Role of rhinovirus infections in asthma

David Proud

Summary
Human rhinoviruses are not only the main pathogens responsible for the common cold, but are now recognized to have a major impact on asthma pathogenesis. There is evidence that rhinovirus infections play a role in asthma development, asthma exacerbations and, potentially, airway remodeling. Children who experience repeated rhinovirus-induced wheezing episodes in infancy have a significantly increased risk of developing asthma, even when compared to children who experience wheezing induced by respiratory syncytial virus. Rhinovirus is also the dominant virus type associated with acute exacerbations of asthma. The epithelial cell is the principal site of rhinovirus infection in both the upper and lower airways and there is strong evidence that virus-induced alterations of epithelial cell biology play a critical role in regulating clinical outcomes. This includes rhinovirus-induced epithelial generation of a variety of chemokines, cytokines and growth factors that likely play a role in viral modulation of airway inflammation. It has also become clear, however, that epithelial cells play an important role in the innate antiviral response to rhinovirus infection, raising the possibility that the relative induction of epithelial host innate antiviral responses versus proinflammatory responses may be one factor regulating the susceptibility of asthmatic subjects to virus-induced disease exacerbations. Recent evidence has also highlighted that rhinovirus infection induces epithelial production of a number of growth factors and other mediators that could contribute to the development and progression of airway remodeling processes in asthma. The current article reviews our current state of knowledge in these areas. (Asian Pac J Allergy Immunol 2011;29:201-8)

Key words: Rhinovirus, epithelial cell, chemokines, inflammation, host defense, remodeling

Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>ENA-78</td>
<td>Epithelial neutrophil activating protein 78</td>
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<tr>
<td>ERK</td>
<td>Extracellular signal-regulated kinase</td>
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<tr>
<td>G-CSF</td>
<td>Granulocyte colony-stimulating factor</td>
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<tr>
<td>GM-CSF</td>
<td>Granulocyte-macrophage colony-stimulating factor</td>
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<td>HAE</td>
<td>Human airway epithelial cell</td>
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<td>HRV</td>
<td>Human rhinovirus</td>
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<tr>
<td>ICAM-1</td>
<td>Intercellular adhesion molecule-1</td>
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<tr>
<td>IFN</td>
<td>Interferon</td>
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<tr>
<td>IL</td>
<td>Interleukin</td>
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<tr>
<td>iNOS</td>
<td>Inducible nitric oxide synthase</td>
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<tr>
<td>IP-10</td>
<td>Interferon-induced protein of 10 kilodaltons</td>
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<tr>
<td>IRF</td>
<td>Interferon regulatory factor</td>
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<tr>
<td>LDLR</td>
<td>Low density lipoprotein receptor</td>
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<tr>
<td>MAP kinase</td>
<td>Mitogen activated protein kinase</td>
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<tr>
<td>MEK</td>
<td>Mitogen activated protein kinase kinase</td>
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<tr>
<td>Mda-5</td>
<td>Melanoma differentiation-associated gene</td>
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<tr>
<td>MMP</td>
<td>Matrix metalloprotease</td>
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<tr>
<td>MUC5AC</td>
<td>Mucin 5AC</td>
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<tr>
<td>NF-κB</td>
<td>Nuclear factor kappa-B</td>
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<tr>
<td>NO</td>
<td>Nitric oxide</td>
</tr>
<tr>
<td>OCT-1</td>
<td>Octomer transcription factor-1</td>
</tr>
<tr>
<td>PI-3 kinase</td>
<td>Phosphoinositol 3-kinase</td>
</tr>
<tr>
<td>RANTES</td>
<td>Regulated upon activation, normal T-cell expressed, and secreted</td>
</tr>
<tr>
<td>RIG-I</td>
<td>Retinoic acid-inducible gene-I</td>
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<tr>
<td>RSV</td>
<td>Respiratory syncytial virus</td>
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<tr>
<td>RT-PCR</td>
<td>Reverse transcription, polymerase chain reaction</td>
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<tr>
<td>Src</td>
<td>Sarcoma tyrosine kinase</td>
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<tr>
<td>Syk</td>
<td>Spleen tyrosine kinase</td>
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<tr>
<td>TIMP-1</td>
<td>Tissue inhibitor of matrix metalloproteinases-1</td>
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<tr>
<td>TLR</td>
<td>Toll-like receptor</td>
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<tr>
<td>URI</td>
<td>Upper respiratory tract viral infection</td>
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<tr>
<td>VEGF</td>
<td>Vascular endothelial growth factor</td>
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From the University of Calgary Faculty of Medicine, HRIC 4AC06, 3280 Hospital Drive NW, Calgary, Alberta T2N 4Z6, CANADA
E-mail: dproud@ucalgary.ca
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Introduction

There are over 100 strains of human rhinovirus (HRV) that have been identified to date. They are all members of the picornaviridae family of single strand, positive sense RNA viruses with a genome of approximately 7.2kb that encodes for all of the viral proteins. In the infectious virion, this RNA genome is enclosed in a protein capsid that is approximately 27 nm in diameter. Human rhinoviruses are classified in several ways. The two most useful methods are the classification into genetic clades based upon sequence homologies, and the classification based upon receptor usage. Genetically, rhinoviruses are classified into HRV-A, containing 74 serotypes, HRV-B, comprising 25 serotypes, and the more recently discovered HRV-C clade for which members continue to be found. In terms of receptor usage, 11 serotypes of HRV-A gain cellular entry via members of the low-density lipoprotein receptor (LDLR) family. The remaining serotypes of HRV-A and all members of HRV-B use intercellular adhesion molecule-1 (ICAM-1) to infect cells1. The receptor for HRV-C serotypes remains to be identified, but it is clear that this group of viruses also cause symptomatic responses2.

In healthy individuals, HRV infections are the major cause of the common cold. In susceptible individuals, however, HRV infections play a significant role in both the development of asthma and in triggering exacerbations of asthma. Recent data also point to a potential role for HRV infections in the development and progression of the airway remodeling that is characteristic of asthma. This article will review the role of HRV infections in each of these aspects of asthma.

HRV and asthma development:

Respiratory viral infections are the leading cause of wheezing episodes in infants and young children. Initial studies focused on the role played by respiratory syncytial virus (RSV) as the dominant pathogen associated with bronchiolitis, particularly during the winter months. Virtually all children experience RSV infections within the first two years of life, but only a susceptible subset develop bronchiolitis requiring hospitalization. Infants who are hospitalized with RSV bronchiolitis tend to be younger than children hospitalized with other respiratory viruses. Other risk factors associated with hospitalization for RSV bronchiolitis include prematurity, attendance at daycare, and cigarette smoke exposure1. Several studies have linked early RSV induced bronchiolitis with an increased risk of continued wheezing illness by age 6. There is conflicting evidence, however, regarding how long this association persists. Sigurs and colleagues used a case-control approach to infer that severe RSV bronchiolitis was associated with an increased risk of asthma and allergy at age 13, but it must be noted that the prevalence of allergy and asthma in the control population was lower than may be expected4. By contrast, although the Tucson Children’s Respiratory Study showed that RSV bronchiolitis was an independent risk factor for asthma at age 6, this relationship decreases progressively with age and was no longer significant by age 135. As such, early RSV-induced bronchiolitis may not be a significant risk factor for life-long asthma.

With the advent of improved molecular assays for the detection of respiratory viruses it has become apparent that wheezing illnesses associated with HRV infections during infancy are a strong predictor for asthma development5. Indeed, data from the Childhood Origins of Asthma (COAST) birth cohort study showed that while the occurrence of RSV induced bronchiolitis during the first year of life increased the risk of asthma at age six, episodes of wheezing associated with HRV infections over the same time frame was a significantly more robust predictor6. Furthermore, the odds ratio for development of asthma was not further enhanced when wheezing episodes linked to both HRV and RSV were compared to those due to HRV alone.

Virtually all children are infected with HRV during infancy, and the factors that predispose a subset to develop wheezing illnesses are not fully understood. It is possible that the timing and frequency of infections may be important. Indeed, a proportion of children in the COAST study had more than 5 HRV-associated wheezing illnesses in a given year7. It is also possible that genetic factors underlie susceptibility to HRV-induced wheezing illnesses. For example, children in the COAST cohort are high-risk, having at least one parent with allergy or asthma. Consistent with this, a recent study found that a maternal or family history of atopy or asthma was associated with severity of HRV-associated bronchiolitis8. Both HRV-associated bronchiolitis and allergic sensitization are independent risk factors for asthma development, but the importance of the relative timing of these events in predisposing to asthma development is not well understood.
**HRV and asthma exacerbations:**

Acute exacerbations of asthma are a major healthcare burden. Not only do they account for about half of the total health care costs associated with the disease\(^9\), but they have a major impact on quality of life and, in rare instances, can cause death. Growing evidence implicates upper respiratory tract viral infections (URI) as the predominant risk factor associated with exacerbations of asthma. There is a clear temporal relationship between outbreaks of URI and increases in hospitalizations for asthma exacerbations, with a marked peak in the northern hemisphere in September\(^10\). Moreover, prospective monitoring studies using reverse transcription, polymerase chain reaction (RT-PCR) indicate that as many as 85% of acute asthma exacerbations in children, and about 60% in adults are associated with the presence of URI\(^11\), \(^12\). Although several virus types can be linked to asthma exacerbations, HRV is the dominant viral pathogen detected, being found in some 60% of viral exacerbations in both children and adults\(^13\)-\(^14\). Consistent with this, HRV was also the major viral pathogen detected in children and adults hospitalized for asthma exacerbations\(^15\), \(^16\).

Despite overwhelming evidence linking HRV infections with exacerbations of asthma, the mechanisms by which HRV infections trigger asthma attacks are not completely understood. Moreover, the relationship between allergic status and responses to HRV is not entirely clear. No difference was observed in the frequency, duration or severity of rhinovirus infections between asthmatic and non-asthmatic subjects, but lower airway symptoms were more common in asthmatics\(^17\). On the other hand, comparison of patients hospitalized with asthma exacerbations and subjects with stable asthma found sensitization and exposure to allergens to be an independent risk factor for hospitalization, suggesting that allergens and viruses can act synergistically to exacerbate asthma\(^18\). Despite this, studies of the interactions between experimental HRV infections and experimental allergen exposure have yielded mixed results. Subjects with experimental HRV infections showed enhanced lower airway inflammatory responses to subsequent allergen exposure\(^19\). By contrast, chronic, low dose allergen exposures did not alter lower airway responses to subsequent experimental HRV infection\(^20\). This implies that the relative order or timing of allergen versus viral exposure may have a significant impact on clinical outcomes.

The human airway epithelial cell (HAE) is the principal site of HRV infection. Studies using immunohistochemistry and in situ hybridization during in vivo infections indicate that infection is somewhat patchy in nature and can spread to lower airway epithelial cells\(^21\), \(^22\). In contrast to some other types of viruses, HRV does not cause overt epithelial toxicity. This has led to the general concept that virus-induced alterations in epithelial biology must regulate the development of exacerbations in susceptible individuals. In support of this, there is clear evidence that HRV infection of epithelial cells induces expression of a wide range of products, including not only proinflammatory cytokines and chemokines, but also a range of molecules with innate antiviral and host defense properties\(^23\).

**Epithelial proinflammatory responses to HRV infection:**

Infection of cultured human airway epithelial cells with HRV induces expression of a wide range of pleiotropic cytokines, including IL-1\(\beta\), IL-6, and IL-11; growth factors, such as G-CSF and GM-CSF that could regulate granulocyte survival and activation; and chemokines, including CXCL8 (IL-8), CXCL5 (ENA-78), CXCL10 (IP-10), and CCL5 (RANTES), that could contribute to inflammatory cell recruitment to the airways\(^23\). Many of these molecules have also been detected in airway secretions during in vivo HRV infections and would be expected to increase lower airway inflammation. In subjects who already have on-going inflammatory processes due to their asthma, particularly those whose inflammation is not well controlled, the extra inflammatory burden due to HRV infections may be adequate to cause severe disease exacerbation. One issue of interest is that, although epithelial cells have the capacity to produce a range of chemokines that would be expected to recruit multiple cell types to the airways, experimental HRV infections, and viral exacerbations of asthma, are usually associated with selective recruitment of neutrophils and lymphocytes to the airways\(^24\). Indeed, neutrophil numbers, and neutrophil degranulation, correlate with disease severity during viral exacerbations of asthma\(^25\). This implies that mechanisms must exist to selectively limit the cell types recruited, but these mechanisms are not well understood. It may be that the activation, or “priming”, status of specific cell populations plays a role, but other factors in the
airway microenvironment may also influence responses. For example, increased IL-10 gene expression is observed during viral exacerbations of asthma, and the immunoregulatory effects of IL-10 include suppression of eosinophil influx\textsuperscript{26}. In addition, both IL-17A and cigarette smoke have been reported to alter HRV-induced epithelial chemokine production in a manner that would enhance neutrophilic recruitment, while reducing production of chemokines linked to recruitment of eosinophils and natural killer cells\textsuperscript{27-29}. 

At least \textit{in vitro}, HRV-induced generation of various chemokines and cytokines occurs with different kinetic profiles. Some molecules, such as CXCL8 and IL-6, are induced rapidly after HRV exposure\textsuperscript{30}. This occurs even with purified HRV that has been rendered replication deficient, indicating that cytokine generation must be triggered directly by viral binding to its receptor\textsuperscript{31}. This was perplexing given that ICAM-1, the receptor for many of the HRV serotypes, contains no inherent receptor kinase, nor does it have known kinase recognition motifs. It was recently shown, however, that engagement of HRV with ICAM-1 leads to an association of ICAM-1 with the cytoskeletal linker protein, ezrin. Ezrin serves as a linker that also binds the spleen tyrosine kinase, Syk. Assembly of this complex not only appears to play a role in viral internalization, but also initiates downstream activation of other signalling molecules, including p38 MAP kinase and PI-3 kinase, leading to chemokine production\textsuperscript{31,32}. Although knockdown of Syk reduces production of CXCL8 from cells exposed to HRV, it does not eliminate chemokine release, implying a role for other kinases. The Src tyrosine kinase has also been linked to HRV-induced generation of CXCL8\textsuperscript{33}, but it is unclear whether Src also links to ICAM-1 via ezrin, or if alternative mechanisms are involved. At this point little is known about early signalling by serotypes of HRV that use the LDLR for cellular entry.

A second group of cytokines and chemokines, including CXCL10 and CCL5, are not produced until several hours after viral exposure and generation of these molecules is absolutely dependent upon the ability of HRV to replicate, since UV-treated, replication-deficient HRV does not generate these products from epithelial cells. This indicates that a product (or products) produced during rhinovirus replication is necessary for “late” generation of these epithelial products. Although viral proteins, such as the rhinovirus 3C protease, have been shown to have biological effects in cells, including alteration of nucleocytoplasmic transport and cleavage of the transcription factor, OCT-1, the majority of studies on the role of replication products in cytokine/chemokine generation have focused on viral double-stranded RNA (dsRNA). Three pattern recognition receptors are known to be able to bind to dsRNA, endosomal toll-like receptor (TLR)3 and the cytoplasmic RNA helicases, retinoic acid-inducible gene-I (RIG-I) and melanoma differentiation-associated gene (mda)-5. Viral dsRNA from different types of virus and of differing lengths are preferentially recognized by different receptors\textsuperscript{34}, and there is conflicting data from several studies on which molecules recognize HRV in human airway epithelial cells. This may be due to use of various crude or purified HRV serotypes and differing culture conditions, and additional studies may be necessary to resolve these issues\textsuperscript{35}. Regardless of the dsRNA receptors used by HRV, downstream signalling pathways from RIG-I, mda-5 or TLR3 converge to result in activation of both NF-κB and interferon (IFN) regulatory factors (IRF) that regulate transcription of a wide variety of genes. Although current dogma is that virus infection of cells leads to phosphorylation and/or activation of IRF-3 and IRF-7 triggering production of type I IFNs with subsequent induction of a wide range of “IFN stimulated genes”, this may be an oversimplification. First, it is clear that viruses can induce so-called IFN stimulated genes independently of generation of IFNs\textsuperscript{36}. Indeed, HRV infection actually impairs IRF-3 activation but strongly upregulates IRF-1, which plays a central role in induction of a number of IFN stimulated genes in HRV-infected epithelial cells\textsuperscript{23}. Interestingly, in HRV-infected epithelial cells, activation of the MEK1/ERK pathway selectively reduces viral-induction of several genes, including CXCL10 and CCL5, by suppressing activation of IRF-1\textsuperscript{37}.

\textbf{Epithelial innate immunity to HRV infection:}

Although epithelial cells may contribute to HRV-induced airway inflammation via excessive production of chemokines and cytokines, the epithelium can also regulate clinical outcomes to HRV infection by directly producing molecules with potential antiviral and innate immune functions. Attention has focused on innate immunity as a regulator of viral responsiveness because HRV-specific humoral and cellular immune responses are usually not detectable until after respiratory symptoms have resolved. In subjects undergoing
experimental HRV infections, gene array analysis of nasal epithelial scrapings showed marked increases in a number of genes, the products of which could play a role in host defense against HRV infection. Thus far, however, it is unclear how many of these gene products actually play a major role in limiting HRV infections, as relatively few of these proteins have been evaluated in this context.

Type 2, or inducible, nitric oxide synthase (iNOS) is the major NOS isoform found in epithelial cells and can generate substantial amounts of nitric oxide (NO). HRV infection leads to marked induction of epithelial iNOS both in vitro and in vivo. Interestingly, NO both inhibits the replication of HRV in airway epithelial cells, and suppresses HRV-induced production of a number of cytokines and chemokines, independently of its effects on viral replication. These properties suggested that NO contributes to host defense against HRV infection. Further support for this concept comes from a study showing that subjects who had the highest levels of exhaled NO had the lowest symptoms during experimental HRV infections and cleared virus more quickly, raising the possibility that exogenous supplementation of NO may be of clinical benefit.

Another protein linked to defense against HRV infection is viperin (virus inhibitory protein, endoplasmic reticulum associated, IFN-inducible). Viperin has been shown to exert antiviral effects towards a number of viruses, and siRNA knockdown of viperin in epithelial cells showed that HRV replication was enhanced in cells in which viperin expression was prevented. The mechanisms by which viperin inhibits HRV replication is unknown, but the characterization of viperin as a radical-S-adenosyl-L-methionine enzyme raises the possibility that this enzyme activity contributes to its antiviral effects.

HRV infection induces epithelial expression of mRNA for both type 1 and type 3 IFNs, and it has been suggested that impaired epithelial production of IFN-β and IFN-λ, in asthmatic subjects may contribute to viral exacerbations of asthma. This remains controversial, however, as others have failed to find differential expression of IFNs in epithelial cells from asthmatic compared to normal subjects. Indeed, despite confirming induction of mRNA for type 1 IFNs, some investigators have been unable to detect release of IFN protein from HRV-infected epithelial cells. It is clear, therefore, that additional studies are needed to fully define key components of host defense against HRV infections.

HRV infection and airway remodeling:

The airways of subjects with asthma are characterized by various structural changes, collectively referred to as airway remodeling. These structural changes include increased smooth muscle mass, enhanced subepithelial matrix deposition, angiogenesis, and altered epithelial integrity as well as goblet cell hyperplasia and excessive mucus production. These structural alterations have been linked to increased airways hyperresponsiveness. Traditionally, remodeling was thought to occur as a result of many years of chronic airway inflammation, but there is now clear evidence that airway remodeling begins in early childhood, and can be present even before the clinical diagnosis of asthma is established. Interestingly, airway remodeling is not present in infants (≤ 12 months old) with symptoms of airflow limitation, so is not a congenital feature predisposing to asthma. This suggests that remodeling occurs in parallel with the development of inflammation, presumably as a consequence of some initiating factor present during early life.

As noted above, HRV infections are a major cause of early childhood wheezing illnesses and are a risk factor for the development of asthma. The fact that a significant proportion of children experience multiple HRV-induced wheezing episodes per year, raises the possibility that HRV infections could contribute to the initiation and subsequent progression of airway remodeling (Figure 1). There is growing evidence to support this concept.

In terms of increased mucus production in asthma, HRV infection of cultured airway epithelial cells leads to enhanced expression of the major epithelial mucin MUC5AC. This induction is dependent upon activation of the epidermal growth factor receptor, as well as ERK mitogen activated protein kinase and NF-κB. This is not simply an in vitro phenomenon, as increased epithelial MUC5AC release has also been detected in vivo during experimental HRV infections.

HRV infection also induces epithelial production of proteins linked to matrix protein deposition and remodeling. These include activin A, a member of the transforming growth factor-β family, and amphiregulin, a member of the epidermal growth factor family. Both amphiregulin and activin A have been linked to subepithelial basement membrane thickening in asthma. Infection with HRV also enhances epithelial expression of matrix
metalloproteinase (MMP)-9, without altering production of tissue inhibitor of matrix metalloproteinase (TIMP)-1, the highest affinity tissue inhibitor of MMP-9. TIMP-1 levels were also unchanged in airway secretions from naturally acquired HRV infections, while MMP-9 levels were significantly enhanced. It has been suggested that an imbalance between levels of MMP-9 and TIMP-1 would enhance matrix protein turnover, thereby playing an important role in airway remodeling.

Finally, HRV infection may also contribute to the increased numbers of blood vessels seen in asthmatic airways via its ability to stimulate epithelial production of vascular endothelial growth factor (VEGF), which is considered the major angiogenic factor in asthma. Induction of VEGF from epithelial cells infected with purified HRV was dependent upon both the ERK and p38 mitogen activated protein kinase pathways but was not dependent upon activation of NF-κB. VEGF production has also been observed in airway secretions during naturally occurring HRV infections in both children and adults.

Although the data described above support the concept that HRV infections could contribute to various aspects of airway remodeling, other variables must be necessary to create susceptibility to remodeling. Virtually all children experience HRV infections, yet most do not develop wheezing illness, asthma or airway remodeling. Thus, it is likely that HRV infections contribute to remodeling in the context of genetic susceptibility. In addition, the timing, frequency or severity of infections may be critical. Further research is needed to clarify this.

Conclusions
The airway epithelial cell is the primary site of infection and replication of HRV, the most common respiratory pathogen experienced by humans. HRV infection causes profound changes in epithelial cell biology that contribute, in susceptible individuals, to
the development of asthma and to the pathogenesis of acute exacerbations of asthma. Current medications used in the treatment of asthma do not inhibit HRV-induced inflammation, so new therapeutic approaches are required. Approaches could involve targeting viral signaling mechanisms, or inhibiting specific chemokines that may play a key role in the pathogenesis of viral inflammation. Alternatively, enhancing induction, or exogenous supplementation, of key epithelial antiviral proteins may provide a novel pathway to limit viral exacerbations of asthma.

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References
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