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RHINOVIRUS IN THE PATHOGENESIS AND CLINICAL COURSE OF ASTHMA

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Abstract:

In healthy individuals, human rhinovirus (HRV) infections are the major cause of the common cold. These are generally uncomplicated infections except for occasional cases of otitis media or sinusitis. In individuals with asthma, however, HRV infections can have a major impact on disease development and progression. HRV-induced wheezing illnesses in early life are a significant risk factor for subsequent development of asthma, and growing evidence supports a role of recurrent HRV infections in the development and progression of several aspects of airway remodelling in asthma. In addition, HRV infections are one of the most common triggers for acute exacerbations of asthma, which represent a major burden to health care systems around the world. None of our currently prescribed medications for asthma are effective in preventing or reversing asthma development and airway remodelling, nor are they ideal for treating HRV-induced exacerbations of asthma. Thus, it is important to better understand the role of HRV in asthma if we are to develop more effective therapies. In the past decade, we have gained new insights into the role of HRV infections in the development and progression of airway remodelling. We have also gained a new appreciation for the proinflammatory and host defense responses to HRV infections that may help to regulate the susceptibility to asthma exacerbations. In this article we review our current understanding of the role of HRV infections in the pathogenesis of asthma and identify possible avenues to new therapeutic strategies for limiting the effects of HRV infections in asthma.

Abbreviations:

CDHR3:	cadherin-related family member 3
CXCL:	chemokine (C-X-C motif) ligand
ERK:	extracellular-signal-regulated kinase
GSDMB:	gasdermin B
HRV:	human rhinovirus
ICAM-1:	intercellular adhesion molecule-1
IFN:	interferon
ISG:	interferon stimulated gene
LDLR:	low density lipoprotein receptor
MMP-9:	matrix metalloproteinase-9
MUC5AC:	mucin 5AC
NF-κB:	nuclear factor kappa-light-chain-enhancer of activated B cells
NO:	nitric oxide
OR:	odds ratio
ORMDL3:	orosomucoid like 3
RSV:	respiratory syncytial virus
RT-PCR:	reverse transcription-polymerase chain reaction
VEGF:	vascular endothelial growth factor
ZO-1:	zona occludens-1

Introduction

Human rhinovirus (HRV) is a single strand, positive sense RNA virus of the *picornaviridae* family, with an approximately 7.2kb genome enclosed in a protein capsid of roughly 27 nm in diameter. Over 160 strains of HRV have been identified and are classified into 3 genetic clades based on sequence homology ^{1,2}. Seventy four strains are classified as HRV-A, while the HRV-B clade comprises 25 members. The remaining strains belong to the recently discovered HRV-C clade. HRV strains are also classified based upon receptor usage. Eleven members of the HRV-A clade gain cellular entry via interaction with members of the low-density lipoprotein receptor (LDLR) family. The remaining strains of HRV-A and all members of the HRV-B clade bind to intercellular adhesion molecule-1 (ICAM-1) ¹. It has recently been shown that the asthma susceptibility gene product, cadherin-related family member 3 (CDHR3) mediates HRV-C binding and replication ³. A number of studies have compared the association of the three HRV clades with severity of asthma outcomes. In children, studies suggest that either HRV-C only, or both HRV-A and HRV-C, are more commonly associated with exacerbations of asthma requiring medical intervention ^{4,5}. This has been less well studied in adults, but one study found that severity of disease in hospitalized adults was unrelated to viral genotype ⁶.

The airway epithelium is the primary site of HRV infections in both the upper and lower airways ^{7,8}. Because HRV infection does not cause overt epithelial cytotoxicity, it is believed that alterations of epithelial biology are the initiating events in the pathogenesis of HRV infections. In healthy individuals, HRV infections of the upper airways are the major cause of the common cold, and spread of HRV infection to the lower airway epithelium is generally uneventful. In susceptible individuals, however, increases in asthma symptoms do not occur

until several days after peak nasal symptoms, consistent with the concept that subsequent spread of HRV to infect the lower airway epithelium plays a role in triggering acute exacerbations of asthma. Moreover, recurrent HRV infections in early childhood are a risk factor for asthma development, and may also contribute to the initiation and progression of airway remodelling. This article will review the current state of knowledge regarding the role of HRV in each of these aspects of asthma.

HRV and Asthma Onset

Allergic sensitization and virus-induced wheezing are each independent risk factors for asthma development, and the combination of these two factors markedly enhances the likelihood of asthma development.⁹⁻¹¹ Spread of respiratory viral infections to the lower airway is the major trigger for episodic wheezing in children. With the advent of RT-PCR techniques for improved virus detection, it became clear that HRV-induced wheezing illnesses in early life is a greater risk factor for subsequent asthma development than previously appreciated^{11,12}. In one birth cohort at high risk for developing asthma, HRV-induced wheezing illnesses during the first year of life were the strongest predictor of subsequent wheezing in the third year of life. Moreover, children with HRV-induced wheezing illness during the first 3 years of life had an odds ratio (OR) of 9.8 for development of asthma compared to non-wheezers, while respiratory syncytial virus (RSV)-induced wheezing illness was less strongly associated with asthma development (OR = 2.6)⁹. Although a number of studies have shown a role of HRV-induced wheezing as a particular risk factor for asthma development, a recent report found that it is the cumulative number of respiratory episodes in early life, rather than the particular viral trigger, that is associated with subsequent asthma development¹³. This may still be consistent with a

dominant role of HRV infections, however, since HRV is the most common viral pathogen, and children experience recurrent respiratory illnesses due to serial HRV infections ¹⁴.

HRV infection and genetic susceptibility

Repeated HRV infections in early life are common in all children. The additional risk factors that determine which children are susceptible to developing wheezy bronchiolitis and subsequent asthma upon such infections remain unclear, although host genetic susceptibility and key environmental exposures are likely contributors ^{10,11}. Indeed, gene-environment interactions are recognized as critical for the development of asthma. Disease heritability has been estimated to range from 35-90% ¹⁵, and genome wide association studies have identified a number of candidate genes/loci associated with asthma development. Several such studies of childhood-onset asthma identified a susceptibility locus on chromosome 17q21. The disease-linked variants at this locus are associated with the genes, gasdermin B (GSDMB) and orosomucoid like 3 (ORMDL3) ^{15,16}. It has recently been shown that 17q21 variants are associated with asthma in children who had HRV-induced wheezing illnesses (but not RSV-induced wheezing illnesses) and with expression of ORMDL3 and GSDMB ¹⁷. Also of interest in terms of the role of HRV in asthma development, is the recent description that CDHR3 is a susceptibility locus for early childhood asthma with severe exacerbations. The single nucleotide polymorphism in CDHR3 linked to increased exacerbations and hospitalizations leads to mutation of a cysteine residue at position 529 to a tyrosine residue ¹⁸. This same mutation has been shown to be associated with increased binding and progeny yields for HRV-C strains ³. Paradoxically, asthmatic epithelial cells cultured *ex vivo* show lower gene expression of CDHR3 than cells from normal subjects ¹⁹.

Allergic sensitization and HRV-induced wheezing

If genetic susceptibility factors contributing to asthma development are complex, the role of additional environmental factors is equally so. Other potential environmental early life factors linked to asthma development include allergic sensitization, living on a farm, cigarette smoke exposure, antibiotic usage, daycare attendance and impaired lung development¹⁰. Although the concept that combinations of environmental stimuli may contribute to asthma development is logical, the relative contributions of individual environmental stimuli, and the relative timing or duration of various exposures, necessary to lead to the development of asthma are poorly understood (**Figure 1**). As noted above, both allergic sensitization and virus-induced wheezing are independent risk factors for asthma development⁹⁻¹¹. In a high-risk birth cohort study, Markov modeling suggested that allergic sensitization increased the risk of subsequent development of virus-induced wheezing, while the reverse was not the case²⁰. This does not imply, however, that allergic sensitization **must** precede viral wheezing for subsequent development of asthma. Approximately three times more patients experienced viral wheezing by 1 year of age than developed allergic sensitization, and it is unclear how many of those with virus-induced wheeze went on to develop asthma and whether other environmental exposures play a role in determining the risk of asthma development in such children. It should also be noted that the cohort in question may not be representative of the general population, as having one or both parents with respiratory allergies was an entry requirement for the study. Additional studies are needed, therefore, to try to further delineate the temporal and pathogenic interactions of environmental risk factors for asthma development.

HRV and Airway Remodelling/Phenotypic Change

The airways of patients with asthma show characteristic structural changes that are referred to, collectively, as airway remodelling. These changes include reticular basement membrane thickening, increased smooth muscle mass, angiogenesis and an altered epithelial phenotype that is reflected by altered gene expression, impaired barrier function, increased fragility and goblet cell hyperplasia/metaplasia leading to increased mucus production^{21,22}. Combined, these changes narrow the airway lumen and result in a thickened airway wall; changes that are believed to underlie the development and persistence of airways hyperresponsiveness²³. Although the traditional dogma held that remodelling occurred only after years of chronic airway inflammation, it is now clear that structural changes occur early in disease progression. Bronchial biopsy studies demonstrated that several components of remodelling are present in pre-school children with airflow limitation and wheezing, even prior to the formal diagnosis of asthma^{24,25}, implying that remodelling occurs in parallel with inflammation. Interestingly, these structural changes were not observed in infants with symptoms of airflow limitation²⁶, suggesting that remodelling is induced in susceptible individuals in response to some stimuli experienced in early childhood. Children experience serial HRV infections that can lead to recurrent episodes of wheezing illness¹⁴, and, as described above, HRV-induced wheezing is a risk factor for asthma development⁹. This led to the hypothesis that recurrent HRV infections in early life may play a role in the development and progression of airway remodelling in asthma. In support of this concept, infection of cultured human airway epithelial cells with HRV induces gene and protein expression for a range of molecules associated with remodelling processes.

Epithelial changes

A number of studies have shown that HRV infection increases expression of the epithelial mucin, MUC5AC^{19,27,28}. Induction of this protein depends upon activation of the epidermal

growth factor (EGF) receptor and downstream activation of the ERK mitogen activated protein kinase and NF- κ B^{27,28}. Increased epithelial MUC5AC expression was also seen following experimental HRV infection of both normal and asthmatic volunteers²⁸. HRV infection of cultured epithelial cells also reproduces other features of the asthmatic epithelium. These include disruption of normal epithelial barrier function, reflected both by reduction in resistance and by concomitant loss of expression of zona occludens-1 (ZO-1), a component of the junctional plaque of tight junctions²⁹. Reduction of ZO-1 is also observed in both bronchial biopsies and differentiated bronchial epithelial cell cultures derived from patients with severe asthma³⁰. HRV infection also decreased ciliated cell numbers³¹, and expression of ciliogenesis and junction-related genes *in vitro*¹⁹.

Production of growth factors and matrix remodelling

In addition to the modulation of epithelial phenotype, HRV infection also triggers increased epithelial expression of a number of growth factors and proteins linked to other aspects of remodelling. These include activin A and amphiregulin, members of the transforming growth factor- β and EGF families, respectively, that have been linked to subepithelial fibrosis³². Gene expression for other growth factors with similar activity, such as fibroblast growth factor-2 and heparin-binding-EGF is also induced³¹. In parallel with the production of growth factors, HRV-infected epithelial cells also release a number of extracellular matrix proteins³³. Other proteins induced upon HRV infection include matrix metalloproteinase-9 (MMP-9)³⁴, which regulates turnover of extracellular matrix proteins, and vascular endothelial growth factor (VEGF)³², the major angiogenic factor in the airways of asthmatic patients. Both MMP-9 and VEGF are also increased in airway secretions during HRV infections^{32,34}. Although it has been shown *in vitro* that airway smooth muscle cells and fibroblasts also can produce airway remodelling factors and

matrix proteins^{33,35}, there is no direct evidence that these cell types are infected *in vivo*. Thus, the epithelial cell is likely the major source of such proteins *in vivo*. Overall, these data support the concept that recurrent HRV infections may play a role in the initiation and progression of airway remodelling in asthmatic airways (**Figure 2**), but clinical studies are needed to definitively establish effects of HRV infection on airway remodelling *in vivo*.

HRV and Asthma Exacerbations

Asthma exacerbations and associated hospitalizations are a major cause of morbidity, and their management accounts for about 50% of health care costs for the disease. Upper respiratory tract viral infections are associated with at least 80% of acute asthma exacerbations in children and adolescents, and about 60% of exacerbations in adults. In each case, HRV is the dominant viral pathogen, accounting for some 60% of all virus-induced exacerbations^{11,36,37}.

Susceptibility to HRV infection in asthma

The occurrence of HRV-induced lower respiratory tract illnesses in asthmatic, compared to healthy, individuals raised the possibility that the lower airways of asthmatics may be more susceptible to HRV infection, or may experience higher viral loads. However, quantitative studies found no differences in the frequency of lower airway infection, or in viral loads, in sputum between healthy subjects and those with mild asthma^{8,38}. An alternative explanation for the differential symptomatic response may relate to underlying disease status. In healthy subjects with no basal lower airway inflammation, a modest virus-induced inflammatory response may not cause symptoms. By contrast asthmatic subjects already have active inflammation in the lower airways characterized, in most patients, by the presence of Th2 cytokines and eosinophilia. Thus, when virus-induced inflammation occurs in asthmatic

individuals, particularly those with poorly controlled symptoms and active allergic airway inflammation, it may lead to further loss of symptom control, worsening airway inflammation and, ultimately, a clinical exacerbation.

Although results have varied somewhat, a number of studies have shown that HRV can be detected in a significant percentage of patients with stable disease in the absence of exacerbations^{5,39}. The reason for this is unclear. It may be that asymptomatic infections relate to the pathogenicity of various HRV strains, to the presence of pre-existing immunity, or to viral load. The presence or absence of concomitant allergen exposure also may contribute to the level of symptomatic responses. The impact and consequences of HRV infections during stable disease remains to be defined.

Inflammation versus host antiviral defenses

Although HRV infections are linked to exacerbations of asthma, the mechanisms underlying such exacerbations, and the factors that regulate susceptibility of a given individual to experience an exacerbation are poorly understood. As noted, the airway epithelium is the primary site of HRV infection and replication, and plays an active role in regulating airway function and inflammation⁴⁰. HRV infections *in vivo* induce modulation of expression of many epithelial genes⁴¹. These genes encode proteins with a range of functions, including numerous proinflammatory cytokines and chemokines, many of which are detected in increased amounts in airway secretions during HRV infections. Levels of chemokines, such as CXCL8 and CXCL10 correlate with increased numbers of neutrophils and lymphocytes, respectively, observed in airway secretions during HRV infections and disease exacerbations^{42,43}. In addition to proinflammatory products, HRV infections also induce epithelial expression of many molecules

with innate antiviral and host defense activities, including many so-called “interferon (IFN) stimulated genes” (ISGs). Although the major antivirals that contribute to specific innate defenses against HRV still need to be fully elucidated, it seems likely that, in addition to virulence factors that may be associated with individual viral strains, the relative balance of proinflammatory versus host defense proteins produced in any given individual may be one factor in determining the susceptibility to developing an asthma exacerbation (**Figure 3**).

Impaired interferon production

In the context of the importance of this potential balance, it has been reported that epithelial cells from asthmatic patients release lower levels of IFN- β and IFN- λ proteins compared to normal individuals^{44,45}. Although this would be expected to impair antiviral immunity and lead to increased viral replication, it must be noted that the issue of impaired epithelial IFN production is controversial. Other studies have failed to observe any difference in IFN induction in cells from healthy subjects and patients with asthma^{46,47}, and a study of naturally-acquired HRV infections found no difference in levels of viral shedding between children with and without asthma⁴⁸. Moreover, a number of studies have failed to detect IFN- β protein release from HRV infected epithelial cells, despite robust induction of IFN- β mRNA and the induction of numerous ISGs⁴⁹⁻⁵¹. This may appear to be contradictory, but there is strong evidence that viruses can induce ISGs in the absence of IFN signalling⁵².

Implications for clinical practice

Given the major role that HRV infections appear to play in asthma development and exacerbations, additional studies are needed to fully elucidate the mechanisms by which HRV

modulates asthma pathogenesis. Several current anti-inflammatory medications, including corticosteroids and monoclonal antibodies targeting IgE or IL-5, are able to reduce asthma exacerbations⁵³⁻⁵⁵. There is no evidence that these treatments directly affect HRV-induced inflammation, so it is likely that they reduce asthma exacerbations by reducing Th2 inflammation and improving baseline asthma control. The inability of these medications to prevent or reverse asthma development and airway remodelling, however, clearly indicates the need for new therapeutic approaches. In general, three basic approaches have been proposed.

a) Limit HRV infection/replication

The first is to limit HRV infection and/or replication. This approach has been the most studied thus far, but with limited success. The sheer number of distinct HRV strains creates unique challenges in trying to develop vaccine approaches that could impact multiple strains⁵⁶. In addition, drugs aimed at preventing receptor binding, either by targeting receptors or the viral capsid, and drugs inhibiting HRV proteases critical to the viral replication process have met with limited success⁵⁷.

b) Reduce HRV-induced inflammation

The second potential approach to therapy would be to modulate viral stimulation of key proinflammatory pathways, or to block the actions of selected inflammatory mediators. It is unclear, however which aspects of viral inflammation could be targeted for inhibition, as some proinflammatory mediators may also contribute to innate immunity. Studies are needed to gain more insight into how HRV infections trigger asthma exacerbations in order to identify key inflammatory pathways or virus-specific signalling pathways that may be of therapeutic benefit.

c) Increase antiviral responses

Perhaps the most conceptually promising approach for treatment is to enhance key epithelial innate antiviral responses. This could be done with a broad based induction of antiviral pathways, or by selectively targeting key aspects of the host antiviral response to HRV infection. Regardless of the controversy concerning impaired IFN responses to HRV infection in asthma, the role of IFNs in antiviral immunity is universally accepted. Several studies in the 1980s showed that type I IFNs can prevent HRV infections if administered prophylactically, and reduced symptoms if given soon after infection; however the use of high doses of IFNs in these early studies led to unwanted adverse effects⁵⁷. A recent study has revisited the use of type I IFN in patients with asthma and a history of exacerbations after colds. The authors used a dose of IFN- β that was substantially lower than those used in earlier studies of HRV infections but that still induced markers of antiviral responses in the airways. Although no significant effects were seen on the primary outcome in the intention-to-treat analysis, subgroup analysis suggested some beneficial effects in the most difficult to treat patients⁵⁸. Additional studies are needed to further validate these observations.

Although a global elevation of antiviral responses is one approach, it may also be feasible to enhance expression of, or exogenously supplement, molecules important in the specific antiviral response to HRV. For example, studies have shown that viperin and nitric oxide (NO) each inhibit HRV replication in epithelial cells^{41,59}, while NO can also reduce HRV-induced chemokine production⁵⁹. Thus, topical administration of NO donor compounds may represent a potential approach to enhancing antiviral immunity to HRV infections. Additional work is needed to fully define other “key players” in host defense to HRV infection but as we gain a greater understanding of host defense pathways, additional therapeutic approaches to treat HRV-induced asthma exacerbations may be revealed.

Conclusions

The past decade has provided important new insights into the host proinflammatory and host defense responses to HRV infection, and has suggested a number of potential factors that may determine susceptibility to asthma exacerbations. These include potential variations in HRV genotype specific virulence, such as the clinical impact of HRV-C and its likely receptor CDHR3, as well as potential impairments in host innate immunity. Additional studies are needed to fully define key aspects of host antiviral immune responses to HRV infection and to establish if HRV infections can contribute to airway remodelling *in vivo*. A number of potential approaches have been suggested to regulate outcomes to HRV infection including regulating allergic airway inflammation to ensure optimal control of disease, blocking key inflammatory pathways, or boosting antiviral responses. Identifying potential therapies and evaluating them in appropriate models will be a key goal in the coming years. Given that there is no predictive animal model for responses to HRV infection, such studies will need to utilize either human experimental infection models or natural exacerbations in patients with asthma.

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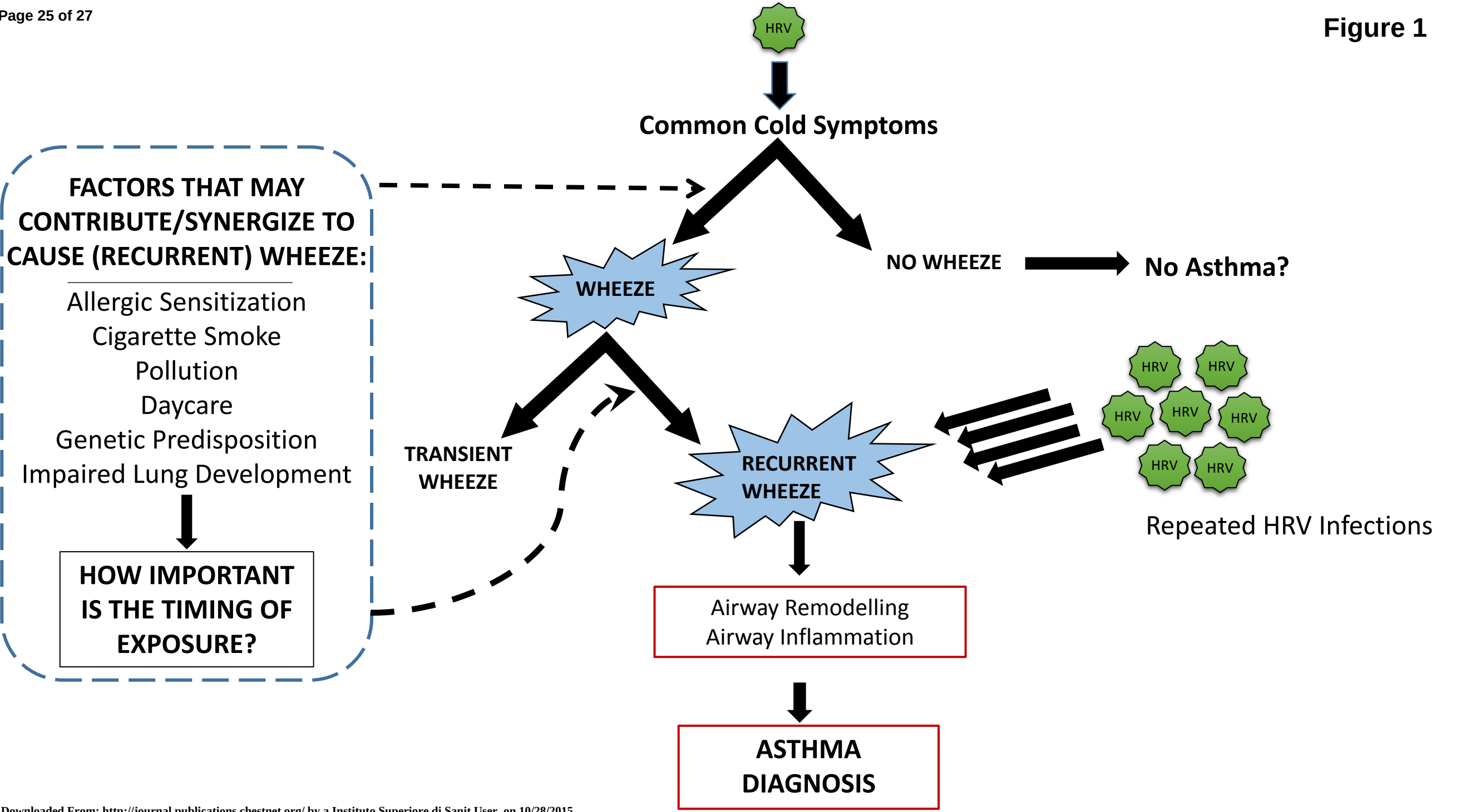
Figure Legends

Figure 1: Role of HRV Infections in Asthma Development. In all children, HRV infections lead to symptoms of the common cold. In susceptible individuals, however, such infections lead to wheezing episodes. A number of genetic, developmental and environmental factors are believed to contribute to wheezing, or to act in concert with wheezing to increase the risk of asthma development. Wheezing may be transient or recurrent, and recurrent wheezing, in particular, is strongly associated with induction of airway remodelling, inflammation and subsequent diagnosis of asthma.

Figure 2: Role of HRV Infections in Development of Airway Remodelling. Growing evidence suggests that HRV infection of epithelial cells causes alterations of epithelial phenotype and triggers the release of matrix proteins and mediators that are linked to various aspects of airway remodelling. In susceptible individuals, recurrent HRV infections could lead to progression of airway remodelling, resulting in a permanently altered epithelial phenotype that can contribute to goblet cell hyperplasia and increased mucus secretion, subepithelial fibrosis, angiogenesis and increased smooth muscle mass.

Figure 3: HRV Infections and Asthma Exacerbations. HRV infection of epithelial cells can trigger both proinflammatory and host antiviral responses. Proinflammatory mediators can recruit a range of inflammatory cells that may be linked to symptom development (e.g. neutrophils) or may contribute to host innate and adaptive immunity. The balance between host antiviral and proinflammatory responses may be one factor that governs the susceptibility of an individual to experience an HRV-induced asthma exacerbation.

Figure 1



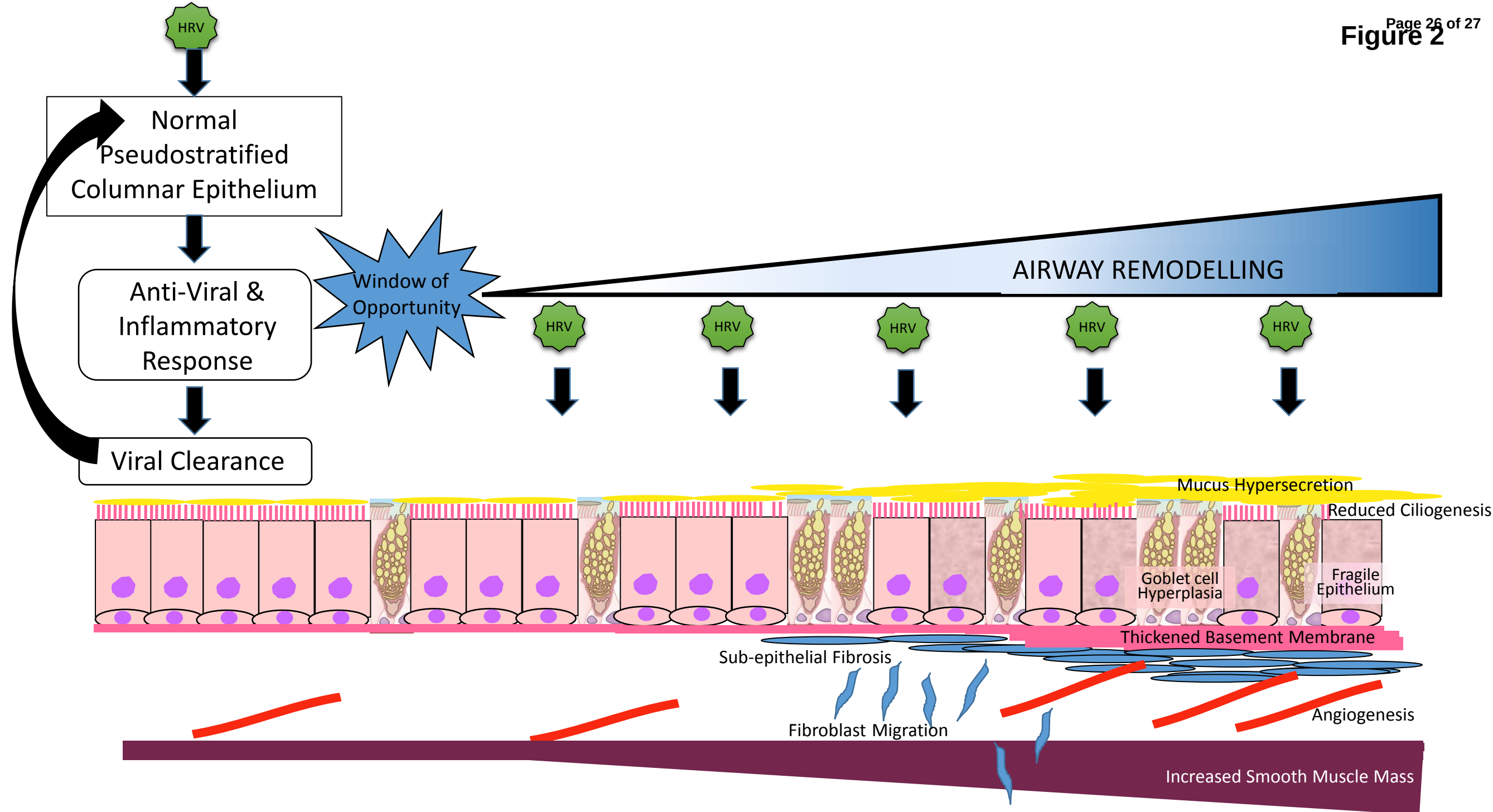


Figure 3

