Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.
CHAPTER 8

In vivo experimental models of infection and disease

Jason Girkin¹, Steven Maltby¹, Aran Singanayagam², Nathan Bartlett¹ and Patrick Mallia²
¹Priority Research Center for Healthy Lungs, Faculty of Health and Medicine, University of Newcastle, Newcastle, Australia
²Faculty of Medicine, National Heart & Lung Institute, Imperial College London, London, United Kingdom

8.1 HUMAN MODELS OF RHINOVIRUS INFECTION

Experimental infection of human subjects with rhinovirus (RV) has long been used to study the pathogenesis of infection. Indeed, such studies were carried out even before RVs were identified as causative agents of the common cold. In 1914 almost 40 years prior to identification of RV, Kruse induced colds in volunteers by inoculating them with a filtrate of nasal washings.¹ Following the identification of RVs, viral challenge studies were used extensively in healthy volunteers to study numerous aspects of RV biology including viral infectivity, modes of transmission, role of environmental factors, host immune responses, and the effects of treatments. In the 1990s these studies were extended to subjects with asthma to study the pathogenesis of RV-induced asthma exacerbations, and over the last decade experimental RV infection has also been carried out in patients with chronic obstructive pulmonary disease (COPD). These studies have provided unique insights into the pathogenesis of RV infection that would have been difficult to obtain using studies of naturally acquired infections or in animal models.

8.2 RATIONALE FOR HUMAN INFECTION STUDIES

Viral respiratory tract infections are the commonest infectious syndrome in humans with adults experiencing two to four infections per annum. Given the frequency of viral infections it is pertinent to ask why studies
that deliberately expose humans to an infectious agent are required, when there are more than enough naturally acquired infections to yield sufficient subjects for studies. Despite the ubiquity of viral colds there are a number of factors that make studies of naturally acquired infections problematic. Although RVs are the commonest etiological agents of viral colds there are several other viral causes (and nonviral causes of upper airway symptoms), and the clinical syndromes caused by different virus types are indistinguishable.\textsuperscript{2,3} Other factors contributing to variability in naturally acquired infections include different routes of inoculation (eye, nasopharynx, direct contact, aerosol, etc.), different inoculation doses, variability in the perception of symptoms leading to differences in time to presentation and host factors (immune status, smoking, age, etc.) that influence viral pathogenicity. Further, the heterogeneous nature of naturally occurring infections requires that large patient numbers are needed to identify statistically significant effects of treatment. Therefore human experimental infection studies are an attractive proposition as they allow for a known etiological agent to be administered at a standard dose, route of inoculation, and time point to a selected group of recipients with similar characteristics (e.g., age, smoking history, health/disease, and antibody status). Detailed follow up can be carried out in a controlled clinical setting with sample collection at defined time points in relation to the time of infection. As the clinical syndrome induced by RV challenge in young healthy volunteers is benign and self-limiting, experimental RV infection in this group is relatively uncontroversial. Perhaps the only risk to subjects was the possibility of additional infectious agents present in the inoculum and good manufacturing practice (GMP)-prepared stocks are now required by regulators for experimental infection studies in humans to contravene this risk.\textsuperscript{4} Studies in healthy volunteers have been central to establishing the key aspects of the biology of RV infection including routes of acquisition of infection,\textsuperscript{5–8} clinical symptoms,\textsuperscript{8,9} inflammatory and immune responses,\textsuperscript{10} involvement of the lower airway,\textsuperscript{10,11} and correlates of immune protection of RV infection.\textsuperscript{12}

Virus challenge studies have also been used in healthy volunteers to evaluate a vast array of potential treatments. However, none of these studies have led to the licensing of a single treatment for the common cold.\textsuperscript{13–51} Licensing approval was sought for an antiviral drug, pleconaril, for the treatment of RV infection.\textsuperscript{21} Approval was denied by the Food and Drug Administration as the adverse effects outweighed the
benefits in healthy subjects with self-limiting colds. The lack of approval of any antiviral treatments casts doubt on whether continued investment in viral challenge studies is justified. However, the recognition that RV infection is associated with more severe clinical manifestations in people with chronic lung diseases such as asthma and COPD provided a new impetus to research and a new direction to human experimental infection studies.

8.3 RHINOVIRUS INFECTION AND EXACERBATIONS OF ASTHMA AND CHRONIC OBSTRUCTIVE PULMONARY DISEASE

Up until the early 1990s the prevailing view was that RV infection resulted in a self-limiting, mild upper respiratory tract syndrome only. There were occasional reports of RV infection associated with more severe clinical illness such as pneumonia but both scientific and pharmacological research tended to be focused on other respiratory viruses such as influenza and respiratory syncytial virus, as these were considered to be more serious human pathogens. Colds had long been associated with asthma exacerbations but early studies investigating virus infection in asthma and COPD exacerbations reported low detection rates. The consensus was that asthma exacerbations were predominantly triggered by allergen exposure and COPD exacerbations by acute bacterial infection. The development of highly sensitive and specific molecular diagnostic techniques using polymerase chain reaction (PCR) technology led to a revolution in viral diagnostics and a reevaluation of the role of respiratory viruses in a range of clinical syndromes. This was particularly pertinent to human RVs, which are either difficult or impossible to culture (e.g., RV-C strains) and due to the large number of serotypes diagnostic serology testing is not feasible. PCR-based diagnostic tests have a much greater sensitivity for the detection of RVs and studies using PCR revealed that the range of clinical illness associated with RV infection was much broader than previously recognized and included more severe disease syndromes such as pneumonia, bronchiolitis, acute rhinosinusitis, and influenza-like illness. In addition RVs could be detected in most asthma exacerbations and in a substantial proportion of COPD exacerbations. Asthma is estimated to affect 360 million people worldwide and COPD affects 174.5 million people and was the cause of 3.2 million deaths in 2015. Much of
the enormous morbidity, mortality, and healthcare costs associated with asthma and COPD are related to acute exacerbations. Therefore the recognition that RVs are a major cause of asthma and COPD exacerbations stimulated new interest in their biology and treatment. As part of this research investigators considered whether experimental infection studies in humans could be extended from healthy volunteers to patients with asthma and COPD.

8.4 EXPERIMENTAL RHINOVIRUS INFECTION IN ASTHMA

Respiratory viruses can be detected in up to 80% of asthma exacerbations in children and 60%–80% of exacerbations in adults, with RVs the commonest virus detected. The recognition of the role of RVs in asthma exacerbations stimulated research into their biology in an attempt to develop treatments for virus-induced exacerbations. This research included investigating whether experimental RV infection could be used in people with asthma in the same way it had been used in healthy individuals. The first experimental RV challenge of subjects with asthma was carried out in 1985 at Dalhousie University, Canada. Of the 21 volunteers inoculated, 19 became infected but only 4 had $\geq 10\%$ decrease in forced expiratory volume in 1 second (FEV$_1$) and an increase in airway hyperactivity (AHR). The authors felt that these findings suggested “that other viral pathogens may play a more important role in precipitating asthma attacks.” It is unclear why this study failed to induce features of asthma exacerbations but it would be almost another decade before further experimental RV infection studies in people with asthma were attempted. Experimental infection studies in allergic (nonasthmatic) subjects suggested that RV infection could induce changes in lower airway physiology similar to that seen in asthma. In 1994 an experimental infection study from the University of Southampton, United Kingdom included a small group of people with allergic asthma and reported that upper respiratory symptoms were more severe in atopic subjects but did not report on lower airway symptoms or physiology. Concurrently a study using PCR to detect viruses in naturally acquired asthma exacerbations strongly supported a role for RV. Subsequent studies were carried out by research groups in the United Kingdom, the Netherlands, and the United States in volunteers with mild, intermittent asthma. These studies demonstrated that RV infection induced airway obstruction, increased AHR, and airway inflammation and RV could be
detected in the lower airways, thereby supporting a causative role for RVs in asthma exacerbations.

Having established that respiratory viruses are a trigger for asthma exacerbations, research focused on investigating why RVs cause a benign, self-limiting illness in healthy subjects but result in more severe manifestations in people with asthma. A study of naturally acquired infections in cohabiting couples discordant for asthma suggested that people with asthma were not more susceptible to virus infection but had more lower respiratory tract symptoms. Airway inflammation during naturally acquired infections was greater in people with asthma compared with those without asthma but the number of subjects in this study was small and the viruses detected were different between the two groups. Viral challenge studies are ideally suited to addressing this research question as people without asthma matched for characteristics such as age and gender can be infected simultaneously. Most of the earlier infection studies did not include a control group of healthy volunteers and therefore could not address the question as to whether host responses to infection differ in people with asthma. Studies that did include nonasthmatic controls produced somewhat inconsistent results with one study reporting no differences in lower airway inflammatory cells, another reporting increased nasal inflammatory mediators in asthma and discrepant results regarding virus-induced respiratory symptoms. These divergent results were likely related to differences in sampling methods and timing, antibody status of subjects, and choice of healthy controls (e.g., atopic vs nonatopic).

The first study to show clear differences between subjects with and without asthma in their responses to RV infection was published in 2008. In this study, RV challenge induced more respiratory symptoms, greater lung function impairment, increased bronchial hyperreactivity, and eosinophilic and neutrophilic lower airway inflammation in asthmatic compared with normal subjects with direct correlations between loss of lung function and the degree of neutrophilic, eosinophilic inflammation, and nasal viral load. In addition, the study provided insights into potential mechanisms of differential responses to viral infection in people with asthma. Despite being infected with the same dose of virus, postinoculation virus loads tended to be higher in the asthmatic subjects compared with the healthy controls, suggesting that antiviral immunity may be impaired in people with asthma with subsequent failure to control viral replication. Virologic and clinical outcomes were related to deficient
interferon (IFN)–γ and interleukin (IL)–10 responses and to augmented T-helper type 2 (T\textsubscript{H}2) responses (IL-4, IL-5, and IL-13), indicating that excessive T\textsubscript{H}2 or impaired T\textsubscript{H}1 (or IL-10) immunity may be important mechanisms. When infected with RV in vitro, alveolar macrophages and bronchial epithelial cells from subjects with asthma demonstrated deficient production of IFN\textsubscript{β} and IFN\textsubscript{λ}, and this was related to the severity of virus-induced asthma exacerbations.\textsuperscript{91} Other reports subsequently confirmed that IFN production is deficient in asthma,\textsuperscript{92,93} but this finding has not been replicated in all studies.\textsuperscript{94–96} It may be that this phenomenon only occurs in a subset of people with asthma, or that it is seen in more severe or poorly controlled asthma. Such patients were not initially included in challenge studies as these were limited to mild, well-controlled asthma not requiring inhaled corticosteroids. In 2014 RV challenge was shown to be safe in a small group of people with well-controlled asthma requiring long-term use of inhaled corticosteroids.\textsuperscript{97} A larger study confirmed this and reported significantly more upper and lower respiratory symptoms, greater reduction in peak expiratory flow and FEV\textsubscript{1}, increased viral loads, increased bronchoalveolar lavage (BAL) eosinophils, and increased nasal IL-4, IL-5, and IL-13 in subjects with moderate asthma using inhaled corticosteroids.\textsuperscript{98} This study also identified novel mediators of virus-induced asthma exacerbations including IL-33,\textsuperscript{98} IL-25,\textsuperscript{99} and IL-18.\textsuperscript{100} Poor asthma control was associated with more severe virus-induced exacerbations, greater T\textsubscript{H}2 inflammation and higher virus load.\textsuperscript{101} Therefore responses to virus infection may differ depending on asthma severity and control, which may account for some of the discrepant results in earlier experimental infection studies. These successful viral challenge studies should pave the way for further studies in subjects with moderate asthma that should reveal new insights into the pathogenesis of exacerbations that may not have been obtained from studies in mild asthma.

The evidence that emerged from research, including experimental infection studies, that asthma is associated with deficient IFN responses led to the development of inhaled IFN\textsubscript{β} as a treatment for asthma exacerbations. A clinical trial of inhaled IFN\textsubscript{β} reported that treatment can reduce the severity of virus-induced exacerbations in a subgroup of patients assessed with more severe asthma.\textsuperscript{102} The development of inhaled IFN as a novel asthma treatment is a clear demonstration of the potential of experimental RV infection studies to contribute to the discovery of new treatments for virus-induced asthma exacerbations.
8.5 EXPERIMENTAL RHINOVIRUS INFECTION IN CHRONIC OBSTRUCTIVE PULMONARY DISEASE

The Global Initiative for Obstructive Lung Disease defines COPD as “a common preventable and treatable disease, characterized by persistent airflow limitation that is usually progressive and associated with an enhanced chronic inflammatory response in the airways and the lung to noxious particles or gases. Exacerbations and comorbidities contribute to the overall severity in individual patients.”

Acute exacerbations of COPD are the major drivers of morbidity, mortality, and healthcare costs in COPD and prevention of exacerbations a major unmet need. Acute bacterial infection was believed to be the main cause of COPD exacerbations and this is reflected in the widespread use of antibiotics in COPD exacerbations. Although COPD exacerbations are preceded by upper respiratory symptoms in up to two-thirds of cases, virus detection rates in the pre-PCR era were low. As with asthma, the role of viruses in COPD exacerbations was reexamined using PCR-based detection methods. Although detection rates of respiratory viruses in COPD exacerbations are more variable than in asthma, respiratory viruses can be detected in 50%—64% of COPD exacerbations, with RVs the predominant virus type detected. Despite this emerging evidence implicating respiratory viruses in a significant proportion of COPD exacerbations, both scientific and clinical research continued to focus on bacterial infection. As evidence of this, it was almost two decades after the first experimental RV infection study was carried out in asthma that a similar study in COPD was attempted. Despite the excellent safety record of experimental infection studies in asthma, caution was warranted in repeating these studies in COPD as there are major differences between these two populations. COPD patients are older, current or ex-smokers, and have impaired lung function with irreversible airflow obstruction. All these factors have the potential to result in a more severe response to experimental RV challenge, compared with the younger, nonsmoking patients with relatively normal baseline lung function recruited to the asthma infection studies.

The first experimental infection study in COPD was a small pilot study published in 2006 that established the safety of RV infection in four patients with moderate airflow obstruction (FEV₁ 50%—80% predicted) and not using regular inhaled therapy. The subjects developed symptoms consistent with a COPD exacerbation following RV inoculation, together with objective markers of exacerbation with falls in lung function.
and increases in upper airway inflammatory markers. All the subjects recovered completely without treatment and no adverse events were reported. Subsequently the same research group carried out two larger studies of experimental RV infection in subjects with COPD and non-COPD control subjects. These studies replicated the findings of the pilot study, wherein RV infection manifested in cold symptoms, lower respiratory symptoms consistent with exacerbations of COPD, including airway inflammation and worsened airflow obstruction. These studies provided important causal evidence linking virus infection to COPD exacerbations. Studies from naturally acquired infection were supportive of this link but do not provide definitive evidence as PCR evaluation detects viral nucleic acid and therefore does not prove the presence of live virus and samples are only collected after exacerbation onset. Respiratory virus nucleic acid can also be detected in COPD patients with stable disease, although is usually elevated during COPD exacerbations.

In experimental infection models, RV was present in airway samples prior to exacerbation onset, virus load increased in parallel with the increase in symptoms, airflow obstruction and inflammation, and clearance of virus was associated with exacerbation resolution. Strong correlations were observed between virus load and airway neutrophil numbers, neutrophil elastase, IL-8, IL-6, and tumor necrosis factor-alpha (TNFα), granulocyte macrophage colony stimulating factor, most of which of also correlated with levels of epigenetic regulator, histone deacetylase 2 and these inflammatory responses were greater in patients with COPD. In these studies, RV infection is the sole experimental agent responsible for increased inflammatory markers in patients with COPD, providing strong evidence that RV infection directly causes exacerbations in COPD patients.

Another advantage of virus challenge studies over naturally acquired infections is the ability to carry out detailed and repeated lower airway sampling during the course of the exacerbations, including the use of bronchoscopy. This has provided a wealth of mechanistic data regarding the pathogenesis of virus-induced exacerbations including the presence of inflammatory mediators, inflammatory cells, oxidative and nitrosative stress, and impaired antiviral IFN responses. A novel observation that emerged from these studies was that secondary bacterial infections occurred in 60% of experimental virus-induced exacerbations, whereas coinfection was rarely reported in naturally acquired exacerbations. Analysis of the respiratory microbiome following
experimental RV infection suggest that secondary infection occurs due to an outgrowth of previously present airway bacteria. Potential mechanisms of secondary bacterial infection include reduced antimicrobial peptides and increased glucose in the airways. A subsequent study of naturally acquired exacerbations sampling at multiple time points during exacerbations confirmed the validity of this observation. Therefore although the numbers of COPD subjects recruited to virus challenge studies to date is small, RV infection appears to be safe in this population and replicates the features of naturally acquired infection. Further studies including larger numbers of patients are needed to validate these findings and further investigate the mechanisms of virus-induced exacerbations in COPD.

8.6 FUTURE DIRECTIONS FOR HUMAN INFECTION MODELS

Since the first studies in healthy volunteers, experimental RV infection has been extended to patients with asthma and COPD and has contributed enormously to expanding our understanding of the biology of RV infection and how it affects patients with chronic airway diseases. A summary of the key findings from human experimental infection studies in people with asthma and COPD is provided in Table 8.1. These studies have tended to have a narrow focus on RV infection and host immune responses. It is clear from both in vivo and in vitro studies that there are interactions between respiratory virus infections and other factors that exacerbate asthma and COPD such as bacteria, allergens, and air pollution. These factors have been somewhat neglected in viral challenge studies and are a promising field of future research that is starting to be addressed.

As mentioned previously, the use of the viral challenge model in asthma is much further advanced compared with COPD. One study identified IFN-deficiency in COPD but this has not been replicated. With the development of inhaled IFN as a therapy option for asthma, the role of IFN in COPD requires urgent further investigation. Other areas of future research include the effects of virus infection on novel pathways such as lipidomics and metabolomics in asthma and COPD.

Respiratory viruses have also been identified as triggers of exacerbations in other airway diseases such as cystic fibrosis and bronchiectasis and there is evidence of impaired antiviral immunity in these diseases. Experimental infection studies may help to define the role of virus infection in these patient populations.
| Study                  | Patient population                                      | Controls           | Main outcomes                                                                 |
|------------------------|---------------------------------------------------------|--------------------|-------------------------------------------------------------------------------|
| Halperin et al. (1985) | 21 AA, 12 using SABA, 1 using ICS                       | No change in FEV₁ or AHR |
| Bardin et al. (1994)   | 6 AA, mild asthma, SABA only                           | 11 NANA, 6 ANA     | More severe colds in atopic subjects                                         |
| Fraenkel et al. (1995) | 6 AA, SABA only                                         | 11 NANA            | No change in in FEV₁ or AHR, increase in bronchial mucosal lymphocytes in all groups but no difference between groups, increased eosinophils at convalescence in AA |
| Cheung et al. (1995)   | 14 AA, SABA only. 7 infected, 7 sham infected           | No change in FEV₁ or AHR, increased cold and asthma symptoms, increased blood neutrophils and reduced blood lymphocytes in the infected group |
| Grunberg et al. (1997, 1999) | 27 AA, SABA only. 19 infected, 8 sham infected   | No change in laboratory FEV₁, fall in home FEV₁, increase in AHR, increased nasal IL-8, sputum ECP, IL-8 and IL-6, blood neutrophils and reduced blood lymphocytes in the infected group |
| Gern et al. (1997)     | 5 AA, SABA only                                         | Detection of RV in the lower airway |
| de Gouw et al. (1998)  | 14 AA, SABA only. 7 infected, 7 sham infected           | No change in FEV₁ or AHR, increase in FeNO in the infected group |
| Parry et al. (2000)    | 17 AA                                                   | No significant differences in symptom scores, viral shedding, or cytokine responses between groups |
| Jarjour et al. (2000)  | 8 AA, SABA only                                         | Increased blood neutrophils and reduced blood lymphocytes, increased nasal IL-8 and G-CSF; no change in bronchial lavage IL-8, TNFα, IL-5, IL-1β, IFN-γ, LTB₄, or EDN; increase in BAL neutrophils and MPO |
| Study Authors               | Subjects | Intervention | Outcomes |
|----------------------------|----------|--------------|----------|
| Gern et al. (2000)         | 15 AA, SABA only | 7 ANA. All 22 subjects analyzed together | No change in FEV₁, sputum inflammatory cells, eosinophils, lymphocytes, IL-8, IFN-γ and IL-5 protein; increased sputum neutrophils, G-CSF protein, IFN-γ and IL-5 mRNA, increased nasal neutrophils, IL-8 and G-CSF |
| Bardin et al. (2000)       | 6 AA     | 11 NANA, 5 ANA | |
| Grunberg et al. (2000, 2001), de Kluijver et al. (2003) | 25 AA, SABA only. 12 received budesonide prior to inoculation and 13 placebo | 12 NANA | No change in FEV₁ or AHR, increased bronchial biopsy T cells, increased biopsy expression of ICAM-1, nasal IL-8 and IL-1β in AA; increased nasal IL-1ra in NANA; no effect of budesonide |
| de Kluijver 2003           | 11 AA exposed to allergen + placebo, 10 AA exposed to RV, 9 AA exposed to RV and allergen | 12 NANA | |
| Zambrano et al. (2003)     | 16 AA SABA only; 6 high IgE, 10 low IgE | 9 NANA | No change in FEV₁ or AHR; lower respiratory symptoms and FeNO greater in AA with high IgE |
| Mosser et al. (2005)       | 13 AA    | 6 NANA       | No difference in upper or lower airway viral load between groups |
| Christiansen et al. (2008) | 4 AA, mild or intermittent asthma | 4 ANA | Increased human tissue kallikrein activation activity in BAL in AA |

(Continued)
| Study                  | Patient population                        | Controls | Main outcomes                                                                                                                                                                                                 |
|-----------------------|-------------------------------------------|----------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Message et al. (2008) | 10 AA, SABA only                          | 15 NANA  | Increased chest symptom scores, sputum and BAL eosinophils, AHR in AA; significant falls in FEV₁ and PEF in AA; reduced blood CD4⁺, CD8⁺, and B cells in AA |
| Adura (2014)         | 11 asthmatics using ICS                   |          | Increased cold and asthma scores, no adverse events                                                                                                                                                           |
| DeMore et al. (2009) | 15 AA, SABA only                          | 18 ANA   | No change in PEF from baseline; between groups no differences in cold scores, PEF, virus load, sputum or nasal lavage neutrophil, monocyte and lymphocytes, nasal lavage IL-6, IL-10, CXCL8, CCL2, and CCL5, serum CXCL10; increased sputum eosinophils in AA |
| Kloepfer et al. (2011) | 19 AA SABA only, 8 received montelukast, 11 placebo |          | No effect of montelukast on symptoms, PEF, viral load, sputum eosinophils, or neutrophils                                                                                                                   |
| Majoor et al. (2014) | 13 AA                                     | 11 NANA  | Coagulant TF-exposing microparticles in BAL fluid reduced in AA                                                                                                                                              |
| Jackson (2014)       | 28 AA, 15 using ICS                       | 11 NANA  | Significantly greater upper and lower respiratory symptoms, greater reduction in PEF and FEV₁, increased viral loads, increased BAL eosinophils, increased nasal IL-4, IL-5, and IL-13 in AA |
| Silkoff et al. (2018) | 63 mild-to-moderate asthmatics, 63.5% using ICS, 32 randomized to CNTO3157 |          | CNTO3157 had no effect on FEV₁, PEF, symptom scores, viral load, or FeNO; more moderate and severe asthma exacerbations reported in subjects receiving CNTO3157                                                      |
Mallia et al. (2006)\textsuperscript{112}

- 4 COPD, FEV\textsubscript{1} 50\%–80\% predicted
- Increased upper and lower respiratory symptoms, falls in PEF and FEV\textsubscript{1}, increased nasal IL-8

Mallia et al. (2011)\textsuperscript{113}

- 11 COPD, FEV\textsubscript{1} 50\%–80\% predicted
- 12 smokers with normal lung function
- Upper and lower respiratory symptoms, falls in PEF, sputum neutrophils and NE, BAL lymphocytes, nasal lavage virus load higher in COPD

Footitt et al. (2016)\textsuperscript{114}

- 9 COPD, FEV\textsubscript{1} 50\%–80\% predicted
- 10 smokers, 11 nonsmokers with normal lung function
- Sputum inflammatory cells, neutrophils, NE, IL-1\textbeta, GM-CSF, IL-8, TNF\textalpha, MMP-9, 8-OHdG, 3-NT, nitrite and 8-isoprostane higher in COPD; sputum HDAC2 activity reduced in COPD

Key findings from studies of experimental RV infection in human subjects with asthma and COPD, including information on the patient and control populations assessed and key experimental findings. 3-NT, 3-Nitrotyrosine; AA, people with atopic asthma; AHR, airway hyperreactivity; ANA, atopic nonasthmatic; BAL, bronchoalveolar lavage; ECP, eosinophil cationic protein; EDN, eosinophil derived neurotoxin; FeNO, fraction of exhaled nitric oxide; FEV\textsubscript{1}, forced expiratory volume in 1 second; G-CSF, granulocyte colony stimulating factor; GM-CSF, granulocyte macrophage colony stimulating factor; HDAC, histone deacetylase; ICAM-1, intercellular adhesion molecule 1; ICS, inhaled corticosteroid; IFN, interferon; IgE, immunoglobulin E; IL, interleukin; MMP, matrix metalloprotease; MPO, myeloperoxidase; NANA, nonatopic nonasthmatic; NE, neutrophil elastase; PEF, peak expiratory flow; RV, rhinovirus; SABA, short-acting \textbeta\textsubscript{2}-agonist; TNF\textalpha, tumor necrosis factor-alpha.
Perhaps the most promising use of the virus challenge model will be to accelerate the process of drug development. Virus challenge studies have been used to evaluate the effects of existing asthma therapies on virus-induced exacerbations. Recently the first study using viral challenge to evaluate a novel, unlicensed drug in asthma was also published. Although these studies had negative results they demonstrate the potential of the viral challenge model in drug development.

The key to successful drug development is the identification of clinically relevant mechanisms of RV infection or immunopathology that can be experimentally manipulated for therapeutic benefit. This is where human experimental RV infection is complemented by work in animal models. There are a number of options for modeling human RV infection in animal models and they provide the ability to investigate specific disease components and mechanisms that would be otherwise impossible in humans. Human experimental infection models have the advantage of identifying disease correlates, but the degree of experimental manipulation possible is extremely limited and the safety and effectiveness of interventions must first be evaluated in animals.

8.6.1 Animal models of rhinovirus infection

Animal models have proven useful for mechanistic studies across a range of diseases. Models of RV infection have been reported in several animal species, including mouse, cotton rat, and nonhuman primates. Each of these experimental systems provides its own advantages and disadvantages and a substantial contribution to the knowledge base of the biology of RV infection.

Animal models provide a range of benefits to complement human experimental approaches. Experimental animals can be readily manipulated to induce consistent disease outcomes, such as the induction of allergic airway disease (AAD) to model asthma or cigarette smoke—induced COPD. Animal models have less variability than human populations providing more consistent experimental outcomes and statistical power in intervention studies. Experimental environment, exposures (e.g., previous infection history), endpoints, and interindividual variability can be controlled. Further, a broad array of tools are available to characterize disease outcomes, including reagents, genetically modified animal strains, experimental protocols, and assessment techniques (e.g., lung function testing).
Sample tissues can be easily isolated at experimental endpoints, which are difficult or impossible to sample in humans (e.g., lung tissues, draining lymph nodes, bone marrow). Further, animal models serve as a valuable preclinical system for the assessment of novel interventions, to provide proof-of-principle safety and efficacy findings prior to exposure of healthy human volunteers.

### 8.7 MOUSE MODELS

Mice in particular have been extensively used to model disease, including virus infection and exacerbations of asthma and COPD. As a result, a wealth of tools and techniques are available to study immune mechanisms and pathophysiology in mice. Well-characterized protocols and reagents support the induction of disease states and allow detailed characterization of immune responses (e.g., fluorescently tagged monoclonal antibodies to quantify immune cell subsets). A range of transgenic and knockout mouse strains are available that allow for the careful dissection of relevant disease mechanisms. Further, reagents are available to assess the effects of novel interventions on disease outcomes (e.g., blocking antibodies and various forms of innate immunity activators; see Chapter 9: Emerging therapeutic approaches).

The expansion of mouse RV infection models has paralleled our understanding of RV biology in humans. Initial approaches focused on understanding the effects of RV infection in isolation, identifying the mechanisms and cell types mediating lung pathology. Increasingly complex experimental models are now being used to characterize long-term effects of infection on airway function and the effects of RV infection on preexisting airway disease (e.g., asthma exacerbations).

One of the biggest developments in mouse RV infection models was the protocol for isolation of highly purified, concentrated (high titters) of RV from Henrietta Lacks (HeLa) immortalized human epithelial cell lines and later, the intracellular adhesion molecule 1 (ICAM-1) transfected rhabdomyosarcoma cell lines. Prior to this, clarified infected HeLa cell lysates were used for in vitro experiments. Some investigators also used infected HeLa cell lysates in mouse models. Efforts to improve the quality and validity of the model made use of a partial purification protocol to generate viral stocks. For mouse models of RV infection or exacerbations, the refined, high-titer, RV purification protocol is the gold standard.
8.8 ORIGINS OF RHINOVIRUS MOUSE MODELS

A major barrier that hindered the early development of mouse models was the species specificity of RV infection. “Major-group” RV strains, which make up approximately 90% of all RV strains, enter the cell through binding of ICAM-1.\textsuperscript{147,148} RV binding to ICAM-1 is limited to human and chimpanzee and does not occur in other species, including mouse.\textsuperscript{149} As a result, major-group RV strains cannot infect mouse cells and fails to replicate or induce pathology in mouse models. Early attempts to develop RV mouse models failed to detect sufficient viral replication to induce disease.\textsuperscript{150}

A major advance enabling the development of mouse RV infection models was the initial recognition that the minor-group RV-A1, which use the host cell receptor low-density lipoprotein receptor, can infect the mouse epithelial cell line LA-4.\textsuperscript{151} This recognition suggested that minor-group RV viruses (e.g., RV-A1) may be useful to model infections in mice in vivo. Indeed, inoculation of wild-type BALB/c mice with RV-A1 induced lung pathology, mucus production, and inflammatory cytokine production.\textsuperscript{152} Notably, RV infection was also sufficient to induce exacerbations of preexisting asthma in sensitized and challenged mice (as discussed in more detail below).\textsuperscript{152}

An alternate approach was also sought to allow modeling of major-group RV infection in mice. The same study by Tuthill et al. demonstrated that transfection of LA-4 cells with a chimeric ICAM-1 receptor containing the human extracellular receptor domains allowed infection and replication by the major-group virus RV-A16.\textsuperscript{151} This finding provided the basis for developing a transgenic mouse strain expressing a chimeric mouse—human ICAM-1 receptor. Chimeric receptor expression in hu-ICAM\textsuperscript{Tg} mice is sufficient to support in vivo infection with RV-A16, resulting in airway inflammation, mucus production, viral replication, and inflammatory cytokine production.\textsuperscript{152} Of note, the hu-ICAM\textsuperscript{Tg} mouse was generated by random insertion of a chimeric transgene and little is known about the transgene insertion site within the genome. Use of this transgenic strain requires additional experimental considerations (e.g., genotyping and use of heterozygous animals in experiments), which has limited its broad utility.

Additional variations have also been reported in the literature, which aim to broaden the available mouse models. Genetic RV-A1 variants have been generated by serial passage through mouse embryonic fibroblasts in vitro and lung epithelial cells in vivo, which exhibit increased
growth in mouse cells. Inoculation of BALB/c mice with the RV-A1/M2M7 variant \( [8 \times 10^6 \text{ plaque forming units (PFU)}] \) allowed for recovery of virus from mouse lung after 24 hours, when mice were also pretreated with intranasal hydrochlorous acid to increase epithelial permeability.

Successful use of mouse models also depended on the development of streamlined RV isolation protocols that have allowed consistent and rapid isolation of virus stocks, to limit variability between experiments. The current gold-standard protocols for RV isolation, RV-A1 infection of wild-type BALB/c mice, and the infection of transgenic mice expressing chimeric mouse–human ICAM-1 receptor with RV-A16 are published and readily available.

### 8.9 TECHNICAL DETAILS AND MAIN FINDINGS FROM MOUSE RHINOVIRUS INFECTION MODELS

A range of studies have assessed the effects of primary RV infection in mice, contributing to our understanding of the mechanisms underlying disease pathogenesis. Studies have used similar protocols, typically performing intranasal inoculation of \( \sim 10^6-10^8 \text{ TCID}_{50} \) (tissue culture infective dose in 50% of culture, a titration of infection units of pathogens that do not form plaques in culture) RV-A1 and assessing responses over 1 week following infection in BALB/c or C57Bl.6 mice.

In the initial publication by Bartlett et al., intranasal inoculation of wild-type BALB/c mice with \( 5 \times 10^6 \text{ TCID}_{50} \) RV-A1 induced a range of disease features similar to human disease. RV infection induced airway inflammation characterized by increased neutrophil numbers at 24 and 48 hours postinfection and increased lymphocyte numbers persisting for 1-week postinfection. Tissue pathology was characterized by perivascular and peribronchial inflammation, increased mucus production, and elevated inflammatory cytokine production (including MIP-2, KC, MIP-3α, IP-10, RANTES, ITAC, IL-6, IL-1β, IFNα/β/λ/γ). Furthermore, RV infection resulted in the development of an RV-specific adaptive antibody response by 7 days postinfection.

Further studies have provided insights into the effects of RV infection alone in mice. Following inoculation of wild-type C57Bl/6 mice with \( 1 \times 10^8 \text{ TCID}_{50} \) RV-A1, detectable RV positive- and negative-strand RNA were recovered from the lung, indicative of active viral replication and viral RNA was detectable up to 7 days postinfection. The study also noticed a small increase in airway neutrophils and lymphocytes in the
presence of UV-inactivated RV-A1, although UV-inactivated RV-A1 (and major-group virus RV-39) failed to induce inflammatory cytokine production. RV-A1 infection also increased airway responsiveness to methacholine challenge at days 1 and 4 postinfection. Inoculation with RV-A1 or UV-inactivated RV-A1 induced PI3K activation in airway epithelial cells and pretreatment with the PI3K inhibitor LY294002 in vivo dampened neutrophilic inflammation and inflammatory cytokine production (KC, MIP-2, MIP-1α, IFNγ). Inoculation of C57Bl/6 mice with RV-A1 (5 × 10^7 TCID_50) leads to discontinuous expression of zonula occludens-1, suggesting that infection disrupts airway epithelial barrier function. RV infection also stimulated IL-15 production and release into the airways, which is dependent on type I IFN production and stimulates activation of natural killer (NK) and CD8^+ T cell responses. Treatment with an IL-15–IL-15Rα complex increased expression of IL-15, IL-15Rα, IFNγ, and CXCL9 and stimulated increased NK, CD8^+, and CD4^+ T cell recruitment and activation.

CCL7 and IRF-7 are the most upregulated lung transcripts following RV-A1 infection. Blocking CCL7 or IRF-7 function reduced lung neutrophil and macrophage accumulation and IFN responses and blocking CCL7 also reduced AHR. This publication also delineated AHR from inflammatory infiltrates showing instead a relationship between classical proinflammatory transcription factors (NFκB) and AHR.

As alluded to previously, the use of knockout mice in particular has provided insights into a number of mechanisms regulating RV-induced pathology. Key roles for neutrophils and the neutrophil chemokine receptor CXCR2 in mediating RV-induced pathology were identified. Inoculation of CXCR2^−/− mice with RV-A1 (4.5 × 10^6 TCID_50) resulted in reduced airway neutrophil numbers, reduced inflammatory cytokine production (TNFα, MIP-2, KC), decreased mucus production, and decreased cholinergic responsiveness, with no alteration in viral load, compared with wild-type control animals. Further, antibody depletion of neutrophils and infection of TNFR^−/− mice also reduced AHR, compared with control animals. These findings provide evidence for a role of neutrophilic inflammation, potentially via TNFα production, on downstream pathology following RV infection. Roles for pattern recognition molecules have been demonstrated for MDA5, Toll-like receptor 3 (TLR3) and TLR7. Infection of MDA5^−/− mice resulted in delayed type I IFN (IFNα/β) and suppressed type III IFN expression, with a slight early increase in viral load in the lung. In contrast, inoculation of
TLR3$^{-/-}$ mice resulted in normal IFN responses and no difference in viral yield. Both MDA5$^{-/-}$ and TLR3$^{-/-}$ mice had reduced neutrophil numbers, inflammatory cytokine production (CXCL1, CXCL2, CCL2, CXCL10) and airways responsiveness, compared with wild-type controls. Differing roles for NFκB signaling pathways in RV-induced inflammation and type I INF responses in antiviral immunity have been demonstrated. Disruption of NFκB signaling in p65$^{+/−}$ mice resulted in reduced neutrophil numbers and inflammatory cytokine production (CXCL1, CXCL5, CXCL2), while IFN production and viral loads are unaltered. In contrast, IFNAR1$^{-/-}$ mice have unaltered neutrophilic inflammation, a persistent increase in lymphocyte numbers and cytokines CCL5, CXCL10, and CXCL11, with reduced IFNα production and increased viral load. A pathogenic role for the proinflammatory molecule MUC18 has also been demonstrated, with increased expression of antiviral genes (Mx1, IP-10), reduced neutrophil inflammation and viral load in MUC18$^{-/-}$ mice following RV-A1 inoculation ($1 \times 10^7$ PFU). Studies using Tbet$^{-/-}$ mice (a key regulator of T$_{H_1}$ cell differentiation) have also demonstrated the key role for T$_{H_1}$-polarized T cells in the response to RV infection. Tbet$^{-/-}$ mice developed a T$_{H_2}$/T$_{H_17}$-polarized immune response to RV infection ($5 \times 10^6$ TCID$_{50}$) with increased IL-13 and IL-17A production, deficient NK cell responses, and decreased neutralizing antibody development. CD4$^{+}$ T cells contributed to increased airway eosinophil numbers and mucus production following RV infection in Tbet$^{-/-}$ mice. Studies using TSLP receptor-deficient mice (TSLPR$^{-/-}$) demonstrated that RV-A1 infection interferes with tolerance to an inhaled allergen, via a mechanism requiring TSLP, IL-33, and activation of OX40L on lung dendritic cells.

After observing increased levels of the TNF super family member protein, Tnfsf10 (TRAIL or CD253) production over a time course of RV-A1 infection in mice, Girkin et al. compared RV-A1 infection in Tnfsf10$^{-/-}$ mice to wild-type BALB/c mice and observed an almost complete ablation of inflammatory responses to RV-A1. Following RV infection, peribronchiolar inflammation and lung histopathology were reduced in Tnfsf10$^{-/-}$ mice; neutrophil and lymphocytes in BAL remained at baseline; and CD4$^{+}$ T cells, CD8$^{+}$ T cells, NKs, plasmacytoid dendritic cells (pDCs), and myeloid dendritic cells were all reduced in flow cytometry of total lung cells. Tnfsf10$^{-/-}$ mice were protected from RV-induced AHR, and failed to develop RV-induced exacerbations of allergic airways disease. An interesting proviral effect of TRAIL was
also identified whereby Tnfsf10<sup>−/−</sup> mice had reduced viral load and anti-TRAIL antibodies reduced viral load (whereas recombinant TRAIL administration increased viral load) in BEAS2B cells infected with RV-A1 in vitro.<sup>163</sup> This effect on viral load was independent of IFN responses and may be associated with an unidentified role of apoptosis in RV replication, which remains to be explored.

Some studies have assessed the effect of primary RV infection on clinically relevant sequelae, including secondary bacterial infection and the effects of premature birth on infection. Exposure of epithelial cells in culture to RV-A1 resulted in increased bacterial attachment and translocation through an epithelial monolayer (nontypeable *Haemophilus influenzae* (NTHi), *Pseudomonas aeruginosa*, *Staphylococcus aureus*),<sup>154</sup> suggesting a potential mechanism underlying secondary bacterial infections following viral infection. A subsequent study demonstrated that primary inoculation with RV-A1 (5 × 10<sup>6</sup> TCID<sub>50</sub>) delayed the clearance of NTHi in vivo, associated with suppressed chemokine production (KC, MIP-2) and neutrophilic inflammation through a TLR2-mediated mechanism.<sup>164</sup> The model has also been used to assess immune alterations relevant to premature birth and bronchopulmonary dysplasia, risk factors for viral-induced exacerbations. Exposure of neonatal mice to hyperoxia (75% oxygen) in early life increased inflammatory cytokine expression (IL-12, IFN<sub>γ</sub>, TNFα, CCL2, CCL3, CCL4) and suppressed early IFN responses following RV-A1 infection (9 × 10<sup>6</sup> PFU) at 14 days of age.<sup>165</sup> One study has also assessed the effect of RV infection timing on subsequent development of AAD. Inoculation of 7-day-old mice with RV-A1 and subsequent induction of house dust mite (HDM)-induced allergic airways disease had additive effects with increased neutrophilia and AHR in female mice, although RV inoculation had no additional impact in male mice.<sup>166</sup> These studies extend the use of RV infection in mice to new areas, including mechanisms of early life infection susceptibility, to mechanisms of secondary bacterial infection/compromised antimicrobial immunity and experimental exploration of clinical risk factors associated with increased likelihood to develop virus-induced exacerbations of respiratory diseases.

### 8.10 Preclinical Testing in Mouse Models of Rhinovirus Infection

Mouse models are valuable tools for the preclinical testing of novel treatments. Several studies have used the mouse RV infection model to assess
intervention strategies, including vaccine development and drug treatment. Primary inoculation of BALB/c with RV-A1 \((1 \times 10^6 \text{ TCID}_{50})\) rapidly induced circulating RV-specific IgG antibody production within 4 days, which binds capsid protein VP1 and those antibodies were cross-reactive to another minor strain RV (RV-29). Repeated RV infections were necessary to induce RV-specific IgA responses and neutralizing antibodies, but administration of CpG or subcutaneous immunization with Freund’s adjuvant promoted neutralizing antibody development and may inform potential vaccine strategies. Pretreatment with the plant flavanol quercetin before and during RV-A1 infection effectively reduced viral replication, inflammatory cytokine production (KC, MIP-2, TNF-α, CCL2, IFNα, and IFNλ2), and AHR. Treatment with the cancer therapeutic gemcitabine (20,20-difluorodeoxycytidine) reduced RV load, inflammatory cytokine levels (TNF-α, IL-1β), and reduced lung lymphocyte numbers. Treatment with corticosteroid therapy (fluticasone propionate) suppressed IFN responses to RV and reduced airway inflammation, leading to increased mucus production and reduced antimicrobial responses. Effects on viral load, mucin production, and antibacterial response could be reversed by administration of recombinant IFN-β. Despite promising findings in mouse models, quercetin has not entered clinical trials for the treatment of RV infection, likely due to a previous randomized community clinical trial in 2010 that showed little benefit of quercetin supplementation on upper respiratory infections. These findings may highlight the limitation of mouse models, which (while valuable) do not always fully recapitulate human disease mechanisms.

### 8.11 RHINOVIRUS-INDUCED DISEASE EXACERBATION MODELS IN MICE

Animal models to study RV-mediated exacerbations of airway disease have also been developed. These models combine experimental RV infection with models of airways disease, including asthma, COPD, and chronic rhinosinusitis. Models of asthma typically consist of administration of a sensitizing agent [e.g., ovalbumin (OVA) or HDM] and subsequent challenge in the airways to induce an eosinophilic, allergic airways disease. COPD is typically induced by prolonged and repeated exposure of mice to cigarette smoke or treatment with elastase. After airways disease is established, mice are then inoculated with RV to induce disease...
exacerbations. These models are explained and expanded upon in the following sections.

Researchers have also used double-stranded RNA (dsRNA) administration as a surrogate for virus infection to exacerbate preexisting disease (reviewed in Refs. [139,140]). However, these approaches fail to model the complexity of virus infection and are beyond the scope of the current book chapter.

### 8.11.1 Mouse asthma exacerbation models

Many studies have characterized the effects of RV infection on preexisting asthma and have provided insights into the immune cell types involved, key molecules, and responses to potential therapies. In the initial report of an RV (RV-A1) exacerbation model, OVA-sensitized and challenged BALB/c mice were inoculated with RV-A1 during the allergen challenge phase. The combination of virus and allergen challenge increased airway neutrophil, eosinophil, and lymphocyte numbers; increased cytokine production (IL-4, IL-13, and IFNγ), increased AHR; and increased mucus gene expression.

Subsequent studies have identified key functional roles for macrophages, gamma-delta (γδ) T cells, dendritic cell subsets, and neutrophils in RV-induced immunopathology. In a similar model, RV-A1 inoculation into OVA-sensitized/challenged mice increased macrophage lung infiltration and eotaxin-1/CCL11 expression. Eotaxin was expressed by pulmonary macrophages in the lung after combined virus infection and allergen challenge. Further, macrophage depletion or antieotaxin treatment reduced RV-induced airway eosinophilia and AHR. Macrophage activation state also modulates the response to RV infection in allergen sensitized/challenged mice and shapes the resulting pattern of inflammation. RV infection in asthma exacerbation models induced an IL-13-expressing macrophage population, with M2 polarized phenotype. Depletion of IL-13-producing cells in CD11b-DTR mice or CCR2−/− mice reduced airway inflammation and AHR. Interestingly, while RV infection of OVA-treated wild-type mice contributes to mixed neutrophilic and eosinophilic airway inflammation and M2 macrophage phenotype, IL-4R−/− mice exhibit neutrophil inflammation alone and increased M2 polarization of pulmonary macrophages but still have exacerbated airway responses. γδT cells dampen exacerbation responses. γδT cells are increased in RV-induced asthma exacerbation models and...
blocking responses with anti-γδ TCR antibody worsened exacerbations with increased AHR, and increased numbers of T\(_{H2}\) cells and eosinophils, with no effect on virus load.\(^{175}\) pDCs were recruited to the lung during RV-induced inflammation and subsequently promoted T\(_{H2}\) responses in the lung draining lymph nodes, in a process mediated by IL-25.\(^{176}\) Depletion of pDCs with an antibody or treatment with anti-IL-25 reduced eosinophil numbers, decreased lung pathology, reduced cytokine production (IL-5, IL-13), and reduced AHR.\(^{176}\) Functional roles for neutrophils, and neutrophil extracellular traps (NETs), have also been provided in RV-induced asthma exacerbation models. Chronic low-dose HDM exposure and RV infection have additive effects on neutrophilia and induce AHR.\(^{177}\) A more recent study demonstrated that RV infection in an HDM-mediated asthma model results in double-stranded DNA release into the airways and administration of genomic DNA alone was sufficient to mimic characteristic components of RV-induced exacerbations.\(^{178}\) Further, blocking neutrophil elastase or degrading NETs by applying DNase into the airways reduced eosinophil and lymphocyte numbers, tissue pathology, and cytokine production (IL-5, IL-13).\(^{178}\)

A number of studies have highlighted the functional roles of specific molecules in RV-induced mouse exacerbation models, as potential therapeutic targets to validate in patient populations. In addition to roles during RV infection alone highlighted above, MDA5 and TLR3 are also involved in RV-induced exacerbations. MDA5\(^{-/-}\) and TLR3\(^{-/-}\) mice have decreased inflammatory responses and AHR, while MDA5\(^{-/-}\) also had decreased IFN responses (IFN\(\beta/\lambda2/\lambda3\)).\(^{158}\) Midline 1 (a E3 ubiquitin ligase) is upregulated in an HDM-induced model, and short interfering RNA–mediated inhibition prior to RV inoculation reduced neutrophil numbers and mucus production production.\(^{179}\) The monocyte chemotactic protein CCL2 is produced by epithelial cells and macrophages following RV-induced exacerbation and administration of an anti-CCL2 antibody reduced eosinophil numbers and AHR.\(^{180}\) Foxa3–overexpressing transgenic mice produce excess mucus in their airways and RV infection increased Foxa3 expression.\(^{181}\) In Foxa3–deficient mice (Fosa3\(^{-/-}\)), RV clearance is increased, with increased IFN\(\beta\) activation.\(^{181}\) IL-25 expression is also increased in RV-induced exacerbations and blocking the IL-25 receptor reduced type 2 cytokine production (IL-4, IL-5, IL-13, IL-25, IL-33, TSLP), mucus production, and numbers of eosinophils, neutrophils, T cells, and innate lymphoid type 2 cells.\(^{99}\) Combining dsRNA administration with RV-A1 inoculation worsened preexisting allergic
airways disease. Repeated dsRNA administration after OVA sensitization/challenge resulted in neutrophilic lung inflammation and tissue pathology and combined dsRNA and RV-A1 inoculation increased expression of TSLP, TNFα, and IFNλ in the lung. Key roles for pattern recognition receptors have also been demonstrated in RV asthma exacerbation models. HDM-allergic TLR7−/− mice had a decreased antiviral response, with reduced IFN release (IFNα/β/λ1/λ2/λ3) and increased virus replication, associated with increased eosinophil and lymphocyte numbers, increased IL-5 and CCL11, and AHR. Administration of IFN or transfer of wild-type TLR7-competent pDCs could restore antiviral responses and reduce disease exacerbation. OVA-allergic TLR2−/− mice also had reduced macrophage, neutrophil, and eosinophil numbers and suppressed AHR after RV inoculation. Bone marrow transfer experiments demonstrated that TLR2−/− bone marrow could protect from exacerbations, while transfer of wild-type bone marrow restored responses in TLR2−/− mice. Transfer of wild-type macrophages into TLR2−/− mice could also restore exacerbations. As previously mentioned, a role for TRAIL has also been demonstrated, with HDM-allergic TRAIL-deficient mice (Tnfsf10−/−) protected from RV-induced AHR and induction of airway inflammation. RV-induced asthma exacerbation models have also been used to assess the responses to existing therapies and as preclinical models for novel therapies. Treatment of HDM-allergic mice with the long acting beta-2 agonist salmeterol reduced AHR and eosinophil numbers during RV exacerbation, and limited chemokine levels (CCL11, CCL20, CXCL2) through modulation of PP2A. The findings of this study were focused on PP2A as a novel therapeutic target rather than promoting salmeterol monotherapy (which was associated with adverse events and tolerance to β2-agonists with chronic salmeterol use). An approach to block major-group RV virus infection was assessed through administration of antihuman ICAM-1 antibody, which prevented entry of RV-A16 and RV-14 and reduced neutrophil and lymphocyte numbers, cytokine production (IL-4, IL-5, IL-6, CCL1, CCL11), mucus production, and virus load in human transgenic mice in an OVA-allergic model. Treatment with a nontoxic anthraquinone derivate reduced RV-induced AHR, neutrophil, and eosinophil airway inflammation; inflammatory cytokine production; and mucus hypersecretion while also boosting type 1 IFN response and reducing viral yields, with associated decreased AKT, HIF-1α, and VEGF production. Treatment with an antiinflammatory VAP-1/SSAO
inhibitor, PXS-4728A, or the macrolide antibiotic azithromycin also reduced neutrophil numbers and PXS-4728A reduced AHR following RV-A1 inoculation in HDM-allergic mice. 

8.11.2 Mouse chronic obstructive pulmonary disease exacerbation models

RV also plays an important role in virus-induced exacerbations of COPD. Several studies have assessed the effects of RV inoculation in animal models of COPD. Exposure to elastase and lipopolysaccharide (LPS) once per week for 4 weeks induces features of COPD, including airway inflammation, goblet cell metaplasia, and altered lung function. Addition of RV-A1 led to persistence of viral RNA (>14 days postinfection), deficient IFN responses (IFNα/β/γ) and increased AHR, lung volume, cytokine production (TNFα, IL-5, IL-13), and mucus production, compared with elastase/LPS administration alone. A subsequent study attempting to replicate the elastase/LPS model of COPD found that a single elastase treatment followed by RV-A1 inoculation was enough to increase airway neutrophil and lymphocyte numbers, increased inflammatory cytokine production (TNFα, CXCL10, CCL5), mucus hypersecretion, and AHR. In the same elastase-induced model, fluticasone propionate treatment reduced IFN responses, increased viral load, suppressed airway immune cell numbers (lymphocytes and neutrophils), suppressed inflammatory cytokines (IL-6, TNFα), and increased mucus production, following RV-A1 exacerbation. The differences in the experimental approaches required to elicit an RV-induced exacerbation in these different studies is likely due to the quality of virus inoculum used by the different investigators, which as explained previously, is influenced by purification approach. The first study used only crude virus-infected cell lysates, whereas the later study employed a highly purified virus inoculum.

Several studies have also reported on RV infection in a cigarette smoke-induced COPD model. In an initial study, 8 weeks of cigarette smoke exposure resulted in increased viral persistence, neutrophilia, and increased mucus production following RV infection. Subsequent studies demonstrated that goblet cell gene expression was reduced following treatment with a gamma-secretase inhibitor (GSK L685,458) to limit NOTCH activation. Further, supplementation of feed with quercetin reduced RV-induced lung inflammation (including neutrophilia), goblet cell metaplasia, and AHR.
8.11.3 Mouse chronic sinusitis exacerbation models

To our knowledge, only one study has assessed the effect of RV infection in a chronic sinusitis model. A mouse model of chronic allergic rhinosinusitis was induced by 5 weeks of repetitive nasal OVA challenges.\textsuperscript{194} Increased RV-A1 yields were reported in the nasal tissue of mice with rhinosinusitis, although inflammatory cytokine production and histopathology were unaffected.\textsuperscript{194} This study served to illustrate the range of additional diseases where RV infection has been shown to be relevant in human populations where animal models are available for future research (e.g., cystic fibrosis).

8.12 OTHER RHINOVIRUS ANIMAL MODELS

A limited number of studies reported the use of RV infection in other animals, namely cotton rats and nonhuman primates. We note that historical studies assessing “RV” infection in other animal species are referring to genetically distinct viral genera and should not be confused with human RV (e.g., equine RV and bovine RV). For example, while human RV and equine RV were both originally assigned to the \textit{Rhinovirus} genus, they have been reclassified into \textit{Enterovirus} and \textit{Aphthovirus}, respectively. Equine RV has subsequently been renamed “equine rhinitis virus.”

8.12.1 Cotton rat

The cotton rat (\textit{Sigmodon hispidus}) is a recognized model for human respiratory infection, particularly for respiratory syncytial virus, as well as adenoviruses, parainfluenza virus, measles, and human metapneumovirus (reviewed in Ref. [\textsuperscript{195}]). To date, two studies have reported on RV infection in cotton rats, providing evidence that cotton rats are partially permissive to RV major-group infection. Intranasal inoculation of RV-A16 ($10^7$ PFU) into cotton rats induced lower respiratory histopathology, increased mucus production, and induction of INF-activated genes.\textsuperscript{196} Immunization with live RV-A16-induced high levels of circulating antibodies and protected from subsequent infection, while prophylactic transfer of anti-RV-A16 serum also protected from disease.\textsuperscript{196} Further, this protection was transferred effectively from mother to newborn, limiting viral yields in subsequently infected progeny.\textsuperscript{196} In a later study, the same group provided evidence that infection with RV-B14 ($10^6$ PFU) induced similar disease pathology. Furthermore, immunization with RV-B14 provided protection
from subsequent infection with either RV-B14 (an RV-B group virus) or RV-A16 (an RV-A group virus), demonstrating some degree of cross-reactivity to very different major-group viruses.\textsuperscript{197}

8.13 NONHUMAN PRIMATES

Chimpanzees and gibbons are the only nonhuman primates that support RV infection, although RV infection has also been reported in the vervet monkey cells, with consistent infection requiring high dose exposure.\textsuperscript{198} Initial RV infection studies in chimpanzees were reported in 1968, using RV-B14 and RV-A43\textsuperscript{199} and in gibbons in 1969.\textsuperscript{200} Subsequent studies in chimpanzees and gibbons assessed the antiviral effects of drug treatments on RV infection, using bis-benzimidazole and triazinoindole, respectively.\textsuperscript{201,202} Administration of soluble truncated form of human ICAM-1 can prevent subsequent infection in chimpanzees.\textsuperscript{203} However, it has been noted that neither chimpanzees nor gibbons develop “cold” symptoms following RV infection and the high costs and logistics of these studies has limited further progress.

Chimpanzees are an endangered species and require considerable resources and facilities for research. Current chimpanzee research is limited to the United States and Gabon. However, the National Institutes of Health in the United States have indicated that they are seeking to eliminate the use of chimpanzees in research. All but one species of gibbon are endangered. Thus clinical research using nonhuman primates in the future to characterize RV infection are likely to be limited or nonexistent.

8.14 ANIMAL MODELS USING OTHER VIRUSES

As RV does not normally infect rodents, an attenuated mengovirus infection model has been proposed as an alternative option to model RV infection. Mengovirus also belongs to the \textit{Picornaviridae} family and normally causes systemic infection in rodents. Using an attenuated mengovirus, intranasal inoculation of $10^7$ PFU into rats increased airway neutrophil and lymphocyte numbers, induced lung tissue pathology, and increased expression of CXCL1 and CCL2.\textsuperscript{204} A subsequent report using a genetically attenuated mengovirus vMC(0) in mice also induced lower respiratory tract infection with increased lung neutrophil and lymphocyte numbers, expression of CXCL1, CXCL2, CXCL5, IL-17A, INFs, and chemokines CXCL10 and CCL2.\textsuperscript{205}
Other respiratory virus infections are associated with acute exacerbations of asthma and COPD, including respiratory syncytial virus, influenza, human coronavirus, human parainfluenza virus, human metapneumoviruses, and adenoviruses. Animal models for these infections have largely been limited by the specificity of viruses to humans. It is unclear to what extent the mechanisms causing pathology differ between different viruses (or between strains of the same virus). A detailed discussion of the disease processes induced by each of these different virus infections is beyond the scope of this chapter. A detailed analysis of the relevant disease mechanisms in each infection setting is necessary to inform our understanding of disease exacerbations and ideally to identify common mechanisms between viruses that can be targeted for therapy.

8.14.1 Considerations, cautions, and limitations of animal infection models

No animal model can completely recapitulate naturally occurring human RV infection. While animal models provide important insights into disease mechanisms, it is important to also recognize their limitations.

There are recognized limitations of mice as models of human respiratory disease. These include differences in response/symptoms between other species and humans. There are differences in respiratory tract architecture in human, nonhuman primate, and mouse airways. They range from dichotomas (each airway splits into two), trichotomas (airways split into threes), or monopodial branching (central airway continues while subordinate airways branch out) with differences in airflow inhomogeneities covered in detail by Miller et al. There are also differences in mucus production processes in mouse compared with human airways. The short lifespans of laboratory animals do not capture the life-course of human disease, mice do not naturally develop asthma or COPD, and most current models of asthma represent eosinophilic, allergic patterns of disease. It remains unclear to what extent the current models and pathophysiology truly reflect human disease (particularly considering recognized heterogeneity of the human population).

RV has evolved for efficient replication in the human respiratory tract. Due to the decreased efficiency of RV entry into nonhuman epithelial cells (and likely differences in the nuances of cellular machinery required for replication), a high amount of viral load is required to elicit a biological response to RV in laboratory animals (e.g., $10^6$ TCID$_{50}$ in mouse vs 5–10 TCID$_{50}$ in experimental human infection models). Human RV
strains also demonstrate limited viral replication and different replication kinetic between mouse and man. These differences highlight the importance of confirming findings in relevant patient cohorts/samples and the utility of using human experimental models in parallel with animal models. This point is not purely for academic consideration. More so, it is important to take into consideration clinical trial design and outcomes. For example, mouse models highlighted the key relevance of IL-5 in asthma pathology through use of knockout mice and antibody blockade. However, the initial randomized control trial assessing anti-IL-5 therapy (mepolizumab) in a broad asthma population failed to demonstrate any clinical effect. It was not until subsequent trials limited recruitment to patients with demonstrable eosinophilic asthma (a patient subset that is more closely modeled by the experimental mouse system) that clinical improvements were observed.

8.14.2 Future directions for animal models

In a similar way to experimental human infection models, there has been a narrow focus in animal models. In mice, focus has largely been on RV infection alone with a growing body of literature assessing asthma exacerbations. While difficult to model in mice, RV-induced COPD exacerbations models are emerging through use of elastase administration and cigarette smoke-induced COPD. A summary of key findings from mouse models of RV-induced exacerbations of airway disease is presented in Table 8.2. Limited studies have reported on RV effects or potential interventions in these models. As with human experimental infections, animal infection models may also be relevant to an expanding array of diseases in the future (e.g., CF, bronchiectasis).

To date, there has been limited assessment across different RV strains in both animal and human studies. The primary focus of RV models has been on RV-A1 in mice, or RV-A16 and RV-A39 in human, possibly due to the availability of these strains and the ease of growing these strains in cell culture. In particular (due to its relatively recent discovery), RV-C infection has yet to be assessed in animal models. There has so far been difficulty in generating sufficient quantities of RV-C for research purposes (particularly at infectious titers required for mouse models). With the recent establishment of a suitable cell line (E8 HeLa cells) that supports RV-C replication this gap in the literature will likely be rectified.
### Table 8.2 Key experimental infection studies in mouse models of exacerbations

#### Mouse asthma exacerbations

| Authors                     | Model                        | Interventions                                                                 | Main findings                                                                 |
|-----------------------------|------------------------------|-------------------------------------------------------------------------------|-------------------------------------------------------------------------------|
| Toussaint et al. (2017)     | HDM + RV-A1 infection       | DNAse and NETosis inhibition                                                  | Treatment suppressed type 2 immunopathology                                  |
| Girkin et al. (2017)        | HDM + RV-A1                 | Deletion of TNF-related apoptosis inducing ligand (Tnfsf10−/−)                | Deletion suppressed cellular infiltration and AHR                             |
| Han et al. (2016)           | OVA + RV-A1                 | Gene targeted deletion of TLR2 (TLR2−/−)                                      | Reduced apoptotic cell death and reduced IFN-λ2/3                              |
| Hatchwell et al. (2015)     | HDM + RV-A1                 | Targeted deletion of TLR7 Exogenous IFN                                       | Deletion reduced neutrophilic and eosinophilic inflammation and AHR           |
| Han et al. (2016)           | OVA + RV-A1                 | Deletion of Foxa3 (Foxa3−/−)                                                   | Treatment attenuated eosinophilic inflammation and AHR                         |
| Phan et al. (2014)          | HDM + RV-A1                 | Nil                                                                           | HDM and RV had additive increases in neutrophilia and tissue elastance       |
| Hatchwell (2014)            | HDM + RV-A1                 | Salmeterol                                                                    | Treatment reduced inflammation via increased PPA2 activity                   |
| Chen et al. (2014)          | HDM + RV-A1                 | Deletion of Foxa3 (Foxa3−/−)                                                   | Deletion inhibited RV clearance                                               |
| Beale et al. (2014)         | OVA + RV-A1                 | IL-25 receptor blockade                                                       | Treatment attenuated type 2 cytokine expression, mucus production and inflammatory cell recruitment |
| Hong et al. (2014)          | OVA + RV-A1                 | Gene targeted deletion of IL-4 receptor (IL-4R)                               | Deletion shifted from type 2 to type 1 response and increased neutrophilic inflammation |
| de Souza Alves (2013)       | HDM + RV-A1                 | Anthraquinone (PI3K-mediated AKT phosphorylation inhibitor)                    | Treatment reduced AHR, viral replication, neutrophilic and eosinophilic inflammation |
| Traub et al. (2013)         | OVA + RV-A1                 | Anti-ICAM-1                                                                   | Treatment suppressed T_{H2} cytokine/chemokine production                    |
| Glanville et al. (2013)     | OVA + RV-A1                 | Anti-gamma-delta-T-cell receptor antibody                                      | Treatment increased T_{H2} inflammation and AHR                              |
| Collison et al. (2013)      | OVA + RV-A1                 | Inhibition of the E3 ubiquitin ligase MID1                                    | Treatment suppressed allergic airway inflammation and AHR                     |
| Schneider et al. (2013)     | HDM + RV-A1                 | CCL2 neutralizing antibody                                                     | Treatment reduced airway inflammation and AHR                                 |
| Nagarkar et al. (2010)      | OVA + RV-A1                 | Antieotaxin-1                                                                | Treatment reduced airway eosinophilia and AHR                                 |
| Bartlett et al. (2008)      | OVA + RV-A1                 | Nil                                                                           | RV infection exacerbated allergic airway inflammation and AHR                 |
### Mouse COPD exacerbations

| Study                          | Intervention  | Pharmacology/Intervention | Key Findings                                      |
|--------------------------------|---------------|----------------------------|---------------------------------------------------|
| Jing et al. (2018)\(^{192}\)   | Cigarette smoke + RV-A1 | Gamma-secretase inhibitor (Notch inhibitor) | Attenuated mucin expression                       |
| Farazuddin et al. (2018)\(^{193}\) | Cigarette smoke + RV-A1 | Querceptin                  | Reduced inflammation, goblet cell metaplasia, and AHR |
| Singanayagam et al. (2018)\(^{170}\) | Elastase + RV-A1 | Fluticasone propionate     | Suppressed antiviral immunity                      |
| Singanayagam et al. (2015)\(^{190}\) | Elastase + RV-A1 | Nil                         | Enhanced airway inflammation                       |
| Ganesan, et al. (2014)\(^{191}\) | Cigarette smoke + RV-A1 | Nil                         | Increased mucous production                        |
| Sajjan et al. (2009)\(^{189}\) | Elastase/LPS + RV-A1 | Nil                         | Deficient antiviral immunity                       |

Key findings from studies of RV infection in mouse models with underlying allergic airway disease (asthma) and COPD, including information on the approach used, interventions where applicable, and key experimental findings. AHR, airway hyperresponsiveness; HDM, house dust mite; ICAM-1, intracellular adhesion molecule 1; IL, interleukin; IFN, interferon; LPS, lipopolysaccharide; MID1, midline 1; OVA, ovalbumin; pDCs, plasmacytoid dendritic cells; RV, rhinovirus; T\(_{H2}\), T-helper type 2; TLR, Toll-like receptor.
As is the case for the majority of human virus—mouse infection models, the mouse is semipermissive to RV infection and as such a high-titer inoculum is required to induce prolonged replication and robust, reproducible host immune responses. A mouse-adapted RV strain (RV-1BM) has been generated by serial passage in mouse epithelial cells (LA-4 cells) though this mouse-adapted virus has only been characterized in vitro with primary mouse tracheal epithelial cells\(^{215}\) and has not yet been tested in vivo.

The clinical translation of novel therapies identified in animal models for the treatment of RV infection in humans is yet to come to fruition. However, there are multiple molecules currently in the drug development pipeline, ranging from virus-targeting molecules, drugs targeting host factors of the viral replication cycle, and biologics such as innate immune stimulators and cytokine blocking monoclonal antibodies, all of which are elaborated on in Chapter 9, Emerging therapeutic approaches.

### 8.15 CONCLUSION

There are significant opportunities for further research in both human and nonhuman models, including assessment of infections in various unexplored disease backgrounds that are exacerbated by RV infection (e.g., cystic fibrosis) and expansion of studies using newly identified RV strains (e.g., RV-C strains). Human and mouse RV experimental infection models effectively complement each other and have contributed immensely to our understanding the mechanisms shaping RV-induced pathology. Human experimental RV challenge studies have shed light on the biology of RV infection and the mechanisms associated with RV-induced exacerbations of chronic respiratory diseases. Mouse models of RV infection in particular are readily manipulatable to identify cause and effect between specific molecules and disease outcomes for preclinical testing. An excellent example of how human and mouse models complement each other is the growing understanding of the disease mechanisms during RV-induced asthma exacerbations. Human experimental infection revealed a potential role for induced type 2 immunity following RV infection in individuals with asthma.\(^{90,98}\) Subsequent mouse model studies have demonstrated a causal role for RV-induced type 2 immune effector molecules in exacerbations,\(^{99,152,176,179}\) allowing preclinical assessment of the efficacy and safety of novel therapies. Findings from these studies have not yet resulted in the development of approved therapies for RV infections, cold
symptoms, or exacerbations of respiratory diseases associated with RV infection. However, the wealth of knowledge derived from experimental RV infections has broadened our understanding and identified many potential therapeutic approaches.

REFERENCES

1. Kruse. *Münchener medizinische Wochenschrift*. 1914;61:1547.
2. Ma X, Conrad T, Alchikh M, Reiche J, Schweiger B, Rath B. Can we distinguish respiratory viral infections based on clinical features? A prospective pediatric cohort compared to systematic literature review. *Rev Med Virol*. 2018;28:e1997.
3. Thiberville SD, Ninove L, Vu Hai V, et al. The viral etiology of an influenza-like illness during the 2009 pandemic. *J Med Virol*. 2012;84(7):1071–1079.
4. Fullen DJ, Murray B, Mori J, et al. A tool for investigating asthma and COPD exacerbations: a newly manufactured and well characterised GMP wild-type human rhinovirus for use in the human viral challenge model. *PLoS One*. 2016;11(12):e0166113.
5. Couch RB, Cate TR, Douglas Jr. R.G, Gerone PJ, Knight V. Effect of route of inoculation on experimental respiratory viral disease in volunteers and evidence for airborne transmission. *Bacteriol Rev*. 1966;30(3):517–529.
6. Dick EC, Jennings LC, Mink KA, Wartgow CD, Inhorn SL. Aerosol transmission of rhinovirus colds. *J Infect Dis*. 1987;156(3):442–448.
7. Gwaltney Jr. JM, Hendley JO. Transmission of experimental rhinovirus infection by contaminated surfaces. *Am J Epidemiol*. 1982;116(5):828–833.
8. Douglas Jr R.G. Pathogenesis of rhinovirus common colds in human volunteers. *Ann Otol Rhinol Laryngol*. 1970;79(3):563–571.
9. Halperin SA, Eggleston PA, Hendley JO, Suratt PM, Groschel DH, Gwaltney Jr. JM. Pathogenesis of lower respiratory tract symptoms in experimental rhinovirus infection. *Am Rev Respir Dis*. 1983;128(5):806–810.
10. Bush RK, Busse W, Flaherty D, Warshauer D, Dick EC, Reed CE. Effects of experimental rhinovirus 16 infection on airways and leukocyte function in normal subjects. *J Allergy Clin Immunol*. 1978;61(2):80–87.
11. Cate TR, Couch RB, Fleet WF, Griffith WR, Gerone PJ, Knight V. Production of tracheobronchitis in volunteers with rhinovirus in a small-particle aerosol. *Am J Epidemiol*. 1965;81:95–105.
12. Hendley JO, Edmondson Jr WP, Gwaltney Jr JM. Relation between naturally acquired immunity and infectivity of two rhinoviruses in volunteers. *J Infect Dis*. 1972;125(3):243–248.
13. Winther B, Buchert D, Turner RB, Hendley JO, Tschaikin M. Decreased rhinovirus shedding after intranasal oxymetazoline application in adults with induced colds compared with intranasal saline. *Am J Rhinol Allergy*. 2010;24(5):374–377.
14. Turner RB, Bauer R, Woelkart K, Hulse TC, Gangemi JD. An evaluation of *Echinacea angustifolia* in experimental rhinovirus infections. *N Engl J Med*. 2005;353(4):341–348.
15. Turner RB, Riker DK, Gangemi JD. Ineffectiveness of *Echinacea* for prevention of experimental rhinovirus colds. *Antimicrob Agents Chemother*. 2000;44(6):1708–1709.
16. Sperber SJ, Hendley JO, Hayden FG, Riker DK, Sorrentino JV, Gwaltney Jr JM. Effects of naproxen on experimental rhinovirus colds. A randomized, double-blind, controlled trial. *Ann Intern Med*. 1992;117(1):37–41.
17. Sperber SJ, Hunger SB, Schwartz B, Pestka S. Anti-rhinoviral activity of recombinant and hybrid species of interferon alpha. *Antiviral Res.* 1993;22(2–3):121–129.

18. Sperber SJ, Shah LP, Gilbert RD, Ritchey TW, Monto AS. *Echinacea purpurea* for prevention of experimental rhinovirus colds. *Clin Infect Dis.* 2004;38(10):1367–1371.

19. Sperber SJ, Sorrentino JV, Riker DK, Hayden FG. Evaluation of an alpha agonist alone and in combination with a nonsteroidal antiinflammatory agent in the treatment of experimental rhinovirus colds. *Bull NY Acad Med.* 1989;65(1):145–160.

20. Turner RB, Sperber SJ, Sorrentino JV, et al. Effectiveness of clemastine fumarate for treatment of rhinorrhea and sneezing associated with the common cold. *Clin Infect Dis.* 1997;25(4):824–830.

21. Hayden FG, Turner RB, Gwaltney JM, et al. Phase II, randomized, double-blind, placebo-controlled studies of ruprintrivir nasal spray 2-percent suspension for prevention and treatment of experimentally induced rhinovirus colds in healthy volunteers. *Antimicrob Agents Chemother.* 2003;47(12):3907–3916.

22. Turner RB, Gwaltney JM, et al. Efficacy of brompheniramine maleate for the treatment of rhinovirus colds. *Clin Infect Dis.* 2000;31(5):1202–1208.

23. Abisheganaden JA, Avila PC, Kishiyama JL, et al. Effect of clarithromycin on experimental rhinovirus-16 colds: a randomized, double-blind, controlled trial. *Am J Med.* 2000;108(6):453–459.

24. Turner RB, Wecker MT, Pohl G, et al. Efficacy of tremacamra, a soluble intercellular adhesion molecule 1, for experimental rhinovirus infection: a randomized clinical trial. *JAMA.* 1999;281(19):1797–1804.

25. Pitkaranta A, Wecker MT, Korts DC, Hayden FG. Combined intranasal ipratropium bromide and oxymetazoline in experimental rhinovirus infection. *Am J Rhinol.* 1998;12(2):125–129.

26. Gwaltney Jr JM, Druce HM. Efficacy of brompheniramine maleate for the treatment of rhinovirus colds. *Clin Infect Dis.* 1997;25(5):1188–1194.

27. Gwaltney Jr JM, Park J, Paul RA, Edelman DA, O’Connor RR, Turner RB. Randomized controlled trial of clemastine fumarate for treatment of experimental rhinovirus colds. *Clin Infect Dis.* 1996;22(4):656–662.

28. Gustafson LM, Proud D, Hendley JO, Hayden FG, Gwaltney Jr JM. Oral prednisone therapy in experimental rhinovirus infections. *J Allergy Clin Immunol.* 1996;97(4):1009–1014.

29. Doyle WJ, Riker DK, McBride TP, et al. Therapeutic effects of an anticholinergic-sympathomimetic combination in induced rhinovirus colds. *Ann Otol Rhinol Laryngol.* 1993;102(7):521–527.

30. Turner RB, Dutko FJ, Lockwood G, Hayden FG. Efficacy of oral WIN 54954 for prophylaxis of experimental rhinovirus infection. *Antimicrob Agents Chemother.* 1993;37(2):297–300.

31. al-Nakib W, Higgins PG, Barrow GI, et al. Suppression of colds in human volunteers challenged with rhinovirus by a new synthetic drug (R61837). *Antimicrob Agents Chemother.* 1989;33(4):522–525.

32. Hayden FG, Andries K, Janssen PA. Safety and efficacy of intranasal pirodavir (R77975) in experimental rhinovirus infection. *Antimicrob Agents Chemother.* 1992;36(4):727–732.

33. Hayden FG, Hipskind GJ, Woerner DH, et al. Intranasal pirodavir (R77,975) treatment of rhinovirus colds. *Antimicrob Agents Chemother.* 1995;39(2):290–294.

34. Higgins PG, al-Nakib W, Barrow GI, Tyrrell DA. Recombinant human interferon-gamma as prophylaxis against rhinovirus colds in volunteers. *J Interferon Res.* 1988;8(5):591–596.
35. Higgins PG, Al-Nakib W, Willman J, Tyrrell DA. Interferon-beta ser as prophylaxis against experimental rhinovirus infection in volunteers. J Interferon Res. 1986;6 (2):153–159.

36. Higgins PG, Barrow GI, al-Nakib W, Tyrrell DA, DeLong DC, Lenox-Smith I. Failure to demonstrate synergy between interferon-alpha and a synthetic antiviral, enviroxime, in rhinovirus infections in volunteers. Antiviral Res. 1988;10 (1–3):141–149.

37. Higgins PG, Barrow GI, Tyrrell DA. A study of the efficacy of the bradykinin antagonist, NPC 567, in rhinovirus infections in human volunteers. Antiviral Res. 1990;14 (6):339–344.

38. Higgins PG, Barrow GI, Tyrrell DA, Isaacs D, Gauci CL. The efficacy of intranasal interferon alpha-2a in respiratory syncytial virus infection in volunteers. Antiviral Res. 1990;14(1):3–10.

39. Hsia J, Simon GL, Higgins N, Goldstein AL, Hayden FG. Immune modulation by aspirin during experimental rhinovirus colds. Bull NY Acad Med. 1989;65(1):45–56.

40. Gaffey MJ, Gwaltney Jr JM, Dressler WE, Sorrentino JV, Hayden FG. Intranasally administered atropine methonitrate treatment of experimental rhinovirus colds. Am Rev Respir Dis. 1987;135(1):241–244.

41. Gaffey MJ, Gwaltney Jr JM, Sastre A, Dressler WE, Sorrentino JV, Hayden FG. Intranasally and orally administered antihistamine treatment of experimental rhinovirus colds. Am Rev Respir Dis. 1987;136(3):556–560.

42. Gaffey MJ, Hayden FG, Boyd JC, Gwaltney Jr JM. Ipratropium bromide treatment of experimental rhinovirus infection. Antimicrob Agents Chemother. 1988;32 (11):1644–1647.

43. Hayden FG, Gwaltney Jr JM, Colonno RJ. Modification of experimental rhinovirus colds by receptor blockade. Antiviral Res. 1988;9(4):233–247.

44. Al-Nakib W, Higgins PG, Barrow I, Tyrrell DA, Lenox-Smith I, Ishitsuka H. Intranasal chalcone, Ro 09-0410, as prophylaxis against rhinovirus infection in human volunteers. J Antimicrob Chemother. 1987;20(6):887–892.

45. Al-Nakib W, Willman J, Higgins PG, Tyrrell DA, Shepherd WM, Freestone DS. Failure of intranasally administered 4′, 6-dichloroflavan to protect against rhinovirus infection in man. Arch Virol. 1987;92(4):255–260.

46. Phillpotts RJ, Wallace J, Tyrrell DA, Freestone DS, Shepherd WM. Failure of oral 4′,6-dichloroflavan to protect against rhinovirus infection in man. Arch Virol. 1983;75 (1–2):115–121.

47. Phillpotts RJ, Wallace J, Tyrrell DA, Tagart VB. Therapeutic activity of enviroxime against rhinovirus infection in volunteers. Antimicrob Agents Chemother. 1983;23 (5):671–675.

48. Pachuta DM, Togo Y, Hornick RB, Schwartz AR, Tominaga S. Evaluation of iso-prinosine in experimental human rhinovirus infection. Antimicrob Agents Chemother. 1974;5(4):403–408.

49. Togo Y, Durr FE, Laurenzana DA. Clinical evaluation of prophylactic intranasal 1-phenyl-3-(4-phenyl-2-thiazolyl) guanidine (CL 88,277) medication against rhinovirus 44 challenge. Med Microbiol Immunol. 1977;163(1):37–44.

50. Togo Y, Schwartz AR, Hornick RB. Antiviral effect of 3, 4-dihydro-1-isoquinolineacetamide hydrochloride in experimental human rhinovirus infection. Antimicrob Agents Chemother. 1973;4(6):612–616.

51. Togo Y, Schwartz AR, Hornick RB. Failure of a 3-substituted triazinoindole in the prevention of experimental human rhinovirus infection. Chemotherapy. 1973;18 (1):17–26.

52. George RB, Mogabgab WJ. Atypical pneumonia in young men with rhinovirus infections. Ann Intern Med. 1969;71(6):1073–1078.
53. Buscho RO, Saxtan D, Shultz PS, Finch E, Muñson MA. Infections with viruses and Mycoplasma pneumoniae during exacerbations of chronic bronchitis. *J Infect Dis*. 1978;137(4):377–383.

54. Mitchell I, Inglis JM, Simpson H. Viral infection as a precipitant of wheeze in children. Combined home and hospital study. *Arch Dis Child*. 1978;53(2):106–111.

55. Beasley R, Coleman ED, Hermon Y, Holst PE, O'Donnell TV, Tobias M. Viral respiratory tract infection and exacerbations of asthma in adult patients. *Thorax*. 1988;43(9):679–683.

56. Jain S, Self WH, Wunderink RG, et al. Community-acquired pneumonia requiring hospitalization among U.S. adults. *N Engl J Med*. 2015;373(5):415–427.

57. Mansbach JM, Piedra PA, Teach SJ, et al. Prospective multicenter study of viral etiology and hospital length of stay in children with severe bronchiolitis. *Arch Pediatr Adolesc Med*. 2012;166(8):700–706.

58. Pitkaranta A, Arruda E, Malmberg H, Hayden FG. Detection of rhinovirus in sinus brushings of patients with acute community-acquired sinusitis by reverse transcription-PCR. *J Clin Microbiol*. 1997;35(7):1791–1793.

59. Chang ML, Jordan-Villegas A, Evans A, et al. Respiratory viruses identified in an urban children’s hospital emergency department during the 2009 influenza A(H1N1) pandemic. *Pediatr Emerg Care*. 2012;28(10):990–997.

60. Johnston SL, Pattemore PK, Sanderson G, et al. Community study of role of viral infections in exacerbations of asthma in 9–11 year old children. *BMJ*. 1995;310(6989):1225–1229.

61. Zwaans WA, Mallia P, van Winden ME, Rohde GG. The relevance of respiratory viral infections in the exacerbations of chronic obstructive pulmonary disease—a systematic review. *J Clin Virol*. 2014;61(2):181–188.

62. GBD 2015 Chronic Respiratory Disease Collaborators. Global, regional, and national deaths, prevalence, disability-adjusted life years, and years lived with disability for chronic obstructive pulmonary disease and asthma, 1990-2015: a systematic analysis for the Global Burden of Disease Study 2015. *Lancet Respir Med*. 2017;5(9):691–706.

63. Wark PA, Johnston SL, Moric I, Simpson JL, Hensley MJ, Gibson PG. Neutrophil degranulation and cell lysis is associated with clinical severity in virus-induced asthma. *Eur Respir J*. 2002;19(1):68–75.

64. Grissell TV, Powell H, Shafren DR, et al. Interleukin-10 gene expression in acute virus-induced asthma. *Am J Respir Crit Care Med*. 2005;172(4):433–439.

65. Arden KE, Chang AB, Lambert SB, Nissen MD, Sloots TP, Mackay IM. Newly identified respiratory viruses in children with asthma exacerbation not requiring admission to hospital. *J Med Virol*. 2010;82(8):1458–1461.

66. Halperin SA, Eggleston PA, Beasley P, et al. Exacerbations of asthma in adults during experimental rhinovirus infection. *Am Rev Respir Dis*. 1985;132(5):976–980.

67. Lemanske Jr RF, Dick EC, Swenson CA, Vrtis RF, Busse WW. Rhinovirus upper respiratory infection increases airway hyperreactivity and late asthmatic reactions. *J Clin Invest*. 1989;83(1):1–10.

68. Calhoun WJ, Swenson CA, Dick EC, Schwartz LB, Lemanske Jr RF, Busse WW. Experimental rhinovirus 16 infection potentiates histamine release after antigen bronchoprovocation in allergic subjects. *Am Rev Respir Dis*. 1991;144(6):1267–1273.

69. Bardin PG, Fraenkel DJ, Sanderson G, et al. Amplified rhinovirus colds in atopic subjects. *Clin Exp Allergy*. 1994;24(5):457–464.

70. Fraenkel DJ, Bardin PG, Sanderson G, Lampe F, Johnston SL, Holgate ST. Lower airways inflammation during rhinovirus colds in normal and in asthmatic subjects. *Am J Respir Crit Care Med*. 1995;151(3 Pt 1):879–886.
71. Cheung D, Dick EC, Timmers MC, de Klerk EP, Spaan WJ, Sterk PJ. Rhinovirus inhalation causes long-lasting excessive airway narrowing in response to methacholine in asthmatic subjects in vivo. *Am J Respir Crit Care Med.* 1995;152(5 Pt 1):1490–1496.

72. Grunberg K, Timmers MC, Smits HH, et al. Effect of experimental rhinovirus 16 colds on airway hyperresponsiveness to histamine and interleukin–8 in nasal lavage in asthmatic subjects in vivo. *Clin Exp Allergy.* 1997;27(1):36–45.

73. Grunberg K, Kuipers EA, de Klerk EP, et al. Effects of experimental rhinovirus 16 infection on airway hyperresponsiveness to bradykinin in asthmatic subjects in vivo. *Am J Respir Crit Care Med.* 1997;155(3):833–838.

74. Grunberg K, Sharon RF, Hiltemann TJ, et al. Experimental rhinovirus 16 infection increases intercellular adhesion molecule-1 expression in bronchial epithelium of asthmatics regardless of inhaled steroid treatment. *Clin Exp Allergy.* 2000;30(7):1015–1023.

75. Gern JE, Galagan DM, Jarjour NN, Dick EC, Busse WW. Detection of rhinovirus RNA in lower airway cells during experimentally induced infection. *Am J Respir Crit Care Med.* 1997;155(3):1159–1161.

76. Parry DE, Busse WW, Sukow KA, Dick CR, Swenson C, Gern JE. Rhinovirus-induced PBMC responses and outcome of experimental infection in allergic subjects. *J Allergy Clin Immunol.* 2000;105(4):692–698.

77. Grunberg K, Timmers MC, de Klerk EP, Dick EC, Sterk PJ. Experimental rhinovirus 16 infection causes variable airway obstruction in subjects with atopic asthma. *Am J Respir Crit Care Med.* 1999;160(4):1375–1380.

78. Bardin PG, Fraenkel DJ, Sanderson G, van Schalkwyk EM, Holgate ST, Johnston SL. Peak expiratory flow changes during experimental rhinovirus infection. *Eur Respir J.* 2000;16(5):980–985.

79. de Gouw HW, Grunberg K, Schot R, Kroes AC, Dick EC, Sterk PJ. Relationship between exhaled nitric oxide and airway hyperresponsiveness following experimental rhinovirus infection in asthmatic subjects. *Eur Respir J.* 1998;11(1):126–132.

80. Grunberg K, Smits HH, Timmers MC, et al. Experimental rhinovirus 16 infection. Effects on cell differentials and soluble markers in sputum in asthmatic subjects. *Am J Respir Crit Care Med.* 1997;156(2 Pt 1):609–616.

81. Jarjour NN, Gern JE, Kelly EA, Swenson CA, Dick CR, Busse WW. The effect of an experimental rhinovirus 16 infection on bronchial lavage neutrophils. *J Allergy Clin Immunol.* 2000;105(6 Pt 1):1169–1177.

82. Gern JE, Vrtis R, Grindle KA, Swenson C, Busse WW. Relationship of upper and lower airway cytokines to outcome of experimental rhinovirus infection. *Am J Respir Crit Care Med.* 2000;162(6):2226–2231.

83. Christiansen SC, Eddleston J, Bengtson SH, et al. Experimental rhinovirus infection increases human tissue kallikrein activation in allergic subjects. *Int Arch Allergy Immunol.* 2008;147(4):299–304.

84. Majoor CJ, van de Pol MA, Kamphuisen PW, et al. Evaluation of coagulation activation after rhinovirus infection in patients with asthma and healthy control subjects: an observational study. *Respir Res.* 2014;15:14.

85. Mosser AG, Vrtis R, Burchell L, et al. Quantitative and qualitative analysis of rhinovirus infection in bronchial tissues. *Am J Respir Crit Care Med.* 2005;171(6):645–651.

86. Corne JM, Marshall C, Smith S, et al. Frequency, severity, and duration of rhinovirus infections in asthmatic and non-asthmatic individuals: a longitudinal cohort study. *Lancet.* 2002;359(9309):831–834.

87. Pizzichini MM, Pizzichini E, Efthimiadis A, et al. Asthma and natural colds. Inflammatory indices in induced sputum: a feasibility study. *Am J Respir Crit Care Med.* 1998;158(4):1178–1184.
88. de Kluijver J, Grunberg K, Pons D, et al. Interleukin-1beta and interleukin-1ra levels in nasal lavages during experimental rhinovirus infection in asthmatic and non-asthmatic subjects. *Clin Exp Allergy*. 2003;33(10):1415–1418.

89. Zambrano JC, Carper HT, Rakes GP, et al. Experimental rhinovirus challenges in adults with mild asthma: response to infection in relation to IgE. *J Allergy Clin Immunol*. 2003;111(5):1008–1016.

90. Message SD, Laza-Stanca V, Mallia P, et al. Rhinovirus-induced lower respiratory illness is increased in asthma and related to virus load and Th1/2 cytokine and IL-10 production. *Proc Natl Acad Sci USA*. 2008;105(36):13562–13567.

91. Contoli M, Message SD, Laza-Stanca V, et al. Role of deficient type III interferon-lambda production in asthma exacerbations. *Nat Med*. 2006;12(9):1023–1026.

92. Sykes A, Edwards MR, Macintyre J, et al. Rhinovirus 16-induced IFN-alpha and IFN-beta are deficient in bronchoalveolar lavage cells in asthmatic patients. *J Allergy Clin Immunol*. 2012;129(6):1506–1514.e6.

93. Parsons KS, Hsu AC, Wark PA. TLR3 and MDA5 signalling, although not expression, is impaired in asthmatic epithelial cells in response to rhinovirus infection. *Clin Exp Allergy*. 2014;44(1):91–101.

94. Sykes A, Edwards MR, Macintyre J, et al. TLR3, TLR4 and TLRs7-9 induced interferons are not impaired in airway and blood cells in well controlled asthma. *PLoS One*. 2013;8(6):e65921.

95. Sykes A, Macintyre J, Edwards MR, et al. Rhinovirus-induced interferon production is not deficient in well controlled asthma. *Thorax*. 2014;69(3):240–246.

96. DeMore JP, Weisshaar EH, Vrtis RF, et al. Similar colds in subjects with allergic asthma and nonatopic subjects after inoculation with rhinovirus-16. *J Allergy Clin Immunol*. 2009;124(2):245–252. 252 e241–e243.

97. Adura PT, Reed E, Macintyre J, et al. Experimental rhinovirus 16 infection in moderate asthmatics on inhaled corticosteroids. *Eur Respir J*. 2014;43(4):1186–1189.

98. Jackson DJ, Makrinioti H, Rana BM, et al. IL-33-dependent type 2 inflammation during rhinovirus-induced asthma exacerbations in vivo. *Am J Respir Crit Care Med*. 2014;190(12):1373–1382.

99. Beale J, Jayaraman A, Jackson DJ, et al. Rhinovirus-induced IL-25 in asthma exacerbation drives type 2 immunity and allergic pulmonary inflammation. *Sci Transl Med*. 2014;6(256):256ra134.

100. Jackson DJ, Glanville N, Trujillo–Torralbo MB, et al. Interleukin–18 is associated with protection against rhinovirus-induced colds and asthma exacerbations. *Clin Infect Dis*. 2015;60(10):1528–1531.

101. Jackson DJ, Trujillo–Torralbo MB, Del-Rosario J, et al. The influence of asthma control on the severity of virus-induced asthma exacerbations. *J Allergy Clin Immunol*. 2015;136(2):497–500.e3.

102. Djukanovic R, Harrison T, Johnston SL, et al. The effect of inhaled IFN-beta on worsening of asthma symptoms caused by viral infections. A randomized trial. *Am J Respir Crit Care Med*. 2014;190(2):145–154.

103. Vogelmeier CF, Criner GJ, Martinez FJ, et al. Global strategy for the diagnosis, management, and prevention of chronic obstructive lung disease 2017 report: GOLD executive summary. *Am J Respir Crit Care Med*. 2017;195(5):557–582.

104. Halpin DM, Miravitlles M, Metzdorf N, Celli B. Impact and prevention of severe exacerbations of COPD: a review of the evidence. *Int J Chron Obstruct Pulmon Dis*. 2017;12:2891–2908.

105. Beasley V, Joshi PV, Singanayagam A, Molynieux PL, Johnston SL, Mallia P. Lung microbiology and exacerbations in COPD. *Int J Chron Obstruct Pulmon Dis*. 2012;7:555–569.
106. Amin AN, Bollu V, Stensland MD, Netzer L, Ganapathy V. Treatment patterns for patients hospitalized with chronic obstructive pulmonary disease. *Am J Health Syst Pharm*. 2018;75(6):359–366.

107. Stenhouse AC. Rhinovirus infection in acute exacerbations of chronic bronchitis: a controlled prospective study. *Br Med J*. 1967;3(5563):461–463.

108. Wilkinson TMA, Aris E, Bourne S, et al. A prospective, observational cohort study of the seasonal dynamics of airway pathogens in the aetiology of exacerbations in COPD. *Thorax*. 2017;72(10):919–927.

109. Biancardi E, Fennell M, Rawlinson W, Thomas PS. Viruses are frequently present as the infecting agent in acute exacerbations of chronic obstructive pulmonary disease in patients presenting to hospital. *Intern Med J*. 2016;46(10):1160–1165.

110. Hosseini SS, Ghasemian E, Jamaati H, Tabaraie B, Amini Z, Cox K. Association between respiratory viruses and exacerbation of COPD: a case-control study. *Infect Dis (Lond)*. 2015;47(8):523–529.

111. Seemungal T, Harper-Owen R, Bhowmik A, et al. Respiratory viruses, symptoms, and inflammatory markers in acute exacerbations and stable chronic obstructive pulmonary disease. *Am J Respir Crit Care Med*. 2001;164(9):1618–1623.

112. Mallia P, Message SD, Kebadze T, Parker HL, Kon OM, Johnston SL. An experimental model of rhinovirus induced chronic obstructive pulmonary disease exacerbations: a pilot study. *Respir Res*. 2006;7:116.

113. Mallia P, Message SD, Gielen V, et al. Experimental rhinovirus infection as a human model of chronic obstructive pulmonary disease exacerbation. *Am J Respir Crit Care Med*. 2011;183(6):734–742.

114. Footitt J, Mallia P, Durham AL, et al. Oxidative and nitrosative stress and histone deacetylase-2 activity in exacerbations of COPD. *Chest*. 2016;149(1):62–73.

115. Rohde G, Wiethge A, Borg I, et al. Respiratory viruses in exacerbations of chronic obstructive pulmonary disease requiring hospitalisation: a case-control study. *Thorax*. 2003;58(1):37–42.

116. Mallia P, Message SD, Contoli M, et al. Lymphocyte subsets in experimental rhinovirus infection in chronic obstructive pulmonary disease. *Respir Med*. 2014;108(1):78–85.

117. Mallia P, Message SD, Contoli M, et al. Neutrophil adhesion molecules in experimental rhinovirus infection in COPD. *Respir Res*. 2013;14(1):72.

118. Mallia P, Footitt J, Sotero R, et al. Rhinovirus infection induces degradation of antimicrobial peptides and secondary bacterial infection in chronic obstructive pulmonary disease. *Am J Respir Crit Care Med*. 2012;186(11):1117–1124.

119. Dimopoulos G, Lerikou M, Tsiodras S, et al. Viral epidemiology of acute exacerbations of chronic obstructive pulmonary disease. *Pulm Pharmacol Ther*. 2012;25(1):12–18.

120. Molyneaux PL, Mallia P, Cox MJ, et al. Outgrowth of the bacterial airway microbiome after rhinovirus exacerbation of chronic obstructive pulmonary disease. *Am J Respir Crit Care Med*. 2013;188(10):1224–1231.

121. Mallia P, Webber J, Gill SK, et al. Role of airway glucose in bacterial infections in patients with chronic obstructive pulmonary disease. *J Allergy Clin Immunol*. 2018;142(3):815–823.e6.

122. George S, Garcha DS, Patel A, et al. Human rhinovirus infection and secondary bacterial infection in COPD exacerbations. *Am J Respir Crit Care Med*. 2013;187: A2164.

123. Grunberg K, Sharon RF, Sont JK, et al. Rhinovirus-induced airway inflammation in asthma: effect of treatment with inhaled corticosteroids before and during experimental infection. *Am J Respir Crit Care Med*. 2001;164(10 Pt 1):1816–1822.
124. de Kluijver J, Evertse CE, Sont JK, et al. Are rhinovirus-induced airway responses in asthma aggravated by chronic allergen exposure? *Am J Respir Crit Care Med*. 2003;168(10):1174—1180.

125. Kloepfer KM, DeMore JP, Vrtis RF, et al. Effects of montelukast on patients with asthma after experimental inoculation with human rhinovirus 16. *Ann Allergy Asthma Immunol*. 2011;106(3):252—257.

126. Silkoff PE, Flavin S, Gordon R, et al. Toll-like receptor 3 blockade in rhinovirus-induced experimental asthma exacerbations: a randomized controlled study. *J Allergy Clin Immunol*. 2018;141(4):1220—1230.

127. Kloepfer KM, Lee WM, Pappas TE, et al. Detection of pathogenic bacteria during rhinovirus infection is associated with increased respiratory symptoms and asthma exacerbations. *J Allergy Clin Immunol*. 2014;133(5):1301—1307. e1301—1303.

128. Akbarshahi H, Menzel M, Ramu S, Mahmutovic Persson I, Bjerner L, Uller L. House dust mite impairs antiviral response in asthma exacerbation models through its effects on TLR3. *Allergy*. 2018;73(5):1053—1063.

129. Capistrano SJ, Zakarya R, Chen H, Oliver BG. Biomass smoke exposure enhances rhinovirus-induced inflammation in primary lung fibroblasts. *Int J Mol Sci*. 2016;17(9):1—12.

130. Lehtinen MJ, Hibberd AA, Mannikko S, et al. Nasal microbiota clusters associate with inflammatory response, viral load, and symptom severity in experimental rhinovirus challenge. *Sci Rep*. 2018;8(1):11411.

131. Nguyen A, Guedan A, Mousnier A, et al. Host lipidome analysis during rhinovirus replication in human bronchial epithelial cells identifies potential therapeutic targets. *J Lipid Res*. 2018;59(9):1671—1684.

132. Stelzer-Braid S, Liu N, Doumit M, et al. Association of rhinovirus with exacerbations in young children affected by cystic fibrosis: preliminary data. *J Med Virol*. 2017;89(8):1494—1497.

133. Kieninger E, Singer F, Tapparel C, et al. High rhinovirus burden in lower airways of children with cystic fibrosis. *Chest*. 2013;143(3):782—790.

134. Gao YH, Guan WJ, Xu G, et al. The role of viral infection in pulmonary exacerbations of bronchiectasis in adults: a prospective study. *Chest*. 2015;147(6):1635—1643.

135. Kapur N, Mackay IM, Sloots TP, Masters IB, Chang AB. Respiratory viruses in exacerbations of non-cystic fibrosis bronchiectasis in children. *Arch Dis Child*. 2014;99(8):749—753.

136. Schogler A, Stokes AB, Casaulta C, et al. Interferon response of the cystic fibrosis bronchial epithelium to major and minor group rhinovirus infection. *J Cyst Fibros*. 2016;15(3):332—339.

137. Lambkin-Williams R, Noulin N, Mann A, Catchpole A, Gilbert AS. The human viral challenge model: accelerating the evaluation of respiratory antivirals, vaccines and novel diagnostics. *Respir Res*. 2018;19(1):123.

138. Del Vecchio AM, Branigan PJ, Barnathan ES, Flavin SK, Silkoff PE, Turner RB. Utility of animal and in vivo experimental infection of humans with rhinoviruses in the development of therapeutic agents for viral exacerbations of asthma and chronic obstructive pulmonary disease. *Pulm Pharmacol Ther*. 2015;30:32—43.

139. Maltby S, Tay HL, Yang M, Foster PS. Mouse models of severe asthma: understanding the mechanisms of steroid resistance, tissue remodelling and disease exacerbation. *Respirology*. 2017;22(5):874—885.

140. Kumar RK, Foster PS, Rosenberg HF. Respiratory viral infection, epithelial cytokines, and innate lymphoid cells in asthma exacerbations. *J Leukoc Biol*. 2014;96(3):391—396.

141. Bartle NT, Singanayagam A, Johnston SL. Mouse models of rhinovirus infection and airways disease. *Methods Mol Biol*. 2015;1221:181—188.
142. Lee W-M, Chen Y, Wang W, Mosser A. Growth of human rhinovirus in H1-HeLa cell suspension culture and purification of virions. Methods in Molecular Biology In: Jans DA, Ghildyal R., eds. *Rhinoviruses: Methods and Protocols*. vol. 1221. New York: Springer Science + Business Media; 2015:49–61.

143. Bartlett NW, Singanayagam A, Johnston SL. Mouse models of rhinovirus infection and airways disease. In: Jans DA, Ghildyal R, eds. *Rhinoviruses: Methods and Protocols*. New York: Springer New York; 2015:181–188.

144. Papi A, Johnston SL. Rhinovirus infection induces expression of its own receptor intercellular adhesion molecule 1 (ICAM-1) via increased NF-kappaB-mediated transcription. *J Biol Chem*. 1999;274(14):9707–9720.

145. Newcomb DC, Sajjan US, Nagarkar DR, et al. Human rhinovirus 1B exposure induces phosphatidylinositol 3-kinase-dependent airway inflammation in mice. *Am J Respir Crit Care Med*. 2008;177(10):1111–1121.

146. Han M, Chung Y, Young Hong J, et al. Toll-like receptor 2-expressing macrophages are required and sufficient for rhinovirus-induced airway inflammation. *J Allergy Clin Immunol*. 2016;138(6):1619–1630.

147. Greve JM, Davis G, Meyer AM, et al. The major human rhinovirus receptor is ICAM-1. *Cell*. 1989;56(5):839–847.

148. Tomassini JE, Graham D, DeWitt CM, Lineberger DW, Rodkey JA, Colombo RJ. cDNA cloning reveals that the major group rhinovirus receptor on HeLa cells is intercellular adhesion molecule 1. *Proc Natl Acad Sci USA*. 1989;86(13):4907–4911.

149. Register RB, Uncapher CR, Naylor AM, Lineberger DW, Colombo RJ. Human-murine chimera of ICAM-1 identify amino acid residues critical for rhinovirus and antibody binding. *J Virol*. 1991;65(12):6589–6596.

150. Yin FH, Lomax NB. Establishment of a mouse model for human rhinovirus infection. *J Gen Virol*. 1986;67(Pt 11):2335–2340.

151. Tuthill TJ, Papadopoulos NG, Jourdan P, et al. Mouse respiratory epithelial cells support efficient replication of human rhinovirus. *J Gen Virol*. 2003;84(Pt 10):2829–2836.

152. Bartlett NW, Walton RP, Edwards MR, et al. Mouse models of rhinovirus-induced disease and exacerbation of allergic airway inflammation. *Nat Med*. 2008;14(2):199–204.

153. Rasmussen AL, Racaniello VR. Selection of rhinovirus 1A variants adapted for growth in mouse lung epithelial cells. *Virology*. 2011;420(2):82–88.

154. Sajjan U, Wang Q, Zhao Y, Gruenert DC, Hershenson MB. Rhinovirus disrupts the barrier function of polarized airway epithelial cells. *Am J Respir Crit Care Med*. 2008;178(12):1271–1281.

155. Jayaraman A, Jackson DJ, Message SD, et al. IL-15 complexes induce NK- and T-cell responses independent of type I IFN signaling during rhinovirus infection. *Mucosal Immunol*. 2014;7(5):1151–1164.

156. Girkin J, Hatchwell L, Foster P, et al. CCL7 and IRF-7 mediate hallmark inflammatory and IFN responses following rhinovirus 1B infection. *J Immunol*. 2015;194(10):4924–4930.

157. Nagarkar DR, Wang Q, Shim J, et al. CXCR2 is required for neutrophilic airway inflammation and hyperresponsiveness in a mouse model of human rhinovirus infection. *J Immunol*. 2009;183(10):6698–6707.

158. Wang Q, Miller DJ, Bowman ER, et al. MDA5 and TLR3 initiate pro-inflammatory signaling pathways leading to rhinovirus-induced airways inflammation and hyperresponsiveness. *PLoS Pathog*. 2011;7(5):e1002070.

159. Bartlett NW, Slater L, Glanville N, et al. Defining critical roles for NF-kappaB p65 and type I interferon in innate immunity to rhinovirus. *EMBO Mol Med*. 2012;4(12):1244–1260.
Berman R, Jiang D, Wu Q, Stevenson CR, Schaefer NR, Chu HW. MUC18 regulates lung rhinovirus infection and inflammation. *PLoS One*. 2016;11(10):e0163927.

Glanville N, Peel TJ, Schroder A, et al. Tbet deficiency causes T helper cell dependent airways eosinophilia and mucus hypersecretion in response to rhinovirus infection. *PLoS Pathog*. 2016;12(9):e1005913.

Mehta AK, Duan W, Doerner AM, et al. Rhinovirus infection interferes with induction of tolerance to aeroantigens through OX40 ligand, thymic stromal lymphopoietin, and IL-33. *J Allergy Clin Immunol*. 2016;137(1):278–288.e6.

Girkin JL, Hatchwell LM, Collison AM, et al. TRAIL signaling is proinflammatory and proviral in a murine model of rhinovirus 1B infection. *Am J Physiol Lung Cell Mol Physiol*. 2017;312(1):L89–L99.

Unger BL, Faris AN, Ganesan S, Comstock AT, Hershenson MB, Sajjan US. Rhinovirus attenuates non-typeable *Haemophilus influenzae*-stimulated IL-8 responses via TLR2-dependent degradation of IRAK-1. *PLoS Pathog*. 2012;8(10):e1002969.

Cui TX, Maheshwer B, Hong JY, Goldsmith AM, Bentley JK, Popova AP. Hyperoxic exposure of immature mice increases the inflammatory response to subsequent rhinovirus infection: association with danger signals. *J Immunol*. 2016;196(11):4692–4705.

Phan JA, Kicic A, Berry LJ, Sly PD, Larcombe AN. Early life rhinovirus infection exacerbates house-dust-mite induced lung disease more severely in female mice. *Exp Lung Res*. 2016;42(1):24–36.

McLean GR, Walton RP, Shetty S, et al. Rhinovirus infections and immunisation induce cross-serotype reactive antibodies to VP1. *Antiviral Res*. 2012;95(3):193–201.

Ganesan S, Faris AN, Comstock AT, et al. Quercetin inhibits rhinovirus replication in vitro and in vivo. *Antiviral Res*. 2012;94(3):258–271.

Song JH, Kim SR, Heo EY, et al. Antiviral activity of gemcitabine against human rhinovirus in vitro and in vivo. *Antiviral Res*. 2017;145:6–13.

Singanayagam A, Glanville N, Girkin JL, et al. Corticosteroid suppression of antiviral immunity increases bacterial loads and mucus production in COPD exacerbations. *Nat Commun*. 2018;9(1):2229.

Heinz SA, Henson DA, Austin MD, Jin F, Nieman DC. Quercetin supplementation and upper respiratory tract infection: a randomized community clinical trial. *Pharmacoep Res*. 2010;62(3):237–242.

Nagarkar DR, Bowman ER, Schneider D, et al. Rhinovirus infection of allergensensitized and -challenged mice induces eotaxin release from functionally polarized macrophages. *J Immunol*. 2010;185(4):2525–2535.

Chung Y, Hong JY, Lei J, Chen Q, Bentley JK, Hershenson MB. Rhinovirus infection induces interleukin-13 production from CD11b-positive, M2-polarized exudative macrophages. *Am J Respir Cell Mol Biol*. 2015;52(2):205–216.

Hong JY, Chung Y, Steenrod J, et al. Macrophage activation state determines the response to rhinovirus infection in a mouse model of allergic asthma. *Respir Res*. 2014;15:63.

Glanville N, Message SD, Walton RP, et al. γδT cells suppress inflammation and disease during rhinovirus-induced asthma exacerbations. *Mucosal Immunol*. 2013;6(6):1091–1100.

Chairakaki AD, Saridaki MI, Pyrillou K, et al. Plasmacytoid dendritic cells drive acute asthma exacerbations. *J Allergy Clin Immunol*. 2018;142(2):542–566.e12.

Phan JA, Kicic A, Berry LJ, et al. Rhinovirus exacerbates house-dust-mite induced lung disease in adult mice. *PLoS One*. 2014;9(3):e92163.

Toussaint M, Jackson DJ, Swieboda D, et al. Host DNA released by NETosis promotes rhinovirus-induced type-2 allergic asthma exacerbation. *Nat Med*. 2017;23(6):681–691.
179. Collison A, Hatchwell L, Verrills N, et al. The E3 ubiquitin ligase midline 1 promotes allergen and rhinovirus-induced asthma by inhibiting protein phosphatase 2A activity. *Nat Med*. 2013;19(2):232–237.

180. Schneider D, Hong JY, Bowman ER, et al. Macrophage/epithelial cell CCL2 contributes to rhinovirus-induced hyperresponsiveness and inflammation in a mouse model of allergic airways disease. *Am J Physiol Lung Cell Mol Physiol*. 2013;304(3):L162–L169.

181. Chen G, Korfhagen TR, Karp CL, et al. Foxa3 induces goblet cell metaplasia and inhibits innate antiviral immunity. *Am J Respir Crit Care Med*. 2014;189(3):301–313.

182. Mahmutovic-Persson I, Akbarshahi H, Bartlett NW, et al. Inhaled dsRNA and rhinovirus evoke neutrophilic exacerbation and lung expression of thymic stromal lymphopoietin in allergic mice with established experimental asthma. *Allergy*. 2014;69(3):348–358.

183. Hatchwell L, Collison A, Girkin J, et al. Toll-like receptor 7 governs interferon and inflammatory responses to rhinovirus and is suppressed by IL-5-induced lung eosinophilia. *Thorax*. 2015;70(9):854–861.

184. Hatchwell L, Girkin J, Dun MD, et al. Salmeterol attenuates chemotactic responses in rhinovirus-induced exacerbation of allergic airways disease by modulating protein phosphatase 2A. *J Allergy Clin Immunol*. 2014;133(6):1720–1727.

185. Morales DR. LABA monotherapy in asthma: an avoidable problem. *Br J Gen Pract*. 2013;63(617):627–628.

186. Traub S, Nikonova A, Carruthers A, et al. An anti-human icam-1 antibody inhibits rhinovirus-induced exacerbations of lung inflammation. *PLoS Pathog*. 2013;9(8):e1003520.

187. de Souza Alves CC, Collison A, Hatchwell L, et al. Inhibiting AKT phosphorylation employing non-cytotoxic anthraquinones ameliorates TH2 mediated allergic airways disease and rhinovirus exacerbation. *PLoS One*. 2013;8(11):e79565.

188. Schilter HC, Collison A, Russo RC, et al. Effects of an anti-inflammatory VAP-1/SSAO inhibitor, PXS-4728A, on pulmonary neutrophil migration. *Respir Res*. 2015;16:42.

189. Singanayagam A, Ganesan S, Comstock AT, et al. Elastase- and LPS-exposed mice display altered responses to rhinovirus infection. *Am J Physiol Lung Cell Mol Physiol*. 2009;297(5):L931–L944.

190. Ganesan S, Comstock AT, Kinker B, Mancuso P, Beck JM, Sajjan US. Combined exposure to cigarette smoke and nontypeable *Haemophilus influenzae* drives development of a COPD phenotype in mice. *Respir Res*. 2014;15:11.

191. Jing Y, Gimenes JA, Mishra R, et al. NOTCH3 contributes to rhinovirus-induced goblet cell hyperplasia in COPD airway epithelial cells. *Thorax*. 2018;83:18–32.

192. Farazuddin M, Mishra R, Jing Y, Srivastava V, Comstock AT, Sajjan US. Quercetin prevents rhinovirus-induced progression of lung disease in mice with COPD phenotype. *PLoS One*. 2018;13(7):e0199612.

193. Lee SB, Song JA, Choi GE, Kim HS, Jang YJ. Rhinovirus infection in murine chronic allergic rhinosinusitis model. *Int Forum Allergy Rhinol*. 2016;6(11):1131–1138.

194. Blanco JC, Boukhvalova MS, Perez DR, Vogel SN, Cajon A. Modeling human respiratory viral infections in the cotton rat (*Sigmodon hispidus*). *J Antivir Antiretrovir*. 2014;6:40–42.

195. Blanco JC, Core S, Pletneva LM, March TH, Boukhvalova MS, Cajon AE. Prophylactic antibody treatment and intramuscular immunization reduce infectious
human rhinovirus 16 load in the lower respiratory tract of challenged cotton rats. *Trials Vaccinol*. 2014;3:52–60.

197. Patel MC, Pletneva LM, Boukhvalova MS, Vogel SN, Kajon AE, Blanco JCG. Immunization with live human rhinovirus (HRV) 16 induces protection in cotton rats against HRV14 infection. *Front Microbiol*. 2017;8:1646.

198. Martin GV, Heath RB. Rhinovirus infection of vervet monkeys. A model of human rhinovirus disease. *Br J Exp Pathol*. 1969;50(5):516–519.

199. Dick EC. Experimental infections of chimpanzees with human rhinovirus types 14 and 43. *Proc Soc Exp Biol Med*. 1968;127(4):1079–1081.

200. Pinto CA, Haff RF. Experimental infection of gibbons with rhinovirus. *Nature*. 1969;224(226):1310–1311.

201. Shipkowitz NL, Bower RR, Schleicher JB, Aquino F, Appell RN, Roderick WR. Antiviral activity of a bis-benzimidazole against experimental rhinoviruses in chimpanzees. *Appl Microbiol*. 1972;23(1):117–122.

202. Pinto CA, Bahnsen HP, Ravin LJ, Haff RF, Pagano JF. The antiviral effect of a triazinoindole (SK&F 40491) in rhinovirus infected gibbons. *Proc Soc Exp Biol Med*. 1972;141(2):467–474.

203. Huguenel ED, Cohn D, Dockum DP, et al. Prevention of rhinovirus infection in chimpanzees by soluble intercellular adherence molecule-1. *Am J Respir Crit Care Med*. 1997;155(4):1206–1210.

204. Rosenthal LA, Amineva SP, Szakaly RJ, Lemanske Jr RF, Gem JE, Sorkness RL. A rat model of picornavirus-induced airway infection and inflammation. *Virol J*. 2009;6:122.

205. Rosenthal LA, Szakaly RJ, Amineva SP, et al. Lower respiratory tract infection induced by a genetically modified picornavirus in its natural murine host. *PLoS One*. 2012;7(2):e32061.

206. Jackson DJ, Johnston SL. The role of viruses in acute exacerbations of asthma. *J Allergy Clin Immunol*. 2010;125(6):1178–1187. quiz 1188–1187.

207. Persson CG. Con: mice are not a good model of human airway disease. *Am J Respir Crit Care Med*. 2002;166(1):6–7. discussion 8.

208. Miller FJ, Mercer RR, Crapo JD. Lower respiratory tract structure of laboratory animals and humans: dosimetry implications. *Aerosol Sci Technol*. 1993;18(3):257–271.

209. Foster PS, Hogan SP, Ramsay AJ, Matthaei KI, Young IG. Interleukin 5 deficiency abolishes eosinophilia, airways hyperreactivity, and lung damage in a mouse asthma model. *J Exp Med*. 1996;183(1):195–201.

210. Hogan SP, Koskinen A, Foster PS. Interleukin-5 and eosinophils induce airway damage and bronchial hyperreactivity during allergic airway inflammation in BALB/c mice. *Immunol Cell Biol*. 1997;75(3):284–288.

211. Leckie MJ, ten Brinke A, Khan J, et al. Effects of an interleukin-5 blocking monoclonal antibody on eosinophils, airway hyper-responsiveness, and the late asthmatic response. *Lancet*. 2000;356(9248):2144–2148.

212. Nair P, Pizzichini MM, Kjarsgaard M, et al. Mepolizumab for prednisone-dependent asthma with sputum eosinophilia. *N Engl J Med*. 2009;360(10):985–993.

213. Pavord ID, Korn S, Howarth P, et al. Mepolizumab for severe eosinophilic asthma (DREAM): a multicentre, double-blind, placebo-controlled trial. *Lancet*. 2012;380(9842):651–659.

214. Bochkov YA, Watters K, Basnet S, et al. Mutations in VP1 and 3A proteins improve binding and replication of rhinovirus C15 in HeLa-E8 cells. *Virology*. 2016;499:350–360.

215. Foxman EF, Storer JA, Fitzgerald ME, et al. Temperature-dependent innate defense against the common cold virus limits viral replication at warm temperature in mouse airway cells. *Proc Natl Acad Sci USA*. 2015;112(3):827–832.