Virus-induced modulation of lower airway diseases: Pathogenesis and pharmacologic approaches to treatment

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ARTICLE INFO

ABSTRACT

Uncomplicated upper respiratory viral infections are the most common cause of days lost from work and school and exert a major economic burden. In susceptible individuals, however, common respiratory viruses, particularly human rhinoviruses, also can have a major impact on diseases that involve the lower airways, including asthma, chronic obstructive pulmonary diseases (COPD) and cystic fibrosis (CF). Respiratory virus-induced wheezing illnesses in early life are a significant risk factor for the subsequent development of asthma, and virus infections may also play a role in the development and progression of airway remodeling in asthma. It is clear that upper respiratory tract virus infections can spread to the lower airway and trigger acute attacks of asthma, COPD or CF. These exacerbations can be life-threatening, and exert an enormous burden on health care systems. In recent years we have gained new insights into the mechanisms by which respiratory viruses may induce acute exacerbations of lower airway diseases, as well as into host defense pathways that may regulate the outcomes to viral infections. In the current article we review the role of viruses in lower airway diseases, including our current understanding on pathways by which they may cause remodeling and trigger acute exacerbations. We also review the efficacy of current and emerging therapies used to treat these lower airway diseases on the outcomes due to viral infection, and discuss alternative therapeutic approaches for the management of virus-induced airway inflammation.

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Contents

1. Introduction

Upper respiratory tract viral infections are one of the most common acute respiratory illnesses experienced by humans. In healthy individuals, such infections are usually self-limiting and are associated with the relatively mild symptoms of the clinical syndrome referred to as the common cold. According to the Centers for Disease Control and Prevention, colds result in 22 million lost school days each year in the United States alone. Moreover, Americans experience more than 500 million upper respiratory tract viral infections per year (excluding infections due to influenza) leading to a total annual economic impact estimated at $40 billion in 2003 (Fendrick et al., 2003). A number of different viral types, including coronaviruses, respiratory syncytial virus (RSV), and parainfluenza viruses can induce upper respiratory symptoms, but more than half of all colds are caused by human rhinovirus (HRV) infections (Arruda et al., 1997; Makela et al., 1998).

Although most healthy individuals experience simple common colds, a relatively small percentage of cases develop complications including acute sinusitis or otitis media (Arola et al., 1990; Turner et al., 1992; Gwaltney et al., 1994). More serious complications, however, can occur in subjects with pre-existing lower airway diseases, such as asthma, chronic obstructive pulmonary disease (COPD) or cystic fibrosis (CF), where upper respiratory virus infections are a major trigger for acute disease exacerbations that can be life-threatening and are a major cause of emergency room visits or hospitalizations. Moreover, there is now considerable evidence that virus induced wheezing illnesses in early childhood are a major risk factor for the subsequent development of asthma. These latter observations have also prompted investigations into whether respiratory viral infections may also contribute to the development of the structural changes, referred to as "airway remodeling", that occur in the airways of subjects with asthma. The fact that several viruses are able to exacerbate lower airway diseases suggests that common mechanisms underlie the pathophysiology of these effects. Recently, new insights have been gained into pathways mediating the effects of viruses on lower airway diseases. This review places particular emphasis on how components of these pathways may provide possible new targets for therapeutic interventions. As will be discussed, new therapeutic approaches are clearly required, as currently available medications for the treatment of lower airway diseases are less than optimal for the treatment of viral exacerbations of lower airway diseases. Moreover, none of our current treatment options prevent the development of airway remodeling in asthma or reverse existing features of remodeling.

2. Viral wheezing as a risk factor for asthma development

Respiratory viral infections are the major trigger for episodic wheezing in children. Although a number of virus types, including parainfluenza, metapneumovirus and influenza can trigger bronchiolitis and wheezing (Heymann et al., 2004; Jartti et al., 2004; Williams et al., 2004), initial studies focused on RSV as the major cause of bronchiolitis in young infants, particularly during the winter months. These studies showed that children who develop RSV induced bronchiolitis in early childhood have impaired lung function in later life (Strope et al., 1991). Such children also were at increased risk for recurrent wheezing and development of asthma by ages 6–10 (Stein et al., 1999; Sigurs et al., 2000). Although data from the Tucson Children's Respiratory Study showed that this risk decreases progressively with age and was no longer significant by age 13 (Stein et al., 1999), other studies indicate that RSV bronchiolitis that is severe enough to cause hospitalization in infancy is still a risk factor

for asthma into adolescence (Sigurs et al., 2005). While these studies focused on RSV, the improved sensitivity of viral detection that resulted from the introduction of reverse transcription-polymerase chain reaction (RT-PCR), has generated strong evidence for a much greater role for HRV-induced wheezing illnesses in infancy as a major risk factor for the subsequent development of asthma than was previously appreciated. Outside of the winter months, and in children older than 6 months of age, HRV appears to be the dominant viral pathogen associated with wheezing illnesses (Heymann et al., 2004; Jartti et al., 2004; Korppi et al., 2004). It has been reported that children hospitalized with HRV-induced bronchiolitis are at particularly high risk for the subsequent development of asthma (Reijonen et al., 2000; Kotaniemi-Syrjänen et al., 2003). In addition, a series of publications from the high risk **C**hildhood **O**rigins of **Ast**hma (COAST) birth cohort study, showed not only that HRV induced wheezing illness during the first year of life was the strongest predictor of subsequent wheezing in the third year of life (Lemanske et al., 2005), but also that 90% of children who wheezed in the third year of life had confirmed asthma by age 6 (Jackson et al., 2008). Interestingly, in both of these studies, children who wheezed with rhinovirus-associated illnesses had a significantly higher risk of developing chronic wheeze or asthma than those who wheezed due to illnesses associated with RSV. Moreover, there is now clear evidence that children can experience recurrent respiratory illnesses due to serial HRV infections (Jartti et al., 2008).

Although viruses can induce wheezing illnesses in children that predispose to development of asthma, all children experience RSV infections before age 2, and all children experience repeated HRV infections. What renders a subset of children susceptible to developing wheezy bronchiolitis and subsequent asthma is not yet entirely clear. There is undoubtedly a genetic component to asthma susceptibility, and genome wide association studies have identified multiple candidate genes/loci linked to asthma (Ober & Yao, 2011). It is clear, however, that the underlying genetic susceptibility is complex, with heritability accounting for as little as 10–35% of disease according to some estimates (Ober & Yao, 2011). Thus, it seems likely that additional environmental factors play a role in determining susceptibility to asthma development. This has led to the paradigm of a "two-hit" or, conceivably, a "multiple-hit" thesis for the development of asthma, in which recurrent viral infections in the setting of an additional risk factor(s) may be necessary to trigger the subsequent development of asthma. Other relevant factors in early childhood that could contribute to regulating susceptibility to developing asthma include impaired lung development and reduced lung function (Martinez et al., 1988; Gern et al., 2005), living on a farm (von Mutius & Vercelli, 2010), cigarette smoke exposure (Gilliland et al., 2006), daycare attendance in early life (Nystad, 2000), breastfeeding (Gdalevich et al., 2001), antibiotic use (Kozyrskyj et al., 2007) and allergic sensitization (Rakes et al., 1999). Although the concept that two (or more) independent risk factors may interact to induce the development of asthma is attractive, we have limited understanding of the importance of the relative timing and duration of exposures in regulating asthma susceptibility. In the case of allergic sensitization and viral-induced wheezing, which are recognized as independent risk factors for asthma development (Jackson et al., 2008), a recent study examined the temporal relationship between these risk factors in the COAST study birth cohort (Jackson et al., 2012). Markov modeling of the data indicated that allergic sensitization increased the risk of developing HRV-induced wheezing, but did not alter the risk of RSV-induced wheezing. By contrast, viral wheeze did not lead to increased risk of subsequent allergic sensitization. While these data could be interpreted to suggest that allergic sensitization must precede viral wheezing in the development of asthma, a note of caution is warranted.

First, the study cohort was a high-risk population, selected because at least one parent had documented respiratory allergies. Thus, they may not be fully representative of the broader population. Second, while only 10% of patients were sensitized to allergen by 1 year of age, approximately 30% of children already had experienced a wheezing episode by that time, implying that viral wheezing illnesses do not necessarily require prior allergen sensitization. This may be a virus specific reflection of a prevalence of RSV in these early months, but this is not clear. Finally, the impact of other potential risk factors, listed above, was not examined. Thus, while these data are intriguing, additional studies are needed to examine the temporal relationship between viral-induced wheezing episodes and other risk factors in leading to the subsequent development of asthma.

3. HRV and airway remodeling

The lower airways of individuals with asthma show a series of structural changes that are characteristic of the disease and are collectively referred to as airway remodeling. These changes include an increase in the mass of smooth muscle, angiogenesis, thickening of the lamina reticularis, increased numbers of myofibroblasts and enhanced matrix protein deposition, thickening and fragility of the epithelium, as well as goblet cell metaplasia and accompanying excessive mucus production (Jeffery, 2004). Together, these changes increase the thickness of the airway wall while narrowing the airway lumen (Sobonya, 1984; Laitinen et al., 1985; Jeffery et al., 1989; Siddiqui et al., 2007), and are believed to underlie the development and persistence of the airways hyperresponsiveness that is a hallmark of asthma (McParland et al., 2003; James & Wenzel, 2007; Paré et al., 2007). Although asthma can occur at any age, it is usually already evident in childhood in the majority of patients (Gern et al., 1999; Sears et al., 2003). Despite this, the long-standing paradigm held that airway remodeling occurs only after years of chronic airway inflammation. A number of bronchial biopsy studies conducted in children, however, have now clearly established that, while airway remodeling is not present in infants (≤12 months old) with symptoms of airflow limitation (Saglani et al., 2005), several major components of remodeling are already present in pre-school children with symptoms or airflow limitation even before a formal clinical diagnosis of asthma is made (Pohunek et al., 2005; Saglani et al., 2007; O'Reilly et al., 2013). The absence of airway remodeling in infants, but presence in pre-school children, raises the prospect that remodeling is induced as a consequence of some initiating stimuli experienced in early childhood, and that remodeling occurs in parallel to airway inflammation. In considering potential stimuli that could contribute to the initiation and progression of remodeling in susceptible individuals, HRV infections should be considered. As noted above, HRV induced wheezing illnesses in early life are a major risk factor for subsequent asthma development. Longitudinal analysis has shown that pre-school age children have about six HRV infections per year (Winther et al., 2006), and it has been confirmed that serial viral infections can lead to recurrent wheezing episodes (Jartti et al., 2008). This has led to the hypothesis that recurrent HRV infections may not only be a stimulus for asthma development but may also play a key role in the development and continued progression of airway remodeling in asthma. In support of this hypothesis, studies using cultured human airway epithelial cells, the primary site of viral infection in the airways, have shown that HRV infection can induce epithelial production of a variety of growth factors and proteins linked to remodeling processes in the airways (Fig. 1). These include both amphiregulin, a member of the epidermal growth factor family, and activin A, a member of the transforming growth factor-β family (Leigh et al., 2008), proteins that have been linked to airway subepithelial fibrosis in asthma (Enomoto et al., 2009; Gregory et al., 2010). In addition, HRV infection increases epithelial production of matrix metalloproteinase-9 (MMP-9) (Tacon et al., 2010), a protease also linked to extracellular matrix turnover and airway remodeling (Atkinson & Senior, 2003; Broide, 2008), as well as

vascular endothelial growth factor (VEGF) (Psarras et al., 2006; Leigh et al., 2008), considered to be the primary angiogenic factor in asthmatic airways (Simcock et al., 2007). Interestingly, both VEGF and MMP-9 levels were also increased in vivo in airway lining fluid during natural rhinovirus infections (Leigh et al., 2008; Tacon et al., 2010).

Asthma has been associated with an impaired barrier function of the airway epithelium (Holgate, 2007), and infection with any of several HRV serotypes been shown to disrupt barrier function and lead to concomitant loss of the junctional plaque protein, zona occludens-1, in cultures of polarized epithelial cells (Sajjan et al., 2008). By contrast, others have failed to observe this loss of barrier function (Lopez-Souza et al., 2009). Increased production of mucins and mucus hypersecretion are also common features of airway remodeling in asthma and several studies have shown that HRV infection leads to increased expression of the major epithelial mucin MUC5AC (Inoue et al., 2006; Zhu et al., 2009; Hewson et al., 2010). Increased epithelial MUC5AC release has also been detected in vivo during experimental HRV infections (Hewson et al., 2010). The induction of MUC5AC in HRV infected epithelial cells has been linked to activation of the epidermal growth factor receptor, as well as to activation of the ERK mitogen activated protein kinase and of the transcription factor, NF-κB (Zhu et al., 2009; Hewson et al., 2010).

Taken together, these observations support the concept that HRV infections may be a key trigger for airway remodeling changes in young children as they develop asthma.

4. Viral exacerbations of lower airway diseases

Acute exacerbations of asthma, COPD and CF may be life-threatening, and are a major cause of emergency room visits and hospitalizations. As such, they account for 50–70% of the total costs associated with these diseases and represent a huge health care burden (Weiss et al., 1992; Smith et al., 1997; Pauwels et al., 2001). Indeed, the total cost of these diseases exceeds $40 billion dollars annually in the United States alone.

Upper respiratory viral infections are a major risk factor associated with exacerbations of asthma, COPD and CF (van Ewijk et al., 2005; Traves & Proud, 2007). Prospective studies have shown that viral pathogens were detected in up to 85% of acute asthma exacerbations in children and adolescents (Johnston et al., 1995). Although a number of RNA viruses have been linked to exacerbations of asthma, HRV was the dominant viral pathogen, being detected in 60% of viral exacerbations (Johnston et al., 1995; Khetsuriani et al., 2007). In adults, viral infections were detected in up to 60% of exacerbations, with HRV again being the most common virus detected (Nicholson et al., 1993; Kistler et al., 2007). Bacterial and viral infections are the major triggers in the etiology of exacerbations of COPD and CF (Wedzicha & Donaldson, 2003; van Ewijk et al., 2005). Studies suggest that about half of exacerbations of COPD or CF are associated with viral infections, and that HRV is, again, the most common viral pathogen detected (Greenberg et al., 2000; Seemungal et al., 2001; McManus et al., 2008; Wat et al., 2008). In exacerbations of both CF and COPD, viral infections can occur either alone or as a co-infection with bacteria.

Despite strong evidence linking respiratory viral infections to exacerbations of lower airway diseases, the mechanisms by which such respiratory virus infections trigger such exacerbations are not fully understood. The ability of multiple respiratory virus species to induce exacerbations suggests that at least some mechanisms involved are common to all of the RNA viruses that are linked to disease exacerbations. HRV is the most common virus linked to lower airway exacerbations of asthma, COPD and CF, and the airway epithelial cell is the primary site of HRV infection. Although infection usually begins in the nasal and/or oropharyngeal airways, there is now clear evidence that HRV can spread to the lower airway epithelium (Papadopoulos et al., 2000; Mosser et al., 2005). In contrast to other viruses, such as influenza, HRV is not overtly cytotoxic to airway epithelial cells (Winther et al.,
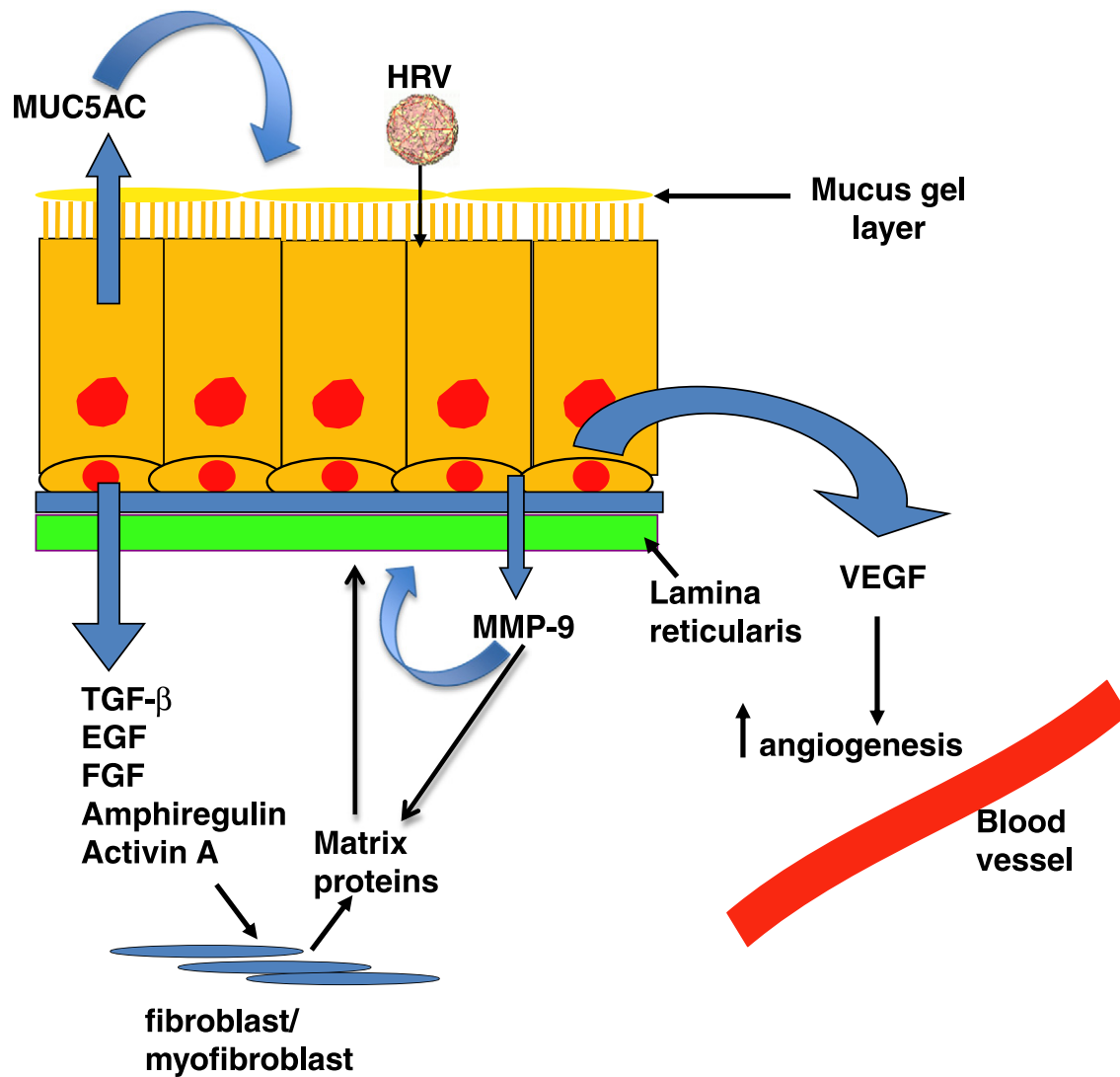
Fig. 1. Release of remodeling mediators from human airway epithelial cells upon infection with HRV. Release of growth factors of the transforming growth factor-β (TGF-β) and epidermal growth factor (EGF) families could contribute to remodeling via regulation of fibroblast/myofibroblast release of matrix proteins that can lead to thickening of the lamina reticularis. Matrix metalloproteinase-9 (MMP-9) may regulate matrix protein turnover. Vascular endothelial growth factor can regulate angiogenesis, while increased release of MUC5AC may enhance mucus production.

1984; Subauste et al., 1995). This implies that virus-induced alterations in epithelial biology initiate the development of exacerbations in susceptible individuals, although, conceivably, in those cases where viruses do induce cytotoxicity, such damage may further contribute to disease severity. Given the major role played by HRV in clinical exacerbations, however, we will use responses induced by this virus as the prototype to further discuss how the status of lower airway diseases may be regulated. In support of the hypothesis that HRV alters epithelial biology, gene array analyses of airway epithelial cells, obtained either during in vivo HRV infections or during such infections in cultured epithelial cells, confirm altered expression not only of multiple proinflammatory cytokines and chemokines, but also of a range of molecules with innate antiviral and host defense properties (Proud et al., 2008; Proud et al., 2012).

5. Proinflammatory responses to HRV infection

Both mRNA and protein analyses have shown that HRV infection of human airway epithelial cells induce expression and release of a broad range of growth factors, including (i) granulocyte colony-stimulating factor (G-CSF) and granulocyte–macrophage colony-stimulating factor (GM-CSF) that could regulate granulocyte maturation, survival and activation; (ii) pleiotropic cytokines, including IL-1β, IL-11 and IL-6 that can regulate several aspects of inflammation; and (iii) chemokines, including CXCL1, CXCL5, CXCL8, CXCL10, CCL5 and CCL20 that could contribute to the recruitment to, and activation of, several types of inflammatory cells in the airways (Leigh & Proud, 2011). Moreover, many of these proinflammatory molecules are also increased in airway secretions during in vivo HRV infections (Leigh & Proud, 2011). Taken together, the increased generation of these chemokines and cytokines would be expected to lead to recruitment and activation of a variety of inflammatory cell types within the airways. In healthy individuals, it is reasonable to speculate, based on responses in the nasal airways, that virus infections of lower airway epithelial cells would trigger a modest inflammatory response in the airways that would cause no lower airway disease or overt clinical problems in the vast majority of individuals. By contrast, individuals with diseases such as asthma, COPD or CF already have pre-existing lower airway inflammation. In these subjects, particularly those where the inflammation is not well controlled, the extra inflammatory burden due to HRV infections may be sufficient to cause disease exacerbations. In addition, as will be discussed below, it is possible that subjects with lower airway

inflammatory diseases may also have some level of impaired host defense that could also lead to more severe virus-induced inflammatory responses.

Understanding the mechanisms by which viruses induce epithelial production of proinflammatory cytokines and chemokines may permit strategies for new therapeutic interventions. In the case of HRV, it is known that some molecules, such as CXCL8 and IL-6, can be induced with virus that has been rendered replication deficient, suggesting that viral binding to its receptor is sufficient to induce production of these cytokines (Sanders et al., 1998). This was surprising, as the receptor for the largest group of HRV family members is intercellular adhesion molecule-1 (ICAM-1) and this receptor has neither endogenous kinase activity nor any known kinase recognition sites (Greenwood et al., 2003). Studies have shown that binding of HRV to ICAM-1 leads to formation of a complex in which the cytoskeletal protein ezrin serves as a linker that binds both to ICAM-1 and to the spleen tyrosine kinase, syk. This complex not only appears to play a role in virus internalization (Lau et al., 2008), but syk then leads to activation of both p38 mitogen activated protein kinase and PI-3 kinase to trigger downstream cytokine production (Wang et al., 2006). The Src tyrosine kinase has also been associated with early ICAM-1 linked HRV signaling (Bentley et al., 2007). At this point, the capacity for direct signaling of other HRV receptors to trigger epithelial cytokine production is unknown.

Although early HRV signaling may contribute to the inflammatory response in the airways, it is unlikely that signaling via a single virus receptor explains the ability of multiple RNA viruses to trigger exacerbations of lower airway diseases. The majority of cytokine production from HRV infected epithelial cells appears to be dependent upon viral internalization and replication, indicating that a molecule(s) generated during viral replication is necessary for cytokine production. Although virus specific proteins, such as the rhinovirus 3C protease, have been shown to modulate cell function by exerting effects on nucleocytoplasmic transport and cleavage of the transcription factor, OCT-1 (Amineva et al., 2004), it would be logical to assume that a product(s) common to the replication of multiple viruses is central to the regulation of this inflammatory response. Double-stranded RNA (dsRNA) is normally not present in eukaryotic cells but is generated during the replication cycle of RNA viruses, with one strand being used as a template for generation of new genomic RNA. Consequently, dsRNA is a recognized by pattern recognition receptors (PRR) as a pathogen associated molecular pattern (PAMP). A number of PRR are known to recognize dsRNA. The first such PRR identified was toll-like receptor (TLR)3 (Alexopoulou et al., 2001). Although initially considered to be essential for recognition of viral dsRNA, subsequent studies showed that TLR3-deficient mice did not show impaired pathogenesis or antiviral immunity to a number of viruses (Edelmann et al., 2004). This suggested that other PRRs must be able to recognize dsRNA and led to the demonstration that members of the DEXD/H-box family of cytoplasmic RNA helicases fill this role. The first helicase family members linked to viral recognition were retinoic acid-inducible gene-I (RIG-I) and melanoma differentiation-associated gene (mda)-5 (Yoneyama et al., 2004; Kato et al., 2006). Studies showed that viral dsRNA from different types of virus and of differing lengths are preferentially recognized by these two PRR (Kato et al., 2008; Takeuchi & Akira, 2009), and that utilization may be cell type specific (Kato et al., 2005). More recently, additional members of the DEXD/H-box family of cytoplasmic RNA helicases, including DDX1, DDX21, DHX36 and DHX15 have also been shown to sense dsRNA, at least in dendritic cells (Zhang et al., 2011; Lu et al., 2014). Even within the same cell type for a specific virus, such as HRV, there can be controversy regarding the relative role and temporal use of TLR-3, RIG-I and mda-5 in mediating dsRNA signaling (Wang et al., 2009; Slater et al., 2010; Triantafilou et al., 2011; Hudy et al., 2014). While the growing number of PRR that can potentially recognize dsRNA makes selecting suitable targets for therapeutic intervention difficult, this may be obviated by the fact that most of these PRR ultimately converge in the common downstream activation of NF-κB and interferon (IFN) regulatory factors (IRF) to regulate transcriptional activation of multiple proinflammatory and host defense genes. Current dogma emphasizes that phosphorylation and activation or IRF-3 and IRF-7 are key to viral host defense responses (Nakhaei et al., 2009), but this may prove to be an oversimplification. For example, HRV infection has actually been reported to inhibit IRF-3 activation (Kotla et al., 2008), while strongly inducing activation of IRF-1, which has been shown to play a key role in the induction of a number of HRV-induced genes from epithelial cells (Stirnweiss et al., 2010; Zaheer & Proud, 2010).

In considering the generation of proinflammatory cytokines and chemokines as potential targets for new therapies, it is critical to know which molecules contribute to disease pathogenesis and which also may play a role in requisite host defense. One potential clue may come from the fact that, while HRV infected epithelial cells induce generation of chemokines and cytokines that should, in theory, recruit multiple cell types to the airways, HRV infections, and viral exacerbations of lower airway diseases, are usually associated with selective recruitment of neutrophils and lymphocytes to the airways (Jarjour et al., 2000). Indeed, neutrophil numbers, and neutrophil degranulation, correlate with disease severity during viral exacerbations of asthma or COPD (Wark et al., 2002; Quint & Wedzicha, 2007). Thus, chemokines that recruit and activate neutrophils may be attractive targets for additional study. The selective recruitment of neutrophils also suggests that there may be mechanism(s) by which recruitment of cell types to the airway is selectively regulated. If so, such mechanisms are not yet well defined, although it is known that other factors that can be present in the airways can modulate epithelial responses to HRV infection. These include cigarette smoke and IL-17A, both of which enhance HRV-induced production of chemokines that are chemotactic for neutrophils, while reducing production of chemokines linked to recruitment of eosinophils and natural killer cells (Wiehler & Proud, 2007; Hudy et al., 2010; Eddleston et al., 2011; Hudy & Proud, 2013; Hudy et al., 2014).

6. Epithelial innate defense to HRV infection

In addition to the ability of epithelial cells to produce proinflammatory chemokines and cytokines upon HRV infection, these cells can also regulate clinical outcomes to HRV infection by directly producing molecules with antiviral and host defense functions (Fig. 2). Gene array analyses of human airway epithelial cells infected, either in vivo or in vitro, with HRV have shown increased expression of a wide range of genes with potential antiviral activity (Proud et al., 2008, 2012). To date, however, few of these genes have been evaluated to determine which gene products are actually able to limit HRV infections.

Because many of these genes are also classified as "interferon stimulated genes" (ISG), it has been suggested that type I and type III interferons (IFNs) play a key role in regulating innate immunity to HRV infections. Indeed, it has been reported that epithelial production of IFN-β and/or IFN-λ in response to HRV infection is impaired in patients with asthma or COPD, and this has been suggested to contribute to susceptibility to HRV-induced exacerbations of these diseases (Wark et al., 2005; Contoli et al., 2006; Mallia et al., 2011). The concept that impaired IFN production underlies viral exacerbations of lower airway diseases is, however, controversial. Not only have other authors failed to observe impaired IFN responses in epithelial cells from asthmatic, compared to normal, subjects (Lopez-Souza et al., 2009; Bochkov et al., 2010), but recent data from the same authors who reported the original observations, suggest that IFN deficiency is not an inherent feature of asthma, as they did not observe this feature in subjects with asthma whose disease was well-controlled (Sykes et al., 2013). Indeed, a number of investigative groups have been unable to detect any measurable release of type I IFN protein from HRV infected epithelial cells, despite detecting induction of mRNA expression (Spurrell et al., 2005; Khaitov et al., 2009; Schneider et al., 2010; Proud et al., 2012; Bochkov et al., 2013). The failure to detect secretion of type I IFN proteins in the setting of increased expression of a panoply of ISGs may appear to be a paradox.
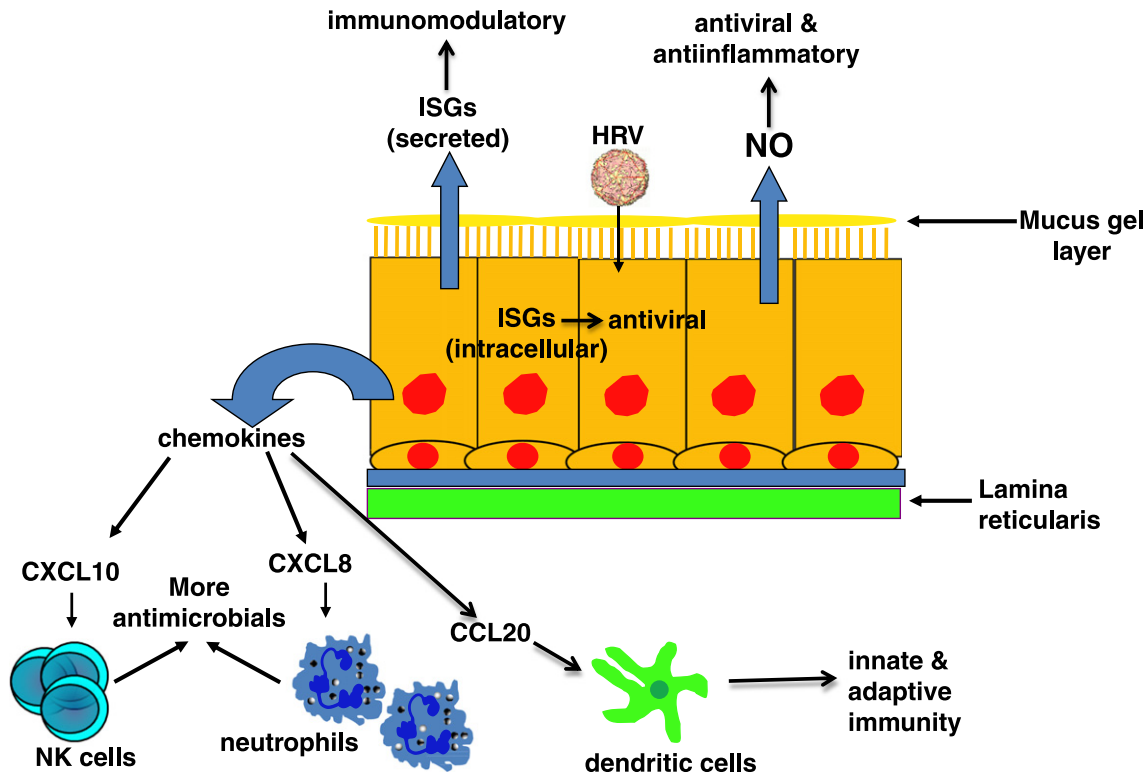
Fig. 2. Regulation of airway epithelial cell antiviral immunity upon infection with HRV. Release of nitric oxide (NO) can inhibit both replication of HRV and viral induced chemokine production. Induction of interferon-stimulated genes (ISG) in the intracellular compartment may regulate viral replication, while secreted ISGs may exert immunomodulatory effects. Chemokine production can be both proinflammatory and antimicrobial via release of secondary mediators or recruitment of dendritic cells.

However, there is clear precedent that many ISGs can be induced independently of IFN via direct activation of key transcription factors by any of a number of viruses, including HRV (Spurrell et al., 2005; Schmid et al., 2010; Stirnweiss et al., 2010; Proud et al., 2012).

As mentioned, signaling induced by dsRNA, or other viral replication intermediates, induces a wide range of potential antiviral genes. Although many antiviral gene products are assumed to be of broad specificity, it is likely this large number of antiviral genes incorporate both the capacity to function against multiple viruses, as well as redundancy in terms of the number of antivirals targeting a given virus. Despite this, there is still a lack of knowledge regarding which antiviral pathways are critical for regulating responses to HRV, and only a few pathways have been examined to date.

HRV is well known to induce marked expression of members of the 2′,5′-oligoadenylate synthetase (OAS) family which produce 2-5A from ATP (Hovanessian et al., 2007). In humans, there are 8–10 isoforms of OAS encoded by 3 genes (OAS1, 2 and 3), and all are induced upon HRV infection of epithelial cells (Proud et al., 2008, 2012). The only well-defined role of 2-5A is activation of RNase L (Silverman, 2007). RNase L then dimerizes and expresses potent RNase activity, thereby degrading single-stranded RNA, such as the genomic RNA of picornaviruses. It must be noted, however, that picornaviruses are not defense-less against RNase L. For example, the 3C protease of poliovirus (which is homologous to that of HRV) is a potent inhibitor of RNase L (Han et al., 2007). Moreover, mice deficient in RNase L (or even triply deficient in RNase L, RNA-dependent protein kinase and myxovirus resistance 1) are still able to mount a significant antiviral response to picornaviruses, such as encephalomyocarditis virus (Zhou et al., 1999). Thus, other pathways must also play a role in antiviral defenses.

Gene array analysis of human airway epithelial genes upregulated upon HRV infection identified viperin (**v**irus **i**nhibitory **p**rotein, **e**ndoplasmic **r**eticulum associated, IFN-**in**ducible) as a potential novel antiviral (Proud et al., 2008). Viperin is known to interfere with the replication of several viruses (Chin & Cresswell, 2001; Wang et al.,

2007; Zhang et al., 2007). Using an siRNA knockdown approach it was confirmed that viperin was also a component of the epithelial antiviral defense against HRV, as prevention of viperin expression led to enhanced replication of HRV (Proud et al., 2008). The mechanisms by which viperin inhibits replication of HRV remain unknown, but the identification of this protein as a radical-S-adenosyl-L-methionine enzyme raises the question of whether this enzyme activity may play a role in antiviral activity (Shaveta et al., 2010).

The free radical nitric oxide (NO) is known to play a role in host defense against a wide range of pathogens (Proud, 2005). Type 2, or inducible, nitric oxide synthase (iNOS) is one of the most highly induced genes in airway epithelial cells upon experimental HRV infection both in vitro and in vivo (Sanders et al., 2001; Proud et al., 2008, 2012). Epithelial induction of iNOS has also been observed upon infection with other viruses, including RSV and influenza (Uetani et al., 2000; Kao et al., 2001). It has been reported that epithelial iNOS is the major determinant of levels of exhaled NO (Lane et al., 2004). Consistent with this, increased epithelial expression of iNOS during experimental HRV infections in vivo correlates with levels of NO in exhaled breath. Subjects who had the highest levels of exhaled NO had the lowest symptoms during experimental HRV infections and cleared virus more quickly (Sanders et al., 2004). These data are further supported by the fact that NO is one of the few agents that can both suppress replication of HRV in airway epithelial cells, as well as inhibit HRV-induced production of a number of cytokines and chemokines, independently of its effects on viral replication (Sanders et al., 1998). Inhibition of HRV-induced epithelial chemokine production by NO is mediated via cGMP-independent reduction of NF-κB and IRF-1 dependent transcriptional pathways (Koetzler et al., 2009a, 2009b). NO is also known to inhibit replication of other respiratory viruses, including RSV and influenza (Rimmelzwaan et al., 1999; Ali-Ahmad et al., 2003). Thus, it appears that generation of NO likely plays an important role in host defense against infection not only by HRV but also by other common respiratory viruses. In this context, it is of interest to note that epithelial cells from subjects with CF, a patient population who do poorly in

response to viral infections, are deficient in their ability to induce iNOS (Zheng et al., 2004).

Determining which other potential antiviral agents may contribute to innate antiviral defenses against HRV infection could identify additional potential novel therapeutic approaches to regulating viral exacerbations of lower airway diseases.

7. Effects of current treatments on viral modulation of lower airway diseases

7.1. Corticosteroids and combination therapies

Inhaled corticosteroids (ICS) are the most effective therapy for the control of asthma and, as such, their use as a primary pharmacological treatment is recommended in all national and international asthma management guidelines, for all patients except those with very mild, intermittent asthma (Bateman et al., 2008; Lougheed et al., 2012). The efficacy of ICS in asthma is due to their ability to suppress allergic inflammation that is characterized by T helper type 2 (Th2)-driven inflammation and airway eosinophilia (Louis et al., 2012). This is the "classical" Th2 inflammatory profile in asthma (Woodruff et al., 2009), and corticosteroids reduce airway expression of a number of chemokines linked to eosinophil recruitment, and also induce eosinophil apoptosis (Cox, 1995), leading to reduced eosinophilic inflammation in the airways (Djukanovic et al., 1992). Despite their overall efficacy, ICS, regardless of dose, do not adequately control asthma in a significant percentage of patients with moderate to severe asthma. This includes patients who smoke, as smoking attenuates the response to ICS (Thomson et al., 2006; Lazarus et al., 2007), and also those who have a different inflammatory profile characterized by neutrophilic inflammation (Barnes, 2007). Indeed, airway neutrophilic inflammation in general responds poorly to ICS treatment, and corticosteroids actually inhibit neutrophil apoptosis (Cox, 1995). This explains why the efficacy of ICS is minimal in diseases that are characterized by neutrophilic inflammation, such as CF or COPD. For COPD, the inhibitory effects of smoking further limit the effectiveness of ICS such that, even at high doses, they do not reduce sputum neutrophil levels in patients with COPD (Keatings et al., 1997). Infection with respiratory viruses, including HRV, also induces inflammation characterized by increased neutrophils, and neutrophil number and neutrophil degranulation correlate with disease severity during viral exacerbations of COPD and asthma and COPD (Wark et al., 2002; Qiu et al., 2003). Neither topical nor systemic corticosteroids significantly reduced symptoms during experimental HRV infections (Farr et al., 1990; Gustafson et al., 1996), so it may be anticipated that these medications may be of limited utility during viral exacerbations of lower airway diseases. Indeed, ICS treatment did not modulate lower airway inflammatory responses to experimental HRV infection (Grunberg et al., 2001). Despite this, the utility of corticosteroids in the management of disease exacerbations remains controversial. In part this may be that the role of corticosteroids in establishing better control of underlying inflammatory status is not usually distinguished from the effects on inflammation triggered by an exacerbating stimulus. In addition, clinical studies rarely identify the inciting stimulus for an exacerbation, or the inflammatory phenotype, prior to initiating treatment.

In the case of asthma, observations vary with the age of the subject group. In infants and pre-school children, the majority of episodes of wheezing illness can be attributed to viral infection. In this population, several months of ICS therapy using moderate doses in infants and young children with recurrent episodic wheezing, did not lead to any improvement in lung function or symptoms compared to placebo (Wilson et al., 1995; Hofhuis et al., 2005). Moreover, although oral corticosteroids are recommended for severe wheezing illness in preschool children, the majority of evidence does not support the utility of such an intervention. Several studies demonstrated that systemic corticosteroids did not reduce the length of hospital stay or symptom severity in preschool

children with virus-induced wheezing (Oommen et al., 2003; Panicker et al., 2009; Beigelman et al., 2013). Consistent with these observations, there is no evidence that corticosteroids prevent the progression of episodic wheezing to chronic wheezing and the development of asthma. Indeed, neither chronic administration of ICS for two years, nor intermittent use of corticosteroids after wheezing episodes, was effective in preventing the development of chronic wheezing (Bisgaard et al., 2006; Guilbert et al., 2006). While we are not aware of any studies specifically looking at the ability of corticosteroids to prevent airway remodeling, the failure of these drugs to prevent asthma development would suggest that airway remodeling is also unaffected.

By contrast, in school age children and young adults, where allergic asthma dominates and exacerbations can be induced by allergen exposure as well as by viral infection, corticosteroids are clearly efficacious. However, in these patients, corticosteroids may have benefits that are unrelated to effects on viral inflammation. Appropriate use of ICS can provide improved control of disease in patients with asthma, and thereby reduce susceptibility to exacerbation triggered by any of a variety of stimuli. In accord with this, there is some evidence that use of ICS early in an exacerbation can reduce admissions (Rowe et al., 2004). The general standard of care during acute exacerbations in school age children and adults relies on the use of short courses of systemic corticosteroids. There is ample evidence that this treatment improves symptoms, reduces admissions, and reduces the risk of relapse (Rachelefsky, 2003; Rowe et al., 2004, 2007). It must be noted, however, that in studies in these age groups, the causative effect of acute exacerbations was not identified, and the efficacy of corticosteroids may be due mainly to effects on allergen-driven, eosinophilic type exacerbations, as well as on better control of underlying chronic allergic inflammation. We are aware of no study that has focused exclusively on virus-induced exacerbations in these age groups.

Although there are conflicting data in the literature, ICS at usual therapeutic levels tend to have, at best, modest effects on reduction of airway remodeling (Jeffery et al., 1992; Trigg et al., 1994; Boulet et al., 2000). Even prolonged treatment with high dose ICS resulted in only a modest reduction of reticular basement membrane thickness after 1 year, while having no effect on tissue collagen staining (Ward et al., 2002).

In patients whose asthma is not sufficiently controlled by inhaled corticosteroids (ICS), guidelines recommend addition of an inhaled long-acting β_2-agonist (LABA) as a combination therapy (Bateman et al., 2008; Lougheed et al., 2012). Combined ICS/LABA therapy can be used either as a controller medication or, when the LABA component has a rapid onset of action, as is the case with formoterol, as both a controller/reliever medication. Both of these treatment regimens have been shown to reduce the number of exacerbations experienced by asthma patients when compared to appropriate control treatments (Pauwels et al., 1997; O'Byrne et al., 2001; Scicchitano et al., 2004; O'Byrne et al., 2005; Rabe et al., 2006). These treatment regimes are also associated with improved asthma control between exacerbations. Thus, it is unclear how much the reduction in exacerbation frequency that is observed is due to improvement of underlying asthma control (perhaps via regulation of Th2-type inflammatory responses) versus reduction of responses to inciting stimuli for triggering exacerbations. Again, there have no studies specifically examining the effects of these treatments on virus-induced exacerbations.

Interestingly, in a recent study of allergen-induced remodeling, allergen challenge led to increased numbers of myofibroblasts in the airways. Although treatment with inhaled corticosteroid alone did not reduce the allergen-induced increase in myofibroblasts, the combination of corticosteroid and LABA significantly reduced myofibroblast numbers (Kelly et al., 2010). This raises the intriguing possibility that combination therapy may prove useful in virus induced airway remodeling, but, thus far, there have been no studies to test this possibility.

In patients with COPD, several large observational studies suggested that ICS treatment reduced mortality and number of exacerbations in

COPD patients (Sin & Tu, 2001; Soriano et al., 2002), presumably by providing improved overall disease control. By contrast, subsequent prospective randomized controlled trials have failed to demonstrate any significant beneficial effects of ICS as a monotherapy on mortality, but do show a modest reduction in exacerbation frequency (Vestbo et al., 1999; Burge et al., 2000; Calverley et al., 2007). It has been argued that most of these randomized trials may fail to find effects due to small sample sizes relative to the observational studies. On the other hand, it has also been argued that the observational studies suffered from a limited assessment of medication use and may suffer from immortal time bias. In an attempt to resolve this issue, a large prospective study incorporated a time-dependent analysis to avoid immortal time bias (Fan et al., 2003). In this study, patients receiving medium or high dose inhaled corticosteroids did not show any significant reduction in exacerbation risk. By contrast, several studies have reported that oral corticosteroids can be beneficial in treating COPD exacerbations (Davies et al., 1999; Niewoehner et al., 1999; Aaron et al., 2003; Wedzicha & Donaldson, 2003). The mechanisms underlying the beneficial effects of oral corticosteroids are unclear and, again, there have been no studies of the effects of these medications during COPD exacerbations of known viral origin. The use of combined ICS/LABA is now standard as maintenance therapy in patients with moderate-to-severe, clinically stable COPD. In a large study of this treatment regimen, the combination therapy failed to achieve statistical significance in reducing all cause mortality, but did show a 25% reduction in exacerbation frequency (Calverley et al., 2007). Again, the mechanism(s) by which exacerbation frequency is reduced remain unknown.

7.2. Leukotriene receptor antagonists and bronchodilators

The potential role of leukotriene receptor antagonists in viral exacerbations of lower airway diseases remains controversial. It has been reported that levels of sulfidopeptide leukotrienes are increased during infections with RSV, influenza, or HRV (Volovitz et al., 1988; Gentile et al., 2003), although data for the latter virus remain controversial. Several studies have reported that the use of leukotriene receptor antagonists, normally added on as a second-line treatment to usual ICS therapy, reduced asthma symptoms or exacerbations due to colds in children (Bisgaard et al., 2005; Horiguchi et al., 2007; Johnston et al., 2007; Robertson et al., 2007). By contrast, in children with RSV bronchiolitis, the use of montelukast did not reduce symptoms, nor did it significantly effect the number, or time to onset, of subsequent exacerbations (Bisgaard et al., 2008; Proesmans et al., 2009). Moreover, during experimental HRV infections of young adults with asthma, treatment with montelukast had no significant effects on asthma or cold symptoms or on virus shedding (Kloepfer et al., 2011). Thus, there is little evidence of any direct effect of leukotriene receptor antagonists on the pathogenesis of virus infections, although in children with asthma, there may be some benefit in terms of reducing exacerbation frequency.

Short-acting bronchodilators, acting as functional smooth muscle relaxants, are essential to maintain airway patency during acute exacerbations of asthma regardless of the inciting trigger. However, there have been few studies directly examining if they have any effects in further control of viral exacerbations. In children with viral bronchiolitis, β_2-adrenergic agonists did not affect rates or duration of hospitalization (Schindler, 2002; Patel et al., 2003). Indeed, Cochrane reviews have concluded that neither β_2-adrenergic agonists nor anticholinergics reduced hospital stay in wheezing children under 2 years of age and that there is insufficient evidence to support their uncritical use in wheezing infants (Chavasse et al., 2002; Everard et al., 2005).

7.3. Monoclonal antibody therapies

Omalizumab is a humanized monoclonal anti-IgE antibody that is approved for treatment of moderate-to-severe asthma that is not controlled by high-dose ICS or by ICS/LABA combination therapy (Humbert et al., 2014). Studies have consistently shown that omalizumab treatment reduces exacerbation rates in both adults and children with moderate-to-severe allergic asthma (Busse et al., 2001; Soler et al., 2001; Lanier et al., 2009; Busse et al., 2011). Omalizumab also improves asthma control and is corticosteroid-sparing. Given that omaluzimab is used primarily for allergic asthma, it is, again, unclear whether omalizumab reduces exacerbation by reducing airway inflammation and improving disease control (Djukanovic et al., 2004), or via direct effects on responses to exacerbating stimuli.

For patients with refractory eosinophilic asthma, the monoclonal antibody to interleukin-5, mepolizumab, has been shown to improve disease control and reduce exacerbations (Haldar et al., 2009; Pavord et al., 2012; Liu et al., 2013; Ortega et al., 2014). This treatment is used almost exclusively for subjects with eosinophilic inflammation that is persistent despite high-dose ICS or oral corticosteroid therapy and, as such, functions via reducing TH2-type inflammatory responses. There are no data available on the ability of mepolizumab to impact virus-induced airway inflammation. Similarly, for the other monoclonal antibody therapies currently in clinical development, including those targeting IL-13 (lebrikizumab and tralokinumab), IL4 (pitrakinra) or the α-subunit of the IL-4 receptor complex thereby inhibiting the biological effects of both IL-4 and IL-13 (dupilumab), there are as yet no data regarding their efficacy in the context of virally-mediated airway inflammation.

8. Approaches based on viral inhibition or blockade of pathogenesis

A number of current or experimental therapeutic approaches target blockade of viral attachment, internalization and/or replication, or focus on proposed modulation of viral pathogenesis or host immune responses (Fig. 3).

Vaccination against influenza has been extremely effective in reducing infection and exacerbations of lower airway disease induced by this virus. However, no vaccine is available for RSV and, given the almost 150 serotypes of HRV, standard vaccination approaches are not feasible for this virus. Although palivizumab, a humanized monoclonal antibody, is available for prophylactic treatment of RSV, cost factors have led its use to be limited to children with specific pre-defined conditions who are at greatest risk of severe disease (Committee, 2014). Ribavirin has been shown to reduce the severity of RSV bronchiolitis if administered early after disease onset, but less encouraging results have been obtained when the ability of ribavirin treatment to reduce subsequent recurrent wheezing or the development of asthma has been examined. Although one study reported a reduction of respiratory morbidity at 1 year follow up (Edell et al., 2002), in general no reduction in asthma development has been observed with longer term follow up (Kimpen, 2002). Thus, additional approaches to treat respiratory viral infections have been sought.

To date, two classes of antivirals have been developed for the treatment of influenza infections. The first group includes rimantadine and amantadine, which are selective inhibitors of the proton channel function of the M2 protein of influenza A. These drugs have been used prophylactically to prevent infections among family members of index cases (Galbraith et al., 1969), but the utility of these drugs has been limited due the rapid emergence of drug resistance (Hayden et al., 1989). The second class of drugs are the neuraminidase inhibitors, such as orally administered oseltamivir and inhaled zanamivir. Neuraminidase activity of influenza is essential for viral budding, and prophylaxis and early treatment with either oseltamivir or zanamivir has demonstrated significant antiviral and clinical effects (Hayden et al., 1999, 2000). Although there has since been a widespread emergence of influenza virus strains resistant to oseltamivir, resistance to zanamivir has, thus far, remained quite infrequent in the clinical setting (Samson et al., 2013).

Several approaches have been taken in trying to limit the deleterious clinical effects of HRV infections. The first is to prevent viral binding to its receptor. HRV members can be classified into three genetic clades
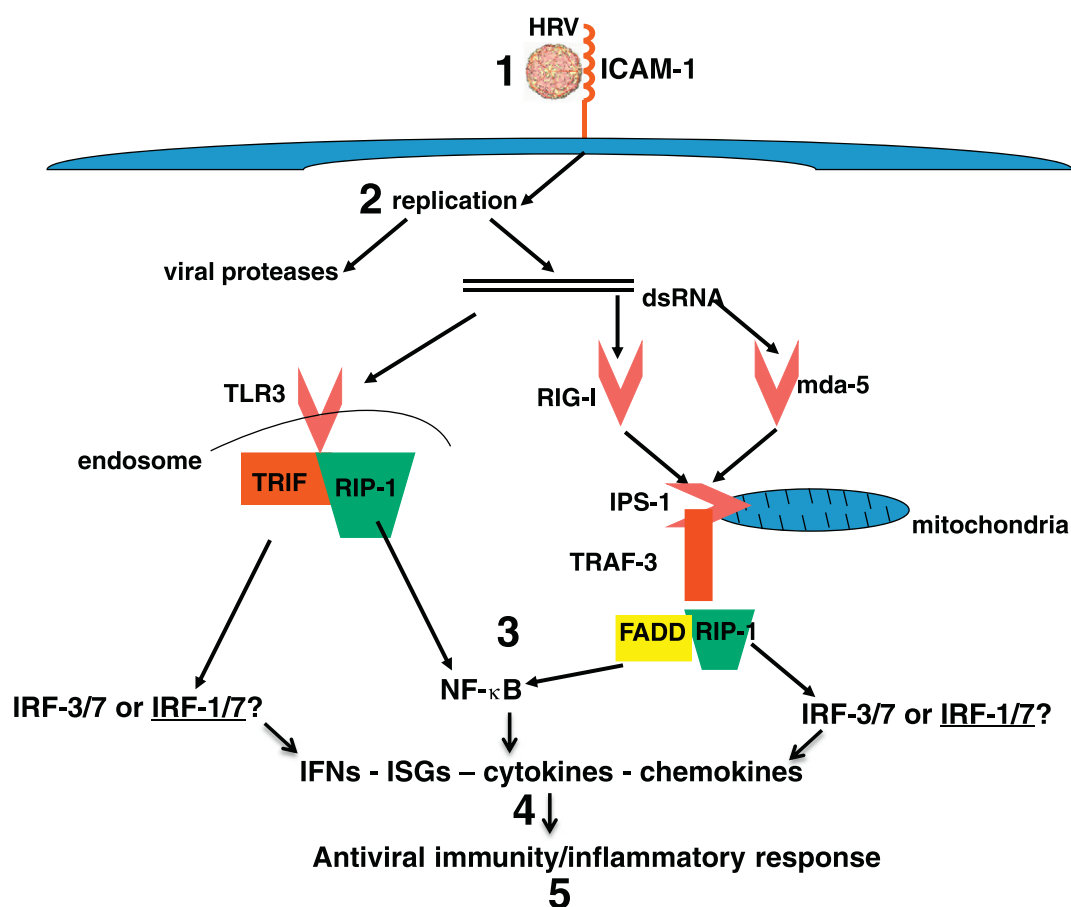
Fig. 3. Potential target sites for the development of therapeutics to regulate viral exacerbations of lower airway diseases. Approaches have targeted: 1) Viral binding or internalization. 2) Viral replication. 3) Signaling downstream from pattern recognition receptors. 4) Release or binding of specific proinflammatory mediators, or supplementation of interferons or specific ISGs and 5) modulation of specific antiviral immune responses. Abbreviations: HRV, Human rhinovirus; ICAM-1, intercellular adhesion molecule-1; dsRNA, double-stranded RNA; TLR3, toll-like receptor 3; RIG-1, retinoic acid inducible gene-I; mda-5, melanoma differentiation-associated gene-5; TRIF, TIR-domain-containing adapter-inducing interferon-β; RIP-1, receptor interacting protein-1; IPS-1, interferon-β promoter stimulating protein-1; TRAF-3, TNF receptor-associated factor-3; FADD, fas-associated protein with death domain; IRF, interferon regulatory factor; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; ISG, interferon-stimulated gene.

(HRV-A, HRV-B, and HRV-C) based upon sequence homologies, particularly in the VP1 and VP2 coat proteins (Palmenberg et al., 2009). There are approximately 100 strains comprising the HRV-A and HRV-B clades. In terms of receptor usage, 11 serotypes of HRV-A gain cellular entry via members of the low-density lipoprotein receptor family. The remaining serotypes of HRV-A and all members of HRV-B use ICAM-1 to infect airway epithelial cells (Greve et al., 1989; Staunton et al., 1989). The receptor for the HRV-C clade members remains to be identified. Treatment with inhaled, recombinant soluble ICAM-1 in experimental HRV infection led to a significant reduction in symptom severity and in reduced viral shedding (R. B. Turner et al., 1999). Although this study effectively established a proof of principle, the cost and dosing regimen of soluble ICAM used in the study proved to be impractical for widespread therapy.

Another approach to prevent HRV entry into cells was the development of capsid-binding drugs. Binding of receptors to HRV occurs at the bottom of a deep "canyon" on the icosahedral faces of HRV. Underneath the canyon is an interior hydrophobic "pocket", formed by the beta-barrel of the VPI capsid protein. Most capsid binding drugs bind within this pocket, which results in inhibition of uncoating and, for some strains of HRV, reduced binding to the receptor (Smith et al., 1986; Pevear et al., 1989). Administration of the novel, orally absorbed capsid-binding drug, pleconaril, beginning within 24 h of symptom development, was shown to reduce symptom duration and severity in naturally-acquired HRV infections (Hayden et al., 2003). Concerns regarding effects on cytochrome P450 3A4, however, precluded further clinical development as an oral antiviral treatment. The drug was

subsequently reformulated as a nasal spray and it is reported on ClinicalTrials.gov (NCT00394914) that a randomized, multicenter, double-blind, placebo-controlled trial of its effects on cold symptoms and asthma exacerbations was completed in 2007, but the results of this study have not yet been reported.

Among the components of the HRV genome is the 3C protease that is essential for cleavage of the viral polyprotein and, thus, for replication. This led to the development of inhibitors of the HRV 3C protease as therapies to reduce viral replication. Multiple intranasal administration of one such compound, ruprintrivir, significantly inhibited symptoms and viral load in experimental HRV infections even when administered beginning 24 h after infection (Hayden et al., 2003). It has since been reported, however, that ruprintrivir did not significantly reduce symptoms or viral load in subsequent natural infection studies and that further clinical development has not been pursued, although the primary data were not presented (Patick et al., 2005). Inhibitors of 3C protease were never evaluated for their ability to limit HRV induced exacerbations of lower airway diseases.

Given the challenges with the development of drugs to inhibit HRV infection and/or replication, alternative considerations could include attempting to modulate aspects of viral pathogenesis of exacerbations of lower airway diseases. In view of the relationship between neutrophil recruitment/activation and viral exacerbations of lower airway diseases, it is feasible that antagonists of CXCR1/2 may be useful in limiting symptoms. Although several such antagonists already exist they have not, to our knowledge, been examined in HRV infections. Moreover, there are concerns regarding their potential for causing deleterious off-target

effects, via impairment of neutrophil functions and, if such concerns prove to be valid, this would negatively impact further development. It is less clear which other aspects of viral inflammation should be targeted for inhibition, as some of the proinflammatory responses observed may also contribute to innate immunity. As we continue to gain greater insights into the mechanisms by which viral infections exacerbate inflammation, it is possible that selective inhibition of key virus-specific signaling could be targeted. One other potential approach is to enhance innate antiviral immune responses. As mentioned above, while the suggestion that patients with asthma and COPD have impaired type I IFN production upon HRV infection remains controversial, the important role of IFNs in antiviral immunity is well established. The concept of administering type I IFNS to reduce symptoms of HRV infections is not new, with several studies showing that either IFN-α2 or IFN-β can prevent HRV infections with prophylactic administration, and modestly reduce cold symptoms if administered soon after infection (Hayden & Gwaltney, 1983, 1984; Hayden et al., 1986; Higgins et al., 1986). These early studies used high doses of IFNs, which were associated with nasal bleeding and, in some patients, transient leukopenia (Hayden & Gwaltney, 1983). It was concluded that type I IFNs would not be useful on their own in treatment of HRV-induced colds, given the modest efficacy relative to cost and side effect profiles. None of these early studies, however, extended to looking at exacerbations of lower airway diseases triggered by viral infection. A recent study has now examined this question using a dose of IFN-β that, while much lower than those used in early cold studies, was shown to induce markers of IFN antiviral activity in the airways. The study examined asthma subjects with a history of exacerbations linked to viral infections, and administered IFN-β for 14 days beginning 24 h after the onset of cold symptoms. The primary outcome was asthma symptoms as assessed by a validated asthma control questionnaire. No significant effects of treatment were observed on the primary endpoint although some modest changes were seen on several secondary outcomes of interest (Djukanovic et al., 2014).

Although these results are disappointing, they do not preclude the concept of enhancing host innate immunity to HRV as a potential treatment approach. Indeed, there is some controversy as to whether type I IFNs are necessary to mediate epithelial antiviral responses to HRV. Although detection of type I IFN mRNA is an almost universal observation on HRV infected epithelial cells, several investigators have failed to detect release of IFN-β protein (Spurrell et al., 2005; Khaitov et al., 2009; Schneider et al., 2010; Proud et al., 2012). Indeed, it has been reported that HRV infection actually suppresses type I IFN induction (Kotla et al., 2008). There is also ample precedent for viral induction of ISGs in the absence of functional IFN responses via direct viral activation of key transcription factors (Schmid et al., 2010). Moreover, while a broad panoply of ISGs are induced upon viral infections, it is likely that select groups of genes may be more important in regulating specific viruses. Thus, a more targeted approach of enhancing the endogenous production of, or exogenously supplementing, specific molecules with demonstrated antiviral activity against key respiratory viruses may be a more promising therapeutic approach. For example, given the evidence that NO can inhibit both replication of respiratory viruses including HRV, influenza and RSV, as well as reduce proinflammatory chemokine production, topical administration of nitric oxide donors may provide a potential approach to limit virus induced inflammation. As we continue to gain greater insights into the mechanisms by which viral infections enhance production of specific proinflammatory cytokines, and into endogenous mechanisms of host defense, alternative targeted therapeutic strategies also may be derived.

Conflict of interest statement

Dr. Leigh has consulted for Almirall, AstraZeneca, Forest, and GlaxoSmithKline, and has received research support from Amgen, AstraZeneca, Genentech, GlaxoSmithKline, Novartis, Ono Pharma and Teva Pharmaceutical Industries (money to institution). He has also received speaker's fees from Almirall, AstraZeneca, Forest, GlaxoSmithKline and Novartis. Dr. Proud has served as a consultant for Janssen and Pfizer, and has received research support from AstraZeneca and MedImmune (money to institution).

Acknowledgments

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