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Pulmonary Pharmacology & Therapeutics 30 (2015) 32–43

Contents lists available at ScienceDirect

Pulmonary Pharmacology & Therapeutics

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

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

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ARTICLE INFO

Article history:
Received 5 August 2014
Received in revised form
24 October 2014
Accepted 29 October 2014
Available online 13 November 2014

Keywords:
Rhinovirus
Exacerbation
Asthma
Chronic obstructive pulmonary disease
Experimental infection

ABSTRACT

There is an association with acute viral infection of the respiratory tract and exacerbations of asthma and chronic obstructive pulmonary disease (COPD). Although these exacerbations are associated with several types of viruses, human rhinoviruses (HRVs) are associated with the vast majority of disease exacerbations. Due to the lack of an animal species that is naturally permissive for HRVs to use as a facile model system, and the limitations associated with animal models of asthma and COPD, studies of controlled experimental infection of humans with HRVs have been used and conducted safely for decades. This review discusses how these experimental infection studies with HRVs have provided a means of understanding the pathophysiology underlying virus-induced exacerbations of asthma and COPD with the goal of developing agents for their prevention and treatment.

1. Introduction

The purpose of this review is to discuss the utility of experimental infection of animals and humans with human rhinoviruses (HRVs) as a means of understanding the pathophysiology underlying virus-induced exacerbations of asthma and chronic obstructive pulmonary disease (COPD) with the goal of developing agents for their prevention and treatment.

1.1. Asthma and COPD represent high unmet need with complex heterogeneous presentations

Asthma and COPD are leading causes of morbidity and mortality. The World Health Organization (WHO) estimates that 300 million people worldwide have asthma with an associated 250,000 asthma deaths annually [1]. Additionally, the WHO estimates that COPD shares fourth and fifth places with HIV/AIDS (after coronary heart disease, cerebrovascular disease, and acute respiratory infection) as a single cause of death [2]. COPD is a very common disease affecting 65 million patients in the G7 countries (U.S., Japan, France, Germany, Italy, U.K. and Canada) and is the third leading cause of death in the U.S. [3]. In 2010, the cost to the U.S. for COPD was projected to be approximately $49.9 billion, including direct health care expenditures, indirect morbidity, and indirect mortality costs [4]. The direct cost of treating COPD exacerbations alone in the U.S. may exceed $18 billion.

Asthma is characterized by the presence of reversible bronchoconstriction, increased sensitivity to specific and nonspecific bronchospastic agents, and excessive mucus production accompanied by an underlying pathology of inflammation associated with airway remodeling [5]. Diagnosis of asthma and classification of its severity are primarily based upon symptoms of cough, wheeze, and shortness of breath combined with an assessment of airflow obstruction and the level of control observed with various therapeutic interventions. Recent data from the Asthma Clinical Research Network suggest that approximately half of patients diagnosed with mild-to-moderate asthma presented with either a persistent or intermittently persistent eosinophilic phenotype that was responsive to combined anti-inflammatory therapy, while the

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http://dx.doi.org/10.1016/j.pupt.2014.10.005
1094-5539/

remaining half of patients presented with a persistently non-eosinophilic asthma phenotype that was less-responsive to similar intervention [6]. With respect to severe asthma, the National Heart, Lung, and Blood Institute Severe Asthma Research Program (SARP) revealed five distinct phenotypic clusters within severe asthma highlighting the heterogeneity of the underlying molecular, cellular, and physiologic manifestations of the disease [7].

COPD is a debilitating chronic respiratory disease associated with cough, dyspnea, excessive sputum production, and a progressive loss of lung function that begins to emerge in middle aged and older patients. Cigarette smoke is the main risk factor for COPD, in addition to air pollution, biofuel, and occupational exposure [8]. Diagnosis of COPD is based on presentation of symptoms of chronic cough, dyspnea, or sputum production in conjunction with spirometry assessment where a post-bronchodilator forced expiratory volume in 1 s/forced vital capacity (FEV_1/FVC) ratio of <0.70 is indicative of COPD. COPD may present as a heterogeneous mixture of physiologic manifestations including chronic bronchitis and parenchymal destruction (emphysema) [2]. During exacerbations, there may be increased hyperinflation and gas trapping leading to a reduction in airflow and enhanced dyspnea [9]. Recent adaptations to the Global Initiative for Chronic Obstructive Lung Disease (GOLD) assessment of disease feature a combined COPD assessment that includes the association between symptoms, spirometric classification, and future risk of exacerbations, with subjects of GOLD grade 3 or 4, or subjects with 2 or more exacerbations per year, considered as at high risk for having an exacerbation [10].

1.2. Virus-induced respiratory exacerbations

Research over several decades has shown that in predisposed individuals, there is an association with acute viral infection of the respiratory tract and the exacerbation of existing chronic pulmonary conditions such as asthma [11–14] and COPD [14–18]. Virus-induced exacerbations contribute significantly to the morbidity and mortality of asthma and COPD, and effective therapeutic intervention for exacerbations remains a major unmet medical need.

The development of agents to prevent and treat exacerbations of asthma and COPD remains a challenge due to the inherent heterogeneity of these diseases which are likely mediated by differences in inflammatory pathways and/or mechanisms modulated by genetic and environmental factors [19,20]. This scenario is complicated by the possibility that additional inflammatory pathways associated with disease exacerbations invoked by viruses may also be different for each of the different disease phenotypes in asthma and COPD. This is further complicated by the possibility that the biological cascades induced by different types of respiratory viruses associated with exacerbations of asthma and COPD may differ for each type or strain of virus. Collectively, this presents an extremely complex pathophysiology with multiple variables.

With respect to the translation of findings from preclinical experiments, animal models of virus-induced exacerbations in the context of asthma and COPD are limited. Rather, experimental infection of healthy adult human volunteers has provided much of our understanding regarding the biology of HRV infections, which are the respiratory viruses most commonly associated with pulmonary exacerbations. Initially, HRV challenge studies in healthy volunteers have been used to study and develop therapeutics for the common cold. In order to better understand the underlying mechanisms of virus induced exacerbations of asthma and COPD, and to develop agents to prevent and treat them, studies in which selected subject populations with asthma or COPD are challenged with a characterized virus strain have been more recently employed. By building on this experience, small controlled viral challenge interventional studies in asthma and COPD early in clinical development could help triage agents that have the potential to attenuate the manifestations of naturally-acquired HRV infections leading to disease exacerbations. This review will summarize common respiratory viruses, the role they play in acute exacerbations of airway diseases, and recent advances in HRV challenge studies with particular emphasis on those studies conducted in subjects with asthma and COPD.

2. The viruses

2.1. Virus infections of the respiratory tract

Upper and lower respiratory tract infections (URTIs and LRTIs) can be caused by a large number (>200) of different viruses from multiple virus families [21]. All these viruses can cause a similar constellation of clinical symptoms (generally referred to as “cold or flu symptoms”) of varying severity depending upon the type of virus, inherent pathogenicity of the strain, and the host's age, immune status, comorbidities, and genetic background. The viruses most commonly isolated from respiratory tract samples collected from patients presenting with cold symptoms are presented in Table 1 (reviewed in Ref. [22]).

2.2. Virus-induced exacerbations of asthma and COPD

In the U.S. alone, there were 1.75 million emergency department visits (1.11 million for adults and 0.64 million for children [ages 0–17 years]), 456,000 asthma hospitalizations (299,000 for adults and 157,000 for children), and 3447 deaths due to asthma in 2007 (3262 among adults and 185 among children), many as the result of acute respiratory viral infections that trigger asthma exacerbations [1,4,23]. The overall risk of death in patients hospitalized for asthma exacerbations in the U.S. is 0.5% [24].

In 2010 in the U.S., there were 1.5 million emergency department visits by adults 25 years and older diagnosed with COPD resulting in 699,000 hospitalizations and 133,575 deaths [25]. The annual rate of COPD exacerbations has been estimated to range

Table 1
Common respiratory viruses.

Genome structure	Virus family (Genus)	Viruses
Single-stranded RNA, positive sense	*Picornaviridae* (Enterovirus)	Rhinoviruses (HRV-A, HRV-B, and HRV-C subgroups containing over 100 serotypes) Enteroviruses (HEV-A, B, C, and D subgroups)
	Coronaviridae (Coronavirus)	Human coronaviruses (hCoV. NL63, HKU1, 229E, OC43)
Single-stranded RNA, negative sense	*Paramyxoviridae* (Pneumovirus)	Human respiratory syncytial virus (HRSV, both A and B) Human metapneumovirus (hMPV)
	(Paramyxovirus)	Parainfluenza viruses (hPIV types 1–4)
Single-stranded RNA, segmented, negative sense	*Orthomyxoviridae* (Influenza virus)	Influenza A and B
Single-stranded DNA	*Parvoviridae* (Bocavirus)	Human bocavirus (HBoV)
Double-stranded DNA	*Adenoviridae* (Mastadenovirus)	Human adenovirus (mostly types B and C)
	Papovaviridae (Polyomavirus)	Human polyomavirus WU, Human polyomavirus KI

from 0.5 to 3.5 exacerbations per patient [16,26,27]. Such virus-induced exacerbations can result in disease worsening that requires increased pharmacologic treatment, visit to the emergency room, hospitalization, admission to intensive care, and death. For example, in-hospital mortality rates associated with acute exacerbations of COPD vary markedly between studies (2.5–30%), depending upon comorbidities and the mode of recruitment and setting (medical ward versus intensive care unit) [24,28–34].

While several types of viruses have been associated with exacerbations of asthma and COPD, it is clear from multiple studies that the vast majority of all virus-induced exacerbations of asthma and COPD are caused by HRVs. Up to 80% of all acute exacerbations of asthma are caused by HRVs which are responsible for up to 85% of all asthma exacerbations in children, and 50% of exacerbations in adults [11–13,35–41]. Factors related to the host, virus, and environment associated with HRV infections in asthma are detailed in a comprehensive recent review [42]. Infection by HRVs has been linked to up to 60% of exacerbations of COPD [15,18,43–49], although human respiratory syncytial virus (HRSV), human parainfluenza virus (hPIV) and influenza virus are also recognized as agents associated with exacerbations of asthma and COPD [17].

2.3. Rhinoviruses

HRVs were first isolated in culture from patients with cold symptoms in the late 1950's [50], and later classified in the 1960's [51,52] as newly discovered infectious agents. HRVs have subsequently been determined to be members of the Enterovirus genus in the *Picornaviridae* family of nonenveloped, positive-sense, single-stranded, RNA viruses. Over 100 types of HRVs have been characterized and classified into 3 groups: HRV-A, HRV-B, and more recently HRV-C [53–55].

HRV infections can occur throughout the year, but typically HRV infections peak in spring and autumn. Interestingly, individuals may be infected several times each year [56]. Recurrent HRV infections may be due to misdirection of antibody responses against a nonprotective epitope associated with the VP1 capsid protein as a mechanism of escape resulting in a lack of immunological protection against HRV infection [57].

HRVs typically infect the upper airways, nasal mucosa, sinuses, and middle ear, and their clinical course of infection has been well characterized [58,59]. Infections produce symptoms of “the common cold” such as sneezing, nasal congestion, rhinorrhea, eye irritation, sore throat, cough, headaches, fever, and chills. In healthy individuals, HRV infections are generally self-limiting and are typically restricted to the upper airways. HRV-induced colds are the leading cause of acute morbidity and missed days from work or school [60], and are associated with nearly 5 hospitalizations/1000 children <5 years of age, particularly in those children with a history of wheezing/asthma [61]. HRV infection can also lead to infection of the lower airways [35,62–69], otitis media (particularly in young children), and sinusitis [70].

In certain predisposed individuals, such as those diagnosed with asthma or COPD, inherent deficiencies in innate antiviral immune response may also contribute to enhanced susceptibility to HRV infection leading to an acute exacerbation of disease. An initial study focused on ex vivo assessment of primary bronchial epithelial cells derived from asthmatic subjects indicated an abnormal innate response to infection by HRV-16. Specifically, an increase in HRV-16 viral replication and cell lysis was associated with resistance to early apoptosis after HRV infection and a deficient type I interferon (IFN-β) response [71]. A subsequent study demonstrated that asthmatic subjects challenged with HRV-16 experienced enhanced lower respiratory symptoms and bronchial hyperreactivity associated with greater bronchoalveolar lavage (BAL) leukocyte concentrations and dysregulated IFNγ and IL-10 responses compared with normal subjects [68].

In certain rare circumstances, HRV infection can also lead to serious complications such as pneumonia, particularly in infants and young children [72,73] and those children with underlying conditions such as bronchopulmonary dysplasia [64], congenital heart disease, prematurity, and neurologic conditions. Rhinoviruses can be detected in 24–45% of children and in 10–18% of adults with pneumonia in upper respiratory or sputum samples (reviewed in Ref. [74]. However, as HRVs are commonly detected from subjects with mild respiratory symptoms, and even from asymptomatic individuals, the role of rhinovirus detected in the upper airways as a causative agent of viral pneumonia is still questioned [72,75]. To further complicate the scenario, HRV is often found together with *S. pneumoniae* or other pathogenic bacteria in patients with pneumonia [47]. Viral-bacterial pneumonia has a poorer response to treatment than pneumonia caused by a single agent.

In certain immunosuppressed (bone marrow transplant recipients) adults, HRVs are associated with more severe disease [76,77], although in general, there are no reports of an increased risk of serious complications due to rhinovirus infection in this population [78].

2.4. Prevention and treatment of rhinovirus infections

Due to the extensive sequence variation among the over 100 different types of HRVs, the co-circulation of multiple types at once, recombination, and the subsequent lack of a broadly cross reactive antibody response, the development of a vaccine against HRVs represents a tremendous challenge [79,80]. Due to the generally mild and self-limiting nature of HRV infections in healthy adults, a direct antiviral agent for HRVs would need to be exceptionally safe with little to no safety risks for use in this population. While a number of antiviral agents specifically directed against HRVs (eg, capsid-binding compounds, 3C protease inhibitors, inhibitors of the ICAM-1 receptor) have been in clinical trials, they have typically shown minimal efficacy or toxicities, and their development has been terminated due to the lack of an acceptable benefit–risk profile (reviewed in Refs. [81,82]). Current treatments for rhinovirus infections are palliative and include antipyretics and decongestants. Herbal and natural supplement-based treatments (eg, echinacea, ascorbic acid [vitamin C], zinc) have not been shown to be clinically effective [83–88]. Given the high hurdles for HRV antivirals and the widespread use of over the counter treatments to treat symptoms, it is not surprising that there are no direct antivirals for HRVs currently in clinical development (Clinicaltrials.gov, February 2014).

The greatest unmet need for specific therapeutic agents targeting HRVs or HRV-associated inflammation is for the prevention and treatment of acute viral exacerbations of asthma and COPD. While the development of preventative vaccines for HRV remains to be a significant challenge, recent efforts toward the development of specific therapeutic agents to treat or prevent HRV-induced exacerbations have shifted focus to defining the underlying mechanisms associated with virus-induced exacerbations. This includes improving inherent deficiencies in the innate host antiviral defense in at-risk populations or reducing the excessive or inappropriate inflammatory host immune response triggered by HRV in the context of active pulmonary disease, in addition to efforts focusing on direct acting anti-HRV antiviral therapeutic interventions.

2.5. Other viruses in exacerbations of airway diseases

While several viruses other than rhinoviruses, including human respiratory syncytial virus (HRSV), influenza, human coronavirus

(HCoV), enteroviruses (EVs), human parainfluenza viruses (hPIVs), human adenoviruses (AdV), and human bocavirus (hBoV), are also associated with exacerbations of asthma and COPD [38], they are found less frequently than HRVs, and the predominant exacerbating agent can vary depending upon the individual's age, geographic location, and season. It is unknown if the biological cascades induced by infection with these different types of respiratory viruses associated with exacerbations of asthma and COPD are similar or different for each type or strain of virus. This picture is further complicated by the different cell tropisms and various forms of cytopathology caused by each type of virus [89–92] and even different strains of a virus [93,94].

3. Animal models of rhinovirus infection

One of the key issues hampering the use of animal models for studying infections by human respiratory viruses is the fact that nonhuman species (with the exception of the chimpanzee) are partially permissive at best for infection with almost all human respiratory viruses including rhinovirus with the noted exception of influenza virus which naturally cross infects diverse species (eg, pigs, birds, and humans).

HRVs are exquisitely species-specific. The only nonhuman primate able to be productively infected by major class HRVs is the chimpanzee [95–99], although the gibbon has also been reported to be able to be infected [100,101]. Chimpanzees are an endangered species, and the restrictions placed on research with chimpanzees makes their use unrealistic based on ethical reasons. Currently, the United States and Gabon are the only countries that allow medical research to be conducted on chimpanzees, and the U.S. National Institutes of Health have recently reassessed the use of chimpanzees in medical research [102] with the intent of eliminating their use in favor of alternate models. In addition, a systematic review of the use of chimpanzees in monoclonal antibody and drug development encompassing almost 2 decades (1981–2010) continue to identify key differences in human and chimpanzee immune functions and highlights the difficulty of predicting results in human patients based on data from chimpanzees [103]. This is supported by the fact that although chimpanzees can be productively infected by HRVs, they do not develop the clinical symptoms associated with the "common cold". In addition, all but 1 species of gibbon are listed as endangered or critically endangered, and gibbons have also been reported not to develop cold symptoms.

Attempts to infect vervet monkeys and a wide variety of other primate species with HRVs have been unsuccessful [104]. This is due to the fact that approximately 90% of HRVs (previously designated as major group HRVs) use the human intracellular adhesion molecule 1 (ICAM-1) as the receptor to infect cells [105,106]. These major group HRVs are only able to use ICAM-1 of human or chimpanzee origin [107,108]. Conversely, minor-group rhinoviruses bind a member of the low-density lipoprotein receptor family which is shared between mouse and human. Infection with minor-group rhinovirus-1B in BALB/c mice has been shown to elicit an acute influx of inflammatory cell types including neutrophils and lymphocytes, neutrophil and T-cell-associated chemokines, type I and type II viral-associated IFN response and mucin production [109,110]. However, HRVs belonging to the minor group represent approximately only 10% of known HRVs. To address these issues, recent efforts from the same group focused on developing transgenic mice engineered to express a chimeric murine-human ICAM-1. This work demonstrated that human chimeric ICAM transgenic mice on a BALB/c background can be infected by HRV-16, and that the results were similar to those observed with minor group rhinovirus-1B in wild type mice [109]. While there are certainly differences between HRV infection in humans and the chimeric ICAM transgenic mouse model with respect to correlation of clinical parameters related to cold symptoms, the model can be used to assess the impact of rhinovirus exacerbation of allergic disease when combined with the ovalbumin model of allergic airway inflammation. Subsequent work has since demonstrated the utility of testing an anti-human ICAM-1 antibody that binds domain 1 of human ICAM-1 in this model, resulting in a reduction in the level of cellular inflammation and cytokine production associated with rhinovirus infection in addition to a reduction in viral load relative to baseline pre-infection and isotype control post-infection [111].

While animal models of HRV infection and exacerbations caused by HRVs do have value to assess specific mechanisms that may be associated with viral exacerbation in humans, they are complicated by several issues such as a lack of clinical symptoms reflective of infections in humans and differences in the physiologies of the respiratory tract between humans and other species typically used for preclinical studies, such as the mouse (where branching occurs after the bronchiole levels) [112]. In addition, since animals such as mice do not naturally develop asthma or COPD, the underlying baseline inflammatory profile and the acute pathology that mice develop in response to viral challenge in these experimental models may not be completely reflective of human disease [104,113]. This, coupled with the inability of these species to be naturally infected with HRVs and other human viral respiratory pathogens, the high titer of viral inoculum required for infection (generally 10^6 50% infectious dose in tissue culture [$TCID_{50}$] versus 5 to 10 $TCID_{50}$ in humans), and minimal replication of virus make these models less than ideal for studying virus-induced exacerbations of asthma and COPD. Translation of observations from in vivo animal models to human disease with respect to assessing potential candidates for therapeutic intervention remains to be clinically validated. For all these reasons, experimental infection of humans with HRV in controlled challenge models may have greater utility in the development of therapeutic agents to intercept and treat viral exacerbations of asthma and COPD.

4. Experimental infection of humans with rhinoviruses

HRV infections in human subjects, including those with asthma and COPD, can be studied in an observational manner (naturally occurring wild type respiratory viral infections) or using experimental infection (controlled viral challenge studies). Controlled experimental infection studies have several advantages over natural wild type observational studies including a controlled clinical setting with a defined subject population, the use of a characterized inoculum with regard to strain and serotype, the use of a uniform challenge dose, uniform timing of infection, and exclusion or identification of coinfection by other respiratory viruses. A planned and detailed biospecimen collection and observational follow-up in the context of a controlled clinical setting is also possible. For the development of therapeutic agents to intercept and treat HRV induced exacerbations, an experimental infection study also has the benefit of allowing the selection of a small and focused population minimizing the number of subjects exposed to a novel agent that may have limited data with respect to a risk–benefit profile.

Since their initial isolation and identification in the mid-1950's, numerous experimental infection studies with HRVs have been safely conducted in healthy adult subjects, as well as in asthmatic- and more recently, COPD-subject populations for more than 60 years. Guidelines for the development and safety testing of suitable rhinovirus challenge stocks manufactured and characterized according to Good Manufacturing Practices (GMP) standards were initially available in the early 1960's [114], although most challenge pools used in studies did not meet this standard. Since then, these guidelines have been updated [115], and GMP-prepared stocks are

now required by regulators for experimental infection studies in humans. Controlled experimental infection studies should also include measures to limit the spread of virus to study personnel and others, and in these studies there has been no evidence of the spread of infection to personnel conducting the study.

4.1. Experimental rhinovirus challenge studies in healthy subjects

Due to the lack of permissive animal species, much of our understanding of the clinical course of HRV infection is derived from experimental infection of healthy human volunteers. Since the initial isolation and characterization of HRVs, numerous studies have been conducted in adult healthy subjects to understand the basic biology of HRV infection including clinical symptoms, the natural course of infection, host immune response to infection, and the person-to-person transmissibility [14,35,58,62,63,65–67,69,116–163]. In addition, experimental infection studies have also been used to evaluate potential agents intended for the prevention and/or treatment of the "common cold" [81,82,86–88,107,126,128,150,164–199]. In these experimental infections, healthy adult subjects were inoculated intranasally with up to 10,000 $TCID_{50}$ of rhinovirus (most commonly HRV-16 or HRV-39), and the course of infection was observed over approximately 1–2 weeks. No serious adverse events (eg, pneumonia) have been reported during these studies in healthy adult subjects, and experimental infections were self-limiting as observed in naturally occurring HRV infections.

4.2. Experimental rhinovirus challenge studies in asthmatic subjects

Experimental viral challenge studies, in which atopic or mild to moderate asthmatic subjects are inoculated with a defined HRV stock in an effort to elicit a productive respiratory infection, typically induce respiratory symptoms similar to those observed during mild acute exacerbations in atopic or mild asthmatics. This includes lower respiratory symptoms, airflow obstruction (change in peak expiratory flow [PEF]), and systemic and airway inflammation that are greater and more prolonged when compared with the control (generally nonasthmatic or healthy subjects) group [62,63,65,67,68,118,124,125,127,130,150–152,200–223]. Such studies have been used as surrogate clinical models of naturally occurring rhinovirus-induced exacerbations of airway diseases in severe asthma as a means of providing greater insight into the underlying mechanisms of host response to respiratory viral infection in asthmatics compared with healthy individuals.

When conducting experimental HRV challenge studies in subjects with asthma and COPD, subject safety is of the utmost concern. To minimize risk, these studies typically enroll subjects with mild to moderate stable disease and exclude those with poor lung function, poorly controlled disease, and a history of recent hospitalizations due to exacerbations. The experimental HRV infection studies completed to date have been quite helpful in defining underlying mechanisms associated with the host response to HRV infection. However, as the magnitude of symptoms in these experimental infection studies can be less marked than those in naturally occurring exacerbations in patients with severe disease, it can be more difficult to detect a significant treatment effect when assessing potential therapeutic candidates in these studies.

Initial studies in asthmatic subjects were designed in a similar fashion to experimental infections in healthy subjects. Atopic or mild asthmatic subjects were inoculated intranasally with 10 to 10,000 $TCID_{50}$ of HRV (again, most commonly HRV-16 or HRV-39), and clinical symptoms, lung function, and biomarker host immune response data were collected. In these experimental viral challenge studies, infection was generally well tolerated, and the subjects experienced clinical symptoms of the common cold similar to that observed in healthy control subjects; some experienced increased chest symptoms (eg, cough, phlegm production, and wheezing) compared with healthy subjects. Some subjects with mild to moderate asthma exhibited enhanced host immune response to the viral challenge including increased BAL lymphocyte concentrations and enhanced lower respiratory symptoms [68]. Typically, viral replication peaked at Day 3 post infection and local inflammatory response and symptoms peaked at 5–6 days post infection. In atopic individuals and mild asthmatic subjects, lung function did not appear to be impaired to a significant degree. However, in some studies, more pronounced effects upon lung function (eg, statistically significant decreases in FEV_1 and PEF) have been observed [118,124,223]. The majority of these studies are performed in mild asthmatics to reduce the potential for inducing severe acute exacerbations.

A limited number of studies have also incorporated an allergen challenge together with HRV infection in atopic and asthmatic subjects to further provoke inflammatory responses associated with exacerbations in the context of an allergic response to a relevant allergen [124,209,224]. Even in these dual provocation studies which could result in an amplified response, reports of clinical exacerbations and the need for medical intervention are rare. In one of the allergen/RV challenge studies [124,125], 2 out of 24 subjects withdrew due to an asthma exacerbation. One subject had a spontaneous exacerbation during the placebo-allergen period prior to HRV-16 infection, and another subject developed an HRV-induced exacerbation on the second day after inoculation with a high dose ($0.4–8.6 \times 10^4$ $TCID_{50}$) of HRV-16 after 2 weeks of exposure to allergen (house dust mite extract). The subject's PEF decreased to 28% of their personal best. The subject was withdrawn from the study, admitted to the hospital for a single night, treated with oxygen, inhaled ipratropium/salbutamol, and oral prednisone, and recovered quickly during the night and following days.

Interventional studies using HRV challenge in asthmatics are limited in number. To assess the impact of steroid therapy on HRV infection in asthma, mild asthmatics were experimentally infected with HRV-16 while being treated with inhaled corticosteroids (budesonide 800 μg twice a day for 4 weeks starting 2 weeks prior to HRV-16 infection). No worsening of airway inflammation based on induced sputum in the asthmatic subjects was observed [212]. In a subsequent study [174], 47 asthmatic male subjects were randomized to receive oral prednisone (20 mg) or placebo 3 times a day for 5 days. Steroid treatment was initiated 11 h prior to inoculation with HRV. No safety concerns were reported, suggesting that treatment with prednisone did not impair the host response to HRV infection. In a recent preliminary study of experimental HRV-16 infection in 11 moderate asthmatic subjects already receiving inhaled corticosteroids (ICS), no safety concerns such as withdrawal due to safety issues, serious adverse events, or requirement for treatment beyond extra bronchodilator use were reported [225]. The response to HRV infection as measured by cold symptom and asthma scores was similar to that previously observed in subjects not receiving ICS therapy, and the host response as measured by CXCL10/IP-10 protein in sputum and nasal lavage was also similar. Interestingly, even in a population of moderate asthmatics, a decrement in lung function was not observed, although this may have been due to increased bronchodilator use during the study.

Collectively, these studies indicate that even in the context of the immunosuppression associated with corticosteroids, experimental HRV infection is safe and well tolerated in asthmatic subjects. Furthermore, experimental HRV infection is a relatively safe and useful tool in defining the underlying immune mechanisms associated with HRV-induced disease exacerbations and can be applied to interventional studies.

4.3. Experimental rhinovirus challenge studies in chronic obstructive pulmonary disease

As HRV infection is the leading viral cause of COPD exacerbations [17,26,27,43,226], experimental infection of COPD subjects with HRV-16 has also been used as a model to better understand the underlying biology of COPD exacerbations. Recently, an emerging series of HRV16 experimental challenge studies have been performed in subjects with mild to moderate disease focusing on examining the underlying mechanisms of HRV-induced exacerbations in COPD [227–232].

The initial study by Mallia et al. [232] employed a dose escalation approach to determine the minimum dose of HRV-16 required for a productive infection in GOLD stage II (moderate) COPD subjects, in addition to assessing safety parameters to establish the model in this new population. Follow up studies addressed the impact of smoking and also mechanistic questions related to host immune response to HRV-16 in COPD. Comparing subjects with COPD and control subjects (non-obstructed subjects with a similar smoking history, but normal lung function) demonstrated that subjects with COPD exhibited increased upper and lower respiratory symptom scores, reduced post-bronchodilator PEF, increased viral load in nasal lavage and sputum, and increased inflammatory markers relative to their baseline values when compared with control subjects following experimental infection with HRV-16. Daily upper respiratory symptom scores peaked earlier at Days 3 and 4 in COPD subjects while lower respiratory symptom scores peaked later at Day 9. The peak expiratory flow trough was observed at Days 5 through 9. Peak viral titers were delayed somewhat relative to that observed in asthmatics (Days 4–6), however a lower inoculum of only 10 $TCID_{50}$ was employed in the study which may have resulted in a longer period of time to reach peak viral titer. Additional investigation provided insight into secondary bacterial infections following rhinovirus infection which develop on average 9–15 days post-inoculation in approximately 60% of COPD subjects, but were observed in only 10% of smoking and non-smoking control subjects. These data highlight the frequency of secondary bacterial infections in COPD and suggest a potential impact of earlier intervention with antiviral therapies to reduce both primary viral-induced COPD exacerbations and complications associated with potential secondary bacterial infections [228]. Evaluation of lymphocyte subsets showed lower frequencies of CD4+ and CD8+ T cells in the periphery and increased frequencies of CD3+ and CD8+ in BAL in COPD subjects relative to controls [110], and also differences in neutrophil adhesion and activation markers in COPD subjects relative to control subjects following infection with HRV-16 [230]. Overall, experimental HRV infection was well tolerated without any serious adverse events in both the COPD and control populations. Additional work exploring specific components of the host innate immune response to acute infection with HRV-16 may help to provide further insight into the association of severe acute viral-exacerbation in COPD with specific clinical phenotypes of disease.

4.4. Biomarker host immune response profiles in experimental rhinovirus infections

Assessment of various biomarker profiles in the airway and in peripheral blood may help to further define the host immune response to HRV infection, provide insight into different mechanisms of susceptibility to virus-induced exacerbations that may be associated with the underlying heterogeneity observed in asthma and COPD, and also assess the potential efficacy of therapeutic compounds in clinical development for prevention or intervention of acute viral exacerbations in respiratory disease. Significant work has been done to date using in vitro systems to assess the impact of HRV infection on primary cells derived from subjects with asthma or COPD, and to further define specific mechanistic components of host innate response at the cellular level [233–235]. This work has been critical in guiding potential biomarker work in subsequent experimental viral challenge studies in humans. Recent advances in propagation of human rhinovirus-C (HRV-C) in human sinus and airway cells differentiated at air-liquid interface (ALI) has provided a means to study additional relevant HRV strains beyond the few serotypes that have been used to date, potentially providing a path forward for evaluating host response to HRV-C in experimental challenge models in humans [236,237]. Additionally, naturally occurring HRV infection in humans has provided additional although retrospective data defining the host immune response to acute HRV infection. Serum CXCL10/IP-10 (interferon-inducible protein 10 [IP10]) has been demonstrated as a biomarker associated with HRV infection in COPD [49,226], and has also been suggested as a potential biomarker in asthma based on in vitro experiments and assessment of acute naturally occurring infection in asthmatics [221].

Experimental HRV infection studies enable a broad assessment of host response biomarkers over a specific defined time period in the context of a well-controlled study with respect to the viral challenge pool and study population. Typically, peak HRV concentration in nasal wash specimens can be detected 2–3 days post infection. Recovery of replicating virus, indicative of productive infection, recovered from nasal wash provides a means to assess the impact of therapeutic intervention on viral replication kinetics [138]. Cold symptom scores increase and peak 3–4 days post infection while related patient-reported outcomes such as the Asthma Control Questionnaire (ACT) scores tend to lag behind and peak 4–5 days post infection. Assessment of underlying biomarkers associated with host-anti viral response tend to peak at days 5–6 and can remain elevated for several days prior to recovery. CXCL10/IP10 protein levels have been demonstrated to have a strong association with HRV-16 response over time in both nasal lavage and airway sputum samples [49,221].

Other biomarkers of interest have also been identified in human experimental HRV challenge studies. For example, a polymorphism in the interleukin 6 (IL-6) promoter at position -174 was associated with greater symptom magnitudes in the context of experimental HRV-39 infection [238]. Assessment of differential gene expression profiles derived from nasal scrapings pre- and post-HRV infection indicated significant changes in chemokines, signaling molecules, interferon-responsive genes, and antivirals. Viperin expression was associated with productive HRV infection [148]. In addition, experimental rhinovirus infection has been associated with increases in human tissue kallikrein activation in allergic subjects [204].

From a biomarker perspective, experimental viral challenge studies provide distinct advantages to following naturally occurring HRV infections over time. The ability to assess acute host response to infection in a focused and controlled manner, using a specific characterized inoculum and a uniform challenge dose, provides a means to comprehensively assess both the innate and adaptive immune response from multiple biologic matrices at the transcriptomic and proteomic level, including at baseline pre-infection, during the acute infection and also during convalescence through a follow up period. Additionally, subjects with specific and predefined clinical phenotypes may be recruited to help further elucidate potential different mechanisms of susceptibility associated with virus-induced exacerbations compared to a broader heterogeneous population. Recent advances in transcriptomic and proteomic platforms, in addition to advances in computational biology approaches to building experimentally-defined networks

of disease, may help to provide additional tools to better understand the relationship between acute viral exacerbation of asthma and COPD and rhinovirus infection.

4.5. Previous and current therapeutic targets in experimental rhinovirus challenge studies

To date, there are no approved vaccines or antiviral agents for preventing or treating HRV infection (reviewed in Refs. [81] and [82]). Results from clinical studies of direct acting antivirals such as pleconaril only showed a 1 day reduction in the median time to alleviation of clinical illness compared with placebo [180]. A study examining the effects of pleconaril, on cold symptoms and asthma exacerbations following HRV exposure has been completed [239]. Results posted on ClinicalTrials.gov indicated that compared with placebo, treatment with pleconaril was not associated with a statistically significant effect ($p = 0.425$) on the percentage of HRV-positive subjects with asthma exacerbations. Collectively, these results suggest that treatment with antivirals alone may not be sufficient.

As infection with rhinovirus is associated with upper respiratory immune-mediated inflammation, several studies have examined the effects of either prophylaxis or treatment with broad acting anti-inflammatory agents, specifically corticosteroids, administered either systemically or by inhalation on experimental rhinovirus infection. Corticosteroids broadly inhibit immune-mediated (eg, cytokines, chemokines, cell-mediated) inflammatory responses. In one study [170], the effects of combined intranasal and systemic corticosteroids on the local inflammatory response and symptoms due to experimental rhinovirus infection in healthy adults was examined. In this study, 45 adults were randomized to prophylaxis with either placebo or steroids. Intranasal beclomethasone (168 μg twice a day) was initiated 4 days before viral challenge and continued 5 days after challenge. Oral prednisone (30 mg twice daily) was given for 3 days starting 1 day prior to challenge. During the first 48 h after viral inoculation, nasal obstruction, nasal mucus weights, and kinin concentrations in nasal lavages were lower in steroid recipients, but subsequent increases in these variables in the steroid group resulted in no significant cumulative differences between treatment groups. These data suggest that steroid prophylaxis may suppress nasal inflammation and cold symptoms during the first 2 days in experimental rhinovirus colds. No adverse events (AEs) or serious adverse events (SAEs) were reported. Recent emerging data has highlighted the impact of rhinovirus infection on steroid responsiveness in an airway epithelial ex vivo cell based system. RV-16 infection impaired dexamethasone-dependent inhibition of IL-1β—induced CXCL8 release, induction of mitogen-activated protein kinase phosphatase 1 gene expression, and binding of GR to GREs in airway epithelial cells, indicating that RV-16 infection of human airway epithelium induces glucocorticoid resistance [240]. Given the large use in the daily clinical practice of inhaled/systemic corticosteroids for viral-induced exacerbations, the pros and cons of therapeutic intervention with corticosteroids must be considered in the context of specific clinical presentation.

Kloepfer and colleagues (2011) conducted a randomized double-blind placebo-controlled trial in mild allergic asthmatics to assess the efficacy of montelukast, a leukotriene receptor antagonist, in lessening asthma symptoms following experimental inoculation with HRV-16. In that population of 20 subjects (8 active, 12 placebo subjects), montelukast, a leukotriene receptor antagonist, did not appear to have any significant impact in asthma control or cold symptom scores caused by experimental rhinovirus infection in patients experimentally infected with HRV-16 [217].

As of 23 October 2014, a search of ClinicalTrials.gov using the keyword “rhinovirus” identified 2 asthma exacerbation studies [241,242] in which subjects were being experimentally infected with HRV-16 after being treated with an anti-inflammatory agent (mepolizumab [an anti-IL-5 monoclonal antibody] or CNTO 3157 (a monoclonal antibody directed against an innate immune response target). These studies are examining the ability of these 2 anti-inflammatory agents to alleviate HRV-induced effects on asthma symptoms and pulmonary functions.

5. Discussion and conclusions

Although initially identified as agents of the common cold, HRVs are significant human pathogens associated with significant respiratory illnesses such as bronchiolitis and pneumonia, and are the predominant virus associated with acute exacerbations of asthma and COPD. Virus-induced exacerbations of asthma and COPD are the result of a complex interaction of a multitude of variables making the development of agents to prevent or treat exacerbations a tremendous challenge. Variability arises from a number of factors that include the infecting virus (type of virus, particular strain, timing of infection, inoculum size), host-related factors (genetics, phenotype of asthma, prior infections and immune response/immunity, inherent microbial flora), host medical history (e.g. comorbidities, concomitant medications), and environmental conditions (e.g. aeroallergens). Due to the lack of an animal species that is naturally permissive for HRVs to use as a facile model system, coupled with the limitations associated with animal models of asthma and COPD, experimental infection studies in humans have been crucial to our understanding of the biology of HRV pathogenesis. The controlled nature of these HRV challenge studies helps to alleviate much of the variability associated with studying naturally occurring disease exacerbations. These experimental infection studies have focused on trying to understand the immune-mediated inflammatory response (i.e. cytokines, chemokines, other mediators, cell types, and signaling pathways) to HRV infection in asthmatics and patients with COPD often by comparing the responses between healthy and affected populations, or the responses among patients with different severities of disease. In addition, although the development of GMP manufactured HRV challenge stocks is not trivial, experimental infection with serotypes of HRV other than HRV-16 or HRV-39 will be critical in determining if the results obtained with the isolates of HRV-16 and HRV-39 used most frequently can be extended to other serotypes such as those in the HRV-C group, which may be a relevant group for individuals with asthma [243]. Experimental challenge studies in subjects with specific and predefined clinical phenotypes associated with asthma or COPD may help to further elucidate potential different mechanisms of susceptibility associated with virus-induced exacerbations compared to a broader heterogeneous population.

Such experimental HRV infection studies have been conducted safely in both normal subjects and in those with chronic respiratory diseases such as asthma and COPD. Although HRVs can be associated with serious complications such as pneumonia in severely immunosuppressed individuals (such as bone marrow transplant recipients), there have been no reports of pneumonia or other serious complications in published studies of experimental infection with HRV in healthy adults, subjects with mild to moderate asthma or subjects with COPD, even in the context of inhaled or systemic corticosteroids. These experimental infection studies with HRVs are crucial to our understanding of the underlying molecular mechanisms involved in acute exacerbations of asthma and COPD triggered by HRV infection, and are essential for the development of agents to prevent and treat exacerbations. Recent advances in transcriptomic and proteomic platforms, in addition to advances in computational biology approaches to building experimentally-

defined networks of disease, may also help to define potential points of interception to either repair inherent deficiencies in innate anti-viral immune response or to reduce exaggerated host response to acute viral infection.

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