Review Article

Rhinovirus and airway allergy

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ABSTRACT

Rhinoviruses cause the majority of common colds, which often provoke wheezing in patients with asthma. The precise mechanisms responsible for the rhinovirus infection-induced exacerbations of bronchial asthma remain uncertain. However, several reports have demonstrated airway hyperresponsiveness, increases in chemical mediators in airway secretions, such as kinin and histamine, and airway inflammation in patients with bronchial asthma after rhinovirus infection. Rhinovirus infection induces the accumulation of inflammatory cells in airway mucosa and submucosa, including neutrophils, lymphocytes and eosinophils. Rhinovirus affects the barrier function of airway epithelial cells and activates airway epithelial cells and other cells in the lung to produce proinflammatory cytokines, including various types of interleukins, granulocyte–macrophage colony stimulating factor and RANTES, and histamine. Rhinovirus also stimulates the expression of intercellular adhesion molecule-1 (ICAM-1) and low-density lipoprotein receptors in the airway epithelium, receptors for major and minor rhinoviruses. Rhinovirus infection is inhibited by treatment with soluble ICAM-1 and by the reduction of ICAM-1 expression in airway epithelial cells after treatment with either glucocorticoid or erythromycin. Both soluble ICAM-1 and erythromycin have been reported to reduce the symptoms of common colds. Herein, we review the pathogenesis and management of rhinovirus infection-induced exacerbation of bronchial asthma and the relationship between rhinovirus infection and airway allergy.

Key words: airway allergy, airway hyperresponsiveness, airway inflammation, bronchial asthma, rhinovirus.

CLINICAL IMPORTANCE OF RHINOVIRUS INFECTION ON BRONCHIAL ASTHMA

Rhinoviruses cause the majority of common colds, which often provoke wheezing in patients with asthma.1–5 Upper respiratory virus infections are associated with hospital admission for asthma6 and the incidence of rhinovirus infections is suggested be higher in patients with asthma compared with control subjects.7 Prospective studies using cell culture techniques and detection of increasing titers of virus-specific antibodies have indicated that asthma attacks are associated with infection by respiratory viruses, including influenza virus, adenovirus, respiratory syncytial viruses and rhinoviruses, in as many as 20–50% of cases.2,4 Recent studies have demonstrated that reverse transcription-polymerase chain reaction assays are more sensitive for detecting viruses, including rhinoviruses, compared with standard cell culture techniques5 and have emphasized the importance of rhinoviruses by demonstrating that rhinoviruses are responsible for 80–85% and 45% of the asthma flairs in 9–11-year-old children and adults, respectively, with rhinovirus being the most commonly implicated pathogen.1,3

EFFECTS OF RHINOVIRUS INFECTION ON HUMAN SUBJECTS

Airway hyperresponsiveness, which is defined as the increased sensitivity of the small airways to bronchoconstriction in response to inhaled histamine or methacholine, is associated with the pathogenesis of bronchial asthma. Various studies report airway hyperresponsiveness

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after respiratory virus infections. Cheung et al. studied the effects of experimentally infected RV16 on airway narrowing and demonstrated increased airway hyperresponsiveness in response to inhaled methacholine at only 2 days after RV16 inoculation. Furthermore, the maximal response to inhaled methacholine in the RV16-infected group was significantly higher than that in the placebo group. Experimentally inoculated RV16 also increases more potent airway hyperresponsiveness to inhaled histamine in volunteers with respiratory allergy or in asthmatic patients, including atopic subjects, compared with non-allergic subjects. Furthermore, rhinovirus infection increases bronchial responsiveness to histamine and ragweed antigen in association with increases in the histamine release from peripheral blood leukocytes in patients with allergic rhinitis.

Peak expiratory flow rate (PEFR) decreases during the exacerbation of bronchial asthma after respiratory virus infection, but does returns to levels seen before the infection (Fig. 1). In contrast, exhaled carbon monoxide, which is suggested to be produced in the alveolar macrophages and airway epithelial cells in the inflamed lung, increases in patients with untreated bronchial asthma and exacerbations of bronchial asthma.

Infection by respiratory viruses, including rhinoviruses, also activates histamine release from basophils in peripheral blood and the plasma histamine content and kinin concentration in nasal lavage fluid increases after rhinovirus infection. Rhinovirus infection causes the infiltration of neutrophils, lymphocytes and eosinophils in nasal and bronchial mucosa. Furthermore, epithelial eosinophil numbers in patients with bronchial asthma after experimental RV16 inoculation remained elevated during convalescence when the number of eosinophils was similar to preinfection baseline counts in non-asthmatic normal subjects.

These findings suggest that rhinovirus infection may induce airway narrowing and airway hyperresponsiveness in association with airway inflammation in human subjects, resulting in exacerbations of bronchial asthma. Airway narrowing may be caused by inflammatory chemical mediators released from inflammatory cells. Similarly, inflammatory cells, including neutrophils, lymphocytes and eosinophils may be accumulated and activated by the inflammatory mediators and various proinflammatory cytokines, as described below, thereby inducing airway inflammation after rhinovirus infections.

**Fig. 1** Time-course of changes in (a) exhaled carbon monoxide (CO) concentrations and (b) peak expiratory flow rate (PEFR) in asthmatic patients before acute asthma exacerbations (baseline) and after treatment with oral glucocorticoids. The arrow indicates the start of treatment of acute asthma exacerbations with oral glucocorticoids. Horizontal bars indicate mean values for each time-point. Reproduced with permission from Yamaya et al.

**Effects of Rhinovirus Infection on Airway Epithelial Cells**

In order to understand the mechanisms of airway inflammation after rhinovirus infection, various studies have been performed on the production of proinflammatory substances, adhesion molecules and chemical mediators from cells in the lung. Rhinovirus infection increases the production of various proinflammatory substances, including interleukin (IL)-1α, IL-1β, IL-6, IL-8, IL-11, tumor necrosis factor (TNF)-α, RANTES and granulocyte–macrophage colony stimulating factor (GM-CSF) in epithelial cells, primary cultures of epithelial cells or cell
Subauste et al.\textsuperscript{17} demonstrated that RV14 infection induced the release of IL-6, IL-8 and TNF-\(\alpha\), and that pre-exposure of a human bronchial epithelial cell line (BEAS-2B) to TNF-\(\alpha\) increased susceptibility to RV14 infection. Subauste et al.\textsuperscript{17} suggested that inflammatory cytokines produced by rhinovirus infection may increase the susceptibility to rhinovirus infection. Interleukin-6 induces antibody production in B cells and T cell activation and differentiation. Interleukin-8 is a major chemoattractant for neutrophils and stimulates neutrophils to cause enzyme release and the production of reactive oxygen species. Similarly, GM-CSF can prime both neutrophils and eosinophils for enhanced activation to chemical stimuli. Rhinovirus infection increases the production of eotaxin and RANTES, which activates eosinophils, in bronchial epithelial cells.\textsuperscript{18,19} Interleukin-11 is suggested to have direct effects on bronchial hyperresponsiveness.\textsuperscript{24} We also demonstrated that RV14, a major rhinovirus, and RV2, a minor type of rhinovirus, can be infected to primary culture of human tracheal epithelial cells and submucosal gland cells through binding to intercellular adhesion molecule (ICAM)-1 and low-density lipoprotein (LDL) receptors, respectively, and produce proinflammatory cytokines, including IL-1\(\alpha\), IL-1\(\beta\), IL-6, IL-8, TNF-\(\alpha\) and GM-CSF, as well as ICAM-1 and LDL receptor (Figs 2,3).\textsuperscript{20–22,26} Activation of the transcription factor nuclear factor (NF)-\(\kappa\)B is associated with the production of proinflammatory cytokines and ICAM-1,\textsuperscript{23,27,28} and the endogenous production of IL-1\(\beta\) is associated with ICAM-1 expression after rhinovirus infection.\textsuperscript{20}

Upregulation of ICAM-1 could increase the susceptibility to major group rhinoviruses\textsuperscript{29} and could lead cells adjacent to infected cells to infection when viruses are released from the cells infected originally. Furthermore, chronic antigen challenge has been shown to increase ICAM-1 expression on airway epithelium, which may be related to airway inflammation in asthma.\textsuperscript{30}

![Fig. 2 Time-course of the release of cytokines into supernatants of human tracheal submucosal gland cells after RV14 infection (\(\bullet\)), sham infection (control). IL, interleukin; TNF-\(\alpha\), tumor necrosis factor-\(\alpha\); GM-CSF, granulocyte–macrophage colony stimulating factor. Data are the mean \(\pm\) SEM from seven samples. *\(P < 0.05\), **\(P < 0.01\), ***\(P < 0.001\) compared with corresponding control values. Reproduced with permission from Yamaya et al.\textsuperscript{21}](image-url)
Inflammatory conditions, such as asthma, smoking and ozone exposure, in which ICAM-1 expression is increased on respiratory epithelial surfaces, may cause a predisposition to rhinovirus infection by increasing the expression of the major group of rhinovirus receptors. The rhinovirus infection would enhance airway inflammation by recruiting neutrophils and, potentially, other inflammatory cells, causing increased mediator release and exacerbation of the underlying reactive airway diseases.

Furthermore, we demonstrated that hydrogen peroxide increases the transepithelial influx of mannitol in cultured human tracheal epithelial layers and rhinovirus infection further increases the mannitol influx in cells treated with IL-1β. These findings suggest that rhinovirus infection may affect the integrity of airway epithelial cells, although rhinovirus infection does not induce airway epithelial cell damage, as induced by influenza virus infection. Infection with Streptococcus pneumoniae after respiratory infection is associated with the severity of illness and more frequent hospitalization. Rhinovirus infection also increases the adherence of S. pneumoniae to human tracheal epithelial cells via increases in platelet-activating factor receptors, suggesting that increased adherence of S. pneumoniae may be one of the reasons that pneumonia develops after rhinovirus infection.
Effects of Rhinovirus Infection on Mast Cells and Cells Other Than Airway Epithelial Cells

Cells other than lung epithelial cells have also been reported to produce proinflammatory substances and chemical mediators, such as histamine. Infection of respiratory viruses, including rhinoviruses, activates histamine release from basophils of the peripheral blood\textsuperscript{14} and the plasma histamine content increases after rhinovirus infection.\textsuperscript{16} Virus infection, including rhinovirus, increases histamine release in basophils stimulated with anti-IgE and calcium ionophore after rhinovirus infection.\textsuperscript{35} Furthermore, rhinovirus infection increases bronchial responsiveness to histamine and ragweed antigen in association with increases in the histamine release from peripheral blood leukocytes in patients with allergic rhinitis.\textsuperscript{8} Mast cells are major sources of histamine release in airways and are associated with the pathogenesis of bronchial asthma.\textsuperscript{36} We demonstrated that rhinovirus infection primes the production of IL-4, IL-6, IL-8, GM-CSF and histamine in response to stimuli including IgE in both a human mast cell line and a human basophilic leukocyte cell line (Fig. 4).\textsuperscript{37}

Airway macrophages secrete TNF-\(\alpha\) after rhinovirus infection.\textsuperscript{38} Tumor necrosis factor-\(\alpha\) increases the expression of ICAM-1 and other adhesion molecules on a number of different cell types and is associated with wheezing illness in infancy\textsuperscript{39} and the development of the late-phase allergic reaction and asthma.\textsuperscript{40}

Neutrophils accumulates in the airway during the acute stage of a cold.\textsuperscript{41} Interleukin-8 and myeloperoxidase levels in nasal aspirate increase in children during rhinovirus infection-induced asthma exacerbations.\textsuperscript{42} Furthermore, myeloperoxidase levels in nasal aspirate correlate with upper respiratory symptom severity.\textsuperscript{42} Thus, IL-8 from neutrophils in the airway after rhinovirus infection may also be associated with asthma exacerbations.

Similarly, eosinophil accumulation is observed in airway mucosa\textsuperscript{11} after experimental rhinovirus infection. Eosinophil granular proteins, including eosinophil cationic protein (ECP), have been detected in the nasal secretions of children with wheezing illness caused by rhinovirus infection\textsuperscript{43} and in the sputum of asthmatic

Fig. 4 (a,c) Release of histamine into the supernatants of HMC-1 cells in the presence of (a) phorbol myristate acetate (PMA) plus A23187, (c) IgE plus anti-IgE (IgE + a-IgE) or vehicle after RV14 or sham infection (control). (b,d) Release of histamine into the supernatants of KU812 cells in the presence of (b) PMA plus A23187, (d) IgE plus anti-IgE (IgE + a-IgE) or vehicle after RV14 or sham infection (control). Data are the mean \(\pm\) SEM from seven samples. **\(P < 0.01\), ***\(P < 0.001\) compared with RV14 infection alone (control); ††\(P < 0.05\), †††\(P < 0.01\) compared with stimulation with PMA + A23187 or stimulation with IgE + a-IgE alone. Reproduced with the permission of The American Association of Immunologists, Inc., from Hosoda et al.\textsuperscript{37}
patients infected experimentally with RV16. Increases in ECP levels and the percentage of eosinophils in sputum correlated with airway hyperresponsiveness. However, RV16 did not induce superoxide production from peripheral blood eosinophils, as shown by Handzel et al. Therefore, inflammatory mediators such as RANTES and GM-CSF released from cells, including airway submucosal cells and mast cells, may activate eosinophils after rhinovirus infection. In fact, we have demonstrated that eosinophil migration through the airway epithelial cell layers increases in response to the addition of supernatants of human tracheal submucosal glands infected with RV14, through the GM-CSF and RANTES in the supernatants.

Rhinovirus stimulates lymphocytes to induce interferon (IFN)-γ production and T cell proliferation through the activation of eosinophils and monocytes. Experimental rhinovirus infection revealed the accumulation of lymphocytes and monocytes in the airway mucosa and submucosa. Activated lymphocytes may also be associated with exacerbations of bronchial asthma.

Furthermore, the direct effects of rhinovirus on airway smooth muscle contraction were demonstrated by Hakonarson et al. Rhinovirus infection increased rabbit and human airway smooth muscle constrictor responsiveness to acetylcholine and attenuated the dose-dependent relaxation of the smooth muscle to β-adrenergic receptor stimulation with isoproterenol.

**INHIBITION AND TREATMENT OF RHINOVIRUS INFECTION**

In contrast with influenza virus, an effective vaccination for rhinoviruses has not been developed because there are more than 100 serotypes of rhinoviruses. A variety of antiviral agents has been studied on the inhibition of rhinovirus infection or common colds, including vitamin C, zinc gluconate lozenges, WIN compounds, soluble ICAM-1, rhinovirus 3C protease inhibitors, the compound R77975, IFN-α, dexamethasone, macrolide antibiotics, bafilomycin, and erythromycin. However, the effect of vitamin C supplementation has been controversial. Similarly, controlled trials have revealed that zinc gluconate is not effective and the nasal toxicities of INF-α may impose some limitations for its clinical use. WIN 52084 and R77975, antiviral agents that inhibit viral structural dynamics, were not effective at all in reducing cold symptoms. Soluble ICAM-1 inhibits the rhinovirus from adhering to the cells and inhibits rhinovirus infection in chimpanzees. So far, soluble ICAM-1 is the only possible agent that may be useful in alleviating the symptoms of the common cold. However, other WIN compounds and a rhinovirus proteinase enzyme inhibitor are undergoing phase III clinical trials. Two viral proteases designated 2A and 3C have been viewed as excellent targets for antiviral intervention for the picornavirus family, including human rhinovirus.

The specific vacuolar H+-ATPase inhibitor and macrolide antibiotic bafilomycin A₁ blocks the infection of influenza virus and rhinovirus in HeLa cells and Mardin-Darby canine kidney (MDCK) cells and uncoating of rhinovirus type 2 and type 14 (RV14) from late endosomes. We demonstrated the inhibitory effects of bafilomycin A₁ on rhinovirus infection in human tracheal epithelial cells. Bafilomycin A₁ reduced the viral titer of RV14 and inhibited the production of cytokines, including IL-1β, IL-6, IL-8 and TNF-α, and ICAM-1 before and after RV14 infection. Bafilomycin A₁ reduced the susceptibility of epithelial cells to RV14 infection. Infection with RV14 increased activated NF-κB in the cells and bafilomycin A₁ reduced the activated NF-κB. Bafilomycin A₁ decreased the number of acidic endosomes in the epithelial cells.

Furthermore, we have shown that erythromycin, a clinically used macrolide antibiotic, reduces the supernatant RV14 titer, RV14 RNA, the susceptibility to RV14 infection and the production of ICAM-1 and cytokines (Fig. 3). Erythromycin also reduces the supernatant RV2 titer, RV2 RNA, the susceptibility to RV2 infection and cytokine production, although the inhibitory effects of erythromycin on the expression of the LDL receptor, the minor rhinovirus receptor, were small. Erythromycin reduced NF-κB activation by RV14 and decreased the number of acidic endosomes in epithelial cells. These results suggest that the macrolide antibiotics erythromycin and bafilomycin inhibit infection by the major rhinovirus subgroup by reducing ICAM-1, a receptor for a major subgroup of rhinoviruses, and infection by both major and minor rhinovirus subgroups by blocking the entry of rhinovirus RNA into the endosomes in human tracheal epithelial cells.

We also showed that dexamethasone inhibits infection by the major rhinovirus subgroup by reducing ICAM-1 in human tracheal epithelial cells. Dexamethasone also reduced the production of cytokines in epithelial cells.

Furthermore, we showed that erythromycin therapy has beneficial effects on the prevention of the common cold.
cold and exacerbations in patients with chronic obstructive pulmonary disease (COPD). Low-dose and long-term erythromycin therapy has been reported to be effective in treating patients with diffuse panbronchiolitis or bronchiectasis via mechanisms other than antibacterial activity.

To examine whether erythromycin therapy lowers the frequency of the common cold and subsequent exacerbations in patients with COPD, a prospective, randomized, controlled, but not blinded, trial was performed. One hundred and nine patients with COPD were enrolled into the study. Patients were assigned randomly to erythromycin therapy or to no active treatment in September 1997. Patients were then observed for 12 months, starting in October, during which time the risk and frequency of catching common colds and COPD exacerbations were investigated. Fifty-five patients received erythromycin at study entry (erythromycin group). The remaining 54 patients received no active treatment (control group). The number of common colds for 12 months was significantly lower in the erythromycin group than in the control group. Similarly, the number of exacerbations for 12 months was significantly lower in the erythromycin group than in the control group. Furthermore, significantly more patients were hospitalized due to exacerbations in the control group than in the erythromycin group. These findings suggest that erythromycin has beneficial effects on the prevention of common colds and exacerbations in COPD patients. However, this intervention should be restricted to patients who are at high risk for exacerbations of COPD because of the potential risk for the emergence of erythromycin-resistant pathogens.

**Summary of Rhinovirus Infection-Induced Airway Inflammation, Airway Allergy and Bronchial Asthma**

Although the precise mechanisms remain uncertain, various findings suggest the effects of rhinovirus infection on the airway immune response and airway inflammation, which are associated with the exacerbations of bronchial asthma. Rhinovirus infection affects the barrier function of airway epithelial cells, whereas rhinovirus infection has been reported not to induce epithelial damage. Rhinovirus infection also stimulates the production of various types of proinflammatory mediators from the airway epithelial cells, including IL-1, IL-6, IL-8, GM-CSF, and RANTES. These mediators may induce the accumulation of inflammatory cells, such as neutrophils, eosinophils and lymphocytes, in the airway mucosa and submucosa and may activate these cells to release reactive oxygen species and elastase, thereby resulting in airway epithelial cell damage. Direct and indirect airway epithelial damage and airway inflammation may be associated with airway hypersensitivity after rhinovirus infection.

Although the role of rhinovirus infection on airway secretion and airway smooth muscle contraction are also uncertain, levels of kinin in nasal secretion and serum histamine are increased after experimental rhinovirus infection. Rhinovirus infection stimulates histamine release from human mast cells in response to various stimuli, including IgE, whereas the source of kinin remains unknown, suggesting the association of rhinovirus infection with airway allergy. Rhinovirus infection can stimulate the production of mucin mRNA in airway epithelial cells and neutrophil elastase is suggested to induce mucus production from airway submucosal glands. Increased permeability in airway epithelial cells may also be associated with airway hypersecretion. Rhinovirus infection directly stimulates airway smooth muscle contraction. Thus, the mucus hypersecretion, airway contraction and airway allergy may be caused by various mechanisms in the exacerbations of bronchial asthma after rhinovirus infection.

**References**


