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Antiviral Research 102 (2014) 95–105

Contents lists available at ScienceDirect

Antiviral Research

journal homepage: www.elsevier.com/locate/antiviral

Review

Experimental rhinovirus infection in COPD: Implications for antiviral therapies

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ARTICLE INFO

Article history:
Received 16 October 2013
Revised 28 November 2013
Accepted 14 December 2013
Available online 24 December 2013

Keywords:
Chronic obstructive pulmonary disease (COPD)
Exacerbations
Respiratory viruses
Rhinovirus

ABSTRACT

Chronic obstructive pulmonary disease (COPD) is a major public health problem and will be one of the leading global causes of mortality over the coming decades. Much of the morbidity, mortality and health care costs of COPD are attributable to acute exacerbations, the commonest causes of which are respiratory infections. Respiratory viruses are frequently detected in COPD exacerbations but direct proof of a causative relationship has been lacking. We have developed a model of COPD exacerbation using experimental rhinovirus infection in COPD patients and this has established a causative relationship between virus infection and exacerbations. In addition it has determined some of the molecular mechanisms linking virus infections to COPD exacerbations and identified potential new therapeutic targets. This new data should stimulate research into the role of antiviral agents as potential treatments for COPD exacerbations. Testing of antiviral agents has been hampered by the lack of a small animal model for rhinovirus infection and experimental rhinovirus infection in healthy volunteers has been used to test treatments for the common cold. Experimental rhinovirus infection in COPD subjects offers the prospect of a model that can be used to evaluate the effects of new treatments for virus-induced COPD exacerbations, and provide essential data that can be used in making decisions regarding large scale clinical trials.

Contents

1. Chronic obstructive pulmonary disease: aetiology and pathophysiology

Chronic obstructive pulmonary disease (COPD) is defined as a treatable and preventable disease characterised by progressive airflow limitation and an enhanced airway inflammatory response (Vestbo et al., 2013). It is the most common chronic respiratory condition in adults and it is estimated that 65 million people have moderate to severe COPD resulting in 3 million deaths in 2005. COPD develops in response to cumulative exposure to inhaled noxious particles or gases that trigger pathological responses in the lungs that eventually lead to the development of the disease. There are a number of aetiological agents that are associated with the development of COPD. In Western countries the prevalence of

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http://dx.doi.org/10.1016/j.antiviral.2013.12.006

COPD is strongly related to cigarette smoking as tobacco smoke is the main aetiological agent. From surveys carried out in developing nations it has become apparent that the relationship between cigarette smoking and COPD is less strong in these countries (Buist et al., 2007; Menezes et al., 2005), and other risk factors contribute to the development of COPD including exposure to burning of biomass fuels, outdoor air pollution and respiratory infections.

COPD is characterised by a number of pathological changes in the lungs that include parenchymal destruction (emphysema), inflammation of large airways (chronic bronchitis), inflammation and destruction of small airways (bronchiolitis) and mucous hypersecretion (Hogg and Timens, 2009). These pathological changes lead to the characteristic physiological abnormalities of airflow obstruction (manifested by a reduction in the forced expiratory volume in 1 s (FEV_1), and a reduction in the ratio of the FEV_1 to the forced expiratory volume (FVC)), hyperinflation and impaired gas exchange that eventually lead to respiratory failure.

There are three main processes in the lungs that drive development and progression of the disease namely pulmonary inflammation, oxidative stress and protease/antiprotease imbalance. Exposure to inhaled irritants such as cigarette smoke triggers an inflammatory response in the lungs and in those individuals that develop COPD this response is exaggerated. Studies comparing smokers with and without COPD have demonstrated greater numbers of neutrophils, macrophages and CD8+ T cells in the lungs of COPD patients (Decramer et al., 2012; Di Stefano et al., 1996, 1998). These inflammatory cells release a host of biological mediators including proteases such as neutrophil elastase and matrix metalloproteases, whilst at the same time the antiprotease defences of the lung are impaired (Pons et al., 2005). This protease/antiprotease imbalance results in uninhibited proteolytic activity and destruction of lung parenchyma. High levels of reactive oxygen species are generated in COPD from both exogenous sources (tobacco smoke) and endogenous sources (inflammatory cells). When these overwhelm the lungs' anti-oxidant defences oxidative stress results and induces multiple biological effects including induction of pro-inflammatory cytokines and chemokines, mucous hypersecretion, activation of proteases and damage to cellular components including phospholipids, proteins and nucleic acids (Chiba et al., 2012). Therefore the processes of airway inflammation, oxidative stress and protease excess are interlinked and contribute to the development of COPD.

Although COPD develops in response to inhaled noxious agents, once the disease has developed it appears to be autonomous of the original stimulus. Studies of ex-smokers with COPD have demonstrated that the airway inflammation is indistinguishable from COPD patients who continue to smoke (Gamble et al., 2007). Therefore it has been suggested that other mechanisms such as autoimmunity and infection may perpetuate the on-going inflammation in COPD, even after exposure to the initiating agent has been removed (Decramer et al., 2012).

2. Public health importance of COPD and its current and future economic impact

COPD is an enormous public health problem and its impact is expected to increase in the future. In 2002 COPD was the fifth leading cause of death worldwide and is predicted to be the 4th leading cause of death by 2030 (Mathers and Loncar, 2006). COPD develops after many years of exposure to the relevant aetiological agent and therefore the current prevalence of COPD reflects exposure to risk factors that has occurred in previous decades. In Western countries the prevalence of COPD is expected to remain stable for some years despite reductions in smoking rates. This reflects previous smoking rates, an ageing population and improvements in therapies for respiratory and cardiovascular diseases that have reduced mortality in COPD (Feenstra et al., 2001). Much of the increase in the global prevalence of COPD in the future is expected to occur in middle-income countries with large populations such as China, India, Turkey, South Africa and Indonesia. Smoking rates in these countries remain high, and there is also a high burden of other risk factors such as use of biomass fuels, outdoor pollution and respiratory infections. Therefore in these countries a 'perfect storm' of risk factors will contribute to a continuing global epidemic of COPD for the foreseeable future (Finney et al., 2013; van Zyl Smit et al., 2010).

3. COPD exacerbations

COPD patients experience a varying level of chronic symptoms punctuated by periods of sustained acute deterioration during which they experience increases in dyspnoea, sputum production, sputum purulence and cough. These episodes are termed 'acute exacerbations' and are associated with increased airflow limitation and dynamic hyperinflation which can result in respiratory failure. The occurrence of exacerbations increases with increasing severity of the disease and some patients experience frequent exacerbations (Hurst et al., 2010). Exacerbations have considerable impact on patients and healthcare providers both during and after the acute episode, and reduction of exacerbations is a key therapeutic goal in COPD. COPD exacerbations are associated with considerable mortality with exacerbations requiring hospital admission having an in-hospital mortality rate of 11–24% (Almagro et al., 2002; Connors et al., 1996; Groenewegen et al., 2003), and 2-year mortality rates ranging from 22% to 49% (Almagro et al., 2002; Connors et al., 1996; Groenewegen et al., 2003). Exacerbations are associated with falls in lung function that are frequently prolonged and lung function may not return to baseline values for several weeks in some patients (Seemungal et al., 2000a). Frequent exacerbations are associated with an accelerated decline in lung function (Anzueto et al., 2009; Celli et al., 2008; Donaldson et al., 2002; Kanner et al., 2001), impaired quality of life (Seemungal et al., 1998) and increased likelihood of becoming housebound (Donaldson et al., 2005).

The healthcare costs and economic impact of COPD exacerbations are enormous. In the United States COPD exacerbations accounted for 1,254,703 hospitalizations in 2006 with an estimated cost of US$11.9 billion (Perera et al., 2012). Therefore prevention of COPD exacerbations is a major therapeutic target and a major unmet need in COPD management. Non-pharmacological treatments for prevention of exacerbations include smoking cessation, influenza and pneumococcal vaccination and pulmonary rehabilitation. The mainstays of pharmacological therapy are inhaled bronchodilators and inhaled corticosteroids (ICS). Clinical trials have demonstrated that these treatments reduce exacerbations (Calverley et al., 2007; Wedzicha et al., 2008), although the efficacy of ICS continues to be debated (Barnes, 2010; Suissa and Barnes, 2009). These treatments are not without adverse effects and there is some evidence to suggest that ICS use is associated with an increased risk of pneumonia in COPD (Barnes, 2010; Cates, 2013; Singanayagam et al., 2010). Treatment of the established exacerbation includes bronchodilators, systemic corticosteroids and antibiotics but their clinical benefits are modest and they are associated with considerable side effects. Therefore more effective treatments for both prevention and treatment of COPD exacerbations are urgently needed.

4. Pathophysiology of exacerbations

The pathological features of stable COPD are well described but the pathology of exacerbations is less well defined. Exacerbations

are associated with increased airflow obstruction and hyperinflation, and respiratory failure is a common feature of more severe exacerbations. It is commonly stated that airways inflammation is increased in exacerbations (Mackay and Hurst, 2013) but a close examination of the literature reveals that the results of studies are far from consistent. Some studies have reported increases in inflammatory cells and mediators in exacerbations (Bathoorn et al., 2009; Fujimoto et al., 2005; Mercer et al., 2005; Papi et al., 2006), whereas others have not (Bhowmik et al., 2000; Roland et al., 2001; Seemungal et al., 2000b). The role of oxidative stress in COPD exacerbations is also unclear with both increased (Antczak et al., 2012; Biernacki et al., 2003; Dekhuijzen et al., 1996; Drost et al., 2005; Oudijk et al., 2006; Tsoumakidou et al., 2005), and unchanged levels of markers of oxidative stress reported in exacerbations (Kersul et al., 2011; Koutsokera et al., 2009; van Beurden et al., 2003). There are a number of sources of variability in studies of COPD exacerbations that can account for these conflicting results including different aetiologies and exacerbation severities, variations in time to presentation and the effects of treatment. Overcoming these issues is challenging but in order to develop new, more targeted treatments for exacerbations a much better understanding of the mechanistic pathways of COPD exacerbations is required.

5. Aetiology of COPD exacerbations: role of respiratory virus infections

Most exacerbations are associated with respiratory infections and historically bacterial infections were considered the main infective cause, reflected in the high use of antibiotics in exacerbations (Sandhu et al., 2013). Epidemiological data has long suggested a link between COPD exacerbations and virus infection as exacerbations occur more commonly in the autumn and winter months, and between half and two-thirds of patients report coryzal symptoms with exacerbations (Falsey et al., 2006; Hurst et al., 2005; Seemungal et al., 2001). However in older studies respiratory viruses could only be detected in 10–30% of exacerbations (Buscho et al., 1978; Murphy and Sethi, 1992; Smith et al., 1980), apparently confirming the conventional wisdom that most exacerbations were related to bacterial infections. The studies evaluating the role of virus infection used culture and serological tests as these were the only diagnostic tools available at the time but the sensitivity of these tests is low, particularly for rhinoviruses.

Diagnostic methods based on the polymerase chain reaction (PCR) for amplification and detection of viral nucleic acids are significantly more sensitive in detecting viruses in the respiratory tract compared with culture and serology (Beckham et al., 2005; Sethi, 2011). The development of PCR-based diagnostics led to a re-evaluation of the role of respiratory viruses in COPD exacerbations. More recent studies using PCR have generally reported higher detection rates compared to older studies with the prevalence of viruses in COPD exacerbations varying between 22% and 60% (Table 1). Comparisons between studies are difficult due to differences in exacerbation severity, samples collected (sputum/nasal/nasopharyngeal) and the inclusion of PCR for new viruses such as human metapneumovirus and bocavirus in the more recent studies. However in the majority of studies the most frequently identified viruses are rhinoviruses, with influenza and respiratory syncytial virus (RSV) also commonly detected. Other viruses identified but much less frequently include parainfluenza viruses, coronaviruses, adenoviruses, and human metapneumoviruses. In all studies carried out in Europe apart from one (Dimopoulos et al., 2012) rhinoviruses are the most common viruses, whereas the viral aetiology of exacerbations in Asia and North America appears more diverse. Whether this is due to differences in circulating viruses or differences in influenza vaccination uptake between these regions is unknown.

Although these studies demonstrate an association between virus infection and COPD exacerbations they do not prove a causal relationship, as PCR detects viral nucleic acid and therefore does not prove the presence of live, replicating virus. Demonstration of lower airways inflammation in response to virus infection would provide further evidence for a causative role of viruses in COPD exacerbations, as it would provide evidence of a host immune response to infection. Conversely lack of an inflammatory response would suggest that the presence of a virus is simply a secondary event or epiphenomenon. The few studies investigating this have had conflicting results, with some reporting that virus infection is associated with an inflammatory response (Rohde et al., 2008; Seemungal et al., 2000b) whereas others do not (Bafadhel et al., 2011; Hurst et al., 2006; Pant et al., 2009). Evidence in favour of a causative relationship has been provided by case control studies demonstrating higher virus detection rates and higher virus loads in exacerbations compared to the stable state (Quint et al., 2010), but not all studies have included a control group (Table 1). Therefore although respiratory viruses can be detected in COPD exacerbations generally at greater frequency than in stable COPD, a definitive causal relationship between virus infection and exacerbations has not been established. Improved diagnostics and antiviral therapies make treatment of virus infections a realistic possibility in the near future, and therefore establishing the role of respiratory viruses in COPD exacerbations is no longer solely of academic interest.

6. Experimental rhinovirus infection in COPD

Definitive proof that respiratory viruses cause exacerbations will always be difficult to obtain with studies of naturally-occurring virus infections. Asthma is a respiratory disease that shares many of the features of COPD including the occurrence of acute exacerbations that are commonly associated with the detection of respiratory viruses (Johnston et al., 1996). The role of respiratory virus infections in asthma exacerbations is well established and this is in part due to the use of experimental rhinovirus infection as a model of virus-induced asthma exacerbations. Inoculation of carefully selected asthmatic volunteers with rhinovirus induces the clinical features of an asthma exacerbation (Bardin et al., 2000) and these studies have provided important insights into the mechanisms linking virus infections with asthma exacerbations (Contoli et al., 2006; Gern et al., 2000; Message et al., 2008). Moreover experimental rhinovirus infection studies have contributed to the and to the development of a novel treatment for asthma exacerbations with the identification of interferon deficiency in asthmatics (Contoli et al., 2006). This observation led to the development of inhaled interferon-β as a treatment for virus-induced asthma exacerbations and this has demonstrated efficacy in a Phase II clinical trial that has been published as a recent conference abstract (Boxall et al., 2013).

Experimental rhinovirus infection in asthma has been carried out by a number of different groups and has an excellent safety record (Bardin et al., 2000; Gern et al., 2000; Grunberg et al., 2001). In view of the frequent detection of rhinoviruses in COPD exacerbations our group aimed to establish whether experimental rhinovirus infection could be used to develop a similar model in COPD. However despite the excellent safety record of experimental rhinovirus infection in asthma there existed a number of concerns about experimental infections in COPD. COPD patients differ markedly from asthma patients in that they are older, are current or ex-smokers, have impaired lung function and their airflow obstruction is not reversible, all factors that have the potential to result in a

Table 1
Studies of respiratory virus infection in COPD exacerbations using PCR. The detection frequencies of the individual viruses are expressed as percentage of virus positive exacerbations. *Abbreviations:* OP – outpatient, IP – inpatient, ED – emergency department, ITU – intensive care unit, RSV – respiratory syncytial virus.

Country	Site of patient recruitment	Exacerbations with positive virus PCR (%)	Picorna/rhino-viruses (%)	Influenza (%)	RSV (%)	Other viruses (%)	Viruses in stable COPD	References
Hong Kong	IP	22	14	45	10	21	/	Ko et al. (2007)
Australia	OP/IP	22	78	9	3	9	2%	Hutchinson et al. (2007)
USA	ED	25	21	16	32	37	/	Camargo et al. (2008)
Australia	IP	26	70	5	5	20	/	Bozinovski et al. (2008)
Australia	ED	29	14	43	14	29	/	Pant et al. (2009)
UK	OP/IP	29	60	9	33	14	5%	Bafadhel et al. (2011)
Canada	OP/IP		24	29	13	32	4%	De Serres et al. (2009)
UK	IP	37	64	6	6	36	12%	McManus et al. (2008)
UK	OP	39	58	14	29	14	16%	Seemungal et al. (2001)
USA	OP/IP	42	48	20	9	16	/	Beckham et al. (2005)
USA	IP/ICU	30	69	13	6	6%	0%	Singh et al. (2010)
France	IP/OP	44	45	15	5	45	/	Perotin et al. (2013)
Australia	ICU	47	15	46	15	41	/	Cameron et al. (2006)
Italy	IP	48	55	23	13	23	6%	Papi et al. (2006)
Switzerland	IP	51	50	5	7	39	11%	Kherad et al. (2010)
Greece	IP	54	9	14	52	22	/	Dimopoulos et al. (2012)
Germany	IP	56	44	40	27	13	19%	Rohde et al. (2003)
Singapore	IP	64	33	56	0	11	/	Tan et al. (2003)

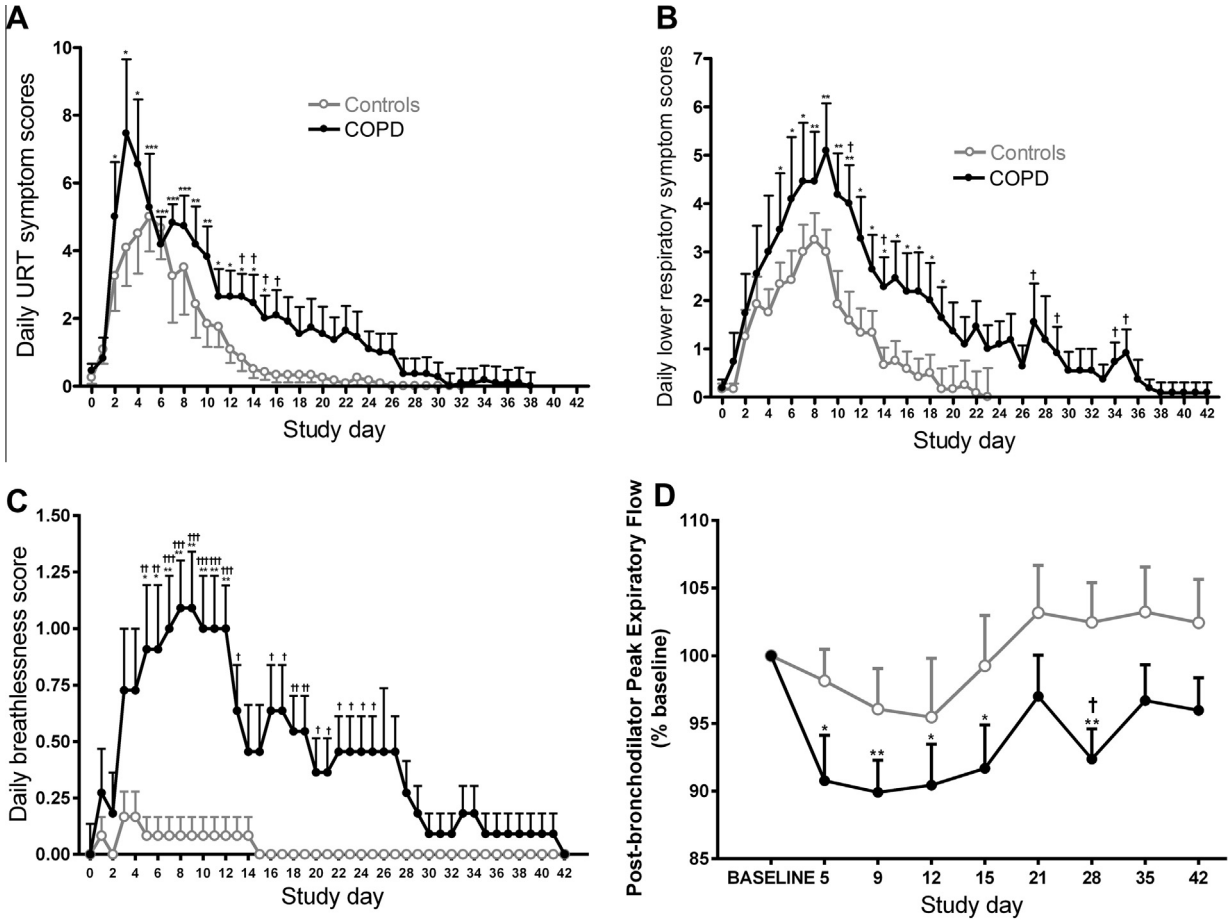

Fig. 1. Time course of symptoms recorded on daily diary cards and lung function during experimental rhinovirus infection in COPD subjects ($N = 11$) and smokers with normal lung function ($N = 12$). (A) Total daily scores for upper respiratory symptoms. (B) Total daily scores for lower respiratory symptoms. (C) Daily scores for the symptom of breathlessness. (D). Post-bronchodilator peak expiratory flow expressed as a percentage of baseline. All values are mean ± SE. $^{*}P < 0.05$ vs. baseline, $^{**}P < 0.01$ vs. baseline, $^{\dagger}P < 0.05$ COPD vs. controls, $^{\dagger\dagger}P < 0.01$ COPD vs. controls, $^{\dagger\dagger\dagger}P < 0.001$ COPD vs. controls (Mallia et al., 2011). Reprinted with permission of the American Thoracic Society.

more severe response to rhinovirus infection. Therefore we carried out a small pilot study to evaluate the effects and the safety of inoculation with a low dose of rhinovirus 16 in subjects with moderate COPD (GOLD stage II). Subjects kept daily diary cards where they recorded upper respiratory symptoms ('cold' symptoms) and the lower respiratory symptoms of cough, sputum volume and purulence, wheeze and breathlessness – the typical symptoms of COPD exacerbations with higher scores indicating more severe

symptoms. Following rhinovirus infection the subjects developed symptomatic colds and increases in lower respiratory symptoms, accompanied by increased airflow obstruction, increased inflammatory markers and the detection of rhinovirus nucleic acid in nasal lavage (Mallia et al., 2006). There were no adverse events such as severe exacerbations requiring treatment, hospitalisations or pneumonia and all the subjects recovered completely. Therefore this was the first demonstration that experimental rhinovirus infection in COPD subjects can safely induce the typical clinical features of a COPD exacerbation.

Following on from this small study we repeated the procedure in a larger group of COPD subjects, together with a group of smokers without COPD, in order to further evaluate the safety of rhinovirus infection and to investigate mechanisms of virus-induced exacerbations. We again successfully demonstrated that rhinovirus inoculation of COPD subjects induced both colds and increases in lower respiratory symptoms. The time course of upper and lower respiratory symptoms are shown in Fig. 1. Cold symptoms occurred early peaking on day 3 post-inoculation (Fig. 1A), whereas lower respiratory symptoms occurred later peaking on day 9, and were more prolonged taking up to 5 weeks to return to baseline levels (Fig. 1B). This mirrors what COPD patients often report in naturally-occurring exacerbations, i.e. that exacerbations are frequently preceded by an upper respiratory tract infection (Seemungal et al., 2001). The non-COPD subjects also had increases in lower respiratory symptoms but these were not as severe or as prolonged as in the COPD group. On analysing the individual symptoms there were no differences between the groups in symptoms of cough and sputum but only the COPD subjetcs reported increases in breathlessness (Fig. 1C).

Breathlessness is the key defining symptom of a COPD exacerbation and therefore rhinovirus infection causes an acute bronchitis in non-COPD subjects, but in patients with COPD it results greater physiological impairment that manifests itself as an acute exacerbation. This was confirmed by significant increases in airflow obstruction in the COPD subjects (Fig. 1D). Using quantitative PCR we measured virus load to analyse the relationships between symptoms and virus replication. Rhinovirus nucleic acid was detectable in nasal lavage fluid on average 2 days post-inoculation peaking on day 6 in nasal lavage and day 5 in sputum (Fig. 2). The kinetics of virus load in the airways provided powerful new evidence of a causative relationship between virus infection and COPD exacerbations, as previously studies had only demonstrated their presence of virus on a single time point. The rise in virus load following inoculation indicates that active viral replication is occurring in the airways. The temporal relationship between appearance of virus in the airways and onset of symptoms and airflow obstruction, and then virus clearance followed by resolution of clinical illness provided strong evidence of a causal relationship between virus infection and exacerbations.

There were significant increases in neutrophils, neutrophil elastase and the neutrophil chemokine CXCL8 in sputum and reduced expression of adhesion molecules on blood neutrophils in the COPD subjects (Mallia et al., 2013b). Lymphocytes in bronchoalveolar lavage in the COPD subjects but not in the non-COPD group increased following rhinovirus infection (Mallia et al., 2011), and these were predominantly CD3+ and CD8+ T cells (Mallia et al., 2013a). There was a significant relationship between peak virus load in sputum and peak levels of inflammatory markers with correlations between sputum virus load and sputum levels of CXCL8, IL-6, TNF-α and sputum neutrophils, indicating that virus infection drives inflammation in exacerbations (Mallia et al., 2011). In a subsequent infection study we replicated these results and again demonstrated consistent induction of exacerbations and airways inflammatory markers by rhinovirus infection, with more severe symptomatic, physiological and inflammatory responses compared to non-COPD subjects (unpublished data).

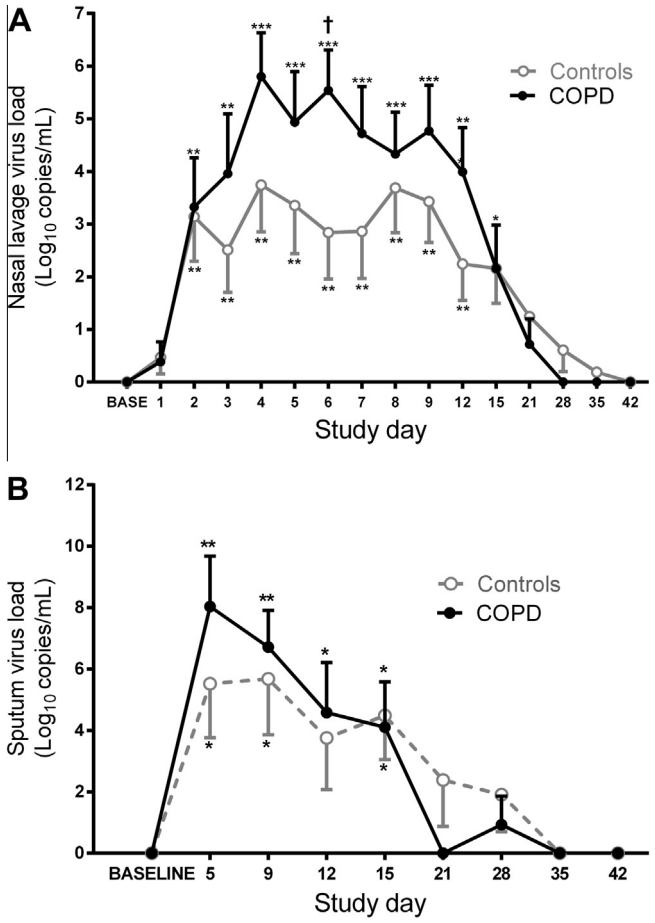

Fig. 2. Virus load in nasal lavage and sputum samples measured with quantitative PCR. (A) The time course of virus load in nasal lavage. (B) The time course of virus load in sputum. All values are mean ± SE. $^{*}P < 0.05$ vs. baseline, $^{**}P < 0.01$ vs. baseline, $^{***}P < 0.01$ vs. baseline, $^{\dagger}P < 0.05$ COPD vs. controls (Mallia et al., 2011). Reprinted with permission of the American Thoracic Society.

7. Susceptibility to virus infection in COPD

All subjects were inoculated with the same dose of virus via the same route of inoculation, but post-inoculation virus loads in the COPD group were higher compared to the non-COPD smokers (Fig. 2). In nasal lavage virus loads were up to 2 logs greater and this was statistically significant on day 6. This suggests that the immune mechanisms that control viral replication are deficient in COPD. The type I interferons (interferon-α and interferon-β) and the type III interferons (interferon-λ) are important mediators of innate immunity to virus infections in the respiratory tract. We examined the responses of bronchoalveolar lavage cells to virus infection by infecting them *ex vivo* with rhinovirus and measuring interferon production. Production of interferons in response to virus infection was impaired in bronchoalveolar lavage cells from COPD patients compared to the controls (Mallia et al., 2011). Our group has previously identified deficient interferon production in asthma, and related this to outcomes following experimental rhinovirus infection (Contoli et al., 2006). The main site of virus replication in the airway are airway epithelial cells and *in vitro* studies have not demonstrated impaired production of interferons in response to virus infection in COPD epithelium (Baines et al., 2013; Schneider et al., 2010). However impaired interferon pro-

duction in response to rhinovirus infection has also been reported in a mouse model of COPD (Sajjan et al., 2009). Therefore the mechanisms of impaired antiviral immunity in COPD require further investigation as they offer new therapeutic options for COPD exacerbations.

8. Rhinovirus infection and secondary bacterial infection

Both bacterial and viral infections are common in COPD but few studies have examined the role of dual virus-bacterial infection in COPD exacerbations. The studies that are available reported dual infection in a minority of exacerbations, casting doubt on whether it plays a significant role in COPD exacerbations (Table 2). All these studies collected samples on a single time point apart form that by Hutchinson et al. In this study patients were sampled at the onset of exacerbation and again 5–7 days later, and 36% of exacerbations in which a virus was detected at the initial time point developed secondary bacterial infection. 71% of patients with bacterial exacerbations had reported symptoms of a viral upper respiratory tract infection prior to onset (Hutchinson et al., 2007). Therefore the studies in which a single sample was collected may have underestimated the true prevalence of dual infection if viral and bacterial infections do not occur concurrently.

We carried out a second experimental infection study that also included a group of non-smokers and used the samples from the 2 studies to investigate relationships between rhinovirus infection and secondary bacterial infection in COPD. Following rhinovirus infection bacterial infection was detected in sputum in 60% of subjects with COPD, compared to 9.5% of smokers and 10% of non-smokers (Mallia et al., 2012). Moreover whereas the peak of virus load occurred on days 5 and 9 post-inoculation, peak bacterial load was on day 15. Airflow obstruction, breathlessness and airway inflammation occurred irrespective of the presence of secondary bacterial infection and peaked at the time of initial viral infection, but recovery time was more prolonged in subjects with dual infection and persisted to day 21 when viral load was undetectable (Mallia et al., 2012). Therefore secondary bacterial infections may play a role in prolonging the course of virus-induced exacerbations. The predominant organisms cultured were *Haemophilus influenzae* and *Streptococcus pneumoniae*. Using molecular methods of bacterial detection we demonstrated a rise in bacterial burden and a significant outgrowth of *Haemophilus influenzae* from the existing microbiota of COPD subjects (Molyneaux et al., 2013).

To investigate the mechanisms of post-viral secondary bacterial infections we measured levels of antimicrobial peptides in sputum. Those COPD subjects who developed secondary bacterial infection had increased levels of neutrophil elastase and lower levels of the antimicrobial peptides secretory leukoprotease inhibitor (SLPI) and elafin (Mallia et al., 2012). Neutrophil elastase degrades SLPI and elafin and therefore this suggests a mechanism linking virus infection to increased susceptibility to bacterial infection in COPD.

Therefore these results demonstrating that bacterial infection follows an initial virus infection with a delay of 6–10 days between the two, suggests that studies that collect a single sample during an exacerbation will underestimate the true contribution of dual virus/bacterial infections to COPD exacerbations. This was confirmed in a recent conference abstract where George et al. reported that in COPD exacerbations where rhinovirus was detected at exacerbation onset, bacteria were frequently detected in subsequent sputum samples and peaked at day 14 post-exacerbation onset (George et al., 2013).

Therefore we have demonstrated that experimental rhinovirus infection induces the clinical features of exacerbations in COPD subjects and provided novel *in vivo* evidence of the mechanisms of virus-induced exacerbations. However this model does have a number of limitations in that, for ethical reasons, experimental rhinovirus infection can only be carried out in subjects with mild or moderate COPD, whereas the patients most susceptible to exacerbations are those with more severe disease. It is not yet known whether the mechanisms of virus-induced exacerbations are the same irrespective of COPD severity. Also it is not known whether exacerbation mechanisms differ according to the aetiology. There may be a common exacerbation inflammatory pathway or the mechanisms may be different with different viruses, bacteria or non-infectious aetiologies such as air pollution. Establishing this will determine whether the results of this model are more widely applicable beyond rhinovirus infection only or not. Notwithstanding these limitations the evidence that there is a causative relationship between respiratory virus infection and exacerbations in COPD patients indicates that antiviral therapies may be potential treatments for COPD exacerbations. The link between virus infections and secondary bacterial infections also holds out the intriguing prospect that antiviral treatments may reduce bacterial infections and therefore result in less use of antibiotics.

Table 2
Studies of viral/bacterial co-infection in COPD exacerbations.

Percentage of exacerbations with virus infection	Percentage of exacerbations with bacterial infection	Percentage of exacerbations with both viral and bacterial infection	
43	23	6.5	Cameron et al. (2006)
53.5	13.5	7	Dimopoulos et al. (2012)
29	25	8	Pant et al. (2009)
51	64	11.5	Kherad et al. (2010)
21	30	12	Hutchinson et al. (2007)
31	49	13	De Serres et al. (2009)
29	55	13	Bafadhel et al. (2011)
29	38	15	Bozinovski et al. (2008)
24	76	17	Hurst et al. (2006)
48	55	25	Papi et al. (2006)
44	42	27	Perotin et al. (2013)

9. Treatments of COPD exacerbations: unmet clinical need

Current treatments for COPD exacerbations consist of supportive therapies, corticosteroids and antibiotics and no new treatments have been developed over the past 3 decades. The use of antibiotics is based on the assumption that bacteria are the main aetiological agents triggering acute exacerbations but debate about the relative role of bacteria and the efficacy of antibiotics continues (Vollenweider et al., 2012; Wedzicha, 2008). Bacterial infections certainly trigger a proportion of exacerbations and it is likely that these exacerbations will benefit from antibiotic treatment. A study of biomarker-directed antibiotic therapy in COPD exacerbations found that patients with low levels of procalcitonin recovered without antibiotics and using a biomarker-directed approach could reduce antibiotic use by 44% (Stolz et al., 2007). However antibiotics continue to be prescribed in the vast majority of COPD exacerbations and this contributes to the development of antibiotic resistance. Corticosteroids have considerable side effects with 1 adverse effect for every 5 patients treated with oral corticosteroids (Walters et al., 2009). Therefore new treatments for COPD exacerbations that are both more effective and safer are urgently required and antiviral drugs may offer a new therapeutic option for virus-induced exacerbations in COPD patients.

10. Development of antiviral drugs

A better understanding of the life cycle of respiratory viruses such as rhinoviruses has led to the identification of key events in

the viral life cycle and the development of molecules that target these. There are a number of points in the rhinovirus life cycle that offer potential targets for pharmacological intervention including viral attachment and uncoating, viral RNA synthesis and the viral proteases. Rhinoviruses are classified into 2 groups based on their receptor use. The majority of rhinovirus serotypes use intercellular adhesion molecule-1 (ICAM-1) as their receptor (major group rhinoviruses), whereas a minority bind to the low density lipoprotein (LDL) receptor (minor group rhinoviruses) (Kennedy et al., 2012). Recently a new group of rhinoviruses has been identified that has been termed human rhinovirus species C, but the cellular receptor for this group remains unknown (Bochkov and Gern, 2012). Following binding to cellular receptors conformational changes occur in the capsid and viral entry occurs via endocytosis. After uncoating, rhinovirus proteins are synthesized by translation of the viral nucleic acid and the resulting polyprotein is cleaved by viral proteases into 4 structural (VP1-VP4) and 7 non-structural proteins. The viral capsid is composed of 3 outer structural proteins VP1, VP2, and VP3 and an interior protein VP4 that anchors the RNA core to the capsid.

A number of molecules have been identified that demonstrate activity against rhinoviruses *in vitro* however the majority of these molecules have never progressed to use in man. One reason for this is the lack of robust small animal models of rhinovirus infection. Major group rhinoviruses only bind to human ICAM-1 and therefore do not infect mice. Our group has developed an experimental animal model of rhinovirus infection using minor group viruses in wild-type mice and major group viruses in transgenic mice expressing human ICAM-1 (Bartlett et al., 2008). Infected mice demonstrated increased bronchoalveolar lavage neutrophils and lymphocytes, chemokines and cytokines and increased viral RNA. In addition combining rhinovirus infection with ovalbumin sensitisation of mice provides a model of virus-induced asthma exacerbations. We have recently demonstrated that use of an anti-ICAM-1 antibody in the mouse model decreased inflammatory cells and cytokines in bronchoalveolar lavage and lung homogenate, and significantly reduced rhinovirus replication (Traub et al., 2013). This model has also been used to evaluate the effect of immunization with a recombinant rhinovirus capsid protein on cellular and humoral immune responses to rhinovirus infection (Glanville et al., 2013). Therefore this was the first successful demonstration of a therapeutic intervention in a small animal model of rhinovirus infection and holds out the prospect of testing compounds with *in vitro* activity in animals prior to use in humans.

Due to the lack of animal models the experimental human challenge model has been used to investigate the *in vivo* effects of agents that have demonstrated antiviral activity *in vitro* (Hayden and Gwaltney, 1982, 1984; Hayden et al., 1988, 2003b; Levandowski et al., 1982; Phillpotts et al., 1983; Turner et al., 1993, 1999). The majority of these have not been successful, or have been limited by adverse effects and have not undergone further development. However studies using experimental rhinovirus infection were essential in providing *in vivo* clinical data regarding the efficacy and adverse effects of these drugs. The active site of viral proteases is highly conserved amongst serotypes and has no homology with mammalian proteases making it an attractive target for antiviral therapy.

11. Studies of antirhinovirus agents

The viral proteases are attractive targets for antiviral drug development due to their essential roles in viral replication and their unique protein structures. The 3C protease inhibitor rupintrivir was identified as having highly active antiviral activity *in vitro* and underwent clinical assessment in experimental infection studies. Rupintrivir prophylaxis reduced the proportion of subjects with positive viral cultures by 26% and reduced viral titres, but had no effect of the frequency of symptomatic colds. Rupintrivir treatment commenced 24 h after inoculation reduced the mean total daily symptom score by 33% and significantly reduced viral titers and nasal discharge weights, but had no effect on the frequency of colds (Hayden et al., 2003b). Based on these results rupintrivir did not undergo further development as an antiviral agent for rhinovirus infections.

Preventing cellular virus attachment by receptor blockade utilising soluble forms of ICAM-1 demonstrates antiviral effects *in vitro* and a soluble ICAM molecule tremacamra was developed for use *in vivo* and underwent clinical trials. In experimental infection studies tremacamra reduced total symptom scores by 45% and the proportion of subjects with clinical colds by 23%, and reduced virus titers and CXCL8 levels in nasal lavage fluid (Turner et al., 1999). The effect was the same when the drug was administered either prior to or after rhinovirus challenge. However the drug required a 6 times daily administration and will not be effective against minor group and group C rhinoviruses as these utilise other receptors for cell entry. Tremacamra has not undergone further clinical development.

Analysis of the 3-dimensional structure of the rhinovirus led to identification of a small hydrophobic pocket within VP1 beneath the ICAM-binding region. Molecules that bind to this area (capsid-binding agents) induce conformational changes in this pocket thus hindering virus-receptor interactions and preventing attachment to host cells. The first drug in this class to be tested was pirodavir that is administered 6 times a day via the nasal route. Intranasal pirodavir administered as prophylaxis reduced both infection rates and clinical colds (54% vs. 8%, $P = 0.03$) in subjects experimentally infected with rhinovirus. When treatment was commenced 24 h after rhinovirus challenge virus shedding was reduced but no clinical benefits were found (Hayden et al., 1992). Intranasal pirodavir was generally well tolerated but was associated with an excess rate of transient unpleasant taste. In a study of naturally-occurring colds no clinical benefits were seen and pirodavir was associated with higher rates of adverse effects including nasal dryness, nasal bleeding and unpleasant taste (Hayden et al., 1995). Pleconaril is an oral capsid-binding agent that is the only anti-picornavirus agent that has undergone large-scale, randomised, double-blinded, placebo-controlled phase III trials in healthy subjects with colds. In an initial experimental infection study with coxsackievirus pleconaril significantly reduced viral shedding and symptom scores (Schiff and Sherwood, 2000). Subsequently in studies carried out in naturally-acquired colds pleconaril resulted in a significant reduction in illness duration, a significantly shorter time to a 50% reduction of symptom severity and a 16% reduction in disturbed nights in subjects with confirmed picornavirus infection (Hayden et al., 2003a). However these modest clinical benefits were outweighed by concerns regarding the risks of drug interactions, particularly with oral contraceptives and pleconaril was not approved for treatment of the common cold by the US Food and Drug Administration (Senior, 2002). An intranasal form of pleconaril has been evaluated as a treatment for asthma exacerbations but the results of this study have not yet been published (ClinicalTrials.gov identifier NCT00394914, http://www.clinicaltrials.gov/ accessed 15th October 2013). The capsid-binding agent vapendavir reduced virus load in healthy subjects experimentally inoculated with rhinovirus 39 (Jacobs et al., 2013) and has undergone a Phase II clinical trial in asthmatics but the results have not yet been published (ClinicalTrials.gov identifier NCT01175226, http://www.clinicaltrials.gov/ accessed 15th August 2013).

12. Antiviral therapies in COPD

To date no drugs for the treatment of rhinovirus infections have been approved for clinical use (apart from use of pleconaril on a compassionate release basis). However the only clinical trials that have been reported have been conducted in healthy volunteers for treatment of the common cold. Pleconaril was not approved as the benefits in healthy subjects were felt insufficient to outweigh the risks. In patients with chronic lung diseases such as COPD the potential benefits are greater as treatment may prevent virus-induced exacerbations rather than just colds. Moreover concerns regarding contraceptive failure are less of an issue in this older patient group, and therefore the risk/benefit ratio may be considerably more favourable. However studies of pleconaril or other antiviral drugs need to be carried out specifically in COPD patients to determine whether they will in fact prevent virus-induced exacerbations. Our data demonstrating relationships between virus infection and lower respiratory symptoms and between virus load and inflammatory markers in the airways provide a strong theoretical basis that antiviral agents will be of clinical benefit in COPD. In addition our observation of frequent secondary bacterial infections following rhinovirus infections hold out the possibility that antiviral therapy may also reduce bacterial infections and antibiotic use.

Clinical studies of treatments for COPD exacerbations can be expensive and time consuming. A recent study of oral corticosteroids in COPD exacerbations recruited patients from 5 hospitals over 5 years and of 717 patients assessed for eligibility only 314 were randomised (Leuppi et al., 2013). A study of the TNF-α antagonist etanercept in COPD exacerbations recruited 81 patients over 3 years from 8 hospitals and had a negative result (Aaron et al., 2013). Therefore even relatively small clinical trials of novel therapeutic agents require a considerable financial investment and this may represent an obstacle to bringing new therapies to market. Moreover evidence is now emerging that exacerbations are heterogeneous with different aetiologies, inflammatory mediators (Bafadhel et al., 2011) and responses to treatment (Bafadhel et al., 2012). In naturally infected COPD patients it may not be possible to detect a beneficial effect of an effective antiviral drug against a background of differences in the route, dose and timing of exposure to a virus, presence or absence of other concurrent infections, effects of other treatments, differences in time to presentation etc.

In the same way that experimental rhinovirus infection in healthy subjects has been used to assess treatments for colds, such an approach could be used to evaluate treatment effects on virus-induced COPD exacerbations. Experimental rhinovirus infection in COPD patients offers a model in which the route, dose and time of infection are the same for all subjects and treatment can be started early in the course of the infection. Therefore many of the sources of variability inherent in clinical trials of naturally-occurring exacerbations can be eliminated. In addition the ability to carry out intensive and frequent clinical sampling allows for analysis of the effects of treatment on a large number of outcomes including symptoms, lung function, virus load, inflammatory markers and secondary bacterial infections. Demonstration that a treatment reduces symptoms, virus load or inflammation would provide evidence of a biological effect that may translate into a clinically relevant effect. Conversely if a treatment had no effect on these parameters a clinically significant effect is unlikely. In addition safety data can be obtained in the specific patient group that the drug is aimed at. Therefore testing a new treatment in the experimental infection model has the potential to demonstrate potential efficacy (or lack of) in a small number of subjects in a relatively short time period with a much smaller financial commitment. Data obtained from such studies can then be used to inform decisions as to whether to proceed to larger clinical trials in naturally-occurring exacerbations. As experimental rhinovirus infection in COPD is a relatively new model such studies have not yet been carried out, but our work has established this as a robust, valid and safe model and therefore provided the basis on which such studies can be carried out in the future.

13. Conclusions

New treatments for COPD exacerbations are urgently needed and the link between respiratory virus infections and COPD exacerbations has highlighted the potential of antiviral agents as treatments for virus-induced exacerbations. To date there are no published studies of antiviral agents in COPD and therefore their effect is unknown. Experimental rhinovirus infection in healthy subjects has been used to evaluate the effects of antiviral drugs due to the lack of robust small animal models. We have developed a model of COPD exacerbation using experimental rhinovirus infection in COPD and established this as a valid model of virus-induced COPD exacerbations. This model could be used to evaluate new antiviral treatments in COPD and provide evidence regarding their effectiveness in treating virus-induced COPD exacerbations. Therefore experimental rhinovirus infection in COPD is a potential tool that can aid translational development of new therapies for COPD exacerbations.

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