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CHAPTER 8

In vivo experimental models of infection and disease

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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

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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.[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.[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,[5–8] clinical symptoms,[8,9] inflammatory and immune responses,[10] involvement of the lower airway,[10,11] and correlates of immune protection of RV infection.[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.[13–51] Licensing approval was sought for an antiviral drug, pleconaril, for the treatment of RV infection.[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[52] 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.[53–55] 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,[56] bronchiolitis,[57] acute rhinosinusitis,[58] and influenza-like illness.[59] In addition RVs could be detected in most asthma exacerbations,[60] and in a substantial proportion of COPD exacerbations.[61] 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.[62] 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,[60,63–65] 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 hyperreactivity (AHR). The authors felt that these findings suggested "that other viral pathogens may play a more important role in precipitating asthma attacks."[66] 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.[67,68] 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.[69] Concurrently a study using PCR to detect viruses in naturally acquired asthma exacerbations strongly supported a role for RV.[60] Subsequent studies were carried out by research groups in the United Kingdom,[70] the Netherlands,[71–74] and the United States[75,76] in volunteers with mild, intermittent asthma. These studies demonstrated that RV infection induced airway obstruction,[77,78] increased AHR,[70–73,79] and airway inflammation[70,72,79–84] and RV could be

detected in the lower airways,[75,85] 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.[86] 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.[87] 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,[70] another reporting increased nasal inflammatory mediators in asthma[88] and discrepant results regarding virus-induced respiratory symptoms.[88,89] 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.[90] 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.[90] 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_H2) responses (IL-4, IL-5, and IL-13), indicating that excessive T_H2 or impaired T_H1 (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 IFNβ and IFNλ, and this was related to the severity of virus-induced asthma exacerbations.[91] Other reports subsequently confirmed that IFN production is deficient in asthma,[92,93] but this finding has not been replicated in all studies.[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.[97] A larger study confirmed this and reported significantly more upper and lower respiratory symptoms, greater reduction in peak expiratory flow and FEV_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.[98] This study also identified novel mediators of virus-induced asthma exacerbations including IL-33,[98] IL-25,[99] and IL-18.[100] Poor asthma control was associated with more severe virus-induced exacerbations, greater T_H2 inflammation and higher virus load.[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 IFNβ as a treatment for asthma exacerbations. A clinical trial of inhaled IFNβ reported that treatment can reduce the severity of virus-induced exacerbations in a subgroup of patients assessed with more severe asthma.[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."[103] Acute exacerbations of COPD are the major drivers of morbidity, mortality, and healthcare costs in COPD and prevention of exacerbations a major unmet need.[104] 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.[105,106] 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.[53,107] 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.[108−111] 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 (FEV_1 50%−80% predicted) and not using regular inhaled therapy.[112] 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.[113,114] 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.[113,114] 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.[115] 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.[113,114] 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,[113,114] inflammatory cells,[113,116,117] oxidative and nitrosative stress,[114] and impaired antiviral IFN responses.[113] A novel observation that emerged from these studies was that secondary bacterial infections occurred in 60% of experimental virus-induced exacerbations,[118] whereas coinfection was rarely reported in naturally acquired exacerbations.[119] Analysis of the respiratory microbiome following

experimental RV infection suggest that secondary infection occurs due to an outgrowth of previously present airway bacteria.[120] Potential mechanisms of secondary bacterial infection include reduced antimicrobial peptides[118] and increased glucose in the airways.[121] A subsequent study of naturally acquired exacerbations sampling at multiple time points during exacerbations confirmed the validity of this observation.[122] 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,[127] allergens,[128] and air pollution.[129] These factors have been somewhat neglected in viral challenge studies and are a promising field of future research that is starting to be addressed.[124,130]

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[131] and metabolomics[121] in asthma and COPD.

Respiratory viruses have also been identified as triggers of exacerbations in other airway diseases such as cystic fibrosis[132,133] and bronchiectasis[134,135] and there is evidence of impaired antiviral immunity in these diseases.[136] Experimental infection studies may help to define the role of virus infection in these patient populations.

Table 8.1 Human experimental infection studies in asthma and chronic obstructive pulmonary disease (COPD)

Study	Patient population	Controls	Main outcomes
Halperin et al. (1985)[66]	21 AA, 12 using SABA, 1 using ICS		No change in FEV_1 or AHR
Bardin et al. (1994)[69]	6 AA, mild asthma, SABA only	11 NANA, 6 ANA	More severe colds in atopic subjects
Fraenkel et al. (1995)[70]	6 AA, SABA only	11 NANA	No change in in FEV_1 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)[71]	14 AA, SABA only. 7 infected, 7 sham infected		No change in FEV_1 or AHR, increased cold and asthma symptoms, increased blood neutrophils and reduced blood lymphocytes in the infected group
Grunberg et al. (1997, 1999)[77,80]	27 AA, SABA only. 19 infected, 8 sham infected		No change in laboratory FEV_1, fall in home FEV_1, 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)[75]	5 AA, SABA only	3 ANA	Detection of RV in the lower airway
de Gouw et al. (1998)[79]	14 AA, SABA only. 7 infected, 7 sham infected		No change in FEV_1 or AHR, increase in FeNO in the infected group
Parry et al. (2000)[76]	17 AA	5 ANA	No significant differences in symptom scores, viral shedding, or cytokine responses between groups
Jarjour et al. (2000)[81]	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, LTB_4, or EDN; increase in BAL neutrophils and MPO

Gern et al. (2000)[82]	15 AA, SABA only	7 ANA. All 22 subjects analyzed together	No change in FEV_1, 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)[78]	6 AA	11 NANA, 5 ANA	
Grunberg et al. (2000, 2001), de Kluijver et al. (2003)[74,88,123]	25 AA, SABA only. 12 received budesonide prior to inoculation and 13 placebo	12 NANA	No change in FEV_1 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[124]	11 AA exposed to allergen + placebo, 10 AA exposed to RV, 9 AA exposed to RV and allergen		Increase in cold scores, sputum neutrophils, neutrophil elastase, IL-8, nasal lavage neutrophils and IL-8; drop in FEV_1; no change in AHR or FeNO; no differences between RV only and RV + allergen groups
Zambrano et al. (2003)[89]	16 AA SABA only; 6 high IgE, 10 low IgE	9 NANA	No change in FEV_1 or AHR; lower respiratory symptoms and FeNO greater in AA with high IgE
Mosser et al. (2005)[85]	13 AA	6 NANA	No difference in upper or lower airway viral load between groups
Christiansen et al. (2008)[83]	4 AA, mild or intermittent asthma	4 ANA	Increased human tissue kallikrein activation activity in BAL in AA

(*Continued*)

Table 8.1 (Continued)

Study	Patient population	Controls	Main outcomes
Message et al. (2008)[90]	10 AA, SABA only	15 NANA	Increased chest symptom scores, sputum and BAL eosinophils, AHR in AA; significant falls in FEV_1 and PEF in AA; reduced blood CD4 + , CD8 + , and B cells in AA
Adura (2014)[97]	11 asthmatics using ICS		Increased cold and asthma scores, no adverse events
DeMore et al. (2009)[96]	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)[125]	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)[84]	13 AA	11 NANA	Coagulant TF-exposing microparticles in BAL fluid reduced in AA
Jackson (2014)[98]	28 AA, 15 using ICS	11 NANA	Significantly greater upper and lower respiratory symptoms, greater reduction in PEF and FEV_1, increased viral loads, increased BAL eosinophils, increased nasal IL-4, IL-5, and IL-13 in AA
Silkoff et al. (2018)[126]	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)[112]	4 COPD, FEV_1 50%–80% predicted		Increased upper and lower respiratory symptoms, falls in PEF and FEV_1, increased nasal IL-8
Mallia et al. (2011)[113]	11 COPD, FEV_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)[114]	9 COPD, FEV_1 50%–80% predicted	10 smokers, 11 nonsmokers with normal lung function	Sputum inflammatory cells, neutrophils, NE, IL-1β, GM-CSF, IL-8, TNFα, 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_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 β_2-agonist; *TNFα*, tumor necrosis factor-alpha.

Perhaps the most promising use of the virus challenge model will be to accelerate the process of drug development.[137,138] Virus challenge studies have been used to evaluate the effects of existing asthma therapies on virus-induced exacerbations.[123,125] Recently the first study using viral challenge to evaluate a novel, unlicensed drug in asthma was also published.[126] 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.[139] 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.[138–140] 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.[141] 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).[138–140]

One of the biggest developments in mouse RV infection models was the protocol for isolation of highly purified, concentrated (high titers) of RV from Henrietta Lacks (HeLa) immortalized human epithelial cell lines and later, the intracellular adhesion molecule 1 (ICAM-1) transfected rhabdomyosarcoma cell lines.[142,143] Prior to this, clarified infected HeLa cell lysates were used for in vitro experiments.[144] Some investigators also used infected HeLa cell lysates in mouse models.[145] Efforts to improve the quality and validity of the model made use of a partial purification protocol to generate viral stocks.[146] 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.[147,148] RV binding to ICAM-1 is limited to human and chimpanzee and does not occur in other species, including mouse.[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.[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.[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.[152] Notably, RV infection was also sufficient to induce exacerbations of preexisting asthma in sensitized and challenged mice (as discussed in more detail below).[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.[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^{Tg} mice is sufficient to support in vivo infection with RV-A16, resulting in airway inflammation, mucus production, viral replication, and inflammatory cytokine production.[152] Of note, the hu-ICAM^{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.[153] Inoculation of BALB/c mice with the RV-A1/M2M7 variant [8×10^6 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.[153]

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.[141]

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 $\sim 10^6 - 10^8$ $TCID_{50}$ (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 5×10^6 $TCID_{50}$ RV-A1 induced a range of disease features similar to human disease.[152] 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.[152] 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, IFN$\alpha/\beta/\lambda/\gamma$).[152] Furthermore, RV infection resulted in the development of an RV-specific adaptive antibody response by 7 days postinfection.[152]

Further studies have provided insights into the effects of RV infection alone in mice. Following inoculation of wild-type C57Bl/6 mice with 1×10^8 $TCID_{50}$ 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.[145] 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.[145] RV-A1 infection also increased airway responsiveness to methacholine challenge at days 1 and 4 postinfection.[145] 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).[145] 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.[154] 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.[155] Treatment with an IL-15—IL-15Ra complex increased expression of IL-15, IL-15Rα, IFNγ, and CXCL9 and stimulated increased NK, $CD8^+$, and $CD4^+$ T cell recruitment and activation.[155] CCL7 and IRF-7 are the most upregulated lung transcripts following RV-A1 infection.[156] Blocking CCL7 or IRF-7 function reduced lung neutrophil and macrophage accumulation and IFN responses and blocking CCL7 also reduced AHR.[156] 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.[157] Further, antibody depletion of neutrophils and infection of $TNFR^{-/-}$ mice also reduced AHR, compared with control animals.[157] 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.[158] In contrast, inoculation of

TLR3$^{-/-}$ mice resulted in normal IFN responses and no difference in viral yield.[158] Both MDA5$^{-/-}$ and TLR3$^{-/-}$ mice had reduced neutrophil numbers, inflammatory cytokine production (CXCL1, CXCL2, CCL2, CXCL10) and airways responsiveness, compared with wild-type controls.[158] 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.[159] 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.[159] 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 (1×10^7 PFU).[160] Studies using Tbet$^{-/-}$ mice (a key regulator of T_H1 cell differentiation) have also demonstrated the key role for T_H1-polarized T cells in the response to RV infection. Tbet$^{-/-}$ mice developed a T_H2/T_H17-polarized immune response to RV infection (5×10^6 $TCID_{50}$) with increased IL-13 and IL-17A production, deficient NK cell responses, and decreased neutralizing antibody development.[161] CD4$^+$ T cells contributed to increased airway eosinophil numbers and mucus production following RV infection in Tbet$^{-/-}$ mice.[161] 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.[162]

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.[163] 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.[163] Tnfsf10$^{-/-}$ mice were protected from RV-induced AHR, and failed to develop RV-induced exacerbations of allergic airways disease.[163] An interesting proviral effect of TRAIL was

also identified whereby Tnfsf10$^{-/-}$ 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.[163] 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*),[154] suggesting a potential mechanism underlying secondary bacterial infections following viral infection. A subsequent study demonstrated that primary inoculation with RV-A1 (5×10^6 $TCID_{50}$) delayed the clearance of NTHi in vivo, associated with suppressed chemokine production (KC, MIP-2) and neutrophilic inflammation through a TLR2-mediated mechanism.[164] 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, IFNγ, TNFα, CCL2, CCL3, CCL4) and suppressed early IFN responses following RV-A1 infection (9×10^6 PFU) at 14 days of age.[165] 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.[166] 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 (1×10^6 $TCID_{50}$) 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).[167] 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.[167] Pretreatment with the plant flavanol quercetin before and during RV-A1 infection effectively reduced viral replication, inflammatory cytokine production (KC, MIP-2, TNFα, CCL2, IFNα, and IFNλ2), and AHR.[168] Treatment with the cancer therapeutic gemcitabine (20,20-difluorodeoxycytidine) reduced RV load, inflammatory cytokine levels (TNFα, IL-1β), and reduced lung lymphocyte numbers.[169] Treatment with corticosteroid therapy (fluticasone proprionate) suppressed IFN responses to RV and reduced airway inflammation, leading to increased mucus production and reduced antimicrobial responses.[170] Effects on viral load, mucin production, and antibacterial response could be reversed by administration of recombinant IFN-β.[170] 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.[171] 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.[152] 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.[152]

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.[172] Eotaxin was expressed by pulmonary macrophages in the lung after combined virus infection and allergen challenge.[172] Further, macrophage depletion or antieotaxin treatment reduced RV-induced airway eosinophilia and AHR.[172] 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.[173] Depletion of IL-13-producing cells in CD11b-DTR mice or $CCR2^{-/-}$ mice reduced airway inflammation and AHR.[173] Interestingly, while RV infection of OVA-treated wild-type mice contributes to mixed neutrophilic and eosinophilic airway inflammation and M2 macrophage phenotype, $IL\text{-}4R^{-/-}$ mice exhibit neutrophil inflammation alone and increased M2 polarization of pulmonary macrophages but still have exacerbated airway responses.[174] γδT cells dampen exacerbation responses. γδT cells are increased in RV-induced asthma exacerbation models and

blocking responses with anti-γδTCR 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 (IFNβ/λ2/λ3).[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 ($Foxa3^{-/-}$), RV clearance is increased, with increased IFNβ 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.[182] 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.[183] Administration of IFN or transfer of wild-type TLR7-competent pDCs could restore antiviral responses and reduce disease exacerbation.[183] OVA-allergic TLR2$^{-/-}$ mice also had reduced macrophage, neutrophil, and eosinophil numbers and suppressed AHR after RV inoculation.[146] 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.[146] Transfer of wild-type macrophages into TLR2$^{-/-}$ mice could also restore exacerbations.[146] 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.[163]

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.[184] 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[185]). 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.[186] 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.[187] 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.[188]

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.[189] 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.[189] 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.[190] In the same elastase-induced model, fluticasone proprionate 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.[170] 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.[191] Subsequent studies demonstrated that goblet cell gene expression was reduced following treatment with a gamma-secretase inhibitor (GSK L685,458) to limit NOTCH activation.[192] Further, supplementation of feed with quercetin reduced RV-induced lung inflammation (including neutrophilia), goblet cell metaplasia, and AHR.[193]

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.[194] Increased RV-A1 yields were reported in the nasal tissue of mice with rhinosinusitis, although inflammatory cytokine production and histopathology were unaffected.[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 *Rhinovirus* genus, they have been reclassified into *Enterovirus* and *Apthovirus*, respectively. Equine RV has subsequently been renamed "equine rhinitis virus."

8.12.1 Cotton rat

The cotton rat (*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. [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.[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.[196] Further, this protection was transferred effectively from mother to newborn, limiting viral yields in subsequently infected progeny.[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.[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.[198] Initial RV infection studies in chimpanzees were reported in 1968, using RV-B14 and RV-A43[199] and in gibbons in 1969.[200] Subsequent studies in chimpanzees and gibbons assessed the antiviral effects of drug treatments on RV infection, using bis-benzimidazole and triazinoindole, respectively.[201,202] Administration of soluble truncated form of human ICAM-1 can prevent subsequent infection in chimpanzees.[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 *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.[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.[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.[206] 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.[207] 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.[208] 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[209] and antibody blockade.[210] However, the initial randomized control trial assessing anti-IL-5 therapy (mepolizumab) in a broad asthma population failed to demonstrate any clinical effect.[211] 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.[212,213]

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.[214]

Table 8.2 Key experimental infection studies in mouse models of exacerbations

Mouse asthma exacerbations

Authors	Model	Interventions	Main findings
Toussaint et al. (2017)[178]	HDM + RV-A1 infection	DNAse and NETosis inhibition	Treatment suppressed type 2 immunopathology
Girkin et al. (2017)[163]	HDM + RV-A1	Deletion of TNF-related apoptosis inducing ligand ($Tnsfs10^{-/-}$)	Deletion suppressed cellular infiltration and AHR Reduced apoptotic cell death and reduced IFN- λ2/3
Han et al. (2016)[146]	OVA + RV-A1	Gene targeted deletion of TLR2 ($TLR2^{-/-}$)	Deletion reduced neutrophilic and eosinophilic inflammation and AHR
Hatchwell et al. (2015)[183]	HDM + RV-A1	Targeted deletion of TLR7 Exogenous IFN Adoptive transfer of TLR7-competent pDCs	Treatment attenuated eosinophilic inflammation and AHR
Phan et al. (2014)[177]	HDM + RV-A1	Nil	HDM and RV had additive increases in neutrophilia and tissue elastance
Hatchwell (2014)[184]	HDM + RV-A1	Salmeterol	Treatment reduced inflammation via increased PPA2 activity
Chen et al. (2014)[181]	HDM + RV-A1	Deletion of Foxa3 ($Foxa3^{-/-}$)	Deletion inhibited RV clearance
Beale et al. (2014)[99]	OVA + RV-A1	IL-25 receptor blockade	Treatment attenuated type 2 cytokine expression, mucus production and inflammatory cell recruitment
Hong et al. (2014)[174]	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)[187]	HDM + RV-A1	Anthraquinone (PI3K-mediated AKT phosphorylation inhibitor)	Treatment reduced AHR, viral replication, neutrophilic and eosinophilic inflammation
Traub et al. (2013)[186]	OVA + RV-A16	Anti-ICAM-1	Treatment suppressed T_H2 cytokine/chemokine production
Glanville et al. (2013)[175]	OVA + RV-A1	Anti-gamma-delta-T-cell receptor antibody	Treatment increased T_H2 inflammation and AHR
Collison et al. (2013)[179]	HDM + RV-A1	Inhibition of the E3 ubiquitin ligase MID1	Treatment suppressed allergic airway inflammation and AHR
Schneider et al. (2013)[180]	OVA + RV-A1	CCL2 neutralizing antibody	Treatment reduced airway inflammation and AHR
Nagarkar et al. (2010)[172]	OVA + RV-A1	Antieotaxin-1	Treatment reduced airway eosinophilia and AHR
Bartlett et al. (2008)[152]	OVA + RV-A1	Nil	RV infection exacerbated allergic airway inflammation and AHR

Mouse COPD exacerbations

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 Impaired virus clearance Mucus hypersecretion Increased bacterial loads
Singanayagam et al. (2015)[190]	Elastase + RV-A1	Nil	Enhanced airway inflammation Increased mucus production Exaggerated AHR
Ganesan, et al. (2014)[191]	Cigarette smoke + RV-A1	Nil	Viral persistence Increased neutrophilia Increased mucus production
Sajjan et al. (2009)[189]	Elastase/LPS + RV-A1	Nil	Deficient antiviral immunity Increased inflammation Exaggerated AHR Increased mucin expression

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.

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