

How Rhinovirus Infections Cause Exacerbations of Asthma

James E. Gern MD

Professor of Pediatrics and Medicine

University of Wisconsin-Madison

Address correspondence to:

K4/918 CSC, 600 Highland Avenue

Madison, WI 53792-9988

Phone 608 263-6201

Fax 608 265-2207

Email gern@medicine.wisc.edu

Abstract

Rhinovirus (RV) infections are closely linked to exacerbations of asthma, and yet most RV infections of patients with asthma cause only upper respiratory symptoms. These findings suggest that RV and other viral infections contribute to the causation of acute exacerbations of asthma, but that additional cofactors are generally required. In fact, factors related to the host, virus, and environment have been identified that affect the severity of RV infections, and propensity to develop lower respiratory tract symptoms. This review will discuss these factors and how their effects may act alone or in combination to increase the probability of RV-induced exacerbations of asthma.

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Introduction

Rhinoviruses (RVs) are frequently detected in respiratory secretions during acute exacerbations of asthma. These relationships were noted many years ago, but became clearer with the advent of molecular diagnostics. Studies conducted in the 1990s to date have reported that viruses can be detected in 60-90% of exacerbations in children and 50-80% of exacerbations in adults. Control specimens obtained from the same subjects during periods of health or from healthy individuals had lower rates of RV detection. RVs account for the majority of viruses identified, and in case control studies RV infections, but not infections with other viruses, were significantly associated with exacerbations. Together these findings strongly suggest that RV infections cause the majority of exacerbations of asthma.

More recent studies utilizing prospective monitoring of nasal secretions of school-aged children during high prevalence months indicate that RV infections are nearly ubiquitous in children regardless of asthma. Many of these infections are either asymptomatic or mild, even in children with asthma. When considered together, these findings strongly suggest that viral infections, most often due to RV, are necessary but not often sufficient to cause acute exacerbations of asthma. These findings also suggest that there are cofactors that either increase the severity of RV infections, or else have additive effects on airway physiology to promote airway obstruction and acute symptoms of asthma.

Of course, exposure to pollutants, irritants and allergens are known to cause acute exacerbations of asthma, along with acute stress and nonadherence with asthma controller therapy. Recently, studies of upper and lower airway secretions have also provided evidence that infections with bacteria can also contribute to acute wheezing illnesses and possibly even the pathogenesis of asthma. Finally, viral factors need to be considered, especially since there are three RV species containing approximately 160 types that have considerable genetic

variation. In the sections that follow, effects of virus, host and environment to the pathogenesis of virus-induced exacerbations of asthma will be considered.

Viral factors

The RV family tree. RVs were first discovered in the 1950s. At the time, RV serotypes were identified by inoculation of nasal secretions into cultured cells, observation of cytopathic effects, and then using serologic neutralization techniques to establish the identity of specific isolates.

The viruses numbered approximately 100 by the 1980s, and were classified into A and B species viruses based on susceptibility to antiviral drugs and partial genetic sequences. In 2006, the first C species virus was reported after a virus detected by molecular techniques was found to have distinct sequence characteristics [1, 2]. Additional studies using similar approaches reported a plethora of new C types, as well as a few additional A and B types [3-5]. The 50-year delay in the recognition of the C types was due to the inability to culture these viruses, and it is clear that these viruses have been circulating for many years [6]. RV-C viruses have now been grown in organ culture [7] and in airway epithelial cells differentiated at air-liquid interface (ALI) [8, 9]. In addition, stocks of C types can be produced by reverse genetics techniques: viral sequences are cloned into bacterial plasmids, and RNA transcribed from these plasmids can be transfected into HeLa or other cell lines to produce infectious virus [10]. C utilizes a unique receptor to enter cells,[10] and the identity of this receptor is so far unknown. To date, there are no cell lines (other than ALI cells) that are known to express the RV-C receptor ([8, 9] and unpublished findings).

Nearly all of the A and B species RVs were sequenced in 2009 [11], and over 350 complete or nearly complete sequences have been published since then. Many of these sequences were obtained using shotgun sequencing directly from genetic material extracted from nasal secretions [12-14]. These studies have clearly established the three species of viruses within

the enterovirus genus, and demonstrate that the RV species are about as different from each other as they are from other enteroviruses (Figure 1). Structural models based on sequence analysis of capsid proteins show that RV-C types compared to other RVs have a relative loss of mass in the VP1 capsid protein in the putative receptor binding region, consistent with the fact that it binds to a unique receptor [15, 16].

RV species and virulence. The high degree of genetic diversity suggests that there could be type- or species-specific effects on severity of RV illness and/or exacerbations of asthma. This hypothesis has been tested in a variety of settings around the globe. In hospitalized children or those who present to acute care settings for acute respiratory illness or exacerbations of asthma, RV-C and in some studies RV-A appear to be overrepresented compared to the prevalence of these viruses in children who are healthy or who have cold symptoms [6, 17-24].

Routine monitoring of infants through age one year who were participating in the Childhood Origins of Asthma (COAST) demonstrated that RV-A and RV-C infections were 7-8 times more likely than RV-B infections to cause moderate to severe respiratory illness [25]. Notably, the majority of RV infections regardless of species produced mild or asymptomatic illness, and RV-B viruses very rarely were detected in children with more significant illnesses. Finally, young children who experience a wheezing illness due to RV-C compared to other viruses are more likely to develop recurrent wheezing [18].

There are many genetic and structural differences among the RV species, and there are emerging data to explore mechanisms of differential virulence. Most RV grow best at cooler temperatures (33-35°C) [26, 27], which likely limits their replication in the lungs. Temperatures in the nose and in large and medium airways are conducive to RV growth [28], but warmer temperatures in the smaller airways and parenchyma may limit the potential for RV LRI, as has been observed with vaccine strains of influenza viruses that are “cold adapted”. In contrast,

three types of RV-C (C2, C15 and C41) were tested for temperature sensitivity in ALI cultures, and all of the viruses grew equally well at 33, 35, and 37°C [8]. The lack of inhibition at warmer temperatures may promote increased risk for RV-C to cause LRI and wheezing illnesses. In addition, the relative virulence of three examples of each RV species was recently examined *in vitro*. RV growth characteristics *in vitro* may not always reflect their performance *in vivo*, in part because laboratory strains of RV readily mutate and adapt to specific cell lines, and also because the responses of cell lines can differ markedly from those of differentiated and nontransformed airway epithelial cells, which serve as the primary host for RV infections. To minimize artifacts, Nakagome et al cloned 7 different viruses from clinical isolates, and produced viral stocks in nontransformed pulmonary fibroblasts [29]. Two additional types were grown from clinical specimens in the same pulmonary fibroblasts. By inoculating these viral stocks into ALI cells, he showed that RV-B replication occurred more slowly and was assessed with reduced cytopathic effects and cell lysis compared to RV-A and RV-C. In addition, RV-B induced lower levels of cytokines, including IFN- λ 1. The lower induction of IFN- λ 1 remained statistically significant even after accounting for lower levels of RV-B replication.

RV encode two proteases (2A and 3C) that help to process structural and nonstructural proteins of the virus, and also interfere with cellular metabolism to the advantage of the viral replication cycle. For example, 2A protease cleaves the host cell ribosomal protein elongation initiation factor 4G (EIF4G) which cripples cellular but not viral translation, and also cleaves nuclear pore proteins that are necessary for transcription factors to enter the nucleus and mRNA to depart for the cytoplasm. In fact, there is considerable species-specific variation in the structure and function of 2A protease that may affect RV virulence. Watters et al cloned 2A proteases from multiple RV types, and tested their relative effects on cleavage of host cell proteins [30]. Interestingly, the 2A proteases from RV-B types were least adept at cleaving EIF4G and nuclear

protein-63. These findings suggest that biochemical differences in the RV-B 2A protease could contribute to reduced virulence.

When considered together, these data suggest that lower rates of RV-B replication and cytokine induction could contribute to reduced virulence. Species-related biochemistry of the 2A protease corresponds with reduced replication and inflammatory responses. Finally, the relative insensitivity of RV-C to temperature may give this virus an advantage in causing lower airway infections.

How do RV infections cause respiratory symptoms? RV infections are likely to cause respiratory illness through several mechanisms. First, RV replication causes cell lysis, which is the principal method for releasing progeny virus into the environment. Even so, most RV infections appear to infect a small subset of cells, and cell lysis is generally not extensive. Studies to map infected cells in the airway have generally found evidence of patchy involvement, and this corresponds to 1-5% infection rate of epithelial cells in vitro even after inoculation with high titers of virus [31, 32], even in the absence of mononuclear cell interferon production. Despite the low number of cells infected, RV can impair epithelial cell barrier function [33].

In addition to damaging the epithelium, RV infections also induce immune responses that have antiviral activities, but also contribute to signs and symptoms of a cold. For example, therapy with exogenous interferon can cause malaise and myalgia, and other cytokines induced by RV infections such as IL-1 β can have similar effects. RV causes neural activation to promote sneezing, sore throat and cough through mechanisms that are likely to involve RV-induced cytokines, as well as mediator release from infected cells and leukocytes that are recruited into the airway. RV-infected epithelial cells produce a variety of chemokines [34, 35], which together with cytokine-induced adhesion molecules promote the recruitment of neutrophils and

mononuclear cells. Chemokine secretion can be potentiated by interferons originating in other cells.[36] RV infections also induce release of kinins, prostaglandins, and other mediators, which are also likely to contribute to respiratory illness signs and symptoms [37, 38]. Histamine does not appear to play a significant role in common cold pathogenesis [39]. Additional work is needed to better understand which mediators are induced by RV infections, and their specific roles in pathogenesis.

Analysis of nasal secretions reveals that rhinorrhea during the early part of the cold is due to transudation of serum fluids and proteins (e.g. albumin and IgG) [40]. The transudation and nasal secretion may be initiated by neural mechanisms and pro-inflammatory cytokines. Certain serum proteins (e.g. low-density lipoprotein) may stimulate additional chemokine secretion by epithelial cells [41]. During the latter stages of the acute illness, mucus secretion is increased [40].

RV and lower respiratory illnesses. For years after their discovery RV infections were presumed confined to the upper airway, but there is now conclusive evidence that lower airway infections can also occur and are in fact relatively common. Supporting clinical evidence includes detection of RV as the sole pathogen in upper, and in some cases lower, respiratory secretions of infants and children with pneumonia [42]. Experimental inoculation studies provide a model for obtaining frequent samples that are timed after administering a uniform dose of RV to the upper airways of seronegative volunteers. In these studies, RV is often detectable in secretions from both the upper and lower airways. Upper airway viral shedding peaks 2-4 days following inoculation, and in subjects with lower airway infections, there here appears to be a short time lag (1-2 days) in the kinetics of lower airway viral shedding [43, 44]. In some study subjects, viral shedding in sputum exceeds that in the nasal secretions. Biopsy specimens from large lower airways RV infected cells in large lower airways, and the patchy distribution in the lower

airways resembles that found in studies of upper airways [45]. Quantitative studies including normal individuals and those with mild asthma demonstrate that comparable amounts of RV can be detected in upper and lower airway cells and secretions in about half of the study subjects, whether or not asthma is present [32, 43]. Finally, children with tracheostomies were studied during periods of respiratory illness, and RV was found in lower airway secretions with at least the same frequency as was found in nasal secretions [46].

Once virus makes its way to the lower airways, it is likely that RV infection induces similar pathology to that observed in the upper airway during the common cold, and increased airway secretions, edema, and cellular inflammatory responses may all contribute to airway obstruction. It is also likely that RV infections induce neuroinflammatory responses that contribute to bronchoconstriction and airway hyperresponsiveness, although experimental data in this area are lacking.

Host factors

A number of host factors have been linked to more severe respiratory illnesses. Examples include extremes in age, immunocompromised individuals, and those with chronic respiratory diseases such as asthma, chronic obstructive lung disease, and cystic fibrosis. Variations in immune responses of epithelial cells and leukocytes have been studied extensively with respect to illness outcomes.

Epithelial cell responses. The airway epithelium is the first line of defense against RV infections. Mucins, antimicrobial peptides, and surfactant proteins in the mucus layer nonspecifically deter infection [47]. In addition, well-differentiated epithelial cells are relatively resistant to RV infection [48]. The epithelium may serve as a barrier against RV infection; RV replication is enhanced when apical cells of well-differentiated epithelial cell cultures are either damaged or stripped

away [49]. The epithelial barrier in asthma may be compromised, and allergic inflammation and exposure to pollutants may exacerbate this condition. It is therefore possible that reduced barrier function in asthma could promote more severe RV infections. RV infection itself can also disrupt epithelial barrier function and perhaps this effect contributes to secondary bacterial infection [33].

Interferon responses and asthma. Interferons are one of the cornerstones of antiviral defense, and function by inducing networks of antiviral effectors within airway epithelial cells, and by potentiating antiviral activities of leukocytes and stromal cells in the lung [50, 51]. In clinical studies, the strength of the interferon response has been linked to viral respiratory illness severity. In infancy, low mitogen- or virus-induced IFN- γ responses from blood mononuclear cells are a risk factor for more severe respiratory illnesses and wheezing illnesses [52, 53]. Similar relationships have been reported in children with low IFN- α responses [54], and in infants with reduced numbers of circulating plasmacytoid dendritic cells (pDCs) [55], the principal blood cell source for IFN- α . In models of experimental infection with a safety tested strain of RV, strong IFN- γ responses to virus in blood mononuclear cells or sputum cells were associated with reduced viral shedding [56], milder cold symptoms and more rapid clearance of the virus [57].

These findings suggest that reduced interferon responses could contribute to RV-induced exacerbations of asthma, but the nature and mechanisms of deficient interferon responses and asthma is an area of controversy. In some studies, interferon responses of peripheral blood mononuclear cells [58, 59] and pDCs [60] are reduced in asthma. It has also been reported that RV-induced epithelial cell production of IFN- β and IFN- λ *ex vivo* is also impaired in asthma [61, 62], raising the possibility that asthma is associated with a global defect in interferon production.

However, other studies have reported that asthma does not have significant effects on interferon responses in cultured epithelial cells [63-65]. Moreover, an observational study of naturally-acquired colds found similar viral shedding in children with vs. without asthma [65], while studies of experimentally inoculated volunteers are mixed [43, 65-67].

Additional studies have provided insights into the controversy as to whether or not interferon responses are blunted in asthma. For example, deficient interferon responses may be related to more severe asthma, because interferon responses of subjects with mild asthma appear to be normal [67]. In addition, deficient interferon responses could be compartmentalized to the lung. Comparisons of ex vivo stimulation of mononuclear cells obtained from the blood vs. bronchial lavage from subjects with asthma indicate that types I and III interferon responses are particularly suppressed in airway cells [68]. This finding suggests that asthma-related airway inflammation (potential mechanisms are discussed in the next section) inhibits antiviral responses or promotes virus-induced inflammation to worsen clinical outcomes. This theory could account for variable findings when cells are studied ex vivo, removed from influences of local airway factors. Another point to consider is that relationships between interferon quantity and clinical outcomes in asthma may depend on quantity and stage of infection; too little interferon during the early phases of infection may lead to increased viral replication, while excessive interferon secretion during the acute phase of illness could add to the burden of symptoms [69]. In fact, there is evidence that interferons may be increased in the respiratory secretions of patients with exacerbations compared to those with uncomplicated colds during times of peak symptoms [70, 71]. Finally, many studies of the relationships between interferon responses and outcomes were conducted using a single RV type (RV-A16); additional studies are needed to determine whether relationships could vary by type or species.

Animal models provide an opportunity to identify mechanisms and test new hypotheses that are difficult to address in human studies of viral respiratory illnesses and asthma. In rodent models, reduced interferon responses are associated with increased severity of the acute viral respiratory illness [72], and can promote chronic pulmonary dysfunction following resolution of acute viral illness. For example, Sendai virus infection of young animals can induce recurrent airway obstruction, chronic airway remodeling, and airway hyperresponsiveness [73, 74], and these effects can be prevented by interferon administration [75]. Mouse models of RV infection underscore the importance of type I interferon responses and interferon-induced genes such as IL-15 to control RV replication [76, 77], and also suggest that early life infections with RV can cause airway structural changes (mucus metaplasia) and airway hyperresponsiveness that can last for weeks [78]. These models may provide opportunities to test interventions targeting acute and chronic effects of VRI on lung function.

Inflammatory responses and allergy. Asthma is a disease of chronic airway inflammation, and there is evidence that airway antiviral responses may be secondarily hindered by preexisting airway inflammation. Detailed studies of airway cells and secretions following experimental inoculation with RV have demonstrated that asthma is associated with a greater cellular response to RV infection, including increased neutrophils and CD68-positive macrophages within the airway epithelium [79]. In an observational study of naturally acquired colds, sputum neutrophils were not different in asthma, but exacerbations in the asthma group were associated with greater neutrophilia compared to uncomplicated colds [80].

Respiratory allergy is also a major risk factor for wheezing with RV infections later on in childhood and in adults. In children presenting to an emergency department, individual risk factors for developing wheezing included detection of a respiratory virus, most commonly RV, the presence of allergen-specific IgE, and evidence of eosinophilic inflammation [81]. These

findings provide strong evidence of a synergistic relationship between respiratory allergies, eosinophilia, and acute virus-induced wheezing.

In vitro models indicate that allergic inflammation can inhibit innate immune interferon responses under some conditions [82]. Plasmacytoid dendritic cells are potent sources for type I and III interferons, and are responsive to allergic inflammation because they express the high-affinity IgE receptor (FcεRI) on the cell surface. Notably, cell surface expression of FcεRI is inversely related to the virus-induced interferon secretion [60]. Furthermore, cross-linking FcεRI markedly impairs interferon responses to influenza virus or RV [60, 83], suggesting that the combination of allergy and allergic inflammation inhibits antiviral responses at the mucosal surface. Transcriptional analysis of blood cells during exacerbations in adults provides evidence that IgE receptors are upregulated on monocytes and dendritic cells, and further implicate alternatively activated macrophages as a source of type-2 cytokines during these episodes [84].

Asthma. Observational studies suggest that RV infections in adults indicate that asthma effects on RV infections may be specific for the lower airway; RV colds do not differ in asthma, but lower airway symptoms are worse [85]. This finding suggests that local conditions in the lower airways may account for the difference in illness severity, rather than a systemic impairment of antiviral responses. There are several features of the lower airway that may contribute to increased airway obstruction during RV infections. Structural and physiologic changes associated with asthma include hyperplasia of mucus-secreting cells in the airway, hyperemia, airway responsiveness and airway narrowing [47, 86], and each of these changes could promote more severe manifestations of RV lower respiratory illness. Accordingly, RV effects on mucus secretion could be worse in an airway with more mucus secreting cells, edema and

transudate could be enhanced in the presence of hyperemia, and bronchospasm accentuated in hyperreactive airways. Collectively, these effects could promote increased airway narrowing and closure, leading to reduced airflow and respiratory compromise.

Environmental factors

Pollutants. A number of environmental factors can contribute to the severity of RV illness and exacerbations of asthma. For example, exposure to pollutants such as sulfur dioxide and nitrogen oxide can increase the risk of common cold-induced exacerbations [87]. In children, exposure to high concentrations of nitrogen dioxide increased the subsequent risk of virus-induced exacerbations of asthma [88]. Furthermore, a case-control study of hospitalized adults linked RV-induced exacerbations to cigarette smoking and non-use of inhaled corticosteroids [89]. In children, virus-induced exacerbations in an emergency department were associated with passive smoke exposure and allergy [90].

Allergen exposure. Throughout this review are numerous references to the fact that allergy is a risk factor for virus-induced wheezing and exacerbations of asthma. In a case control study involving groups of children with acute asthma exacerbations, stable asthma and inpatients with other conditions, being sensitized and highly exposed to an allergen was a strong risk factor for virus-induced exacerbation [91]. Similar relationships have been reported with the combination of allergy and allergen exposure in adults [92].

Airway bacteria. There is mounting evidence that the airway microbiome of asthma is distinct, and that airway bacteria and viruses may both contribute to acute symptoms of asthma. For example, culture-independent methods to assess microbial colonization of the airways demonstrate that Proteobacteria, a phylum of bacteria containing a majority of gram-negative bacteria, are overrepresented in both nasal and bronchial samples in stable asthma, and are

linked to increased bronchial hyperresponsiveness [93, 94]. Furthermore, detection of *Streptococcus pneumoniae*, *Haemophilus influenzae* and *Moraxella catarrhalis* within the upper airway in early infancy is associated with an increased risk of developing recurrent wheezing and then asthma [95]. These same bacteria have been linked to acute wheezing illnesses in young children, and the strength of the association with wheezing is similar to that observed with viruses [96]. Prospective monitoring of nasal secretions from children with and without asthma during the fall indicates that RV infections are associated with an increase in detection of these common bacterial pathogens [97]. Moreover, detection of either *S. pneumonia* or *M. catarrhalis* together with RV was associated with greater illness severity, and an increased risk of developing virus-induced exacerbations of asthma, compared to infection with RV infections alone.

Vitamin D. Vitamin D has attracted attention recently because of studies linking low vitamin D levels to both asthma risk and increased numbers of respiratory illnesses [98, 99]. In addition, the severity of RV infections in infants peaks during the winter months [25], when vitamin D levels are at their nadir. Mechanisms for vitamin D effects on respiratory illnesses have been explored. Vitamin D does not affect RV replication [100], but can modulate interferon and other cytokine responses [101]. Since vitamin D induces antibacterial peptides, it is also possible that vitamin D affects respiratory illness severity by reducing the probability of secondary bacterial infection or invasion.

Stress. Stress is a well-recognized trigger for acute symptoms of asthma, and may exert these effects through neural pathways or perhaps by altering cortisol responses. Several studies have provided evidence that stress increases the severity of natural and experimentally-induced colds [102, 103]. Whether the effects of stress are additive or potentiate virus-induced effects on airway obstruction and exacerbations of asthma has yet to be determined.

Implications for novel therapies.

For the majority of patients with asthma, exacerbations are multifactorial. RV and other viral infections are involved in most exacerbations, especially those leading to hospitalization. On the other hand, the majority of RV infections are well tolerated, and additional factors are generally required for exacerbations to occur. Some of these cofactors may simply be additive, while others (like allergic inflammation) may have mechanistic interactions with viral illnesses and exert synergistic effects. Lack of use of an asthma controller or more severe or unstable asthma is likely to lower the threshold for environmental insults to cause an exacerbation.

The complexity inherent in the multifactorial pathogenesis of virus-induced exacerbations also presents opportunities for novel therapeutic approaches. The combination of corticosteroids and inhaled bronchodilators are the standard treatment of exacerbations of asthma, but provide incomplete relief for virus-induced exacerbations and can be associated with unfavorable side effects. Perhaps elimination or amelioration of one or more cofactors could reduce the risk of RV-induced exacerbations, and several interventional studies suggest that this is the case.

Omalizumab specifically targets the Fc portion of IgE to prevent binding to the surface of cells, and is therefore a narrowly focused intervention for type I hypersensitivity. In a placebo-controlled trial of guidelines-based asthma treatment compared to omalizumab added to standard therapy, omalizumab prevented the seasonal increases in exacerbations during the fall and spring, which are peak times for viral exacerbations [104]. Analysis of viruses in nasal secretions during a subset of exacerbations confirmed that the treatment group had fewer viral and nonviral exacerbations. This study provides direct evidence that IgE-mediated inflammation contributes to the risk of virus-induced exacerbations of asthma. It will be of interest to determine whether other drugs targeting specific type-2 cytokines (e.g. mepolizumab and IL-5 [105]) can also reduce the risk of virus-induced exacerbations.

Another new approach has been to boost antiviral defenses in the lung with inhaled IFN- β . In a randomized study, subjects with persistent asthma and a history of exacerbations with colds were treated with either nebulized IFN- β or placebo within 24 hours of the onset of cold symptoms [106]. In the intent-to-treat population, there were no significant effects on the asthma symptoms scores (which was the primary outcome), but IFN- β treatment improved recovery of peak expiratory flow. Notably, IFN- β was well tolerated, and also induced expression of innate antiviral effectors in the blood and sputum. In a subgroup analysis of study subjects with more severe asthma (BTS Step 4 and 5), colds were associated with increased symptoms in the placebo group but not in IFN- β -treated subjects. These exciting new findings, if confirmed, suggest that inhaled IFN- β used at the first sign of a cold could be a useful adjunct to standard therapy in patients with more severe asthma.

Conclusions. Risk factors for virus-induced exacerbations are becoming better defined, yet additional studies are required to inform mechanisms that promote more severe colds and airway obstruction. Which of the cofactors leads to enhanced viral replication or augments inflammatory responses? Are there other interactive mechanisms to be considered? New approaches are needed to fully appreciate how the various risk factors promote virus-induced exacerbations of asthma. Systems biology has provided us with tools to describe the transcriptome, epigenome, metabolome and proteome; similar advances in “environomics” are also needed [107]. These advances are likely to provide additional insights into the multifactorial causation of asthma exacerbations, and may lead to more effective approaches to the prevention or treatment of virus-induced exacerbations of asthma.

Figure Legends

Figure 1. Circle phylogram of relationships for currently recognized genotypes [108] of RV-A, RV-B and RV-C. The tree was calculated with neighbor-joining methods from aligned, VP1 RNA sequences, and rooted with data from four enteroviruses (EV) of the EV-A, EV-B and EV-C species, similar to ref [109]. The Major ("M", ICAM-1) and minor ("m", LDLR) receptor groups are indicated if determined experimentally. The RV-C receptor is unknown. Bootstrap values (percent of 200 replicates) are indicated at key nodes. Used with permission from reference [110].



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3. Lamson D, Renwick N, Kapoor V, Liu Z, Palacios G, Ju J, Dean A, St GK, Briesse T, Lipkin WI, MassTag polymerase-chain-reaction detection of respiratory pathogens, including a new rhinovirus genotype, that caused influenza-like illness in New York State during 2004-2005. *J Infect Dis* 2006;194: 1398-402.
4. Kistler A, Avila PC, Rouskin S, Wang D, Ward T, Yagi S, Schnurr D, Ganem D, DeRisi JL, Boushey HA, Pan-viral screening of respiratory tract infections in adults with and without asthma reveals unexpected human coronavirus and human rhinovirus diversity. *J Infect Dis* 2007;196: 817-25.
5. Lee WM, Kiesner C, Pappas T, Lee I, Grindle K, Jartti T, Jakiela B, Lemanske RF, Shult PA, Gern JE, A diverse group of previously unrecognized human rhinoviruses are common causes of respiratory illnesses in infants. *PLoS ONE* 2007;2: e966.
6. Linder JE, Kraft DC, Mohamed Y, Lu Z, Heil L, Tollefson S, Saville BR, Wright PF, Williams JV, Miller EK, Human rhinovirus C: Age, season, and lower respiratory illness over the past 3 decades. *J Allergy Clin Immunol* 2013;131: 69-77 e1-6.
7. Bochkov YA, Palmenberg AC, Lee WM, Rathe JA, Amineva SP, Sun X, Pasic TR, Jarjour NN, Liggett SB, Gern JE, Molecular modeling, organ culture and reverse genetics for a newly identified human rhinovirus C. *NatMed* 2011;17: 627-32.
8. Ashraf S, Brockman-Schneider R, Bochkov YA, Pasic TR, Gern JE, Biological characteristics and propagation of human rhinovirus-C in differentiated sinus epithelial cells. *Virology* 2013;436: 143-9.
9. Hao W, Bernard K, Patel N, Ulbrandt N, Feng H, Svabek C, Wilson S, Stracener C, Wang K, Suzich J, Blair W, Zhu Q, Infection and propagation of human rhinovirus C in human airway epithelial cells. *J Virol* 2012;86: 13524-32.
10. Bochkov YA, Palmenberg AC, Lee WM, Rathe JA, Amineva SP, Sun X, Pasic TR, Jarjour NN, Liggett SB, Gern JE, Molecular modeling, organ culture and reverse genetics for the emerging respiratory pathogen human rhinovirus C. *Nature Medicine* 2011;17: 627-32.
11. Palmenberg AC, Spiro D, Kuzmickas R, Wang S, Djikeng A, Rathe JA, Fraser-Liggett CM, Liggett SB, Sequencing and Analyses of All Known Human Rhinovirus Genomes Reveals Structure and Evolution. *Science* 2009;324: 55-59.
12. Liggett SB, Bochkov YA, Pappas T, Lemanske RF, Jr., Gern JE, Sengamalay N, Zhao X, Su Q, Fraser CM, Palmenberg AC, Genome sequences of rhinovirus A isolates from wisconsin pediatric respiratory studies. *Genome announcements* 2014;2.
13. Liggett SB, Bochkov YA, Pappas T, Lemanske RF, Jr., Gern JE, Sengamalay N, Zhao X, Su Q, Fraser CM, Palmenberg AC, Genome sequences of rhinovirus B isolates from wisconsin pediatric respiratory studies. *Genome announcements* 2014;2.
14. Liggett SB, Bochkov YA, Pappas T, Lemanske RF, Jr., Gern JE, Sengamalay N, Zhao X, Su Q, Fraser CM, Palmenberg AC, Genome sequences of rhinovirus C isolates from wisconsin pediatric respiratory studies. *Genome announcements* 2014;2.
15. Basta HA, Ashraf S, Sgro JY, Bochkov YA, Gern JE, Palmenberg AC, Modeling of the human rhinovirus C capsid suggests possible causes for antiviral drug resistance. *Virology* 2014;448: 82-90.
16. Basta HA, Sgro JY, Palmenberg AC, Modeling of the human rhinovirus C capsid suggests a novel topography with insights on receptor preference and immunogenicity. *Virology* 2014;448: 176-84.
17. Bizzintino J, Lee WM, Laing IA, Vang F, Pappas T, Zhang G, Martin AC, Geelhoed GC, McMinn PC, Goldblatt J, Gern JE, Le Souef PN, Association between human rhinovirus C and severity of acute asthma in children. *Eur Respir J* 2011;37: 1037-42.
18. Cox DW, Bizzintino J, Ferrari G, Khoo SK, Zhang G, Whelan S, Lee WM, Bochkov YA, Geelhoed GC, Goldblatt J, Gern JE, Laing IA, Le Souef PN, Human rhinovirus species C

- infection in young children with acute wheeze is associated with increased acute respiratory hospital admissions. *Am J Respir Crit Care Med* 2013;188: 1358-64.
19. Iwane MK, Prill MM, Lu X, Miller EK, Edwards KM, Hall CB, Griffin MR, Staat MA, Anderson LJ, Williams JV, Weinberg GA, Ali A, Szilagyi PG, Zhu Y, Erdman DD, Human rhinovirus species associated with hospitalizations for acute respiratory illness in young US children. *J Infect Dis* 2011;204: 1702-10.
 20. Mak RK, Tse LY, Lam WY, Wong GW, Chan PK, Leung TF, Clinical spectrum of human rhinovirus infections in hospitalized Hong Kong children. *Pediatr Infect Dis J* 2011;30: 749-53.
 21. Drysdale SB, Alcazar M, Wilson T, Smith M, Zuckerman M, Lauinger IL, Tong CY, Broughton S, Rafferty GF, Johnston SL, Greenough A, Respiratory outcome of prematurely born infants following human rhinovirus A and C infections. *European journal of pediatrics* 2014;173: 913-9.
 22. Khetsuriani N, Lu X, Teague WG, Kazerouni N, Anderson LJ, Erdman DD, Novel human rhinoviruses and exacerbation of asthma in children. *Emerg Infect Dis* 2008;14: 1793-6.
 23. Arden KE, Chang AB, Lambert SB, Nissen MD, Sloots TP, Mackay IM, Newly identified respiratory viruses in children with asthma exacerbation not requiring admission to hospital. *J Med Virol* 2010;82: 1458-61.
 24. Linsuwanon P, Payungporn S, Samransamruajkit R, Posuwan N, Makkoch J, Theanboonlers A, Poovorawan Y, High prevalence of human rhinovirus C infection in Thai children with acute lower respiratory tract disease. *J Infect* 2009;59: 115-21.
 25. Lee WM, Lemanske RF, Jr., Evans MD, Vang F, Pappas T, Gangnon R, Jackson DJ, Gern JE, Human rhinovirus species and season of infection determine illness severity. *Am J Respir Crit Care Med* 2012;186: 886-91.
 26. Papadopoulos NG, Sanderson G, Hunter J, Johnston SL, Rhinoviruses replicate effectively at lower airway temperatures. *Journal of Medical Virology* 1999;58: 100-04.
 27. Schroth MK, Grimm E, Frindt P, Galagan DM, Konno S, Love R, Gern JE, Rhinovirus replication causes RANTES production in primary bronchial epithelial cells. *ajrcmb* 1999;20: 1220-28.
 28. McFadden ER, Jr., Pichurko BM, Bowman HF, Ingenito E, Burns S, Dowling N, Solway J, Thermal mapping of the airways in humans. *JApplPhysiol* 1985;58: 564-70.
 29. Nakagome K, Bochkov YA, Ashraf S, Brockman-Schneider RA, Evans MD, Pasic TR, Gern JE, Effects of rhinovirus species on viral replication and cytokine production. *J Allergy Clin Immunol* 2014.
 30. Watters K, Palmenberg AC, Differential processing of nuclear pore complex proteins by rhinovirus 2A proteases from different species and serotypes. *JVirol* 2011;85: 10874-83.
 31. Mosser AG, Brockman-Schneider RA, Amineva SP, Burchell L, Sedgwick JB, Busse WW, Gern JE, Similar frequency of rhinovirus-infectable cells in upper and lower airway epithelium. *jid* 2002;185: 734-43.
 32. Mosser AG, Vrtis R, Burchell L, Lee WM, Dick CR, Weisshaar E, Bock D, Swenson CA, Cornwell RD, Meyer KC, Jarjour NN, Busse WW, Gern JE, Quantitative and qualitative analysis of rhinovirus infection in bronchial tissues. *AmJRespirCrit Care Med* 2005;171: 645-51.
 33. Sajjan U, Wang Q, Zhao Y, Gruenert DC, Hershenson MB, Rhinovirus Disrupts the Barrier Function of Polarized Airway Epithelial Cells. *AmJRespirCrit Care Med* 2008.
 34. Proud D, Turner RB, Winther B, Wiehler S, Tiesman JP, Reichling TD, Juhlin KD, Fulmer AW, Ho BY, Walanski AA, Poore CL, Mizoguchi H, Jump L, Moore ML, Zukowski CK, Clymer JW, Gene Expression Profiles During In Vivo Human Rhinovirus Infection: Insights into the Host Response. *AmJRespirCrit Care Med* 2008;178: 962-68.
 35. Lewis TC, Henderson TA, Carpenter AR, Ramirez IA, McHenry CL, Goldsmith AM, Ren X, Mentz GB, Mukherjee B, Robins TG, Joiner TA, Mohammad LS, Nguyen ER, Burns

- MA, Burke DT, Hershenson MB, Nasal cytokine responses to natural colds in asthmatic children. *Clin Exp Allergy* 2012;42: 1734-44.
36. Konno S, Grindle KA, Lee WM, Schroth MK, Mosser AG, Brockman-Schneider RA, Busse WW, Gern JE, Interferon-g enhances rhinovirus-induced RANTES secretion in human airway epithelial cells. *ajrcmb* 2002;26: 594-601.
37. Naclerio RM, Proud D, Lichtenstein LM, Kagey-Sobotka A, Hendley JO, Sorrentino J, Gwaltney JM, Kinins are generated during experimental rhinovirus colds. *J Infect Dis* 1988;157: 133-42.
38. Seymour ML, Gilby N, Bardin PG, Fraenkel DJ, Sanderson G, Penrose JF, Holgate ST, Johnston SL, Sampson AP, Rhinovirus infection increases 5-lipoxygenase and cyclooxygenase-2 in bronchial biopsy specimens from nonatopic subjects. *J Infect Dis* 2002;185: 540-44.
39. Naclerio RM, Proud D, Kagey-Sobotka A, Lichtenstein LM, Hendley JO, Gwaltney JM, Jr., Is histamine responsible for the symptoms of rhinovirus colds? A look at the inflammatory mediators following infection [published erratum appears in *Pediatr Infect Dis J* 1988 Oct;7(10):682]. *Pediatric Infectious Disease Journal* 1988;7: 218-22.
40. Yuta A, Doyle WJ, Gaumond E, Ali M, Tamarkin L, Baraniuk JN, Van Deusen M, Cohen S, Skoner DP, Rhinovirus infection induces mucus hypersecretion. *American Journal of Physiology: Lung Cellular and Molecular Physiology* 1998;274: L1017-L23.
41. Gern JE, Brockman-Schneider R, Bhattacharya S, Malter JS, Busse WW, Serum and Low-Density Lipoprotein Enhance IL-8 Secretion by Airway Epithelial Cells. *AmJRespirCell MolBiol* 2003;29: 483-89.
42. Miller EK, Lu X, Erdman DD, Poehling KA, Zhu Y, Griffin MR, Hartert TV, Anderson LJ, Weinberg GA, Hall CB, Iwane MK, Edwards KM, New Vaccine Surveillance N, Rhinovirus-associated hospitalizations in young children. *J Infect Dis* 2007;195: 773-81.
43. DeMore JP, Weisshaar EH, Vrtis RF, Swenson CA, Evans MD, Morin A, Hazel E, Bork JA, Kakumanu S, Sorkness R, Busse WW, Gern JE, Similar colds in subjects with allergic asthma and nonatopic subjects after inoculation with rhinovirus-16. *J Allergy Clin Immunol* 2009;124: 245-52, 52.
44. Message SD, Laza-Stanca V, Mallia P, Parker HL, Zhu J, Keadze T, Contoli M, Sanderson G, Kon OM, Papi A, Jeffery PK, Stanciu LA, Johnston SL, Rhinovirus-induced lower respiratory illness is increased in asthma and related to virus load and Th1/2 cytokine and IL-10 production. *Proc Natl Acad Sci USA* 2008;105: 13562-67.
45. Mosser AG, Vrtis R, Burchell L, Lee WM, Dick CR, Weisshaar E, Bock D, Swenson CA, Cornwell RD, Meyer KC, Jarjour NN, Busse WW, Gern JE, Quantitative and qualitative analysis of rhinovirus infection in bronchial tissues. *Am J Respir Crit Care Med* 2005;171: 645-51.
46. Simons E, Schroth MK, Gern JE, Analysis of tracheal secretions for rhinovirus during natural colds. *Pediatr Allergy Immunol* 2005;16: 276-78.
47. Holgate ST, Epithelium dysfunction in asthma. *J Allergy Clin Immunol* 2007;120: 1233-44.
48. Lopez-Souza N, Dolganov G, Dubin R, Sachs LA, Sassina L, Sporer H, Yagi S, Schnurr D, Boushey HA, Widdicombe JH, Resistance of differentiated human airway epithelium to infection by rhinovirus. *American Journal of Physiology - Lung Cellular & Molecular Physiology* 2004;286: L373-L81.
49. Jakiela B, Brockman-Schneider R, Amineva S, Lee WM, Gern JE, Basal cells of differentiated bronchial epithelium are more susceptible to rhinovirus infection. *AmJRespirCell MolBiol* 2008;38: 517-23.
50. Yoo JK, Kim TS, Hufford MM, Braciale TJ, Viral infection of the lung: host response and sequelae. *J Allergy Clin Immunol* 2013;132: 1263-76; quiz 77.

51. MacMicking JD, Interferon-inducible effector mechanisms in cell-autonomous immunity. *Nature reviews Immunology* 2012;12: 367-82.
52. Copenhaver CC, Gern JE, Li Z, Shult PA, Rosenthal LA, Mikus LD, Kirk CJ, Roberg KA, Anderson EL, Tisler CJ, DaSilva DF, Heimke HJ, Gentile K, Gangnon RE, Lemanske RF, Jr., Cytokine response patterns, exposure to viruses, and respiratory infections in the first year of life. *ajrccm* 2004;170: 175-80.
53. Gern JE, Brooks GD, Meyer P, Chang A, Shen K, Evans MD, Tisler C, Dasilva D, Roberg KA, Mikus LD, Rosenthal LA, Kirk CJ, Shult PA, Bhattacharya A, Li Z, Gangnon R, Lemanske RF, Jr., Bidirectional interactions between viral respiratory illnesses and cytokine responses in the first year of life. *J Allergy Clin Immunol* 2006;117: 72-8.
54. Isaacs D, Clarke JR, Tyrrell DA, Webster AD, Valman HB, Deficient production of leucocyte interferon (interferon-alpha) in vitro and in vivo in children with recurrent respiratory tract infections. *Lancet* 1981;2: 950-52.
55. Upham JW, Zhang G, Rate A, Yerkovich ST, Kusel M, Sly PD, Holt PG, Plasmacytoid dendritic cells during infancy are inversely associated with childhood respiratory tract infections and wheezing. *J Allergy Clin Immunol* 2009;124: 707-13 e2.
56. Parry DE, Busse WW, Sukow KA, Dick CR, Swenson C, Gern JE, Rhinovirus-induced PBMC responses and outcome of experimental infection in allergic subjects. *J Allergy Clin Immunol* 2000;105: 692-8.
57. Gern JE, Vrtis R, Grindle KA, Swenson C, Busse WW, Relationship of upper and lower airway cytokines to outcome of experimental rhinovirus infection. *ajrccm* 2000;162: 2226-31.
58. Papadopoulos NG, Stanciu LA, Papi A, Holgate ST, Johnston SL, Rhinovirus-induced alterations on peripheral blood mononuclear cell phenotype and costimulatory molecule expression in normal and atopic asthmatic subjects. *Clinical and Experimental Allergy* 2002;32: 537-42.
59. Gehlhar K, Bilitewski C, Reinitz-Rademacher K, Rohde G, Bufe A, Impaired virus-induced interferon-alpha2 release in adult asthmatic patients. *Clin Exp Allergy* 2006;36: 331-37.
60. Gill MA, Bajwa G, George TA, Dong CC, Dougherty II, Jiang N, Gan VN, Gruchalla RS, Counterregulation between the FcepsilonRI pathway and antiviral responses in human plasmacytoid dendritic cells. *J Immunol* 2010;184: 5999-6006.
61. Wark PA, Johnston SL, Bucchieri F, Powell R, Puddicombe S, Laza-Stanca V, Holgate ST, Davies DE, Asthmatic bronchial epithelial cells have a deficient innate immune response to infection with rhinovirus. *JExpMed* 2005;201: 937-47.
62. Contoli M, Message SD, Laza-Stanca V, Edwards MR, Wark PA, Bartlett NW, Keadze T, Mallia P, Stanciu LA, Parker HL, Slater L, Lewis-Antes A, Kon OM, Holgate ST, Davies DE, Kolenko SV, Papi A, Johnston SL, Role of deficient type III interferon-lambda production in asthma exacerbations. *NatMed* 2006;12: 1023-26.
63. Lopez-Souza N, Favoreto S, Wong H, Ward T, Yagi S, Schnurr D, Finkbeiner WE, Dolganov GM, Widdicombe JH, Boushey HA, Avila PC, In vitro susceptibility to rhinovirus infection is greater for bronchial than for nasal airway epithelial cells in human subjects. *J Allergy Clin Immunol* 2009;123: 1384-90.
64. Bochkov YA, Hanson KM, Keles S, Brockman-Schneider RA, Jarjour NN, Gern JE, Rhinovirus-induced modulation of gene expression in bronchial epithelial cells from subjects with asthma. *Mucosal Immunol* 2010;3: 69-80.
65. Kennedy JL, Shaker M, McMeen V, Gern J, Carper H, Murphy D, Lee WM, Bochkov YA, Vrtis RF, Platts-Mills T, Patrie J, Borish L, Steinke JW, Woods WA, Heymann P, Comparison of Viral Load in Individuals with and without Asthma During Infections with Rhinovirus. *Am J Respir Crit Care Med* 2014.

66. Message SD, Laza-Stanca V, Mallia P, Parker HL, Zhu J, Keadze T, Contoli M, Sanderson G, Kon OM, Papi A, Jeffery PK, Stanciu LA, Johnston SL, Rhinovirus-induced lower respiratory illness is increased in asthma and related to virus load and Th1/2 cytokine and IL-10 production. *Proc Natl Acad Sci U S A* 2008;105: 13562-7.
67. Sykes A, Macintyre J, Edwards MR, Del Rosario A, Haas J, Gielen V, Kon OM, McHale M, Johnston SL, Rhinovirus-induced interferon production is not deficient in well controlled asthma. *Thorax* 2014;69: 240-6.
68. Sykes A, Edwards MR, Macintyre J, del Rosario A, Bakhsoliani E, Trujillo-Torralbo MB, Kon OM, Mallia P, McHale M, Johnston SL, Rhinovirus 16-induced IFN-alpha and IFN-beta are deficient in bronchoalveolar lavage cells in asthmatic patients. *J Allergy Clin Immunol* 2012;129: 1506-14 e6.
69. Gern JE, Interferon-lambda1 and viral wheeze in asthma: a Gothic duality? *Am J Respir Crit Care Med* 2012;185: 468-70.
70. Schwantes EA, Manthei DM, Denlinger LC, Evans MD, Gern JE, Jarjour NN, Mathur SK, Interferon Gene Expression in Sputum Cells Correlates with the Asthma Index Score During Virus-Induced Exacerbations. *Clin Exp Allergy* 2014.
71. Miller EK, Hernandez JZ, Wimmenauer V, Shepherd BE, Hijano D, Libster R, Serra ME, Bhat N, Batalle JP, Mohamed Y, Reynaldi A, Rodriguez A, Otello M, Pisapia N, Bugna J, Bellabarba M, Kraft D, Coviello S, Ferolla FM, Chen A, London SJ, Siberry GK, Williams JV, Polack FP, A mechanistic role for type III IFN-lambda1 in asthma exacerbations mediated by human rhinoviruses. *Am J Respir Crit Care Med* 2012;185: 508-16.
72. Mikus LD, Rosenthal LA, Sorkness RL, Lemanske RF, Jr., Reduced interferon-gamma secretion by natural killer cells from rats susceptible to postviral chronic airway dysfunction. *AmJRespirCell MolBiol* 2001;24: 74-82.
73. Kumar A, Sorkness RL, Kaplan MR, Lemanske RF, Jr., Chronic, episodic, reversible airway obstruction after viral bronchiolitis in rats. *AmJRespirCritCare Med* 1997;155: 130-34.
74. Walter MJ, Morton JD, Kajiwarra N, Agapov E, Holtzman MJ, Viral induction of a chronic asthma phenotype and genetic segregation from the acute response. *JClinInvest* 2002;110: 165-75.
75. Sorkness RL, Castleman WL, Kumar A, Kaplan MR, Lemanske RF, Jr., Prevention of chronic postbronchiolitis airway sequelae with IFN-g treatment in rats. *AmJRespirCritCare Med* 1999;160: 705-10.
76. Bartlett NW, Slater L, Glanville N, Haas JJ, Caramori G, Casolari P, Clarke DL, Message SD, Aniscenko J, Keadze T, Zhu J, Mallia P, Mizgerd JP, Belvisi M, Papi A, Kosenko SV, Johnston SL, Edwards MR, Defining critical roles for NF-kappaB p65 and type I interferon in innate immunity to rhinovirus. *EMBO molecular medicine* 2012;4: 1244-60.
77. Jayaraman A, Jackson DJ, Message SD, Pearson RM, Aniscenko J, Caramori G, Mallia P, Papi A, Shamji B, Edwards M, Westwick J, Hansel T, Stanciu LA, Johnston SL, Bartlett NW, IL-15 complexes induce NK- and T-cell responses independent of type I IFN signaling during rhinovirus infection. *Mucosal immunology* 2014.
78. Hong JY, Bentley JK, Chung Y, Lei J, Steenrod JM, Chen Q, Sajjan US, Hershenson MB, Neonatal rhinovirus induces mucous metaplasia and airways hyperresponsiveness through IL-25 and type 2 innate lymphoid cells. *J Allergy Clin Immunol* 2014.
79. Zhu J, Message SD, Qiu Y, Mallia P, Keadze T, Contoli M, Ward CK, Barnathan ES, Mascelli MA, Kon OM, Papi A, Stanciu LA, Jeffery PK, Johnston SL, Airway inflammation and illness severity in response to experimental rhinovirus infection in asthma. *Chest* 2014.
80. Denlinger LC, Sorkness RL, Lee WM, Evans MD, Wolff MJ, Mathur SK, Crisafi GM, Gaworski KL, Pappas TE, Vrtis RF, Kelly EA, Gern JE, Jarjour NN, Lower airway

- rhinovirus burden and the seasonal risk of asthma exacerbation. *AmJRespirCrit Care Med* 2011;184: 1007-14.
81. Rakes GP, Arruda E, Ingram JM, Hoover GE, Zambrano JC, Hayden FG, Platts-Mills TA, Heymann PW, Rhinovirus and respiratory syncytial virus in wheezing children requiring emergency care. IgE and eosinophil analyses. *AmJRespirCritCare Med* 1999;159: 785-90.
82. Tversky JR, Le TV, Bieneman AP, Chichester KL, Hamilton RG, Schroeder JT, Human blood dendritic cells from allergic subjects have impaired capacity to produce interferon-alpha via Toll-like receptor 9. *ClinExpAllergy* 2008;38: 781-88.
83. Durrani SR, Montville DJ, Pratt AS, Sahu S, DeVries MK, Rajamanickam V, Gangnon RE, Gill MA, Gern JE, Lemanske RF, Jr., Jackson DJ, Innate immune responses to rhinovirus are reduced by the high-affinity IgE receptor in allergic asthmatic children. *J Allergy Clin Immunol* 2012;130: 489-95.
84. Subrata LS, Bizzintino J, Mamessier E, Bosco A, McKenna KL, Wikstrom ME, Goldblatt J, Sly PD, Hales BJ, Thomas WR, Laing IA, LeSouef PN, Holt PG, Interactions between innate antiviral and atopic immunoinflammatory pathways precipitate and sustain asthma exacerbations in children. *J Immunol* 2009;183: 2793-800.
85. Corne JM, Marshall C, Smith S, Schreiber J, Sanderson G, Holgate ST, Johnston SL, Frequency, severity, and duration of rhinovirus infections in asthmatic and non-asthmatic individuals: a longitudinal cohort study. *Lancet* 2002;359: 831-34.
86. Meyer N, Akdis CA, Vascular endothelial growth factor as a key inducer of angiogenesis in the asthmatic airways. *Current allergy and asthma reports* 2013;13: 1-9.
87. Tarlo SM, Broder I, Corey P, Chan-Yeung M, Ferguson A, Becker A, Rogers C, Okada M, Manfreda J, The role of symptomatic colds in asthma exacerbations: Influence of outdoor allergens and air pollutants. *JAllergy ClinImmunol* 2001;108: 52-58.
88. Chauhan AJ, Inskip HM, Linaker CH, Smith S, Schreiber J, Johnston SL, Holgate ST, Personal exposure to nitrogen dioxide (NO₂) and the severity of virus-induced asthma in children. *Lancet* 2003;361: 1939-44.
89. Venarske DL, Busse WW, Griffin MR, Gebretsadik T, Shintani AK, Minton PA, Peebles RS, Hamilton R, Weisshaar E, Vrtis R, Higgins SB, Hartert TV, The relationship of rhinovirus-associated asthma hospitalizations with inhaled corticosteroids and smoking. *JInfectDis* 2006;193: 1536-43.
90. Duff AL, Pomeranz ES, Gelber LE, Price HW, Farris H, Hayden FG, Platts-Mills TAE, Heymann PW, Risk factors for acute wheezing in infants in infants and children: viruses, passive smoke, and IgE antibodies to inhalant allergens. *Pediatrics* 1993;92: 535-40.
91. Murray CS, Poletti G, Keadze T, Morris J, Woodcock A, Johnston SL, Custovic A, Study of modifiable risk factors for asthma exacerbations: virus infection and allergen exposure increase the risk of asthma hospital admissions in children. *Thorax* 2006;61: 376-82.
92. Green RM, Cusotvic A, Sanderson G, Hunter J, Johnston SL, Woodcock A, Synergism between allergens and viruses and risk of hospital admission with asthma: case-control study. *British Medical Journal* 2002;324: 763-66A.
93. Hilty M, Burke C, Pedro H, Cardenas P, Bush A, Bossley C, Davies J, Ervine A, Poulter L, Pachter L, Moffatt MF, Cookson WO, Disordered microbial communities in asthmatic airways. *PLoS ONE* 2010;5: e8578.
94. Huang YJ, Nelson CE, Brodie EL, DeSantes TZ, Baek MS, Liu J, Woyke T, Allgaier M, Bristow J, Wiener-Kronish JP, Sutherland ER, King TS, Icitovic N, Martin RJ, Calhoun WF, Castro M, Denlinger LC, DiMango E, Kraft M, Peters SP, Wasserman SI, Wechser ME, Boushey HA, Lynch SV, Airway microbiota and bronchial hyperresponsiveness in patients with suboptimally controlled asthma. *Jaci* 2011;127: 372-81.

95. Bisgaard H, Hermansen MN, Buchvald F, Loland L, Halkjaer LB, Bonnelykke K, Brasholt M, Heltberg A, Vissing NH, Thorsen SV, Stage M, Phipper CB, Childhood asthma after bacterial colonization of the airway in neonates. *N Engl J Med* 2007;357: 1487-95.
96. Bisgaard H, Hermansen MN, Bonnelykke K, Stokholm J, Baty F, Skytt NL, Aniscenko J, Kebabdz T, Johnston SL, Association of bacteria and viruses with wheezy episodes in young children: prospective birth cohort study. *BMJ* 2010;341: c4978.
97. Kloefer KM, Lee WM, Pappas TE, Kang TJ, Vrtis RF, Evans MD, Gangnon RE, Bochkov YA, Jackson DJ, Lemanske RF, Jr., Gern JE, Detection of pathogenic bacteria during rhinovirus infection is associated with increased respiratory symptoms and asthma exacerbations. *J Allergy Clin Immunol* 2014;133: 1301-07 e3.
98. Camargo CA, Jr., Rifas-Shiman SL, Litonjua AA, Rich-Edwards JW, Weiss ST, Gold DR, Kleinman K, Gillman MW, Maternal intake of vitamin D during pregnancy and risk of recurrent wheeze in children at 3 y of age. *Am J Clin Nutr* 2007;85: 788-95.
99. Ginde AA, Mansbach JM, Camargo CA, Jr., Association between serum 25-hydroxyvitamin D level and upper respiratory tract infection in the Third National Health and Nutrition Examination Survey. *Arch Intern Med* 2009;169: 384-90.
100. Brockman-Schneider RA, Pickles RJ, Gern JE, Effects of vitamin D on airway epithelial cell morphology and rhinovirus replication. *PloS one* 2014;9: e86755.
101. Hansdottir S, Monick MM, Vitamin D effects on lung immunity and respiratory diseases. *Vitam Horm* 2011;86:217-37.: 217-37.
102. Stone AA, Bovbjerg DH, Neale JM, Napoli A, Valdimarsdottir H, Cox D, Hayden FG, Gwaltney JM, Jr., Development of common cold symptoms following experimental rhinovirus infection is related to prior stressful life events. *Behav Med* 1992;18: 115-20.
103. Cohen S, Psychological stress and susceptibility to upper respiratory infections. *Am J Respir Crit Care Med* 1995;152: S53-S58.
104. Busse WW, Morgan WJ, Gergen PJ, Mitchell HE, Gern JE, Liu AH, Gruchalla RS, Kattan M, Teach SJ, Pongracic JA, Chmiel JF, Steinbach SF, Calatroni A, Togias A, Thompson KM, Szefer SJ, Sorkness CA, Randomized trial of omalizumab (anti-IgE) for asthma in inner-city children. *N Engl J Med* 2011;364: 1005-15.
105. Haldar P, Brightling CE, Hargadon B, Gupta S, Monteiro W, Sousa A, Marshall RP, Bradding P, Green RH, Wardlaw AJ, Pavord ID, Mepolizumab and exacerbations of refractory eosinophilic asthma. *N Engl J Med* 2009;360: 973-84.
106. Djukanovic R, Harrison T, Johnston SL, Gabbay F, Wark P, Thomson NC, Niven R, Singh D, Reddel HK, Davies DE, Marsden R, Boxall C, Dudley S, Plagnol V, Holgate ST, Monk P, The Effect of Inhaled IFN-beta on Worsening of Asthma Symptoms Caused by Viral Infections. A Randomized Trial. *Am J Respir Crit Care Med* 2014;190: 145-54.
107. Papadopoulos NG, Borres M, Gern J, Nieto A, New visions in respiratory allergy (asthma and allergic rhinitis): an iPAC summary and future trends. *Pediatr Allergy Immunol* 2008;19 Suppl 19: 51-9.
108. McIntyre C, L., Knowles NJ, Simmonds P, Proposals for the classification of human rhinovirus species A, B and C into genotypically assigned types. *J Gen Virol* 2013;in press.
109. Palmenberg AC, Spiro D, Kuzmickas R, Wang S, Djikeng A, Rathe JA, Fraser-Liggett CM, Liggett SB, Sequencing and analysis of all known human rhinovirus genomes reveals structure and evolution. *Science* 2009;324: 55-59.
110. Palmenberg AC, Gern JE, Classification and Evolution in Human Rhinoviruses. In: Ghildyal R ed. *Methods in Molecular Virology*: Springer, 2014:In press.