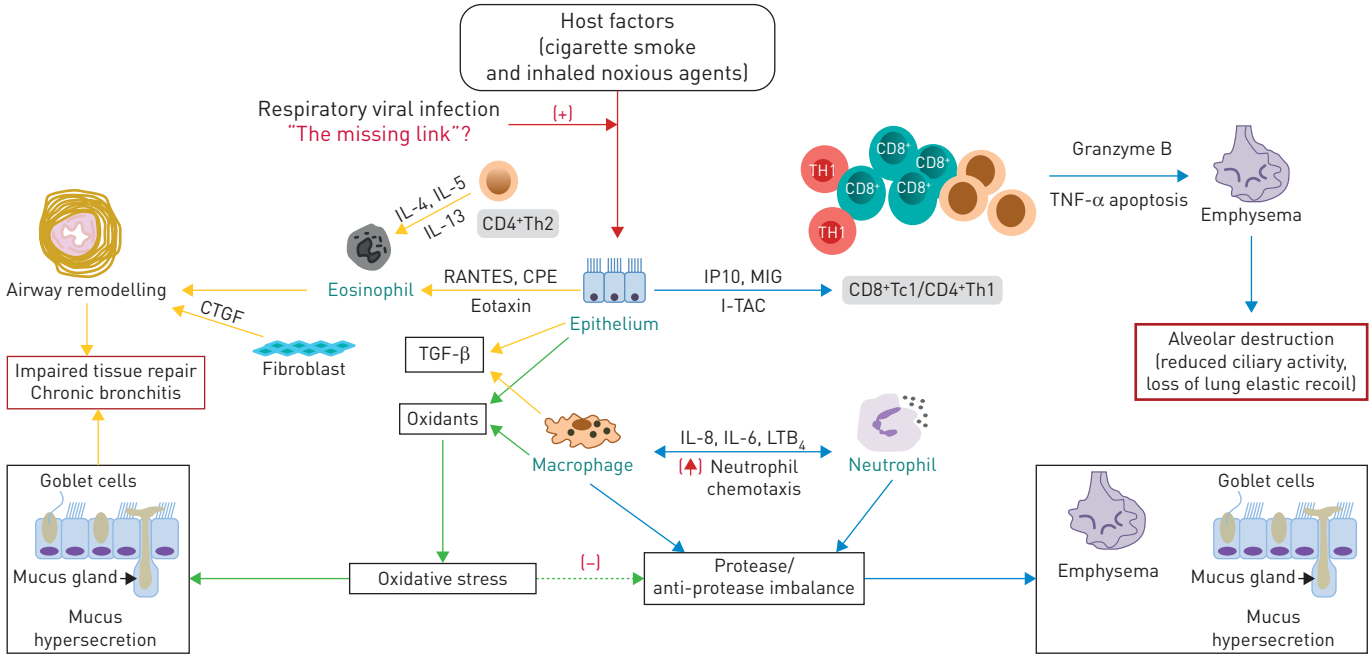

FIGURE 1 Protease/anti-protease imbalance and oxidative stress are viable pro-inflammatory mechanisms that contribute to the pathogenesis of chronic obstructive pulmonary disease (COPD). Cigarette smoke and inhaled noxious agents activate epithelial cells and macrophages to release several chemotactic factors (including interferon-γ-induced protein 10 (IP10), monokine-induced by interferon-γ (MIG), I-TAC, interleukin (IL)-6, IL-8 and leukotriene B_4 (LTB_4)), which attract and activate key inflammatory cells that accumulate within the airway mucosa in COPD. Sustained activation of innate and adaptive immune responses leads to airway influx of neutrophils and CD8 cytotoxic T-cells (TC1 cells)/CD4 T-helper 1 (Th1) cells. In a sub-group of COPD patients, increased Th2 signalling may be present and numerous therapies targeted at Th2 cytokines have been studied. Mepolizumab (IL-5) has shown minor reduction in acute exacerbations of COPD whilst benralizumab (IL5Ra) and navarixin (CXCR2) have shown a modest effect on forced expiratory volume in 1 s. COPD inflammatory mediators sustain the inflammatory process in COPD leading to elastin degradation and emphysema. Neutrophil elastase also causes mucus hypersecretion. Epithelial cells and macrophages also release transforming growth factor (TGF)-β, which stimulates fibroblast proliferation resulting in small airway fibrosis and remodelling. The finding of $CD8^+$ T- and B-cells organised into follicles within the airway epithelium in COPD may represent an increased "immune surveillance" of the airway fuelling the hypothesis that an additional aetiological factor, other than exposure to inhaled noxious agents, is necessary in order to develop COPD. Chronic viral infection might serve as an additional host aetiological factor (the "missing link") in the development of COPD, whilst increased susceptibility to acute viral-induced exacerbations leads to incremental disease progression. CTGF: connective tissue growth factor; CPE: cytopathogenic effect; TNF: tumour necrosis factor.

apoptosis following HRV infection. Singh *et al.* [87] reported that HRV-encoded proteinase 2A induces both T-helper (Th)1 and Th2 responses from $CD4^+$ T-cells and monocyte-derived dendritic cell activation in rhinovirus-initiated acute COPD exacerbation. The subsequent Th2 response results in elevated airway hyperactivity and contributes to more severe dyspnoea and accelerated lung function deterioration [42, 49].

Respiratory syncytial virus in COPD

RSV, a negative single stranded RNA, enveloped virus, is the most common cause of bronchiolitis and is the single most important cause of respiratory illness in the paediatric population. Symptom severity during adult RSV infection is typically milder in adults. However, Falsey *et al.* [88] estimated that the impact of RSV within both elderly and "high-risk" groups was similar to that of non-pandemic influenza A. One retrospective multicentre study reported a 12.6% 60-day mortality during RSV-associated COPD exacerbations [89]. RSV contributes to airway inflammation *via* cytokine release and neutrophil and $CD8^+$ recruitment and has been shown to activate epidermal growth factor receptor leading to suppression of CXCL10 production resulting in impaired viral clearance *via* reduced NK cell and T-lymphocyte recruitment [90, 91]. Mechanistic data demonstrates that RSV infection promotes *P. aeruginosa*-mediated biofilm formation [92]. *In vitro* data show that A549 cells show increased expression of TLR3 following RSV infection [53]. This leads to increased NF-kB activity and IL-8 release [54]. Together these mechanisms sensitise the airway epithelium to ds-DNA exposure. Increased TLR3 expression in COPD is associated with declining lung function and may be a risk factor for RSV-induced AECOPD [53]. It remains unclear which COPD patients are at highest risk from RSV infection. One study (n=379) using logistic regression analysis demonstrated that the presence of "congestive cardiac failure" and/or "exposure to children" were independent risk factors for the development of severe COPD exacerbations [93]. Interestingly, a number of studies have shown high rates of RSV detection during stable COPD [44, 91].

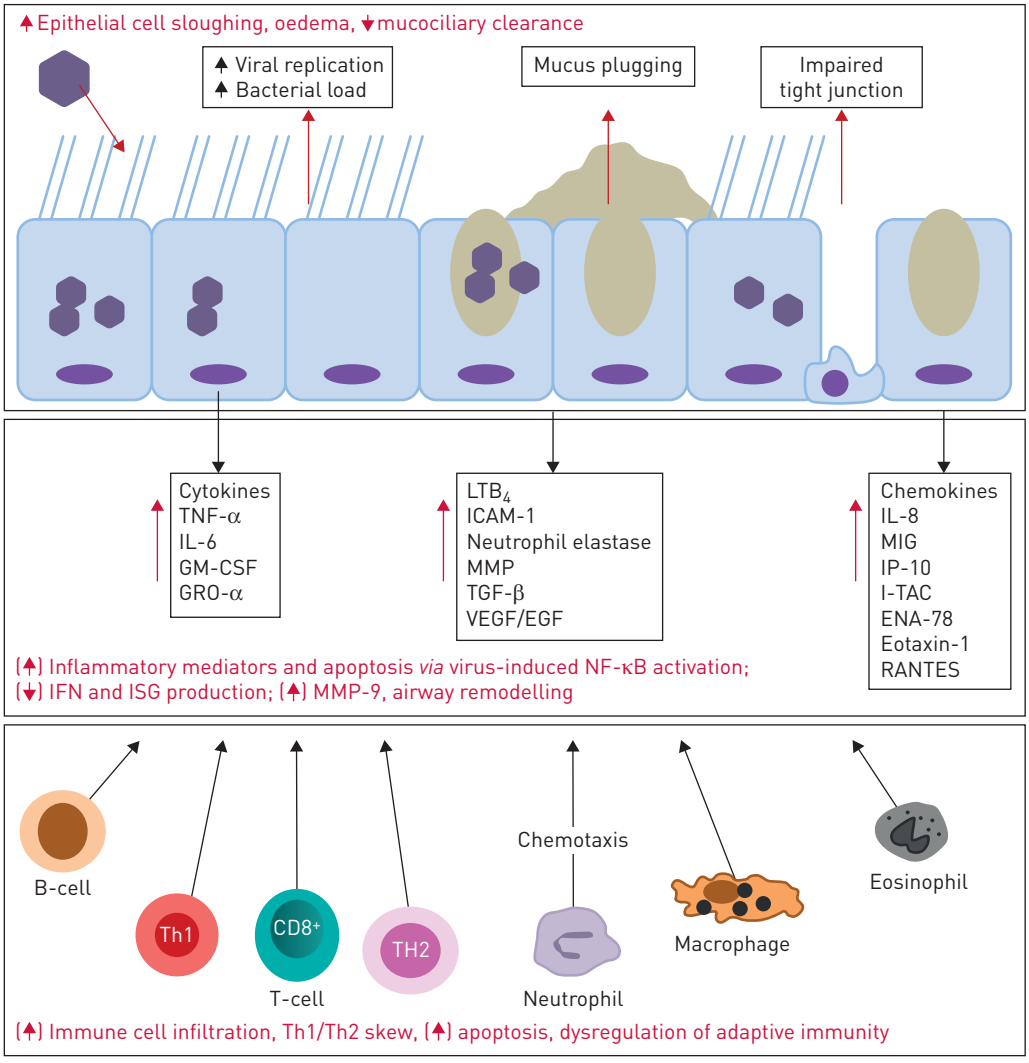

FIGURE 2 Mechanisms of virus-induced airway inflammation in chronic obstructive pulmonary disease (COPD). COPD exacerbations are associated with increased expression of cytokines and chemokines, including tumour necrosis factor (TNF)-α, interleukin (IL)-6, interferon (IFN)-γ-induced protein 10 (IP10), leukotriene B_4 (LTB_4), monokine-induced by IFN-γ (MIG), IFN-inducible T-cell-α chemoattractant and RANTES, growth regulated gene α (GRO-α) and epithelial-neutrophil activating peptide (ENA-78). These cytokines and chemokines attract various inflammatory cells such as neutrophils, T-cells, macrophages and dendritic cells. Vascular endothelial growth factor (VEGF) and epidermal growth factor (EGF) promote the release of matrix proteins from fibroblasts/myofibroblasts, which enhance the production of matrix metalloproteinases (*i.e.* MMP-9) from airway epithelial cells leading to airway remodelling. Respiratory viruses preferentially target airway epithelial cells leading to epithelial cell sloughing, Goblet cell hyperplasia (mucus plug), microvascular dilatation, oedema and immune cell infiltration. Consequently, there is increased impaired mucociliary clearance and increased susceptibility to bacterial infection. Cigarette smoke contributes to impaired host innate antiviral immunity through the reduction of IFNs and IFN-stimulated gene production, and induces inflammatory mediators in the airway and increased epithelial cell apoptosis *via* the activation of nuclear factor (NF)-κB. In addition, dysregulated adaptive immunity may also be an important disease mechanism that may render patients with COPD more susceptible to acute viral infection. GM-CSF: granulocyte-macrophage colony stimulating factor; ICAM: intracellular adhesion molecule; TGF: transforming growth factor; I-TAC: interferon-inducible T-cell alpha chemoattractant; ISG: interferon stimulated genes; Th1: T-helper cell type 1.

Seemungal *et al.* [44] reported detection of RSV in 23.5% of stable COPD patients. These findings led the authors to postulate a potential "chronic low-grade infection with RSV" [44]. There was also a tendency towards higher nasal lavage IL-6 and serum carbon dioxide, however, these findings did not reach statistical significance. Subsequent studies have not demonstrated significant levels of RSV detection in stable COPD [11, 41]. Falsey *et al.* [68] investigated RSV persistence in a study of 112 COPD subjects experiencing AECOPD and detected RSV in none out of 685 routine nasal samples and three out of 315 sputum samples. More recently, McManus *et al.* [41] reported RSV was not detected using real-time PCR

in any stable phase COPD subjects. Giannakaki *et al.* [94] reported that RSV was not detected in the bronchoalveolar lavage or lung biopsy specimens of subjects with COPD (n=31). These findings do not support the hypothesis that chronic RSV infection is implicated in the pathogenesis of COPD, however, RSV may be underestimated as a cause of morbidity and mortality in "at risk" elderly patients and also appears to be a less common trigger of COPD exacerbation.

Influenza infection and COPD

There is a paucity of specific clinical studies relating to influenza infection in the setting of COPD. One study reported that COPD was a statistically significant risk factor for hospital admission during influenza infection whilst asthma was not [95]. Influenza is the second most common respiratory virus detected during AECOPD [11]. Rohde *et al.* [45] reported detection of influenza in 25% of exacerbations whilst Tan *et al.* [96] found influenza in 36%, however this was a small study and patients had not received the influenza vaccination. There is increased lethality following influenza infection in subjects with COPD compared to normal individuals [97]. Numerous randomised control trials (RCT) have shown that influenza vaccination reduces COPD exacerbations [98–100] whilst population-based and observational studies demonstrate that vaccination reduces the incidence of pneumonia, critical care admissions and mortality [101, 102]. Nevertheless, vaccination coverage rates are below target levels in both the USA and EU [103]. One possible reason for this suboptimal vaccine coverage in COPD sufferers is a perceived risk of exacerbation and impaired immune response with subsequent infection has been reported [99, 104]. In rare circumstances influenza vaccination has been reported to be associated with eosinophilic pneumonia and respiratory failure [105]. Numerous studies have shown no evidence of increased exacerbation risk [103, 106, 107] and a systematic review conducted in 2017 reported that influenza vaccination confers a "positive benefit–risk ratio" in the COPD population and that existing data supports annual vaccination in patients with COPD [103].

Secondary bacterial pneumonia may occur as a complication of influenza infection and is the predominant cause of morbidity and mortality during pandemics [108–110]. Chertow *et al.* [109] reported up to 34% of intensive care admissions during the 2009 influenza pandemic were complicated by bacterial co-infection. Viral–bacterial co-infection with *Streptococcus pneumonia* following influenza is an established phenomenon [108, 109, 111]. Bacterial co-infection frequently occurs within 6 days and may be difficult to differentiate from isolated viral infection clinically, however, the consequences of co-infection are more severe [112]. Influenza is capable of suppressing the immune response to *S. pneumonia* and also upregulates pneumococcal adhesion molecules [108, 109, 111]. The presence of influenza neuraminidase leads to epithelial barrier dysfunction with subsequent increase in bacterial adherence and proliferation [113]. Influenza has also been shown to induce neutrophil apoptosis and neutrophil dysfunction [114] and may also inhibit macrophage phagocytosis [112]. Bacterial co-infection may perpetuate inflammation during co-infection *via* increased protease activity and bacterial neuraminidase expression [110]. TNF-α has been identified as a potential biomarker for influenza-bacterial co-infection with *S. pneumonia* [112].

Cochrane reviews published in 2004 [115] and 2006 [116] concluded that amantadine was an effective prophylactic agent. Amantadine and rimantadine both resulted in significant gastrointestinal side-effects [115]. Neither drug has an effect on viral shedding and there is significant resistance to both drugs in H3N2 influenza [117]. Neuraminidase inhibitors such as oseltamivir and zanamivir are also available for prescription in patients with influenza including those with COPD; however, no specific RCTs in COPD patients exist and the treatment efficacy of neuraminidase inhibitors remains dubious. In a systematic review published in 2014, Jefferson *et al.* [118] concluded, "oseltamivir reduces the proportion of symptomatic influenza" and "in treatment studies it also modestly reduces the time to first alleviation of symptoms". However, it was found that oselatmivir resulted in significant gastrointestinal side-effects, headaches, psychiatric symptoms and renal complications [118]. Based on these findings the benefit–risk ratio must be carefully considered prior to making the decision to commence oseltamivir in cases of influenza [118]. The RCT data appraised by Jefferson *et al.* [118] does not relate specifically to patients with COPD and from the current evidence base it is difficult to make recommendations regarding the management of influenza other than to recommend annual vaccination. Some studies of "high-risk" patients including those with COPD have shown modest effects reducing respiratory symptoms and antibiotic usage [119].

Human adenovirus

Human adenovirus is a non-enveloped, ds-DNA virus. There are over 60 reported serotypes with seven known species (human adenovirus-A to -G) [120]. Type 4 (group E), 7 (group B) and 1, 2 and 5 (group C) are the subtypes most frequently implicated in adult respiratory disease [69]. Human adenovirus infection within the lower respiratory tract can result in life-threatening infection in the context of asthma and immunosuppression [69] and is recognised as an uncommon cause of AECOPD [11, 69, 121]. Early

PCR studies reported adenovirus detection in 0.5–1.5% of COPD exacerbations [44, 122] and this is concurrent with more recent systematic review data [11]. However, higher rates of detection have been described. Notably, McManus *et al.* [69] and Kokturk *et al.* [121] reported adenovirus detection during 7–10% of exacerbations.

Latent adenovirus infection has been proposed as a pathogenic mechanism in COPD. Adenovirus is capable of persisting in the airway epithelium without active viral replication and is capable of evading the immune response *via* the 19-kDa protein which delays the expression of class I human leukocyte antigen proteins [70]. The adenoviral E1A gene is capable of integration into human DNA [123]. This E1A protein has been shown to be present in the airway epithelial cells of COPD sufferers at higher levels than in age-matched controls with equivalent smoking history using *in situ* hybridisation [66]. Subjects in whom E1A is positive have also been shown to have lower FEV_1 [70]. One study using bronchial epithelial cells reported significantly higher E1A gene detection *via* PCR in subjects with COPD compared to those with chronic bronchitis [124]. Another study demonstrated up to a 40-fold increase in the number of alveolar epithelial cells expressing the E1A gene when cases of severe emphysema were compared to resected lung tissue from unaffected smokers [125]. Adenovirus has been shown to amplify inflammation following cigarette smoke exposure [70]. Guinea pig lung model data has shown that latent adenovirus-5 infection leads to enhanced inflammation following cigarette smoke exposure [126]. Adenoviral E1A protein alters host gene expression with subsequent increase of inflammatory mediators including IL-8 and ICAM-1 [127]. Transfection of pBECs with adenoviral E1A protein and subsequent stimulation with lipopolysaccharide leads to increased ICAM-1 expression and IL-8 mRNA compared to controls [128]. In addition, E1A transfection of pBECs increases NF-kB binding activity compared to controls [128]. ICAM-1 promoter activity has been shown to increase up to three-fold following stimulation with TNF-α and IFN-γ [129]. In stable E1A transfectants, ICAM-1 promoter activity is 2 to 2.5 times higher than controls [129]. These findings suggest that E1A can modulate ICAM-1 within bronchial epithelial cells and that this modulation differs in cells of alveolar origin [129].

Interestingly, adenovirus significantly reduces IL-6 and IL-8 release following pre-exposure to common bacterial species such as NTHi and *P. aeruginosa* [130]. Adenoviral proteins E1A and E1B have been shown to dysregulate IL-6 and IL-8 in separate experiments [131, 132]. E1A plasmid transfection induces increased expression of connective tissue growth factor (CTGF) and transforming growth factor (TGF)-β1 mRNA from pBECs compared to control cells [67]. This increase in CTGF appears to occur independently from the pathway through which TGF-β1 induces CTGF expression. Mesenchymal cells are the primary source of TGF-β1 within the airway. The presence of E1A also leads to expression of mesenchymal markers, α-smooth muscle actin and vimentin, potentially suggesting the E1A gene may lead to a shift of airway epithelial cells towards a mesenchymal phenotype [67].

Epstein–Barr virus in COPD

EBV is an enveloped, ds-DNA virus estimated to be present in 95% of the global population [133]. The EBV genome consists of a toroid shaped DNA core surrounded by a nucleocapsid, a lipid bilayer envelope embedded with external glycoprotein spikes, and a protein tegument between the nucleocapsid and the envelope [134]. EBV is a gamma herpes virus that has a complex relationship with the human immune system. The virus has a latent and a shedding phase. When latent, it resides in the B-lymphocytes for years at very low copies (20–100 copies per million B-cells) [135]. During the lytic phase, it infects the respiratory epithelial cells, which shed millions of viruses. EBV latent membrane protein-1 induces CD54 (ICAM-1) on epithelial cells [136]. ICAM-1 is the principal ligand for transmigration of neutrophils into the airway by binding neutrophil CD18/CD11b [137]. ICAM-1 is also the receptor for HRV, which has been identified as the commonest cause of AECOPD [11]. Indeed, infection with rhinovirus then further increases ICAM-1 expression [138]. The bacterium *H. Influenzae* binds to ICAM-1 and is the most common bacterial pathogen in COPD. Moreover, EBV infection is associated with increased granulocyte-macrophage colony stimulating factor (GM-CSF) synthesis by monocytes. These monocytes are inhibited from maturing by EBV, adding insult to injury, as the mature pulmonary macrophages are responsible for antigen presentation, a key immune regulatory role for infection. GM-CSF activates neutrophils which more readily migrate across the endothelium and the airway epithelium. However, EBV causes increased apoptosis of these neutrophils. Thus, it is apparent that EBV, in its epithelial shedding phase, sets the scene for increased activation of the epithelium, propensity for further viral infection, adherence of *H. influenzae* and failure of antigen presentation of bacteria and viruses, as well as airway neutrophil recruitment and apoptosis. Furthermore, it has adapted to avoid antiviral responses by expressing LMP-1 (immediate early protein) and BZLF-1 which are located on the cell wall. These inhibit IFN regulatory factor-7, subverting the synthesis of virus inhibiting IFN-α and -β [139, 140].

All the studies to date have focused on high levels of the virus being shed in the airway; the epithelial shedding phase or in the epithelium itself. A study of seroprevalence in military recruits found EBV is more common in poorer socioeconomic groups and in smokers. Poorer smokers are the very group who go on to develop COPD [141]. Furthermore, cigarette smoke promotes viral replication.

EBV has been identified in lung biopsy specimens of subjects with idiopathic pulmonary fibrosis [142]. EBV can frequently be detected in the lower respiratory tract specimens of patients admitted to the intensive care unit with hypoxic respiratory failure [143]. Lower respiratory tract detection of EBV does not appear to be associated with unfavourable clinical outcomes; however, positive EBV serology in this patient group was associated with a significant increase in mortality [143]. Interestingly, the $CD3^+$ T-cell count was reduced below the normal range in EBV-positive cases [143]. These findings may suggest an association between EBV and impaired anti-viral mechanisms within the lower respiratory tract. Impaired host defence mechanisms have been suggested to play a role in the persistent airway epithelial inflammation that occurs in COPD. Polosukhin *et al.* [73] found EBV in the airway epithelium of patients with COPD using *in situ* hybridisation. When examined by severity of disease, 68.2% of patients with Global Initiative for Chronic Obstructive Lung Disease stage I and II COPD compared to 84.4% of stage III and IV patients had EBV in their airway epithelium [73]. A low secretory immunoglobulin (Ig)A was identified and this was attributed to the cause of EBV in the airway [73]. However, IgA is an antibacterial antibody, it is required for inoculation of EBV into lymphocytes. EBV is a lymphotropic virus which affects the B-cells. The relationship of EBV and low IgA could potentially be causal (rather than low IgA causing proliferation of EBV). Indeed, in X-linked aggamaglobulinaemia patients are not infected with EBV [144]. In the search for chronic viruses in COPD, EBV has been found in the sputum of several groups of patients, with differing degrees of disease severity [72] and EBV DNA is more frequently detected in COPD than in unaffected smokers [72]. Furthermore, EBV is detectable both during stable and exacerbated disease states suggesting that EBV has persistence within the airway epithelium in COPD [72].

Conclusion

A new appreciation has emerged for the heterogeneity that exists in COPD. It is clear that historically favoured disease mechanisms such as protease/anti-protease imbalance and oxidative stress can only partially explain COPD pathogenesis. Accumulating evidence demonstrates that the majority of COPD exacerbations are associated with respiratory viruses [11]. Given that exacerbations lead to FEV_1 decline, drive disease progression and increase risk of mortality this directly implicates acute respiratory virus infection in disease pathogenesis. However, despite the huge economic burden of COPD exacerbations, the mechanisms underlying virus-induced exacerbations remain relatively poorly elucidated and current anti-viral therapies are limited. The majority of acute treatments are supportive and antimicrobial prescribing is often empirical in the absence of diagnostic microbiological samples. In the age of personalised medicine a more targeted therapeutic approach is mandated and further research should focus on developing pathogen-specific therapy for viral induced exacerbations There is growing interest in the Th2 signalling pathway in COPD with a degree of emphasis placed on the presence of peripheral blood eosinophilia. Recent clinical trial data indicates that anti-Th2 cytokine therapies offer limited benefit in COPD. However, these treatments are costly and require further evaluation [145–147]. Similarly, the current widespread use of ICS in COPD requires urgent attention as emerging data continues to draw our attention to the potential for harm through impairment of antiviral immunity and risk of pneumonia [64, 65]. $CD8^+$ T-lymphocytes provide adaptive immune defence against viral pathogens; however, they also pose the potential to perpetuate inflammation and may promote tissue injury through direct cytotoxic effects, pro-inflammatory signalling and recruitment of other immune cells (macrophages, neutrophils and possibly eosinophils) [34, 36, 37]. $CD8^+$ T-cells normally undergo Fas-induced apoptosis after a viral pathogen has been eradicated. Consequently, the persistence of these adaptive immune cells within the airway epithelium in COPD has led many to the hypothesis that a chronic virus may be present. In 2004 Hogg *et al.* [13] demonstrated a significant correlation between spirometric COPD severity and the presence of adaptive immune cells within the airway epithelium and their organisation into follicles, leading the authors to postulate that there may be "immune surveillance" of the airway in COPD. However, paradoxically there appears to be increased susceptibility of the airway epithelium to respiratory virus infection. More recent hypotheses have suggested that genetic differences in mucosal immunity between individuals may render subjects with COPD less able to eradicate respiratory viral infections [148]. In conclusion, acute viruses are associated with a significant proportion of COPD exacerbations and appear to play an important role in disease progression whilst the possibility of a chronic viral infection in COPD remains a tempting prospect that requires further exploration.

Conflict of interest: D. Linden has received grants (salary and research costs) from Mater Hospital YP Trustees. H. Guo-Parke has received grants from Pfizer. P.V. Coyle has nothing to disclose. D. Fairley reports grants to support

BHSCT/QUB funding during the conduct of the study, and other support from Hibergene Diagnostics Ltd, outside the submitted work. D.F. McAuley has received a grant from the BHSCT YPC Trust, Mater Hospital for to undertake a clinical trial; personal fees (for consultancy) from GlaxoSmithKline, SOBI, Peptinnovate, Boehringer Ingelheim and Bayer; institutional grants from the UK NIHR, Wellcome Trust and other funding from GlaxoSmithKline; and is one of four named inventors on a patent US8962032 covering the use of sialic acid–bearing nanoparticles as anti-inflammatory agents issued to his institution (Queen's University of Belfast). This has no direct impact on the contents of this work. C.C. Taggart has received grants from Pfizer UK and other support for consultancy from Albumedix. J. Kidney has received grants from Pfizer UK and Mater Hospital YP Trustees. He is also the non-executive director and shareholder of Hibergene Diagnostics Ltd.

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