

The role of rhinovirus in asthma exacerbations

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Rhinoviruses are a major cause of asthma exacerbations in children and adults. With the use of sensitive RT-PCR methods, respiratory viruses are found in approximately 80% of wheezing episodes in children and in approximately one half of such episodes in adults. Rhinovirus is a member of the family Picornaviridae, and acute rhinovirus infections occur predominantly in the upper airway. This virus has also been identified in the lower airway, and it might cause acute wheezing through the production of proinflammatory mediators with a resulting neutrophilic inflammatory response. Precisely how this process leads to increases in airway hyperresponsiveness and airway obstruction is not fully established. However, risk factors for wheezing with colds include asthma and atopy, extremes in age, and perhaps having a deficient T_H1 response to rhinovirus. With the use of *in vitro* models and experimental inoculation studies, significant advances have led to a better understanding of the mechanisms by which rhinovirus infections cause asthma exacerbations. Advances in our understanding of this interaction might provide knowledge that could ultimately lead to specific treatment modalities to prevent and/or treat this significant burden of asthma exacerbations. (*J Allergy Clin Immunol* 2005;116:267-73.)

Key words: Rhinovirus, asthma exacerbations, virology, cytokine response profiles, mechanisms of asthma

Viral upper respiratory tract infections (URIs) are known to cause exacerbations of asthma. A significant and increasing body of evidence demonstrates that in large part the primary respiratory infection causing these exacerbations is rhinovirus (RV), the cause of more than 50% of URIs.¹ The frequency of URI-provoked asthma makes it especially important to understand the role and mechanisms whereby RV infections lead to asthma exacerbations, the basic virologic features of RV, their ability to infect the lower airway, the host susceptibility factors, and mechanisms leading to airflow obstruction.

Abbreviations used

G-CSF: Granulocyte colony-stimulating factor
ICAM: Intercellular adhesion molecule
IL-1ra: IL-1 receptor antagonist
RV: Rhinovirus
URI: Upper respiratory tract infection

WHAT ROLE DOES RV PLAY IN ASTHMA EXACERBATIONS?

Asthma exacerbations are most commonly precipitated by viral URIs, particularly with RV,² and often occur despite concurrent use of appropriate controller medications. Detecting respiratory viruses—in particular, RV—by culture methodology alone has been insensitive and has previously underestimated the role of respiratory viruses in asthma exacerbations, especially in adults. Viral detection rates in asthma exacerbations have significantly increased with the use of sensitive methods and have thus underscored the overwhelming importance of respiratory viruses in asthma exacerbations. When RT-PCR is used to supplement conventional culture techniques, viruses have been found in approximately 80% of wheezing episodes in school-age children and in approximately one half of the acute wheezing episodes in adults. Of the respiratory viruses identified in these circumstances, RV is most commonly found and is detected 65% of the time.^{2,3}

A pivotal study by Johnston and colleagues² in children aged 9-11 years old with histories of asthma symptoms found that 80% to 85% of asthma exacerbations that were associated with reduced peak expiratory flow rates and wheezing were due to viral URIs. Without the use of RT-PCR, the authors reported, the viral detection rate in this study would have been only around 40%.

Similarly, high rates of asthma attacks due to RV were found in adults. Nicholson et al³ reported on 138 young adults with asthma recruited from general practice, the hospital, and the community. In this longitudinal study, 80% of asthma episodes (223 of 280), described as symptoms of wheeze, chest tightness, or breathlessness, were associated with colds. Objectively, viruses were detected in 57% of people with symptomatic colds and asthma exacerbations. In more severe asthma exacerbations with reductions in peak flow measurements of

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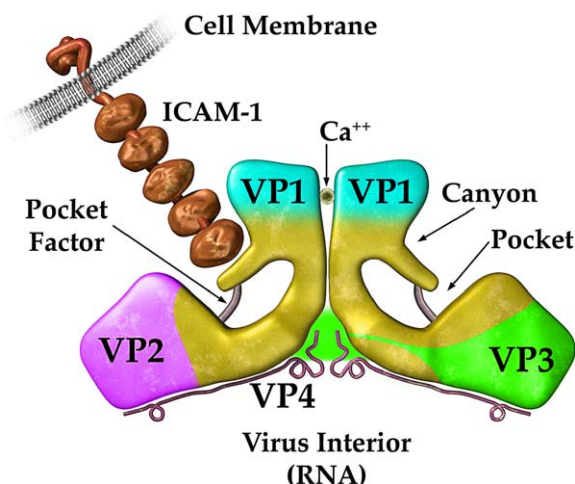


FIG 1. Transverse section through the center of a pentamer depicting entry of its cellular receptor, ICAM-1, and the location of the drug-binding pocket just beneath the canyon floor. An ion, located at each pentamer center in RV-1A, -14, -16 is tentatively identified as calcium, which is necessary for attachment of some RVs. Modified with permission from Gwaltney JM. Rhinovirus. In: Mandell GL, Douglas RG, Bennett JE, Dolin R, editors. Mandell, Douglas, and Bennett's principles and practice of infectious diseases. 6th ed. New York; Elsevier/Churchill Livingstone; 2005. p. 2185-94.

≥ 50 L/min, viruses were detected in 44% of episodes. In comparison with detection rates by cell culture, RT-PCR was 5 times more sensitive in identifying human RV in adults with respiratory infections.³

Further evidence to support the role of viral infection in asthma exacerbations also includes reports that peaks in hospital admissions for asthma significantly correlate with seasonal patterns of viral URIs.⁴ In the United States, RV infection occurs most commonly in the fall and spring.⁵ Thus, current evidence strongly supports the concept that RV respiratory infections are the major cause of acute asthma exacerbations.

WHAT ARE THE VIROLOGIC FEATURES OF RVS?

The genera RV and Enterovirus are classified within the family Picornaviridae. There are more than 100 serotypes of RV; this explains, in part, the lack of an effective vaccine against the major etiologic agent causing the common cold. RV is a small, single-stranded RNA virus whose capsid contains 4 proteins (Fig 1). Three of these proteins, VP1, VP2, and VP3, are located on the surface of the capsid and are responsible for its antigenic diversity; the fourth, VP4, is located inside the virus and anchors the RNA core to the viral capsid.¹ The majority of RV serotypes bind to intercellular adhesion molecule (ICAM) 1, whereas approximately 10% bind to the low-density lipoprotein receptor.^{6,7}

Typically, RV infects small clusters of cells in the epithelial layer with little cellular cytotoxicity. Although

increased polymorphonuclear neutrophils are seen in infected nasal epithelium,⁸ little or no mucosal damage occurs from the infection; this suggests that RV is likely to cause asthma exacerbations by mechanisms other than direct cellular killing.⁹ Even with large inoculating doses of virus, less than 10% of cells in primary airway epithelium cultures become infected. However, although RV-induced cytotoxicity is difficult to detect *in vivo*, an *in vitro* study has demonstrated cytopathic effects when high titers of virus are inoculated with sparsely seeded monolayer cultures of human bronchial epithelial cells.¹⁰ Moreover, the RV serotype might also be an important determinant of this *in vitro*-detected cytotoxicity.

ARE RV INFECTIONS LIMITED TO THE UPPER AIRWAY?

An infection with RV leads to symptoms of the common cold, which is primarily an upper airway illness. Because RV is primarily an infection of the upper airway, early research efforts were directed toward determining whether (a) RV infections could infect the lower airways directly and provoke asthma, (b) their actions on asthma occurred via indirect mechanisms due to the upper airway infection only, or (c) a combination of the 2 methods is responsible. Insight into these questions could suggest potential target areas to act therapeutically to prevent or treat an asthma exacerbation.

Debate initially focused on whether RV could exist and replicate in the lung to directly cause lower airway inflammation. This was based on limited studies demonstrating that RV replication was optimal at 33°C, the temperature of the upper airways. To address this issue, direct thermal mapping of the lower airways was performed. While human subjects breathed room air (26°C), the temperature in the subjects averaged 32°C in the upper trachea and 35.5°C in the subsegmental bronchi. These findings refuted a possible limitation of RV growth due to higher temperatures in the lower airway.¹¹ Moreover, with the use of multiple RV serotypes, it was possible to detect high viral titers in cell cultures at 37°C; little significant difference in replication was found when wild-type RV isolates were used at 33°C compared to 37°C. In fact, some serotypes grew more effectively at the higher temperature.¹⁰ In addition, when primary cultures of lower airway bronchial epithelial cells and upper airway adenoidal epithelial cells were used, RV appeared to infect both upper and lower segments of the respiratory tree with similar ability (Fig 2).⁹

Several additional lines of evidence support the ability of RV to infect the lower airways directly. When bronchoscopy was used to collect samples from subjects with symptomatic experimental infections, RV was detected from bronchial brush specimens.¹² Furthermore, with the use of RT-PCR and Southern blotting, RV genetic material was found in higher amounts in cells of bronchial alveolar lavage fluid than in supernatant; this suggests that the virus was located intracellularly.¹³ However, the role

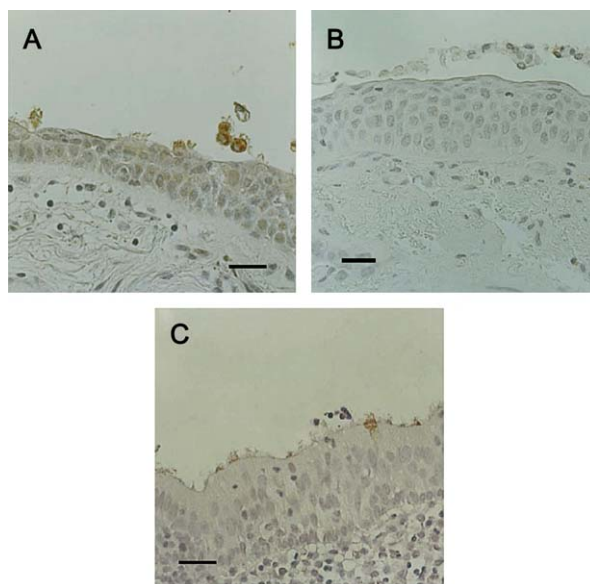


FIG 2. Immunohistochemistry of airway tissue from experimentally infected normal human surgical specimens infected *ex vivo*. **A**, Bronchial tissue specimens were inoculated *ex vivo* and were incubated in tissue culture medium for 24 hours. After washing to eliminate extracellular virus, the tissue was embedded in paraffin, sectioned, and stained for the presence of RV serotype 16. **B**, Uninfected bronchial tissue specimen was processed as in panel A. **C**, Adenoidal tissue specimen infected *ex vivo* for 6 hours. Bar: 50 μ m. Reprinted with permission from Mosser AG, Brockman-Schneider R, Amineva S, Burchell L, Sedgwick JB, Busse WW, et al. Similar frequency of rhinovirus-infectible cells in upper and lower airway epithelium. *J Infect Dis* 2002;185:734-43.

of contamination from the upper airway could not be definitively excluded in these studies.

Another investigation found RV-16 RNA in 50% of bronchial biopsies in experimentally inoculated human volunteers.¹⁰ In this study, *in situ* hybridization was used to localize viral RNA by hybridizing the sequence of interest to the complementary replicative strand of the virus. This technique likely excludes the possibility of contamination of the lower airway with virus from the upper airway. This was further supported by the finding of viral replication in the lower airway as well as increases in viral RNA and the production of new viral proteins. Furthermore, the frequency of lower airway infection was similar to that observed in the upper airway; this indicates that infection of the lower airways might be relatively common as part of the natural history of RV infection. Finally, a recent study showed that an experimental RV infection was associated with virus detection in large lower airways biopsy samples by immunohistochemistry or qPCR in 17 of 19 subjects, but less so in the distal airways.¹⁴ Thus, RV is able to infect both the upper and lower airways.

It is likely that the lower airways are infected as a result of self-inoculation from coughing, sneezing, or perhaps breathing. Whether the concentration of virus in the lower airways is large enough to produce clinically relevant effects is still not established. These studies support the concept that RV is a lower as well as an upper respiratory tract pathogen, and infection of the lower

airway directly likely contributes to viral-induced exacerbations of asthma.

WHAT ARE THE EFFECTS OF RV INFECTION ON THE MECHANISMS OF AIRWAY PHYSIOLOGY IN ASTHMA?

Multiple studies demonstrate the adverse effects of RV on airway physiology in asthma. In school-age children, symptoms of either upper or lower respiratory tract infection were shown to last a week, and during these infectious episodes, the peak flow rates fell for a median duration of 2 weeks.² In another study, asthmatic subjects were experimentally inoculated with RV-16 and found to demonstrate modest changes in increased airway hyper-responsiveness, airway obstruction, and inflammation.¹⁵ Experimental RV-16 infection also has been shown to reduce FEV₁ in patients with mild asthma.¹⁶ In addition, increases in existing airway inflammation have occurred after segmental bronchoprovocation in atopic subjects, suggesting that enhanced airway inflammation is a feature of RV-associated asthma exacerbations.¹⁷

To support this possibility, subjects with allergic rhinitis, but not with active asthma, were inoculated with RV; they were found to have significantly increased airway hyperreactivity as well as a significantly increased incidence of late asthmatic reactions, defined as a 15% decrease in FEV₁ approximately 6 hours after antigen

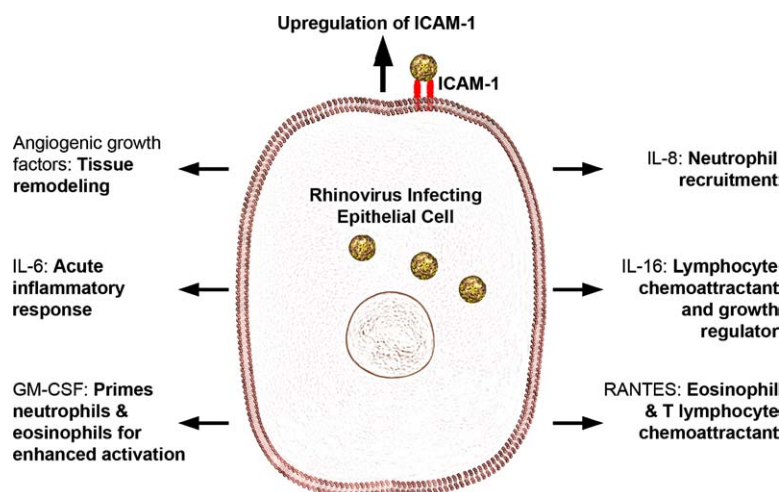


FIG 3. RV induces epithelial cells to produce proinflammatory cytokines leading to airway hyperresponsiveness, neurogenic inflammatory responses, mucous secretion, inflammatory cell recruitment and activation, and plasma leakage. Created with information from Gern JE. Rhinovirus respiratory infections and asthma. *Am J Med* 2002;112(Suppl 6A):19S-27S and from Yamaya M, Sasaki H. Rhinovirus and asthma. *Viral Immunol* 2003;16:99-109.

challenge.¹⁸ Before infection, only 1 patient in this study had a late asthmatic reaction. During the acute infection, this number increased to 8 of 10 subjects ($P = .0085$). The effect occurred independently of the enhancement in airway reactivity experienced during a cold alone. This demonstrates that in addition to causing airway hyperactivity, RV also promotes the development of late-phase responses, even in nonasthmatic patients.

RV infection also promotes eosinophil recruitment to airway segments after antigen challenges. Calhoun et al¹⁷ used segmental bronchoprovocation with antigen after inoculation with RV-16 in subjects with allergic rhinitis. After infection, bronchial alveolar lavage fluid revealed enhanced histamine release immediately and increased eosinophil recruitment 48 hours after antigen challenge. Interestingly, the increase in eosinophils persisted for up to 1 month after infection in some subjects. The effect of RV on airway inflammation appeared to be an augmentation of allergen-specific responses. Thus, enhancement of antigen-induced mediator release from pulmonary mast cells and basophils and eosinophilic recruitment, either directly or via cytokines, could provide one mechanism by which late allergic reactions and airway hyperresponsiveness are enhanced by viral uncoating and might act to intensify the airway inflammatory response to allergen.

Conversely, when nasal allergen challenges in atopic patients were performed before experimental RV inoculation, the onset of cold symptoms was delayed and the responses were less severe in comparison with what was seen in patients without allergies.¹⁹ Delayed nasal inflammation, with attenuation of the increase in IL-6, IL-8, and neutrophils seen in infection, was also found in the group primed with nasal antigen challenge. This might have been due to cytokine profile changes with increased expression of IFN- γ and IL-2, local production of nitric

oxide, or antiviral effects of eosinophil products. Thus, the timing and intensity of antigen exposure play an important role in the severity level and subsequent possible complications of a cold.

HOW DOES RV MODULATE INFLAMMATORY MEDIATORS OF EPITHELIAL CELLS CONTRIBUTING TO ASTHMA EXACERBATIONS?

Epithelial cells are the principal targets of RV infections, allow viral replication, and likely initiate immune responses (Fig 3).^{20,21} Papadopoulos et al¹⁰ found local induction of proinflammatory mediators that could provide a mechanism to explain how lower airway infection can lead to inflammation and asthma. RV infection resulted in an increase in mRNA expression and subsequent translation of IL-6, IL-8, and IL-16. This also occurred with RANTES, a C-C chemokine with chemoattractant activity for eosinophils, monocytes, and T lymphocytes. IL-6 and IL-8 are proinflammatory cytokines, and IL-8 is a specifically potent chemoattractant for neutrophils. IL-16 is a powerful lymphocyte chemoattractant and activator of macrophages and eosinophils and appears to be an important mediator in the pathogenesis of asthma and lower airway inflammation due to RV.¹⁰ The inflammatory actions of RV appear to center on its ability to generate a variety of phlogistic mediators.

Generation of these cytokines correlates with the worsening of respiratory physiology. For example, IL-1 enhances airway smooth muscle contraction in response to bronchospastic agents and attenuates smooth muscle dilation responses to bronchodilators.^{22,23} Differences in immune response, such as the modulation of costimulatory molecules and the induction of antigen presentation,

might explain how RV infections cause acute exacerbations in asthmatic patients.

Virus-induced epithelial damage might cause increased permeability of the mucosal layer and thus increase allergen contact with immune cells to promote neurogenic inflammation. In addition, viruses can enhance vagally mediated reflex bronchoconstriction, possibly by limiting the function of the M2 muscarinic receptor.²⁴ Viral replication activates epithelial cells to initiate innate and adaptive immune responses as well as the generation of oxidative stress.²⁵ Also, double-stranded RNA synthesized in virus-infected cells induces the cytokines IL-8 and RANTES, which initiate proinflammatory and antiviral pathways within the cell.²⁴

Upregulation, or activation, of ICAM-1, the principal receptor for RV, might increase tissue susceptibility to the major group RV and subsequent infection. The asthma phenotype, which is associated with increased ICAM-1 expression, might therefore be associated with increased susceptibility and complications from RV infection.²¹ Chronic antigen challenge can also increase ICAM-1 expression of the airway epithelium, and RV infection itself can increase ICAM-1 expression through production of IL-1 β and a nuclear factor- κ B-dependent mechanism. This might lead to the amplification of airway inflammation after RV infection.^{21,26}

In addition, RV might enhance existing inflammation to a greater degree in asthmatic subjects than in those without the disease. For example, after inoculation with the virus, nasal lavage levels of IL-8 and the proinflammatory mediator IL-1 β were increased in asthmatic patients.²⁷ In this study, a small increase in the anti-inflammatory marker IL-1 receptor antagonist (IL-1ra), a competitive inhibitor of IL-1, also occurred in asthmatic subjects treated with budesonide, whereas lower levels were found in these patients at baseline. These findings suggest that RV might be able to alter the proinflammatory/anti-inflammatory balance of IL-1 β /IL-1ra toward inflammation more markedly in people with existing and active asthma.

Cytokine response profiles generated by RV might translate into neutrophilic inflammation in both the upper and lower airways. Local RV infection is associated with increased levels of IL-8, a potent chemoattractant for neutrophils, and also granulocyte colony-stimulating factor (G-CSF) in nasal secretions and later in the circulation. Increased concentrations of circulatory G-CSF could act on the bone marrow to increase the circulating neutrophils. Thus, a local response in nasal epithelium to RV infection can result in a systemic inflammatory reaction.²⁰

Elevated neutrophil counts are also found in the lower airways with RV infection. Through use of bronchoscopy and bronchial washes, significant increases in airway lumen neutrophils were found 96 hours after inoculation with RV-16 in patients with allergic asthma.²⁸ Infected bronchial epithelium induces the secretion of proinflammatory cytokines, including IL-1, IL-8, TNF- α , IL-10, and IFN- α , as well. This stimulates the recruitment of inflammatory cells and neutrophilia. Products of neutro-

TABLE I. Risk factors for severe lower respiratory tract complications from rhinovirus infection

Asthma
Atopy
Elevated nasal eosinophils or eosinophil cationic protein
Infants and elderly
Low neutralizing antibody titers to rhinovirus
Chronic lung diseases
Smoking
Low IFN- γ producers
High IL-5 producers

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phil activation could cause airway obstruction through the production of elastase, which also upregulates goblet cell mucus secretion.²⁹

WHAT ARE THE RISK FACTORS FOR WHEEZING WITH A COLD?

Various risk factors increase the susceptibility of subjects for more severe lower respiratory complications from an RV infection, such as wheezing, bronchitis, and pneumonia (Table I). These include having low neutralizing antibody titers to RV, being an infant, being elderly, having chronic lung disease, being a smoker, and being an individual with existing asthma.²²

In addition, subjects who are low producers of IFN- γ in response to RV and are atopic appear to be more at risk for wheezing or having a severe respiratory infection. Brooks et al³⁰ demonstrated that whereas RV induces IFN- γ , which is consistent with a strong T_H1-like immune response, those asthmatic patients with diminished, or deficient, T_H1 responses to RV were characterized by increased airway hyperresponsiveness. Moreover, the ratio of RV-16-induced IFN- γ :IL-5, a measure of T_H1:T_H2 balance, correlated with FEV₁. These findings are similar, in general, to what is known about T_H1 and T_H2 responses in asthma. In another study, subjects with persistent and severe asthma displayed a defect in IFN- γ production, whereas their increased IL-5 responses were felt to reflect the presence of atopy but were not specifically linked to asthma itself.³¹ Collectively, these findings demonstrate that a deficiency of the T_H1 response, rather than an increased T_H2 response, is responsible for RV's adverse effect on the airways.

The importance of IgE and eosinophilic airway inflammation was demonstrated by a study showing synergistic interactions between RV infection and allergic airway inflammation.³² In this study, which focused on children aged 2-16 years old, the odds ratios for wheezing with RV detected by RT-PCR in addition to positive radioallergosorbent test results, nasal eosinophilia, and elevated nasal eosinophil cationic protein were 17, 21, and 25, respectively. The odds ratios for wheezing with any of these 4 risk factors alone were much lower, between 3.2 and

8; this shows the importance of IgE and eosinophil-driven inflammatory responses.

Zambrano et al³³ reported that the existence of airway inflammation prior to virus inoculation predisposed subjects to a more deleterious response to RV. Patients were inoculated with RV-16, and compared with those without asthma, those with mild asthma demonstrated increased airway hyperresponsiveness, decreased FEV₁ at baseline, and increased upper and lower respiratory tract symptom scores in response to the infection. Asthmatic patients with elevated IgE profiles also demonstrated higher blood eosinophil counts, increased eosinophil cationic protein in nasal washes, and both an increased expired nitric oxide, a marker of inflammation, and decreased soluble ICAM-1 in nasal washes at baseline and during cold symptoms. These findings suggest that patients with asthma, who are highly atopic, might be more likely to have increased levels of airway inflammation and be at greater risk for asthma exacerbations in response to RV infection.

SUMMARY

The importance of RV in asthma exacerbations is established in both adults and children. The complex mechanisms by which their interaction provokes asthma are becoming better understood. RV appears to have a direct and negative impact on the lower airways and causes an increase in obstructive airway symptoms and physiology. This effect on airway function is felt to occur as the virus upregulates proinflammatory cytokines and predisposes the asthmatic patient to more severe respiratory infections and hence to exacerbations. Defects in T_H1-type immune responses appear to be an important factor in causing airway inflammation in people with asthma. Further work is needed to better explore the mechanisms behind the association between asthma exacerbations and RV infections. This might ultimately lead to treatment modalities to prevent and/or treat the significant burden of asthma exacerbations caused by RV infection.

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