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Review

New treatment regimes for virus-induced exacerbations of asthma

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Abstract

This review will focus on the role of viruses as causes of asthma exacerbations. The article will briefly review the current literature supporting this view, with a special focus on human rhinovirus (RV), the main virus associated with exacerbations of asthma. The review will then refer to possible strategies for treatment, and will include discussion on treatment with specific anti-viral therapy and type I interferon as a treatment for RV. The review will also include a discussion on current therapies for asthma, such as glucocorticosteroid and β_2 agonist therapy alone and in combination and why this may be relevant to virus-induced exacerbations of asthma. Finally, the potential for future anti-inflammatory/immunomodulatory therapies with a focus on NF-κB inhibition will be discussed.

Keywords: Rhinovirus; Asthma; Therapy; NF-κB; Lung; Steroid; Interferon

Contents

Abbreviations: RSV, respiratory syncytial virus; RV, rhinovirus; IFN, interferon; COPD, chronic obstructive pulmonary disease; NIK, NF-κB inducing kinase; IKK, IκB kinase; TBK, TANK binding kinase; PKR, anti-viral protein kinase; TLR3, Toll-like receptor 3; RIG, retinoic acid inducible gene; MIC, minimum inhibitory concentration; GCs, glucocorticoids; GR, glucocorticoid receptor; LABA, long-acting β_2 agonists

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1. Introduction

Asthma exacerbations, the majority of which are caused by respiratory viral infection, are a continuing problem in respiratory medicine worldwide. Most viral exacerbations are due to either respiratory syncytial viruses (RSV), coronaviruses, influenza viruses or human rhinoviruses (RV), with RV being the most frequent causative agent. In vitro and in vivo, RV infects the bronchial epithelium and upregulates a range of pro-inflammatory cytokines, chemokines, adhesion molecules, mucins and growth factors, all of which are thought to contribute to loss of lung function and lower airway inflammation [1–4]. A large number of these mediators are upregulated solely, or in part, by the transcription factor NF-κB. This would suggest that inhibiting the functions of this transcription factor may alleviate symptoms associated with RV-induced exacerbations of asthma. In virus-induced asthma, bronchial biopsies and sputum have neutrophilic and lymphocytic infiltrates [4,5], and these cell types are therefore implicated in exacerbation pathogenesis. Due to the lack of a small animal model, there are many unclarified issues regarding the immunology of RV infection, and how this relates to exacerbations of asthma.

A recent study has estimated the economic impact of asthma in Germany to be in billions of Euros [6]. Although the actual costs of viral exacerbations are not known, it is arguable that they would contribute significantly to this cost, as viral infections account for about 80% of asthma exacerbations in children, and between 40% and 76% in adults [7–12]. In the UK, one study has estimated the cost of asthma exacerbations to be approximately 3.5-fold higher per patient when compared to asthma patients that did not experience exacerbation [13]. Currently, the medical needs of patients suffering from viral exacerbations of asthma are largely unmet. There is no vaccine for RV or RSV, and the use of influenza vaccines in reducing virus-induced exacerbations remain controversial [14]. Steroids so far have been disappointing in their ability to control symptoms in models of experimental RV challenge of asthmatics [15–18], and high-dose steroids remain only partially effective at controlling virus-induced exacerbations of asthma [19,20]. A range of anti-viral, anti-RV compounds and combinations of the above have been used as therapies for RV infection, these have had variable efficacies in controlling RV-associated illnesses. This review will summarise the current understanding of virus-induced exacerbations of asthma, with a special focus on RV, including the epidemiology, host defence and immunology. Studies of treatments aimed at virus-induced exacerbations will also be discussed, with an emphasis on how better understanding the process of infection and upregulation of pro-inflammatory mediator gene expression may be useful in aiding the design of novel therapies for virus-induced asthma exacerbations.

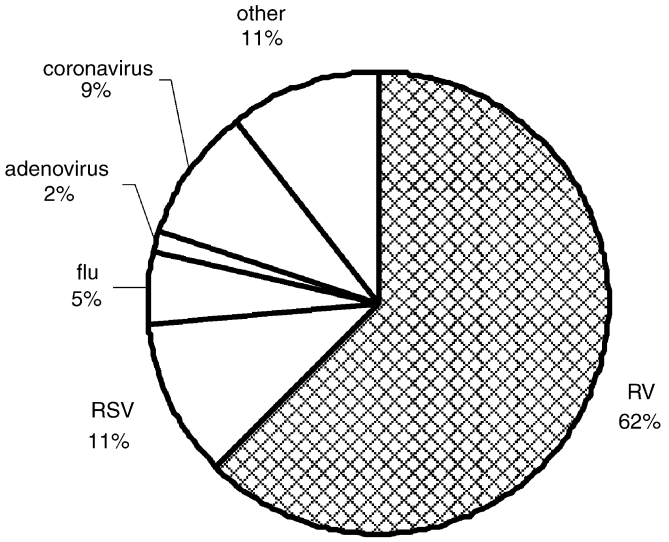

Fig. 1. Relative frequencies of respiratory viral infections in adults and children >2 years with exacerbations of asthma. RV account for up to 62% of exacerbations. Data are taken from three published studies [10,21,22], and presented here as an average. Viral infection was measured in these studies using virus culture, RT-PCR or both.

2. Respiratory virus infections as exacerbators of asthma

There is now overwhelming evidence that respiratory viruses are associated with acute exacerbations of asthma, accounting for up to 80–85% of acute exacerbations [7,10,21,22]. Of the common respiratory viruses, RV have emerged as the most frequent. Other respiratory viruses such as RSV influenza viruses, parainfluenza viruses, coronaviruses, adenoviruses and the newly described metapneumoviruses, may also be associated with exacerbations of asthma. In children <2 years of age, RSV, infection is a common cause of significant morbidity in the form of wheeze or bronchiolitis [9,10,22,23]. In older children and adults, RSV is still implicated in exacerbations of acute asthma, but does not appear to be as important as RV [7,10,21]. Influenza is an important pathogen during winter epidemics [24], but outside these periods it is not a major contributor to exacerbations of asthma. Recent data [25–27] suggest that human metapneumovirus has a minor contribution (<12%) to virus-induced exacerbations of asthma. The relative frequency of detection of each virus type in exacerbations of children >2 years and adults as found in previous studies [10,21,22] are summarised in Fig. 1, and represent approximate estimates for the most common respiratory viruses identified.

3. Virus-induced exacerbations of asthma: similar and distinct pathology and mechanisms of action to persistent asthma

Virus-induced exacerbations of asthma have both similar and distinct properties to persistent asthma. Respiratory virus-induced exacerbations of asthma may occur in patients with phenotypes that differ from the atopic phenotype characteristic of allergen-induced asthma. One

obvious difference between allergen-induced asthma and exacerbations is that inhaled corticosteroids, which are effective for the treatment of persistent asthma, do not work with the same efficacy in asthma exacerbations [19,20]. Evidence for similar mechanisms of action is suggested by the fact that allergen exposure and virus infection may act in a synergistic or additive manner, increasing the risk of asthma exacerbations [28]. Pollutants such as nitrogen dioxide may also increase the risk of virus-induced exacerbations of asthma [29]. Some interesting differences between asthma exacerbations and persistent asthma are highlighted by the following observations.

Virus-induced asthma exacerbations may differ from persistent or allergen-induced asthma in that neutrophils appear to play a more prominent role in exacerbations, while eosinophils predominate in the latter. T lymphocytes appear important to both. The importance of neutrophils and CD4+ and CD8+ T lymphocytes in asthma exacerbations is supported by several studies [4,5,11,15,30–35]. In atopic asthmatics, eosinophils or eosinophil activation are also increased in virus-induced asthma. Given that a mixed aetiology is likely very common [28,36], it is not surprising that there is much overlap in pathogenesis. Differences also exist in the way asthmatics respond to viral infection and may affect the outcome of infection and hence disease severity. Although asthmatics have the same incidence of viral infection as normals, they have increased severity and duration of lower airway symptoms and reduction in lung function than normals [37]. A recent study has also reported persistence of RV in children suffering from exacerbations of asthma, with children showing RV persistence having more severe exacerbations [38]. Also, increased levels of RV replication have also been observed accompanied by lower levels of virus-induced interferon (IFN)-β expression and virus-induced apoptosis compared to normals [39]. In certain studies, greater levels of pro-inflammatory cytokine elaboration and inflammatory cell recruitment are observed [4]. Peripheral blood mononuclear cells from asthmatics when cultured with RV exhibiting lower levels of the T_H1 cytokines IFN-γ and IL-12, suggesting that asthmatics may have a defective T_H1 response to viral infection [40]. These data support two important points; firstly virus exacerbations of asthma have different properties to persistent or allergen-induced asthma, and secondly, researchers are still defining the characteristics of viral exacerbations and the populations in the community that are at risk. These important points must be appreciated prior to discussing new treatments for virus-induced exacerbations of asthma.

4. Human RV—the most common virus associated with exacerbations of asthma

4.1. Epidemiology

RVs belong to the Picornaviridae family of viruses. These viruses have small RNA genomes (approximately 7 kb), [41,42], are non-enveloped and are stable in the environment. There are at least 100 serotypes, which are divided into major or minor groups depending on receptor specificity. Most RV are major group RV, and bind human ICAM-1, minor group RV bind the LDL receptor [43]. The difference in receptor specificity appears to be explained by a charge difference of the H1 loop of structural protein VP1 [44]. RV can replicate efficiently in the upper airway and can be detected in the lower airway although replication remains to be proven in vivo [45,46]. RV infection can be readily observed in the bronchial epithelium [47] in vivo, and ex vivo, detected by sampling both the lower airway [48,49] and upper airway [50,51].

RV are now well established as the major virus associated with exacerbations of asthma and also chronic obstructive pulmonary disease (COPD) [4,8,21,37,52–54]. Using virus-specific RT-PCR and virus culture techniques, epidemiological studies have observed that RV has the highest incidence of all respiratory viruses in exacerbations of asthma in adults and children >2 years of age; approximately 60–65% of viral exacerbations are due to RV infection [7,8,10,22,26], as presented in Fig. 1. RV infection appears to be prevalent in children returning to school, leading to significant epidemics, and increased hospital admissions in the month of September in the Northern Hemisphere [55]. A thorough review of the epidemiology of RV infection of the lower respiratory tract is available elsewhere [56].

4.2. Immunopathology and host defence

Due to the lack of a small animal model, many aspects of immunology and host defence against RV infection remain unelucidated. Experimental and natural infections, and in vitro infection of lung epithelial cells have been useful in the study of RV-induced inflammation, in both asthma and COPD. The immunology of RV infection is a rapidly expanding field, and has been thoroughly reviewed elsewhere. This review will highlight the main findings and discuss some of the unresolved issues; interested readers are directed to the following recent reviews for more information [57–60]. RV upregulate the expression of a range of pro-inflammatory mediators from lung epithelium in vitro and in vivo including the chemokines IL-8/CXCL8 [61–65], ENA78/CXCL5 [66], eotaxin/CCL10 [67,68] RANTES/CCL5 [67,69], IP-10/CXCL10 [70], growth and differentiation factors such as IL-6 [62,64,71], GM-CSF [62,64,65,72,73], IL-11 [74,75] and also adhesion molecules ICAM-1 and VCAM [76–80], and respiratory mucins [81,82].

Virus-induced exacerbations of both asthma and COPD are associated with lower airway infection [47,83], resulting in lower airway inflammatory responses characterised by infiltration of CD4+ and CD8+ T cells, neutrophils, eosinophils and activation of local macrophages and mast cells [5,84–86]. Experimental and natural infections of human subjects have also shown that subjects infected with

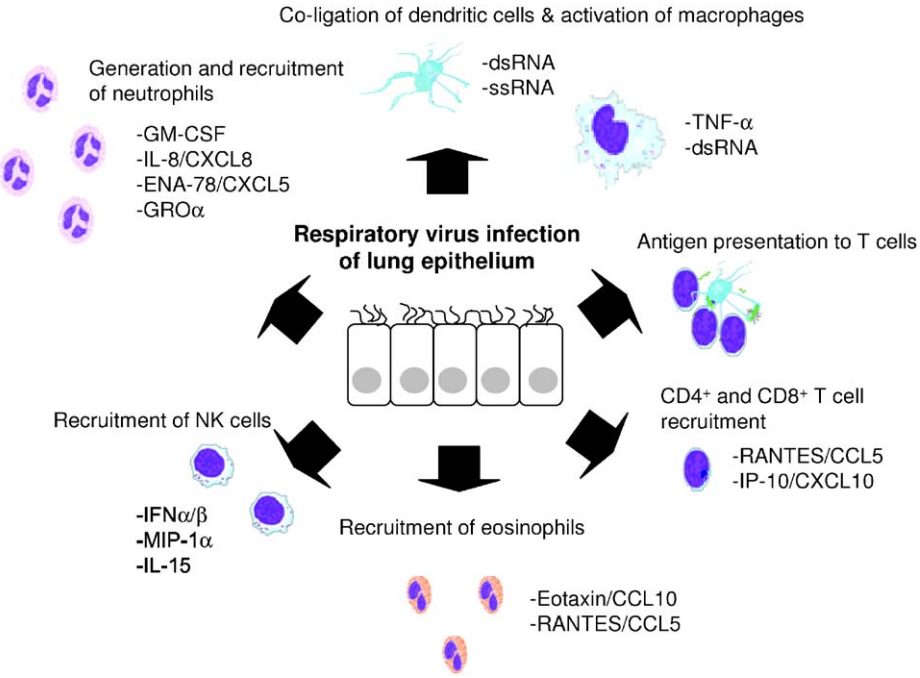

Fig. 2. Immunology of respiratory virus infection. Infection of the bronchial epithelium results in upregulation of a range of pro-inflammatory cytokines, chemokines and growth factors that are involved in the generation of lung inflammation, resulting in exacerbations of asthma.

RV show an increased level of the above cytokines in nasal secretions and sputum [1–4]. In one study, the accumulation of inflammatory cells and molecules correlated with increased symptom scores [2], and in another, decreased lung function of asthmatics [1], giving support to the current hypothesis that the local inflammatory reaction contributes to exacerbations in asthmatics. Therefore, if the upregulation of inflammatory molecules and cells can be prevented, diseases may be prevented. Inhibition of RV-induced inflammatory cytokine/chemokine production therefore represents an important therapeutic target for asthma. Some inflammatory molecules induced by respiratory virus infection and the cells they attract are outlined in Fig. 2.

4.3. Importance of NF-κB signalling

A striking observation is that all the pro-inflammatory molecules and growth factors upregulated by RV so far studied in detail require the transcription factor NF-κB (discussed below). The NF-κB or Rel family of transcription factors (p65, p50, c-Rel) are implicated in the expression of over 100 pro-inflammatory genes (for a review see [87–89]). NF-κB is sequestered in the cytoplasm by its specific inhibitor IκB, when phosphorylated by upstream kinases, IκB is ubiquitinated and degraded by the proteasome, allowing NF-κB to translocate to the nucleus. The upstream kinases responsible for relaying the signal include NF-κB inducing kinase (NIK) [90] and the IκB kinases (IKK)-α/β [91,92], and the more recently identified IKK-ι/ε [93,94] and TANK binding kinase-1 (TBK-1) [95–97]. Once in the nucleus, NF-κB can bind to various *cis*-acting sites within the promoter of NF-κB responsive genes and promote transcription (depicted in Fig. 3).

The promoters of GM-CSF, IL-6, CXCL8, CXCL10, ICAM-1 and VCAM all contain NF-κB binding sequences, and NF-κB is required for the expression of these genes following RV infection in vitro [62,63,70,71,76,80]. We have also extended this analysis for CXCL8 and IL-6 and have shown that NF-κB *cis*-acting sequences are absolutely required for RV-induced promoter activation for these genes in bronchial epithelial cells, and have identified a role for IKK-β, using the specific IKK-β inhibitor AS602868 (Serono, M.R. Edwards and S.L. Johnston, submitted). The role of NF-κB signalling in RV-induced CCL5 and IL-11 gene expression is not yet known, however seems likely to be implicated as both promoters contain NF-κB sequences, and these are important for gene expression following infection with other respiratory viruses [98,99]. The role of NF-κB in RV-induced CCL10 and CXCL5 has not yet been studied; however, both genes also have NF-κB sites within their promoter, that are required for gene expression in various systems [100–102]. Hence inhibition of NF-κB translocation, and NF-κB signalling represents a potential area of therapeutic intervention.

One unresolved question in RV biology is how NF-κB signalling is initiated by virus infection. The dsRNA binding anti-viral protein kinase (PKR) has been widely implicated in NF-κB signalling via interacting with IKK-α/β, or by phosphorylating IκB directly [103–106]. Toll-like receptor 3 (TLR3) [107], and the recently described retinoic acid inducible gene (RIG)-I [108] also activate NF-κB in response to viral infection or dsRNA. Gern et al. [68] have demonstrated a role for PKR in RV-induced

pro-inflammatory mediators from bronchial epithelial cells. PKR would thus seem a potential therapeutic target in RV exacerbations of asthma; however, PKR is also implicated in beneficial anti-viral host responses. Through its ability to phosphorylate eukaryotic initiation factor-2α, PKR causes termination of host protein synthesis following viral infection [109], in an attempt to limit viral replication. PKR is also involved in type I IFN responses [110]. Hence, the possibility of beneficial and/or detrimental affects of PKR inhibition in RV exacerbations of asthma remains an open issue.

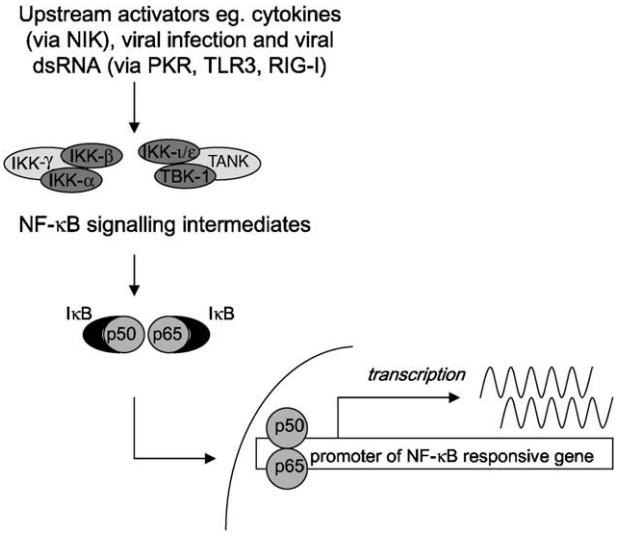

Fig. 3. Activation of NF-κB p65, p50 by the IKK-α/β/γ complex, or an alternative IKK complex consisting of IKK-ι/ε, TBK-1 and TANK. Following viral infection, or dsRNA recognistion via PKR and/or TLR3, RIG-I, or cytokines, e.g. TNF-α or IL-1β (via NIK), IKK phosphorylates IκB leading to its degradation by the proteasome. Once free, NF-κB p65, p50 subunits translocate to the nucleus and upregulate NF-κB responsive genes, such as CXCL8, IL-6 and GM-CSF.

5. Treatment options for virus-induced exacerbations of asthma

5.1. Anti-rhinoviral compounds, RV 3C protease inhibitors and capsid binders

The late 1980s and early 1990s saw great interest in anti-rhinoviral treatments, directed mainly at the control of clinical colds. Compounds which interfere with viral attachment and uncoating by binding to the picornaviral capside protein VP1 were first investigated in the late 1980s [111–113], and RV 3C protease inhibitors such as Ruprintrivir much later [114]. These drugs showed a broad inhibition to RV infection in vitro [115]. The modes of action of all treatment options discussed in this review are shown in Fig. 4.

The viral capsid binder R61837 was first used in 1989, in a model of experimental infection with RV9. R61837 was given in a single dose (2.5 mg) intranasally either 28 or 4 h prior to challenge, and continued up to 4 or 6 days, respectively, post-challenge [112]. Clinical colds were

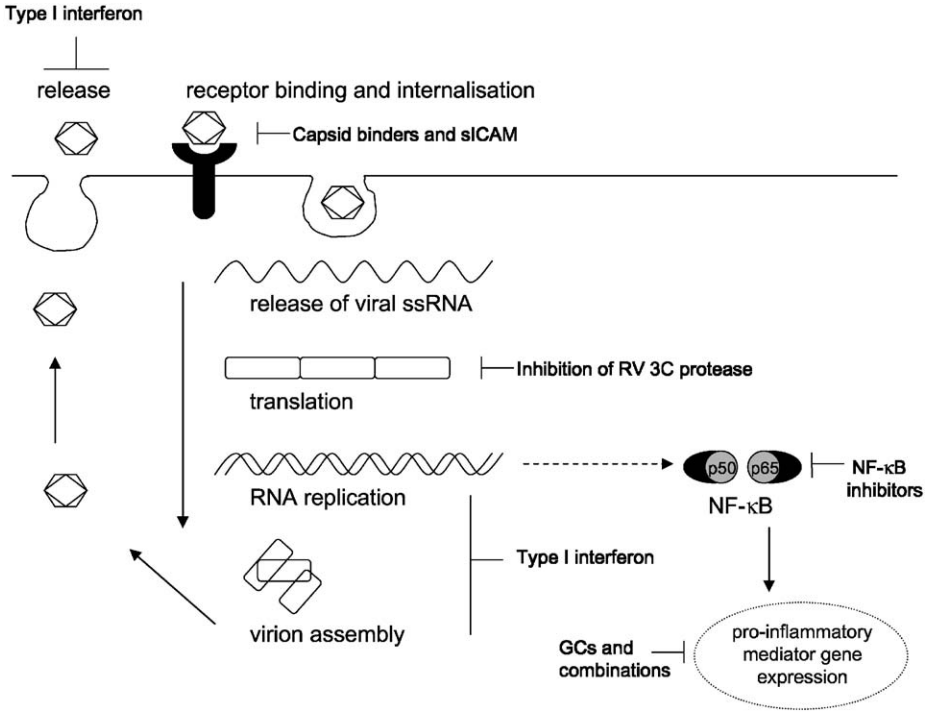

Fig. 4. Mode of action of different treatment options for rhinovirus induced exacerbations of asthma. Capsid binders and sICAM target viral entry, RV 3C protease inhibitors prevent digestion of the polyprotein and generation of RNA polymerase, type I interferon may target RNA replication, virion assembly and release. Other treatment options include NF-κB inhibitors which prevent activation of this transcription factor through targeting the NF-κB signalling pathway. GCs alone or in combination with β_2 agonists are used to prevent expression of pro-inflammatory genes.

defined on the basis of using >4 tissues over the baseline rate plus development of one other relevant symptom (sore throat, sneezing, etc.). When given 4 h prior to challenge, and for 6 days duration, R61837 reduced the incidence of colds, mean total symptom score and weight of nasal secretions compared to a control group given placebo. The effects of R61837 on the duration of the clinical cold, or length of time spent exhibiting symptoms were not studied.

In contrast, the oxazoline WIN54954 was tested in 1993, and showed poor efficacy in experimental models of RV39 and RV23 [111]. Dosing consisted of a 600 mg oral dose, every 8 h for 6 days. Challenge occurred on study day 2. WIN54954 did not reduce any of the parameters tested when compared to placebo, including incidence of colds, mean total symptom score and viral titre. One explanation for the poor performance of WIN54954 was the low levels of drug recovered from saliva and nasal wash after treatment. Few patients had concentrations greater than the minimum inhibitory concentration (MIC) for the test virus. Pirodavir (R77, 975) when given as an intranasal therapy (2 mg), was also disappointing in a study of natural virus infection [113]. Pirodavir reduced only viral titre when compared to control groups treated with placebo, the duration of colds and mean total symptom score were not reduced by Pirodavir.

The anti-picornaviral agent Pleconaril has been used in randomised, placebo-controlled, phase II clinical trials as a treatment of the common cold [116]. Pleconaril acts by preventing uncoating of picornaviruses, including most serotypes of RVs tested in vitro [117]. Participating volunteers commenced treatment 1–1.5 days after a clinical picornavirus infection was established. Individuals taking Pleconaril, when taken as a 400 mg dose in liquid form, or as a 400 mg tablet three times daily showed significant improvement in mean symptoms scores at days 2–5 after commencement of treatment, and decreases in mean duration of illness. Despite promising initial results, Pleconaril has not yet been tested in the setting of RV-induced exacerbations of asthma.

The RV 3C protease inhibitor Ruprintrivir (Agouron Pharmaceuticals, Inc.) has also been tested in a double-blind, placebo-controlled phase II trial of experimental RV39 challenge [114]. RV 3C protease is required for cleavage of the rhinoviral precursor polyprotein into individual components prior to viral assembly. Furthermore, RV 3C protease is required for generation of the RNA polymerase and hence viral RNA replication. RV 3C protease has also been implicated in initiation of host cell signalling leading to pro-inflammatory cytokine production [65]. Ruprintrivir was designed using solved X-ray crystal structures of the RV 3C protease, and was designed to bind irreversibly to the RV 3C active site [118]. Ruprintrivir demonstrated a broad anti-picornaviral spectrum in vitro, with an MIC of 0.023 μM [119]. The above study utilised Ruprintrivir as either two or five times a day as prophylaxis, 6 h prior to infection, or as a treatment five times a day 24 h after infection. As a prophylaxis, Ruprintrivir reduced mean total symptom score, viral titre, nasal secretions but not the incidence or frequency of clinical colds (as assessed by the number of infected subjects that developed clinical colds). As a therapeutic treatment, Ruprintrivir also reduced symptom scores, nasal secretions and viral titre. Ruprintrivir was generally well tolerated, despite this study requiring numerous deliveries via nasal spray.

The above studies provide evidence that treatment of clinical colds with anti-rhinoviral therapies is useful. However, there are potential problems with these approaches, with respect to potential therapies for virus-induced asthma exacerbations. For example, therapy of capsid binding molecules has led to the development of escape mutants [117]. It could also be argued for the RV 3C protease inhibitors, that virus binding, uncoating and entry could be a stimulus for cell signalling events, leading to initiation of pro-inflammatory mediator gene expression [61], and future therapies should aim at preventing the early steps of virus uncoating and entry. In support of this view, a study of Ruprintrivir to prevent RV14-induced IL-6 and CXCL8 suggested that this agent was not 100% effective at reducing mediator production, despite a high dose of 10 μM being used [120]. For therapies such as Ruprintrivir, which were administered via nasal spray, it can also be argued that delivery by this method may not be effective at treating the lower airway, where virus-induced asthma exacerbations are believed to be triggered. Furthermore, dosing regimes must also be suitable for children, as well as adults, in a setting of asthma exacerbations. Perhaps a further criticism of many of these studies is that duration of clinical colds was not affected by most treatments. Pleconaril was the only treatment successful at reducing duration of clinical cold. These therapies, although reducing symptoms may not necessarily affect duration of symptoms, and therefore may not improve the rate of general practitioner consultations, hospital admission rates, and school and work absenteeism for infected individuals with virus-induced exacerbations of asthma.

5.2. *Human or recombinant soluble ICAM-1 and derivatives*

Several versions of human ICAM-1 have been used in clinical studies, including soluble ICAM-1 (Tremacamra/BIRR 4) [121], antibodies to ICAM-1 [122], and fusion proteins of ICAM-1 and IgA [123]. In vitro, soluble ICAM-1 preparations have been shown to reduce titres of a large range of major group RVs [123,124]. Tremacamra has been tested in randomised double-blind placebo-controlled studies both as a therapeutic and prophylactic intervention to RV39 challenge [121]. In these clinical studies, Tremacamra has shown promise as a therapy, reducing the proportion of clinical colds following experimental RV39 challenge, total symptom score, nasal mucus weight, and significantly lowering CXCL8 release on days 3 and 4 post-infection compared to placebo. Tremacamra also

significantly reduced RV39 replication on days 2–4 post-infection. Also, Tremacamra was useful in controlling RV-induced symptoms if given prior to challenge, or after challenge but before establishment of symptoms. Tremacamra holds promise as a therapy for clinical colds, however, has not been tested in a context of exacerbations of asthma.

5.3. Type I IFN therapy

Type I IFNs are potent anti-viral mediators having a range of effects that limit viral infection and dissemination. Type I IFNs include the numerous IFN-αs, IFN-β and the newly identified IFN-λs [125]. Type I IFNs act on virally infected cells or uninfected cells and induce a well-ordered anti-viral programme, involving the upregulation and activation of a range of IFN inducible genes, such as PKR, which phosphorylate eIF-2α and hence blocks translation, also anti-viral RNase L and Mx proteins. IFNs can induce apoptosis, or induce more IFN gene expression in an autocrine or paracrine manner. IFNs also activate NK cells and are required for NK cell survival, and may have other effects on both innate and adaptive immunity (for a review see [126,127]).

Type 1 IFNs-α and β have been extensively studied in the prevention of clinical colds since the 1980s. The potential of IFN-α2 attracted wide interest, culminating in several reports in the early to mid-1980s. In 1984, IFN-α2 was used as therapy for experimental RV39 infection [128]. Previous studies had suggested that effectiveness of IFN-α2 was dose dependent, with high doses ($2–4.5 \times 10^7$ IU per day) effective at reducing both RV infection and illness, and lower doses, 10^6 IU, effective at reducing illness (symptom scores), but not necessarily affecting the incidence of colds [129–131]. Despite the effectiveness of IFN-α2 in models of experimental RV infection at these high doses, significant side effects were observed, including blood-tinged mucus, and even mucosal histopathological abnormalities [131]. This study sought to examine the efficacy of 10^6 IU IFN-α2 delivered three times daily for 5 days via nasal spray or drops. Challenge with RV39 occurred 28 h later. IFN-α2 reduced virus shedding, with the drops having a more potent effect. IFN-α2 delivered via drops also significantly reduced nasal mucus weights, but neither treatment reduced the frequency of clinical colds.

IFN-α2 has also been tested as a prophylactic treatment in natural viral infections. In one study, twice daily IFN-α2 at 10^6 IU for 28 days demonstrated less RV-associated colds, but was not significantly different to placebo [132]. Another study utilised IFN-α2b at either 1.5×10^6 units twice daily or 2.5×10^6 once daily each for 4 weeks; control subjects were given dose-matched placebo [133]. During the medication period, twice daily IFN-α2b greatly reduced the number of RV-associated colds, but had no effect on parainfluenza-associated colds. The effects of IFN were slowly lost after the treatment period, with frequencies of RV-associated colds being about equal after 8 days post-treatment. At both doses, IFN-α2b exhibited side effects, mostly in the form of nasal blood-tinged mucus.

Several studies have investigated the potential of IFN-β in treatment of clinical colds. The first study of intranasally administered recombinant IFN-β (IFN-β Ser) involved 13 doses of 2×10^6 IU over 4 days. This treatment showed promise in control of experimental RV9 or RV14 challenge [134]. This study involved RV challenge occurring after the fourth dose of IFN-β. There were significantly lower symptom scores, nasal secretions and virus release compared with placebo. The next study with IFN-β Ser involved both a tolerance and efficacy study against experimental RV challenge. Volunteers were pre-treated with either IFN-β Ser (12×10^6, or 3×10^6 IU) 36 h before infection, and continued for 25 days [135]. The tolerance study demonstrated numerous side effects particularly with the high-dose regime, including blood-tinged mucus, and an increase in subepithelial lymphocytic infiltrates in nasal biopsies. The efficacy study also reported significant decreases in nasal mucus weights, less development or incidence of clinical colds, but not less viral shedding, and these differences were only significant with the high-dose regime.

A third study involving prophylaxis against natural infections reported quite disappointing results [136]. Two randomised placebo-controlled trials in 1986–87 utilised IFN-β Ser given either 6 days a week for 4 weeks (9×10^6 IU), or a higher dose (24×10^6 IU) for 24 consecutive days. Both studies failed to show a significant reduction in the incidence of clinical colds compared to placebo, and also in patients that received cold, the number of days of illness did not differ between IFN-β Ser treated groups and placebo.

These studies provide a useful background to assess the potential of IFN therapy for viral exacerbations of asthma. An advantage that type I IFN therapy has over anti-RV therapies is that all exacerbations of asthma with viral agents could be controlled. Specific RV therapies would treat 60–65% of virus-induced exacerbations; however, broad-spectrum therapies such as IFN would not have this disadvantage. In particular, the above studies suggest that IFN-α2 is effective when given prior to experimental RV infection, or as a prophylactic therapy in a context of natural RV infections. IFN-β has been less impressive, exhibiting some effect when given prior to experimental RV challenge, but no effect as a prophylactic in natural infections. It is believed that these differences are not due to differences in anti-viral activity, as both IFN-α2 and IFN-β have similar anti-rhinoviral activities in vitro [137]. The relative ineffectiveness of IFN-β has been thought to be due to instability as a nasal spray [136,137]; however, when used as drops, IFN-β also did not perform in a prophylactic study against natural RV infection [136].

Delivery and dose appeared to be contentious issues in these studies, with the effectiveness of IFN-α dependent upon a high enough dose to prevent infection and clinical colds, but low enough to prevent unwanted side effects.

However, most studies reported side effects, even at lower doses of 10^6 IU given daily. It can be argued that such side effects would be even more problematic in asthmatics, particularly the increased inflammation of the nasal passage, which appeared to be a hallmark of intranasal IFN therapy. Another issue for asthmatics would be effective delivery to the upper versus lower airway. While RV when administered by nasal spray can definitely be found in the lower airway during experimental infection, it is currently unknown whether intranasally applied IFN would act at a high enough concentration in the lower airway. An alternative could be IFN delivered via oral inhalation, similar to inhaled corticosteroids, thereby ensuring delivery to the lower airway. The ultimate effectiveness of IFN therapy in RV-induced exacerbations of asthma has yet to be investigated.

IFN has also been tested in conjunction with other treatments such as non-steroidal anti-inflammatory agents. This approach is based on the belief that no single molecule therapy has proved effect for RV-associated clinical colds, as no single therapy can block viral infection and replication, and the associated host response to infection, including the cellular, and humoral inflammatory reactions [138]. These studies aimed to treat the clinical colds with a combination of treatments in the early phase of infection, with the argument that this regime at this time would be effective in not only controlling infection, but the many symptoms associated with the host inflammatory response to infection.

Intranasal IFN-α2b was used along with ipratropium and oral naproxen in a model of experimental RV infection [139]. The treatment was given 24 h post-infection, and continued for 4 days. This treatment effectively reduced days of virus shedding and virus titre compared to placebo, and decreased nasal mucus weights on days 3–4 post-infection. Intranasal IFN-α2b has also been studied with oral chlorpheniramine and ibuprofen, versus treatments consisting of intranasal placebo with oral chlorpheniramine and ibuprofen, or both oral and intranasal placebos, in experimental RV39 infection [138]. The combination reduced the total symptom score by about 22%, compared to 27% with only oral chlorpheniramine and ibuprofen, or 19% achieved with placebos. The combination also significantly reduced nasal mucus weights generated by clinical colds, and also reduced viral titres compared to the other groups on day 3 post-infection. The effects of these treatments on the duration of clinical colds were not studied however. There appeared to be significant side effects associated with the combination treatment regime. One fifth of subjects having received the combination complained of blood-tinged nasal mucus. Drowsiness and nasal dryness were also common for all groups.

5.4. Inhaled GC therapy

Glucocorticosteroids (GC), are the mainstay of current asthma therapy. Steroids when administered topically are potent inflammatory agents, acting to help reduce pro-inflammatory molecule gene expression operating largely at the level of pre-transcription (for a review, see [140]). Steroids can reduce inflammation through glucocorticoid receptor (GR)–DNA interactions, GR–transcription factor interactions, inducing histone deacetylation, and therefore reducing DNA unwinding and hence transcription of inflammatory genes, and finally by inducing anti-inflammatory agents.

Despite evidence that steroids attenuate RV-associated inflammatory responses in vitro [75,77], the use of steroids in virus-induced exacerbations of asthma remains controversial. Several in vivo studies report poor efficacy of steroids in preventing inflammation and reduction of lung function in models of experimental RV infection [15–18].

Farr et al. [18] examined the potential use of prednisone (30 mg twice daily), or intranasal beclomethasone (168 micrograms twice a day), given 3–4 days prior to challenge as a prophylactic treatment in a model of experimental RV infection. Treatment ceased 5 days post-challenge. Up to 48 h, this treatment appeared affective at reducing nasal obstruction, nasal mucus weights, and nasal kinin concentrations; however, this effect was lost when steroid treatment ceased. Another study also examined prednisone (20 mg) given three times daily for 5 days, with treatment commencing 11 h prior to RV infection [141]. The steroids reduced nasal kinin and mucus concentrations but had little effect on other symptoms. Virus load was higher in the steroid-treated group, with significant differences on days 3 and 4 post-infection. The data suggest that an adverse effect of steroids may be suppression of anti-viral mediators that are required for the natural defence against viral infection in vivo. No support for this has yet been observed in vitro; however, the relative effects of steroids on type I IFNs, defensins (for enveloped viruses), or other anti-viral components remains largely unexplored.

Grunberg et al. [15] examined the possible benefits of inhaled budesonide (800 mg, twice daily) treatment in mild asthmatics during experimental RV infection. Treatment commenced 2 weeks prior to challenge and was maintained throughout the study period (until 2 weeks post-challenge). Budesonide improved lung function in the asthmatics, and decreased eosinophil numbers, but did not reduce total inflammatory cell numbers in the lung. The authors concluded that steroids only gave partial protection in RV-associated inflammation of asthma. In another study, it was observed that budesonide treatment caused a small but significant increase in the soluble IL-1R antagonist in nasal secretions. There was no significant difference between CXCL8 and IL-1β levels between asthmatics treated with budesonide and control asthmatics treated with placebo [16]. These data support the idea that steroids may have benefits in RV-associated exacerbations by increasing the level of anti-inflammatory mediators within the airway, rather than having a direct effect on pro-inflammatory mediator expression.

The use of GCs in treating virus-induced wheeze in children under 17 years of age has also been carefully examined. A meta-analysis of compiled data from several studies showed that maintenance low-dose inhaled GCs did not demonstrate any clear reduction over placebo in the proportion of hospital admissions due to viral wheeze, and this treatment did not affect the prescription rate of oral GCs as treatment when these individuals were admitted to hospital [142]. Several other studies report similar findings [143–145]. In contrast, a study investigating emergency room hospital admissions and community cases of viral infection in autumn in Canada revealed that in children, patients admitted to hospital with exacerbations are less likely to have been given prior treatment in the form of inhaled GCs or leukotriene receptor antagonists [55]. Also, two clinical studies consisting of older children (>13 years) and adults have shown that GCs alone have failed to reduce asthma exacerbation rates. In these studies, increasing the GC dose did not reduce the rate of asthma exacerbations [19,20], Currently, there is much evidence suggesting that the use of GCs alone is only partially protective against virus-induced exacerbations of asthma, and that preventive therapy can be improved. Again these data indirectly support the idea that exacerbations of asthma may involve processes that are different to persistent asthma.

5.5. GCs in combination with β_2 agonists

GCs may act in concert with other therapeutics, for example, long-acting β_2 agonists (LABA) in combination therapy. LABAs act via a G protein coupled receptor, activate adenylate cyclase and through the second messenger cAMP, induce intracellular signalling events affecting a broad range of physiological processes, alone and in combination with GCs, such as smooth muscle growth and differentiation [146], inflammation [147–153] and response to bacterial infection [154,155]. In severe or persistent asthma, several studies in vitro and in vivo have shown that the combined use of GCs and LABA has advantages over the use of GCs alone, in terms of alleviating inflammation, controlling smooth muscle remodelling and improving lung function [146,156–160].

Considering the ability of LABA to enhance the anti-inflammatory properties of GCs, and exert some anti-inflammatory effects themselves in vitro [147,152,161], an important question is whether or not combination therapy of LABA and GCs can reduce asthma exacerbation rates. Studies completed thus far suggest a positive effect of β_2 agonists when combined with GCs, in reducing frequencies of asthma exacerbations [162,163]; however, further evidence is required before the use of GCs in this way is generally accepted. These studies may also be of mixed aetiology, as exacerbations in general were investigated, and not specifically virus-induced exacerbations. It is also unclear whether the enhanced effect produced by β_2 agonists in the above studies was due to bronchodilation, or by decreasing inflammation, or both.

There are yet to be clinical studies using combination therapy in models of experimental or natural RV infection in asthma. Until such studies are performed, the potential role of combination therapy in the treatment of RV-induced asthma exacerbations remains open. One caveat to this idea is the general observation that virus-induced exacerbations of asthma involve not only eosinophils, but recruitment of both neutrophils, and activated T cells into the inflamed airway, and also activation of local macrophages [5,84–86]. The ability of combination therapy to control neutrophilic and lymphocytic-induced inflammation is yet to be investigated experimentally.

5.6. Inhibitors of NF-κB signalling

As NF-κB is involved in induction of the majority of pro-inflammatory mediators studied so far in RV infection (see section above), NF-κB signalling components represent possible therapeutic targets. In murine models of allergic asthma, there has been some success with NF-κB inhibition. The redox inhibitor MOL294 and NF-κB decoy oligodeoxynucleotides have been successful in reducing pro-inflammatory molecule expression, lung inflammation and airway hyper-responsiveness to metacholine [164,165].

Recent studies have shown that the RV induction of CXCL8 and IL-6 from bronchial epithelial cells is an IKK-β dependent process, and is sensitive to inhibition with AS602868 (Serono International, [166]), a selective IKK-β inhibitor (M.R. Edwards and S.L. Johnston, unpublished observations), suggesting that IKK-β inhibition may be a useful therapeutic option. A recent review has given a thorough summary of the current range of selective IKK inhibitors available, including both organic-based and small molecule inhibitors [88]; therefore, they will not be discussed in detail here. Importantly, the current range of selective IKK inhibitors have just begun to find their way into clinical trials, after showing promise in cell-based assays and murine models of disease. Inhibitors of these types have not yet been tested in human models of experimental virus infection, or asthma.

Concerning virus exacerbations of asthma, one caveat to the idea of NF-κB inhibition is that protective, anti-viral mediators may also be induced in an NF-κB dependent manner. The obvious example of this is IFN-β, which in many systems, is made initially on viral infection in an NF-κB dependent manner [167–170]. Clearly, the potential role of NF-κB in the expression of mediators that are beneficial to the host response to infection needs to be carefully considered before NF-κB intervention becomes a serious therapeutic option. With the discovery of alternate NF-κB signalling intermediates such as IKK-ι/ε and TBK-1, the proposition of NF-κB inhibition has become more complex (see Fig. 3). However, the role of individual NF-κB signalling intermediates in both harmful pro-inflammatory mediator gene expression as well as in useful anti-viral

Table 1
A summary of the efficacy of some anti-viral therapies used in controlling clinical colds

Therapy	Observed reduction in the following symptoms[a]						Limitation[b]	Reference
	Frequency of colds	Duration of colds	Total symptom score	Viral titre	Nasal secretion weight	Markers of inflammation		
Anti-viral compounds								
Ruprintrivir	×	NS	√	√	√	NS		[114]
Pirodavir	NS	×	×	√	NS	NS	Side effects/ poor efficacy	[113]
WIN54954	×	NS	×	×	×	NS	Poor efficacy	[111
R61837	√	NS	√	NS	√	NS		[112]
Pleconaril	NS	√	√	NS	NS	NS	Side effects	[116]
Soluble ICAM derivatives								
Tremacamra	√	NS	√	√	√	√		[121]
Type I IFN therapy								
IFN-α2	√/×	×	√	√	√	NS	Side effects	[128–133]
IFN-β	×	×	√/×	√/×	√/×	NS	Side effects/ poor efficacy	[134–136]

[a]Refers to the symptoms studied by the authors. √ refers to successful control of the symptom being studied. × refers to unsuccessful control of that particular symptom. NS refers to the symptom not being studied.
[b]Limitations are those as suggested by the authors in each study.

mediator gene expression, is a much understudied field, and whether distinct signalling intermediates exist for IFN expression versus pro-inflammatory cytokine expression is currently unclear. Further research efforts are required before the role of this important transcription factor and its signalling pathways can be fully understood.

6. Summary and conclusions

Many respiratory viruses, in particular RV, cause exacerbations of asthma, and this is a healthcare concern worldwide. RVs upregulate pro-inflammatory mediators and cause local inflammation in the lower airway, and this may precipitate exacerbations of asthma in certain individuals. Individuals suffering from viral exacerbations of asthma are yet to be treated effectively, and there is wide interest in a range of treatment options for these unmet medical needs. Specific anti-rhinoviral therapy, IFN therapy, and steroid-based therapies have all been studied in the past with mixed successes. Anti-viral therapies have been classically applied in the context of clinical colds (Table 1); however, they have not yet been studied in exacerbations of asthma. With the link between RV and asthma exacerbations now more established, the anti-rhinoviral treatments Pleconaril and Tremacamra should be regarded as serious therapeutic options. The study of type I IFN therapy in clinical colds has highlighted many problems with this treatment. Dose, delivery and safety all remain important issues for viral exacerbations of asthma. As a deficiency in IFN-β expression has been recently described in asthmatics [39], type I IFN therapy remains a candidate treatment however. One potential area of further research is combined anti-viral, anti-inflammatory therapy, which may inhibit viral replication as well as treating upper and lower airway inflammation. Another area that has yet to be tested formally is NF-κB inhibition, as many RV-induced inflammatory mediators are NF-κB dependent. The wealth of literature so far reported on RV-induced exacerbations of asthma suggests that the relationship of infection and asthma exacerbation, in particular the cellular and molecular immunological aspects of pathogenesis, is still unclear, and further experimental infection studies are required to better understand these important processes. In particular, how asthmatics may differ from normal individuals who do not suffer from lower airway disease is a priority. There is also a need for further vigorous pursuit of the molecular mechanisms of infection and pro-inflammatory mediator induction, for example, the development of small animal models that will be invaluable for testing these ideas and providing future therapeutic targets.

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