


Experimental Antiviral Therapeutic Studies for Human Rhinovirus Infections

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Abstract: Rhinovirus infection is common and usually causes mild, self-limiting upper respiratory tract symptoms. Rhinoviruses can cause exacerbation of chronic respiratory diseases, such as asthma or chronic obstructive pulmonary disease, leading to a significant burden of morbidity and mortality. There has been a great deal of progress in efforts to understand the immunological basis of rhinovirus infection. However, despite a number of in vitro and in vivo attempts, there have been no effective treatments developed. This review article summarises the up to date virological and immunological understanding of these infections. We discuss the challenges researchers face, and key solutions, in their work to investigate potential therapies including in vivo rhinovirus challenge studies. Finally, we explore past and present experimental therapeutic strategies employed in the treatment of rhinovirus infections and highlight promising areas of future work.

Keywords: antiviral agents, respiratory tract infections, rhinovirus, therapeutics

Introduction

Rhinoviruses (RVs) are among the most abundant of the respiratory viruses known to man. They predominantly cause mild self-limiting illness in healthy individuals but they are associated with significant morbidity in those with chronic lung disease. RVs were first identified in 1956 by WH Price at Johns Hopkins university during a search for the aetiology of the common cold,¹ but have since been implicated as one of the main causes of both asthma² and chronic obstructive pulmonary disease (COPD) exacerbations.³ RVs have also been implicated in lower respiratory tract infections (LRTIs), with one study showing RV-A and RV-C present in around half of all cases of hospitalised LRTIs.⁴ During the SARS-Coronavirus-2 pandemic which resulted in numerous national lockdowns, the transmission of nearly all respiratory viruses was halted with the exception of RV.⁵ There have been numerous attempts at producing an effective anti-rhinovirus therapy, but that goal remains unrealised. In this review, we discuss the structure of RVs, the immune response to infection, development of the human rhinovirus challenge model, and the historical as well as current state of antiviral research.

Background

Virology

RVs are members of the Picornaviridae family and are located within the Enterovirus genus.⁶ Like other enteroviruses, RVs are single stranded and positive-sense viruses.⁷

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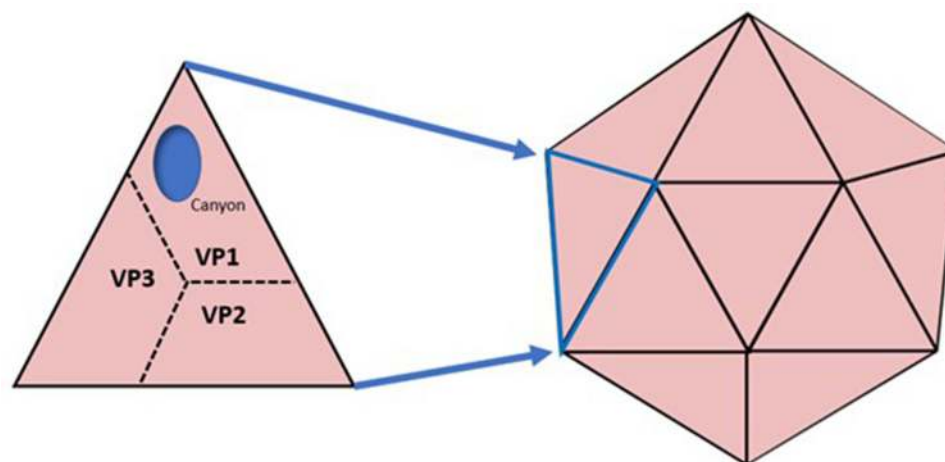


Figure 1 The icosahedron shape of the HRV viral capsid showing the orientation of the VP proteins.

RVs are currently classified into 3 species according to variations within their RNA genome.⁸ The RV-A species currently has 77 strains, whilst RV-B has 29 strains.⁹ In 2006, a novel rhinovirus clade was identified in New York City and, aided by advances in genome sequencing, was found to represent a new genotype termed RV-C;¹⁰ RV-C currently contains 60 strains.¹¹

The RV genome is particularly susceptible to random point mutations due to a lack of traditional RNA proof-reading mechanisms. The result is an enormous amount of antigenic variation which allows individuals to have multiple seasonal RV infections despite each infection stimulating a memory immune response. The large antigenic diversity also provides a hurdle to vaccine and antiviral therapy creation and helps to explain why effective therapies remain elusive.

The RV genome encodes a single gene which is subsequently cleaved to create 11 individual proteins which provide the functions needed for the virus to survive, invade, and replicate.¹² The genome can therefore be divided according to these processes. At the 5-prime end is an untranslated region (5'UTR), followed by the P1, P2, and P3 regions, before terminating with a 3'UTR and a poly-A tail.⁸ The P1 region encodes the structural viral proteins (VP) which form the protective viral capsid and are the main target of the host immune response.⁸ The P2 region performs roles in virus replication. The P3 region is predominantly involved in viral replication but also plays a role in modulation of the host immune response.

The P1 region is divided into sub-sections termed 1A-1D which in turn encode the structural proteins VP4-VP1, respectively. The P2 region (2A-2C) encodes a cysteine

protease (protease 2A) which acts at the P1-P2 junction to cleave the P1 region from the rest of the translated viral polyprotein.⁸ Protein 2B is a viroporin involved in virus efflux from an infected host cell and also assists in recruiting host cell protein machinery to aid viral replication. 2C binds intracellular host proteins but its exact role is not clear. The P3 region (3A-3D) begins with protein 3A, which interacts with the PI4KB protein and assists replication.¹³ The role of 3B remains unclear but appears to also assist with viral replication.¹⁴ Protein 3C is another protease which cleaves and degrades the cytosolic pattern recognition receptor, retinoic acid-inducible gene I (RIG-I) which results in attenuated host type I interferon (IFN) production.¹⁵ The final protein of the P3 region is 3D^{pol}, an RNA-dependent RNA polymerase (RdRps) which synthesises new viral RNA during replication.¹⁶

The VP proteins of the P1 region form an icosahedron shape which becomes the viral capsid (Figure 1). Each triangular face of the icosahedron is formed of a single VP1, VP2 and VP3 on the surface, whilst the VP4 protein is hidden on the internal surface of each face. Within the VP1 protein is a hydrophobic depression, commonly termed a “canyon”, which is important in virus-cell binding.¹⁷ The VP1 canyon is currently postulated to be the ligand for host cell intercellular adhesion molecule 1 (ICAM-1).¹⁸ In RV-C, the VP1 canyon is collapsed and contains hydrophobic residues (such as Trp1080, Phe 1096, and Met1116).¹⁹ Changes in the canyon here make RV-C less susceptible to drugs that target amino acids in this region.

Furthermore, RVs can be classified into major and minor groups based on which host cell receptor they

bind to. Major group RVs bind to ICAM-1 and include most serotypes of HRV-A and HRV-B, whilst the minor group RVs bind to low-density lipoprotein receptor (LDL-R).²⁰ RV-C strains bind to cadherin-related family member 3 (CDHR3).^{21,22}

Understanding the fundamentals of RV virology is key when considering potential therapeutic strategies, but also goes some distance to accounting for the modest success we have had in their implementation.

Immune Response to RVs

RVs primarily infect humans via nasal epithelial cells and are spread through expulsion and inhalation of microdroplets containing the virus. Depending on the RV type, and therefore its target receptor, RVs bind ICAM-1, LDL-R, or CDHR3 expressed on nasal epithelium and macrophages.²³ RVs are engaged by various pattern recognition receptors (PRR), including Toll-like receptor 2 (TLR2) on the cell surface and intracellular TLR3, TLR7, and TLR8.⁷ Other intracellular PRRs that play a role include RIG-I and melanoma differentiation-associated protein 5 (MDA-5) which are both cytosolic. Whilst different receptor groups use separate cellular-signalling pathways, they all result in the transcription and production of type I IFNs (IFN- α , IFN- β) and type III IFNs (IFN- λ 1, IFN- λ 2, IFN- λ 3).²⁴ IFNs appear to be crucial to the early anti-RV immune response and have several roles. IFNs induce Th1 antiviral adaptive responses, but also induce apoptosis of RV-infected cells, inhibit viral transcription and virus entry into cells.²⁵ The inflammatory response is characterised by expression of cytokines (IL-1 β , IL-6, IL-11, TNF), chemokines (CXCL-8, MCP-1, IP-10, RANTES) and various reactive oxygen species and vasoactive peptides.^{8,26,27} This inflammatory milieu contributes to the typical symptoms seen in the common cold of nasal congestion, discharge and sore throat in healthy individuals.

COPD patients are known to have impaired antiviral responses, as demonstrated through the rhinovirus infection model.^{28,29} Individuals are infected with the same quantity and serotype of RV at a controlled time point, allowing differences in the immune response to infection to be compared between COPD patients and healthy controls. Such models show that COPD patients have higher post inoculation viral loads and have exaggerated immune responses, with increased levels of cytokines (IL-1 β , IL-6, TNF, CXCL8), neutrophil elastase, matrix metalloproteinase-9 (MMP-9) and oxidative stress.^{29,30} Cells sampled

by bronchoalveolar lavage from COPD patients post RV challenge showed deficient IFN production.²⁹ The increased virus loads observed, as well as the consequent increased inflammatory responses, likely result from deficient IFN responses to infection in COPD.^{29,31,32} Wark et al demonstrated that pBECs from COPD patients had impaired IFN responses when pre-treated with exogenous IFN- β .³³ A subset of COPD patients with frequent exacerbations has been identified as a potential target group for IFN therapies.³⁴

Asthma patients also have impaired antiviral immune responses. A landmark 2005 study was the first to show impaired control of RV replication within primary human bronchial epithelial cells (HBECs) from people with asthma.³⁵ Further studies have confirmed both deficient IFN- β and IFN- λ responses in people with asthma.^{36,37} In vivo studies have found increased concentrations of IFNs during RV induced asthma exacerbations,^{38,39} likely a result of increased virus loads consequent upon deficient early IFN responses. Work by Kennedy et al found no difference in viral loads in small numbers of subjects (16 with asthma, 8 without) who participated in an experimental challenge with RV,⁴⁰ while a study of larger numbers (28 with asthma and 11 without) found nasal virus load was increased ~250-fold on day 3. RV infection in asthma also stimulates the airway epithelium to produce the cytokines IL-25, IL-33 and thymic stromal lymphopoietin (TSLP) that activate type 2 innate lymphoid cells (ILC2s) and T helper 2 (Th2) cells.^{41,42} RV infection in asthma therefore induces a type 2 response typical of asthma exacerbations.

Experimental RV Infection Model History of the Model

The virus challenge model in humans, also known as experimental infection, has been used to better understand the immune response to RV infection and also the efficacy of various therapeutics. The study of most infections relies upon experimental infection in animal models and natural infection in humans.

Studying natural infections is problematic in the case of RV for a number of reasons: infected individuals are frequently asymptomatic early in the course of infection - the most plausible time that intervention will be effective; infections are usually mild and self-limiting, requiring sensitive and early reporting on the part of patients in order to be identified; and there are other respiratory

viruses clinically indistinguishable from RV infection, so natural infection studies would require large numbers of participants over a long time course in order to sufficiently power analysis. The mild and self-limiting nature of RV infection means that illness is almost always tolerable, safe and temporary in healthy adults. This has allowed pioneering researchers to develop methods of causing RV infections in humans in a predictable manner allowing the study of illness and assessment of therapies.

Initial attempts can be traced back to the Medical Research Council's Common Cold Research Unit in Salisbury, where individuals were isolated in small groups for 'holidays' in order to study viral transmission and work focused on the attempt to isolate and propagate virus in culture.^{43,44} Here, virus inoculation in healthy volunteers was attempted with nasal drippings from symptomatic individuals, with a success rate of 60%.⁴⁵ These efforts paved the way for subsequent efforts in centres in Chicago, Bethesda, Baltimore and Charlottesville, the latter focusing specifically on RV and intranasal IFNs.⁴³ Advances in laboratory culture and genetic identification of virus species progressed gradually until the advent of highly sensitive molecular identification techniques including PCR, notably allowing the detection and classification of the RV-C species.⁸

A large number of different strains have been used in experimental infection models to date, primarily from group A RVs: RV-1,⁴⁶ RV-2,⁴⁷ RV-9,⁴⁸ RV-16,^{49–53} RV-24,⁵⁴ RV-23,⁵⁵ RV-29,⁵⁶ RV-32,⁴⁷ RV-39,⁵⁷ RV-44,^{47,58} and Hank's strain.⁵⁹ RV-14, a group B rhinovirus has also been used in a single experimental infection⁶⁰ (Table 1). No experimental inoculation has occurred with group C RVs to our knowledge.

Whilst many of these RV strains have been well established, cultured and tested to a very high standard and have a track record of safety in clinical trials, most have been developed outside of a formal regulatory framework. This is because the developments occurred at a pace which outstripped that of regulators. In the UK, the Academy of Medical Sciences have published a report which suggests that explicit guidance on the development of controlled human infection models is warranted.⁶²

Findings of Experimental RV Models

The human RV challenge model has allowed both validation of *in vitro* findings and a comprehensive (and clinically relevant) understanding of symptoms, signs and sequelae of RV infection. The same dawn of molecular

mechanisms which identified the breadth of RV species also revealed their importance in chronic lung conditions. In asthma exacerbations two thirds of viral infections, by far the most common cause of exacerbation, are RV species.⁶³ Experimental RV infection in asthma has facilitated the elucidation of inflammatory processes and identified therapeutic targets for therapies in both acute and chronic management of the disease.

Our understanding of COPD exacerbation has similarly progressed with the use of RV models. Viral infections are far more common an aetiology than bacterial infections⁷ and experimental RV infection allows controlled exacerbation which similarly allows for a comprehensive understanding of immune response, identification of therapeutic strategies and evaluation of treatments in a more effective way than natural infection studies.²⁹

RV challenge has become the critical first step for Phase II trials for not just anti-RV therapies but also drug whose aim is to reduction in burden of asthma and COPD exacerbation. Despite the human challenge model presenting a significant improvement in the drug discovery process, and a number of potential treatments being tested with this method, none have been successfully licensed for use in the treatment of RV infection to date.⁶⁴

Antiviral Therapies

Table 2 summarises the actions and evidence of antiviral therapies discussed in this section.

Ribavirin

Ribavirin has a broad spectrum of activity and has been used to treat respiratory syncytial virus (RSV), Lassa fever, influenza and hepatitis C (in combination with pegylated IFN- α).⁷³ It is a synthetic nucleoside (a guanine analogue which acts by inhibiting inosine monophosphate dehydrogenase) and has a range of antiviral properties both direct (impairing viral mRNA synthesis and increasing viral mutation rates) and indirect (including upregulation of the host immune response).⁷⁷ It has been long established that ribavirin is effective *in vitro* against a number of RV strains, especially when used prophylactically.^{71,72}

However, the only *in vivo* evidence has been in a case series treating persisting lower respiratory tract symptoms caused by recurrent RV infection in four patients with hypogammaglobulinaemia. Oral ribavirin was administered with pegylated IFN- α 2 α , and was associated with a marked decrease and clearance of RV RNA. However,

Table I RV Strains Used in Human Experimental Infection Models

RV Group	Strain	Use Examples
A	RV-2	A double-blind placebo controlled trial of chalcone as prophylaxis against RV-2 infection demonstrated no evidence of benefit. ⁶¹
	RV-9	Three trials are reported assessing an experimental antiviral compound, R61837, acting on the virus capsid protein, against RV-9 inoculation in volunteers. The compound demonstrated reduction in symptoms when used prophylactically but not when commenced shortly after infection. ⁴⁸
	RV-16	This strain has been used widely in therapeutics trials but also notably was used in the development of an experimental model of viral COPD exacerbation. ²⁸
	RV-24	Used in early rhinovirus challenge trials, including a 1973 study assessing an antiviral compound, 3,4-dihydro-1-isoquinolineacetamide hydrochloride, as prophylaxis against RV-24 inoculation. This placebo controlled study demonstrated no benefit. ⁵⁴
	RV-23	Echinacea or placebo was given to healthy volunteers 14 days prior to, and for 5 days after, inoculation with RV-23. No significant difference in symptom scores or infections rates was detected. ⁵⁵
	RV-29	Intranasal administration, after inoculation of RV-29, of the antihistamine diphenhydramine hydrochloride was compared against placebo in a double blind randomised controlled trial demonstrating a slight reduction in proportion of cold symptoms. ⁵⁶
	RV-39	The decongestant oxymetazoline was evaluated against placebo when administered soon after infection in a RV-39 experimental model. Mean viral titre was reduced but viral shedding and clinical illness was not affected. ⁵⁷
	RV-44	An antiviral compound, "CL 88,227", or placebo, was given three times daily before and after RV-44 inoculation. It neither prevented illness nor reduced symptom scores. ⁵⁸
	Hank's strain	A three-armed randomised control trial compared oral pseudoephedrine with or without ibuprofen against placebo following either experimental inoculation with Hank's strain or RV-39. Illness severity and rhinorrhoea was reduced in treatment groups compared with placebo. ⁵⁹
B	RV-14	Volunteers were challenged with RV-9 and RV-14 after prophylactic administration of intranasal recombinant human IFN- γ or placebo, with no improvement in outcomes demonstrated. ⁶⁰

there was concomitant use of antibiotics, no clinical outcome data was provided, three of four experienced side effects and all patients had recurrent RV infection within months.⁷³ It is also impossible to know whether the observed effect was due to ribavirin, IFN- α , or a combined synergistic effect. The prospect of this formulation and dose of ribavirin seems limited even within confirmed, symptomatic and high-risk individuals.

Capsid Binding Inhibitors

Pleconaril

Pleconaril is an orally absorbed low molecular weight compound which binds to the hydrophobic pocket of the β -barrel of the VP1 section of RVs, impairing viral capsid functions like attachment to cell receptors and viral RNA uncoating.⁷⁸ Importantly, pleconaril has only weak activity against RV-C due to differences in the VP1 canyon and the amino acid sequences there.⁷⁹ Two double blind

randomised controlled trials have been conducted which evaluated participants experiencing symptoms from picornavirus infection as identified through real time PCR assays. Combined, 681 patients were randomised to pleconaril and 682 to placebo. Analysed individually, only one study met its primary end goal of reduction in time to alleviation of illness (defined by absent or mild cold symptoms in five domains for over 48 hours), although combined analysis suggested a statistically significant reduction overall (a median of 6.3 versus 7.3 days, $P < 0.001$). This effect did not reach statistical significance in all corners – ie, participants with no virus or non-picornavirus isolated – and more side effects were experienced overall in the pleconaril arm.⁷⁰

When submitted to the Food and Drug Administration (FDA) for approval in 2002 for the treatment of the common cold, it was declined as a number of other issues were identified with the results from the above studies and from

Table 2 Summary of Mechanism and Evidence for Key Anti-RV Therapies

Therapeutic	Proposed Mechanism of Action	Existing Evidence
Azithromycin	Induction of IFN- β and IFN- λ in host response to RV	Indirect evidence only via studies in asthma exacerbations, where long term prophylaxis of 420 patients as part of a randomised placebo-controlled trial reduced the number of exacerbations and improved quality of life. ⁶⁵ In a separate study in acute exacerbations, no benefit was demonstrated. ⁶⁶
Budesonide	Reduction in pro-inflammatory cytokines, protection of host cells from cytotoxicity	No evidence from human clinical trials to date.
Gemcitabine	Inhibition of viral proliferation and viral RNA synthesis	No evidence from human clinical trials to date.
Host defence peptides	Exogenous bolstering of the host innate immune response	No evidence from human clinical trials to date.
IFN- β	Exogenous correction of impaired IFN response in asthma and COPD patients	A randomised placebo-controlled trial of 147 people with asthma tested inhaled IFN- β within 24 hours of a naturally occurring cold symptoms. It failed to meet its primary endpoint, likely due to less severe than expected exacerbations. ⁶⁷
Itraconazole	Reduced viral replication and suppression of inflammation	No evidence from human clinical trials to date.
Nitric oxide	Direct inhibition of rhinovirus replication and inhibition of pro-inflammatory cytokine production	No evidence from human clinical trials to date.
Pirodavir	Binding to viral capsid protein, inducing conformational change and preventing adsorption and RNA uncoating	100 patients were enrolled across three randomised placebo-controlled studies assessing pirodavir as prophylaxis against experimental viral challenge of rhinovirus strains, and demonstrated a reduction in symptoms. 32 patients enrolled in a randomised placebo-controlled study treated after inoculation did not demonstrate the same benefit. ⁶⁸ A later randomised controlled trial of 98 patients in the context of naturally occurring colds did not demonstrate a clinical benefit. ⁶⁹
Pleconaril	Binding to and impairing critical viral capsid functions of attachment and RNA uncoating	Combined analysis of two randomised placebo-controlled trials in a total of 1363 patients symptomatic with naturally occurring picornavirus infections demonstrated a reduction in time to alleviation of illness, although more side effects were experienced in the active treatment arm. ⁷⁰
Quercetin	Reduces RV replication and host cytokine response.	No evidence from human clinical trials to date.
Ribavirin	Direct: impairing viral RNA synthesis and increasing viral mutation rates. Indirect: upregulation of host immune response.	Efficacy demonstrated prophylactically in vitro. ^{71,72} In vivo work is limited to a case series of 4 patients with hypogammaglobulinaemia where it was used in combination with IFN- α . ⁷³
Rupintrivir	Inhibits the 3C protease by bonding to the active site on the viral protease	No evidence from human clinical trials to date.
Tremacamra	Blocking viral entry and reduction in pro-inflammatory cytokines	198 adults were randomised across four trials to receive the molecule in either pre- or post- experimental rhinovirus inoculation studies. A reduction in symptom scores, clinical colds and rhinorrhoea was demonstrated. ⁷⁴

(Continued)

Table 2 (Continued).

Therapeutic	Proposed Mechanism of Action	Existing Evidence
Vapendavir	Binding to viral capsid protein, inducing conformational change and preventing adsorption and RNA uncoating	455 asthmatic adults with naturally occurring (presumed) rhinovirus infection in a randomised double-blind placebo-controlled trial received vapendavir for 6 days after symptoms started. Although the trial reported a significant reduction in cold symptoms at 2 to 4 days post infection, the primary endpoint was not met. ⁷⁵
Vitamin D	Supporting innate immune system via increased cathelicidin and IFN stimulated genes	A large recent meta-analysis of 43 trials and 48,488 participants demonstrated that vitamin D supplementation reduces the incidence of acute respiratory infections. ⁷⁶

prior phase II trials, including concerns about interference with the action of oral contraceptives and the lack of a significant reduction in symptoms in non-white participants. Above all, the FDA thought that the requirement to take pleconaril within 24 hours of symptoms was unrealistic; an assessment that suggests a high threshold for licensing for any future RV treatment submissions.⁸⁰

A further randomised, double blind, placebo controlled Phase II trial exploring intranasal pleconaril in asthma patients was completed in 2013.⁸¹ The study did not meet its primary endpoint of reducing asthma exacerbations.

Pirodavidir

By 1991, several compounds had shown in vitro activity against either rhinovirus group A or group B. Pirodavidir is a substituted phenoxy-pyridazinamine and was the first compound to show efficacy against both rhinovirus groups.⁸² Pirodavidir binds to conserved amino acids in the hydrophobic canyon of the VP1 protein. Despite this not all strains of RV are susceptible to pirodavidir. In particular, RV-8, RV-42, RV-45 and RV-87 are resistant strains and this is believed to be due to different amino acid sequences in the canyon. After binding to the VP1 canyon, pirodavidir mediates its effects by inducing a conformational change in the viral capsid protein which prevents adsorption and uncoating of the virus.

Pirodavidir has undergone two clinical trials. This was first used as an experimental rhinovirus infection model where participants were directly infected with RV-39 or RV-Hanks and then allocated to a placebo or pirodavidir group.⁶⁸ Pirodavidir was administered either prophylactically or 24 hours post infection. Significant reductions in viral shedding were seen in the pirodavidir group regardless of the timing of administration. Reduction in common cold symptoms and clinical benefit however was only observed

in the prophylactic group. This was replicated in a subsequent randomised double-blind placebo controlled trial of intranasal pirodavidir which showed no clinical benefit of pirodavidir against naturally occurring common colds, even though a reduction in viral shedding was observed.⁶⁹

Vapendavir

Vapendavir is another capsid binder developed by Biota pharmaceuticals. A Phase IIb trial assessed response to natural upper respiratory tract infection (URTI) in 455 participants with moderate to severe asthma but did not meet its primary end point which was a reduction in symptoms (ACQ-6 questionnaire).⁸³

Novel Capsid-Binding Inhibitors

One of the major drawbacks of capsid binding inhibitors like pleconaril and vapendavir is that they are ineffective against RVs with bulky hydrophobic VP1 canyons. These resistant RVs include RV-B5 and also the RV-C species, which account for a sizeable number of infections. New compounds have emerged that have broad canyon binding activity. OBR-5-340 is a pyrazolopyrimidine that through in vitro work has shown to bind nearer to the entrance of the canyon compared to other capsid binders⁸⁴ and demonstrate inhibition of resistant RV species.⁸⁵ Another compound showing promising cross-species canyon binding is ca603 which has a similar structure to Pirodavidir. In vitro work has shown that ca603 binds to pleconaril resistant RV species and inhibits viral replication through binding at the VP1 canyon site.⁸⁶

Novel RV Inhibitors

Rupintrivir

Rupintrivir is another experimental anti-rhinovirus therapy that acts on the 3C protease. RV-3C protease inhibitors are

based on a tripeptidyl structure and mediate their antiviral effects by forming a covalent bond with the active site cysteine amino acid on the viral protease.⁸⁷ Dragovich et al showed that such antiviral effects could be further enhanced by adding a ketomethylene molecule to the tripeptidyl compound.⁸⁷ This work led to the creation of rupintrivir (originally named AG7088).

Several *in vitro* studies have demonstrated the efficacy of rupintrivir against 48 serotypes of RV in HeLa cells,^{88,89} and in bronchial epithelial cells⁹⁰ and such effects are mediated up to 26 hours after infection. *In vivo* studies of intranasally administered rupintrivir have shown a good safety profile⁹¹ and moderate reduction in symptoms of a common cold and viral titres compared to a placebo.⁹² Due to limited clinical benefit, further trials of rupintrivir have not been undertaken. In more recent years, modification of rupintrivir with proline and azetidine molecules have shown *in vitro* anti-RV effects but are yet to be examined *in vivo*.⁹³

Nitric oxide targeted approaches represent another strategy. Nitric oxide (NO) is produced by the respiratory epithelium as a physiological response to RV infection. NO is primarily synthesised by nitric oxide synthase (NOS) and its active metabolite S-nitroglutathione is conversely metabolised by S-nitrosoglutathione reductase (GSNOR). NO has been shown to inhibit both rhinovirus replication and pro-inflammatory cytokine production.⁹⁴ Yang et al have demonstrated that a specific GSNOR inhibitor, termed C3m, reduced RV replication and also pro-inflammatory cytokines CXCL10, RANTES, and M.⁹⁵ Interestingly, this appears to be due to its effects on ICAM-1 transcription as opposed to its GSNOR activity. NO-based therapies are currently in clinical trials for other respiratory viruses, notably SARS-CoV-2, and remain an unexplored avenue for treatment of RV.

Itraconazole has also exhibited anti-RV effects with reduced viral replication in infected mice lungs. There have currently been no clinical trials of itraconazole against RV.⁹⁶

Host Defence Peptides

Host defence peptides (HDP) are proteins with antimicrobial properties and represent another arm of the innate immune system. They are secreted by leukocytes and epithelial cells, and principally fall into two groups: cathelicidins and defensins. The antiviral mechanism of human cathelicidin is unknown but human and various mammalian cathelicidins have shown efficacy against RV,

including LL-37, protegrin-1, and SMAP-29.⁹⁷ Exogenous administration has not been assessed in clinical trials yet but represents a possible therapeutic approach.

Vitamin D is commonly regarded for its effects on maintaining bone health and calcium homeostasis, but it also plays a role in supporting the innate immune system. Vitamin D is obtained through both diet and by the ultraviolet (UV) radiation conversion of 7-dehydrocholesterol to vitamin D₃. Vitamin D becomes activated via the liver and the kidneys to form the active metabolite calcitriol, which mediates its effects through binding nuclear vitamin D receptors (VDR) within cells. There is also evidence that lung epithelium and various leukocytes can also convert vitamin D to its active form.^{98,99} Calcitriol binding leads to the upregulation of various HDPs, including LL-37 (cathelicidin) in HBECs,^{100,101} β -Defensin-2 (HBD2), and human cationic antimicrobial peptide (hCAP).¹⁰² Furthermore, single nucleotide variants in the VDR gene are associated with increased risk of URTI, again highlighting the importance of calcitriol in antiviral responses.¹⁰³ *In vitro* work showed that HBECs infected with RV in the presence of vitamin D led to decreased viral replication and a rise in cathelicidin and IFN stimulated genes.¹⁰⁰

The exact dosing or treatment duration of vitamin D is less clear. A 2015 literature review concluded that vitamin D metabolites do not consistently reduce RV replication though they do lead to changes within type 1 IFNs and pro-inflammatory cytokines.¹⁰² A 2017 systematic review and meta-analysis covering 10,933 patients found an overall protective effect of vitamin D against URTI.¹⁰⁴ A more recent meta-analysis in 2020 (currently in pre-print form), showed that daily administration of 400–1000 IU vitamin D conferred protection against URTI.⁷⁶ Further data suggests that protective effects are maximal in individuals with pre-existing vitamin D deficiency (baseline 25-hydroxyvitamin D concentration <25 nmol/L).¹⁰⁵ To our knowledge there have been no trials of vitamin D using the human viral challenge model.

Modulation of the Host Response

IFN responses, as previously discussed, are impaired in both asthma and COPD patients. Consequently, exogenous IFN has been explored as a broad-spectrum therapeutic approach against respiratory infection, of which the majority are due to rhinovirus. Numerous trials of intranasal IFN were conducted from the 1960s without success. The first randomised controlled trial using orally inhaled IFN- β in

patients with asthma was completed in 2014.⁶⁷ The trial resulted in a significant improvement in lung function but the study did not meet its primary endpoint of clinical improvement, likely because most patients studied were mild, and asthma control in the placebo treated patients did not deteriorate significantly on virus infection.⁶⁷ A further Phase II trial was terminated early due to an unanticipated low rate of asthma exacerbations but participants did record an improvement in morning peak expiratory flow (PEF) on taking IFN- β .¹⁰⁶ More recent work has suggested that IFN- β may be better administered prophylactically, and work remains ongoing.¹⁰⁷ In November 2020, inhaled INF- β was shown to be of clinical benefit in SARS-CoV-2 positive patients in a hospital-based Phase II clinical trial, suggesting further work against RV is warranted.¹⁰⁸

Azithromycin is a macrolide antibiotic that has been shown to have immunomodulatory effects. In vitro work has shown HBECs pre-treated with azithromycin and then infected with RV-1B and RV-16 had significantly higher levels of RV induced IFNs and IFN stimulated genes.¹⁰⁹ Further work has shown azithromycin induces IFN- β and IFN- λ though the exact mechanism remains unclear.^{110,111} A randomised control trial (AMAZES) found that long-term azithromycin prophylaxis reduced the number of asthma exacerbations that patients with symptomatic asthma experienced and also improved participants asthma-related quality of life.⁶⁵ Despite this, the AZALEA randomised control trial of short-term azithromycin intervention versus placebo in acute exacerbations had no significant difference.⁶⁶ Further work is needed to elucidate the mechanism of azithromycin induced IFN stimulation, for which the experimental infection model would be well suited.

Several positive stranded RNA viruses have been shown to manipulate the membranes of the host cell Golgi apparatus and the endoplasmic reticulum to create a viral replication complex.¹¹² RV appears to bind such membranes using the RV-3A gene region and various host proteins including phosphatidylinositol 4-kinase III beta (PI4KB)¹¹³ and oxysterol binding protein (OSBP).¹¹⁴ PI4KB and OSBP are both substrates of protein kinase D (PKD) suggesting that targeting PKD may be another strategy. In vitro work has shown that inhibition of PKD reduced RV replication in a concentration dependent manner.¹¹⁵ PI4KB and OSBP targets are currently in development¹¹⁶ but have yet to undergo clinical trials.

Human N-myristoyltransferase (NMT) has emerged as another target. Host NMT myristoylates the VP0 region of

the virus polyprotein, which is a key step in the formation of the viral capsid and subsequently creating a viable virion. IMP-1088 was developed as an inhibitor of human NMT1 and NMT2, and has shown promising results in vitro.¹¹⁷ It has yet to reach clinical trials.

Neutralising Antibodies

An alternative approach is to use antibodies that either bind the virion and cause aggregation; disrupt the virion structure; prevent virus entry/attachment; or preventing release of newly formed virions. Two neutralising and immunogenic sites have been identified on VP1 close to the canyon (NIm-1a and NIm-1b), and another two sites on VP2 and VP3 (NIm-2 and NIm-3 respectively) of the RV-2 and RV-14 serotypes.^{118,119} Unfortunately, these NIm's are serotype specific which dramatically limits the possibility of a therapy effective against multiple serotypes. A solution may be to create a "cocktail" of multiple antibodies that provide protection across serotypes.

Antibodies may also be directed against host proteins, such as those critical for virus attachment and binding. Hayden et al demonstrated in 1988 that intranasally administered mouse monoclonal antibody specific for ICAM-1 reduced viral load and symptoms in human participants.¹²⁰

Soluble Proteins

Tremacamra, a recombinant soluble ICAM-1 showed modest improvement in symptom score when given both pre and post RV infection.⁷⁴ One drawback is the intense scheduling of doses, as 6 doses are given per day at 3 hour intervals intranasally. In more recent times, a mouse anti-human ICAM-1 antibody (14C11) has been developed which targets an epitope of ICAM-1 specific for viral entry.¹²¹ When given topically or systemically in mice, 14C11 reduced viral load, pro-inflammatory cytokines and blocked entry of RV-16 and RV-14.¹²¹ An approach to treat all forms of RV would be to create a cocktail of soluble ICAM-1, LDLR and CDHR3 though this remains to be studied and the dosing issue remains.

Other Therapies

Budesonide

Budesonide is a corticosteroid, most frequently used in the treatment of asthma and COPD via the inhaled route. When administered intranasally in mice, budesonide was shown to reduce RV viral load and also levels of pro-inflammatory IL-1 β .¹²²

Gemcitabine

Gemcitabine is a synthetic nucleoside deoxycytidine analogue in use as a chemotherapy agent, which has been demonstrated as efficacious in-vitro against RV-14, RV-21 and RV-71 in H1HeLa cells. Whilst its oncological use is associated with many unpleasant side effects which would surely preclude its use in the same fashion against RV, Kang et al have demonstrated limited cell toxicity and synergistic effects in combination with the guanine analogue ribavirin.¹²³ Gemcitabine's action has been replicated in murine models via intranasal administration, and there is evidence that it acts via inhibition of CTP synthetase.¹²⁴

Quercetin

Quercetin is a flavonoid found in plants and commonly eaten foods like broccoli and berries. A number of immunomodulatory effects have been attributed to the compound, including in vitro antiviral effects. Use in mouse models suggested that the compound reduced RV replication and cytokine responses.¹²⁵ Further work in smoke exposed mouse COPD models suggests that inflammation associated with exacerbation after RV infection was reduced with dietary supplementation of quercetin.¹²⁶

No clinical trials are yet forthcoming, although the groundwork for such a trial appears to be laid with a small safety trial assessing quercetin supplementation in patients with COPD reporting favorably in 2020.¹²⁷

Omalizumab (Anti-IgE)

Recent work has shown that subjects with allergic asthma (as measured by high levels of total IgE) show an increase in their blood eosinophil count and declines in lung function following RV-16 viral challenge.⁵² The same study, by Heymann et al, found that asthma subjects pre-treated with omalizumab (an anti-IgE monoclonal antibody) for 8 weeks before and 3 weeks following virus inoculation showed earlier resolution of lung function, FeNO score, and symptom score.⁵²

Other Approaches

Future efforts in drug discovery against RV infections may be directed by sophisticated computational means as described in Da Costa et al's 2017 paper describing the potential of compound (S)-7f against RV-14 using molecular and in silico models.¹²⁸

Other novel targets have been identified through analysis of the lipidome of human bronchial epithelial cells, and

commercially available enzyme inhibitors have been presented as potential inhibitors of RV infection and replication.¹²⁹

Finally, advances at the nanoscale have allowed a better understanding of the interaction between chemical makeup, structure and mechanics of viruses, and inevitably opened a new door of possibilities for virus inhibition by "mechanical stiffening".¹³⁰ Design, manufacture and evaluation of these compounds is awaited.

Future Work

Whilst an affordable and effective therapy against RVs continues to evade the scientific community, novel experimental therapies that have been uncovered in recent years offer new hope. It is likely that a variety of approaches will be required given the heterogeneity of human rhinoviruses. Antiviral therapies targeting the rhinovirus virion itself are among the oldest approaches but are yet to yield any significant breakthroughs. In vitro work on such therapies continues apace. The work on host defence peptides and in particular vitamin D looks promising and would be an obvious candidate for clinical trials using the rhinovirus challenge model, particularly given the abundance of data on its safety. Many other therapies remain in the experimental phase and should be watched closely.

With the emergence of the COVID-19 global pandemic, international attention has been thrust onto antiviral therapies and the need to produce a diverse array of strategies for this and future pandemics. We hope that with this newfound impetus and the hard work of many groups over the last 6 decades, that new progress will be uncovered. With multiple drug candidates awaiting clinical trials, we may be on the cusp of a new generation of antiviral therapies.

Abbreviation

14C11, anti-human ICAM-1 antibody; 3'UTR, 3-prime untranslated region; 5'UTR, 5-prime untranslated region; ACQ-6, asthma control questionnaire; AMAZES, effect of azithromycin on asthma exacerbations and quality of life in adults with persistent uncontrolled asthma (randomised controlled trial); AZALEA, Azithromycin for acute exacerbations of asthma (randomised clinical trial); COPD, chronic obstructive pulmonary disease; COVID-19, coronavirus disease (caused by SARS-CoV-2); CTP, cytidine triphosphate; FDA, Food and Drug Administration; GSNOR, S-nitroglutathione reductase; HBEC, human bronchial epithelial cells; HRV, (human) rhinovirus; IFN, interferon; ILC2s, type 2 innate lymphoid cells; LDL-R, low-density lipoprotein receptor; MDA-5, melanoma differentiation-

associated protein 5; NMT, human N-myristoyltransferase; NO, nitric oxide; NOS, nitric oxide synthase; PCR, polymerase chain reaction; PI4KB, phosphatidylinositol 4-kinase III beta; RIG-I, retinoic acid-inducible gene I; RNA, ribonucleic acid; TLR, toll-like receptor; TSLP, thymic stromal lymphopoietin; URTI, upper respiratory tract infection; VDR, vitamin D receptors.

Data Sharing Statement

No additional data is held by the authors other than that which appears in the manuscript.

Ethics Approval and Informed Consent

Not required for this review.

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Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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