Allergology International (2004) **53**: 37–45

Review Article

Rhinovirus and airway allergy

Mutsuo Yamaya and Hidetada Sasaki

Department of Geriatric and Respiratory Medicine, Tohoku University School of Medicine, Sendai, Japan

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.

Correspondence: Associate Professor Mutsuo Yamaya, Department of Geriatric and Respiratory Medicine, Tohoku University School of Medicine, 1-1 Seiryo-machi, Aoba-ku, Sendai-city, Miyagi 980-8574, Japan.
Email: yamaya@geriat.med.tohoku.ac.jp
Received 4 December 2003.

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 asthma[6] 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 techniques[5] 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

after respiratory virus infections.[8–10] Cheung *et al*.[9] 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.[9] Experimentally inoculated RV16 also increases more potent airway hyperresponsiveness to inhaled histamine in volunteers with respiratory allergy[10] or in asthmatic patients, including atopic subjects,[11] 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.[8]

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).[1,12] 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[13] and exacerbations of bronchial asthma.[12]

Infection by respiratory viruses, including rhinoviruses, also activates histamine release from basophils in peripheral blood[14] and the plasma histamine content and kinin concentration in nasal lavage fluid increases after rhinovirus infection.[15,16] Rhinovirus infection causes the infiltration of neutrophils, lymphocytes and eosinophils in nasal and bronchial mucosa.[11] Furthermore, epithelial eosinophil numbers in patients with bronchial asthma after experimental RV16 inoculation remained elevated during convalecence when the number of eosinophils was similar to preinfection baseline counts in non-asthmatic normal subjects.[11]

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.

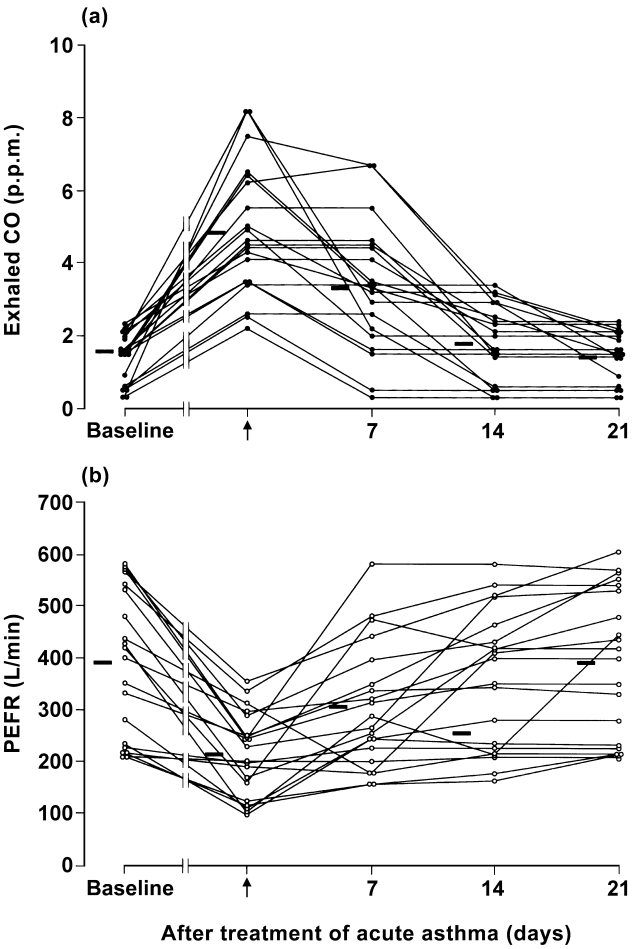

Fig. 1 Time-course of changes in (a) exhaled carbon monoxide (CO) concentrations and (b) peak expiratory flow rate (PEFR) in asthmatic patients before acute asthma exacerbations (baseline) and after treatment with oral glucocorticoids. The arrow indicates the start of treatment of acute asthma exacerbations with oral glucocorticoids. Horizontal bars indicate mean values for each time-point. Reproduced with permission from Yamaya *et al*.[12]

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

lines.[17–25] Subauste *et al*.[17] demonstrated that RV14 infection induced the release of IL-6, IL-8 and TNF-α, and that pre-exposure of a human bronchial epithelial cell line (BEAS-2B) to TNF-α increased susceptibility to RV14 infection. Subauste *et al*.[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.[18,19] Interleukin-11 is suggested to have direct effects on bronchial hyperresponsiveness.[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-1α, IL-1β, IL-6, IL-8, TNF-α and GM-CSF, as well as ICAM-1 and LDL receptor (Figs 2,3).[20–22,26] Activation of the transcription factor nuclear factor (NF)-κB is associated with the production of proinflammatory cytokines and ICAM-1,[23,27,28] and the endogenous production of IL-1β is associated with ICAM-1 expression after rhinovirus infection.[20]

Upregulation of ICAM-1 could increase the susceptibility to major group rhinoviruses[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.[30]

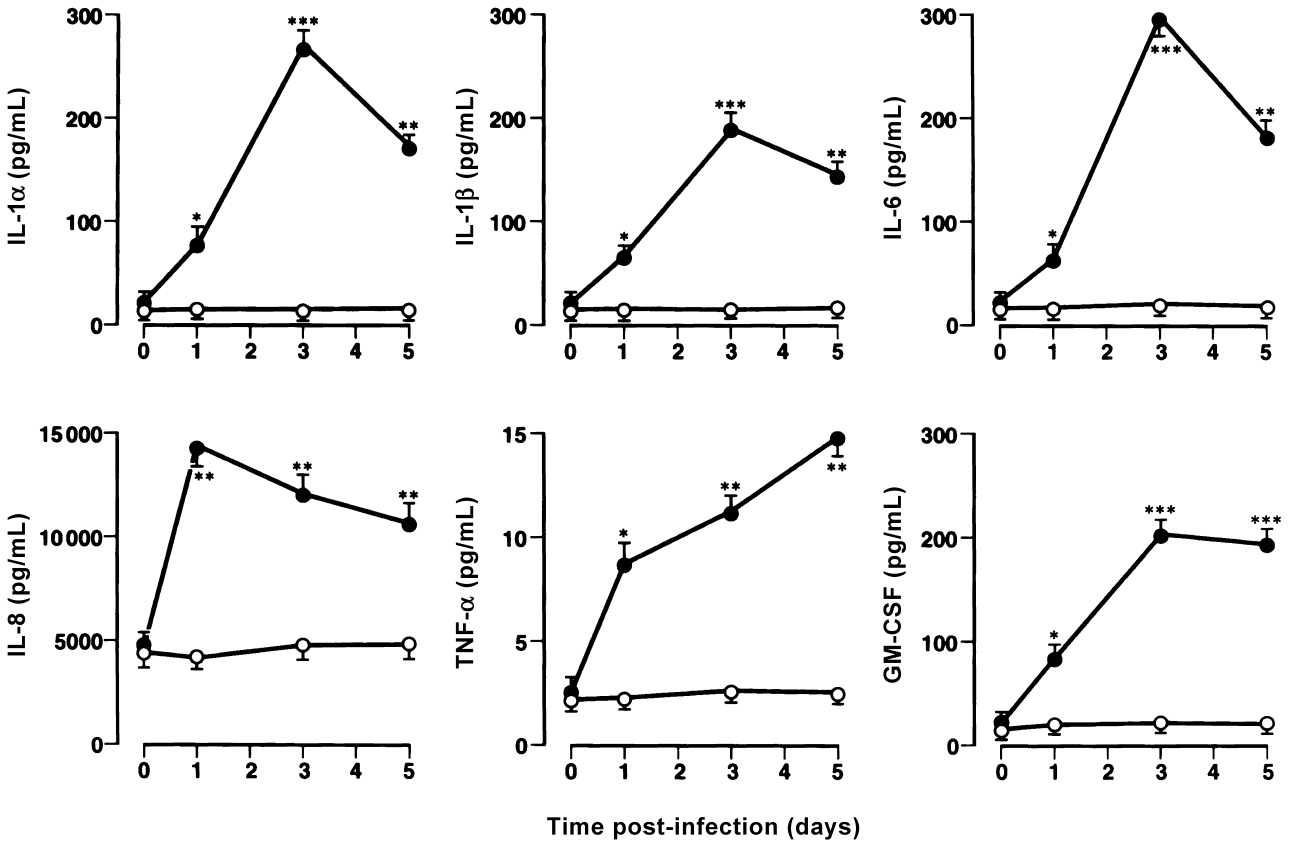

Fig. 2 Time-course of the release of cytokines into supernatants of human tracheal submucosal gland cells after RV14 infection (●). (○), sham infection (control). IL, interleukin; TNF-α, tumor necrosis factor-α; GM-CSF, granulocyte–macrophage colony stimulating factor. Data are the mean ± SEM from seven samples. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ compared with corresponding control values. Reproduced with permission from Yamaya *et al*.[21]

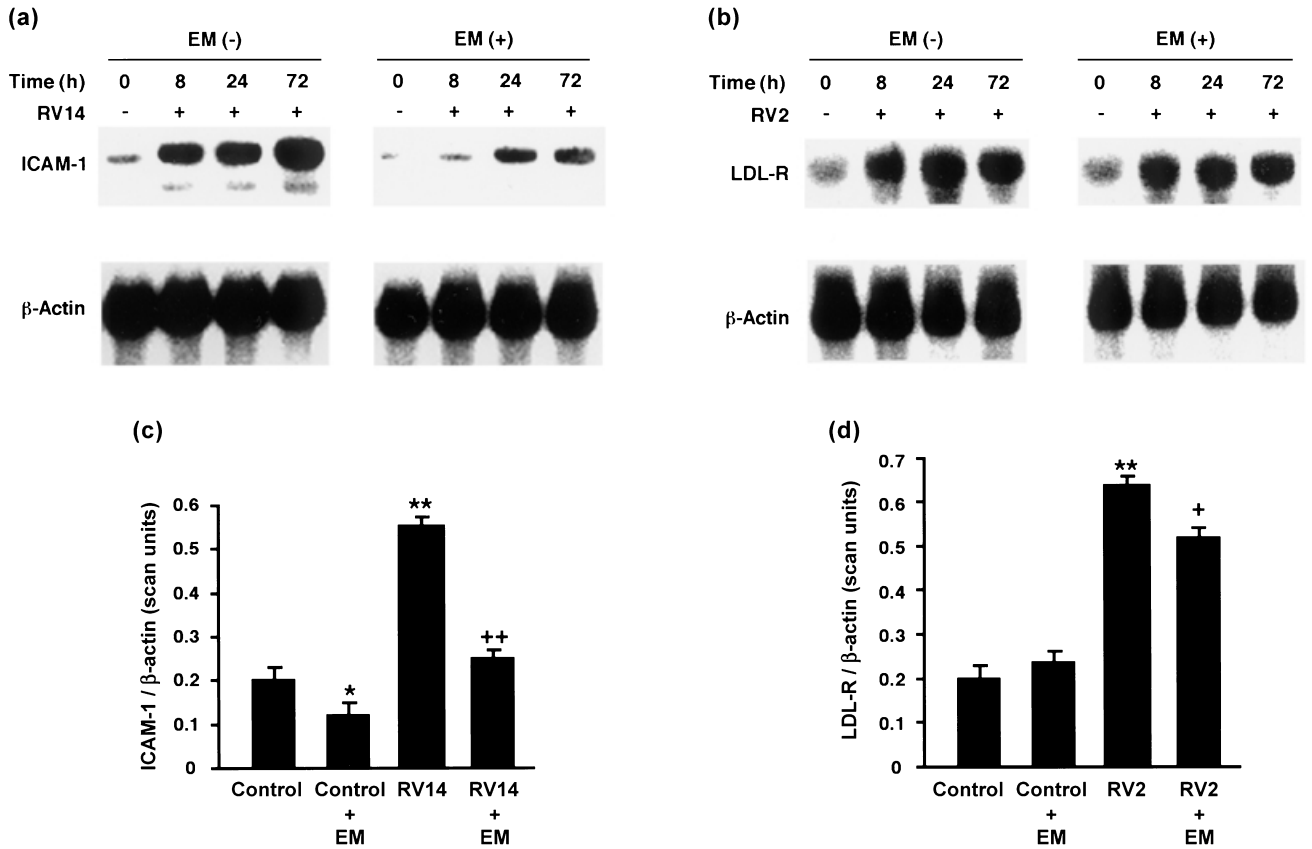

Fig. 3 (a,b) Northern blot analysis for (a) intercellular adhesion molecule (ICAM)-1 and (b) low-density lipoprotein receptor (LDL-R) mRNA levels of human tracheal epithelial cells before (0), 8, 24, and 72 h after RV14 (a) or RV2 (b) infection in the absence (–) or presence (+) of 10 μmol/L erythromycin (EM). β-Actin was used as a housekeeping gene. (c,d) Effects of 10 μmol/L EM on the expression of ICAM-1 (c) and LDL-R (d) mRNA in human tracheal epithelial cells 3 days after RV14 (c), RV2 (d) or sham (control) infection. Both ICAM-1 and LDL-R mRNA was normalized to a constitutive expression of β-actin mRNA. Data are the mean ± SEM from seven samples. Significant differences from corresponding control values are indicated by $^{*}P < 0.05$ and $^{**}P < 0.01$ compared with corresponding control values; $^{\dagger\dagger}P < 0.01$ compared with RV14 infection alone; $^{\dagger}P < 0.05$ compared with RV2 infection alone. To examine the effects of EM on ICAM-1 and LDL-R mRNA expression, human tracheal epithelial cells were treated with EM or the vehicle for EM (ethanol, 0.1%) from 3 days before rhinovirus infection to the mRNA extraction after rhinovirus infection. Reproduced with permission from Suzuki *et al*.[26]

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.[31] These findings suggest that rhinovirus infection may affect the integrity of airway epithelial cells, although rhinovirus infection does not induce airway epithelial cell damage,[32] 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,[33] suggesting that increased adherence of *S. pneumoniae* may be one of the reasons that pneumonia develops after rhinovirus infection.[34]

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[14] and the plasma histamine content increases after rhinovirus infection.[16] Virus infection, including rhinovirus, increases histamine release in basophils stimulated with anti-IgE and calcium ionophore after rhinovirus infection.[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 rhininis.[8] Mast cells are major sources of histamine release in airways and are associated with the pathogenesis of bronchial asthma.[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).[37]

Airway macrophages secrete TNF-α after rhinovirus infection.[38] Tumor necrosis factor-α 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[39] and the development of the late-phase allergic reaction and asthma.[40]

Neutrophils accumulates in the airway during the acute stage of a cold.[41] Interleukin-8 and myeloperoxidase levels in nasal aspirate increase in children during rhinovirus infection-induced asthma exacerbations.[42] Furthermore, myeloperoxidase levels in nasal aspirate correlate with upper respiratory symptom severity.[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[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[43] and in the sputum of asthmatic

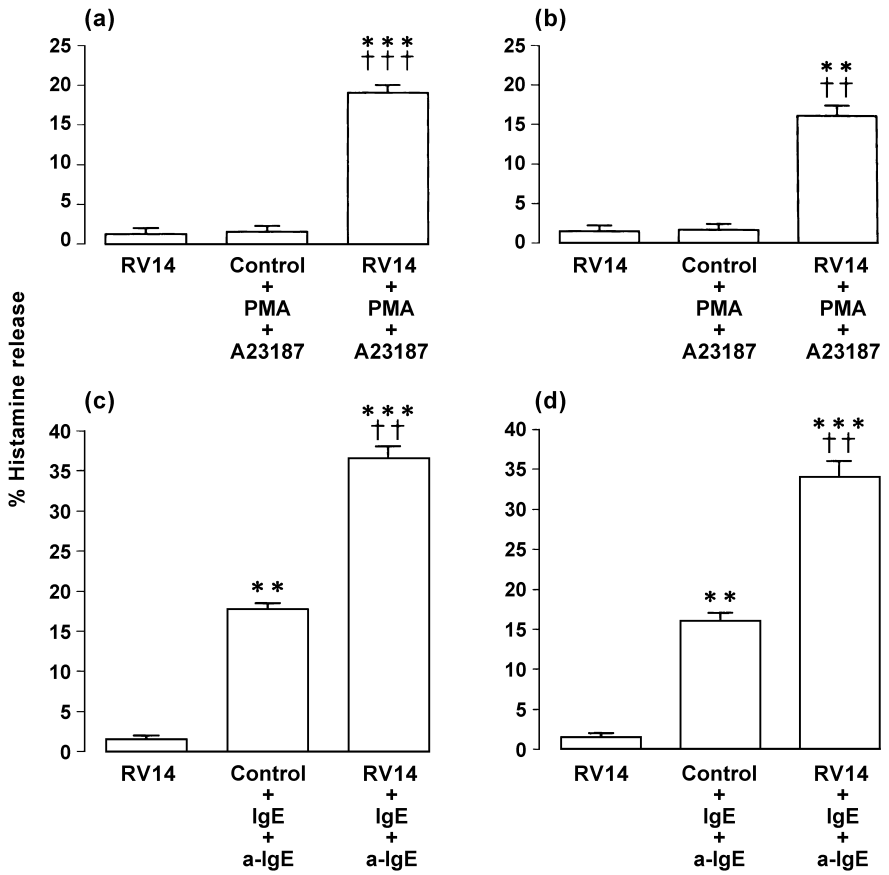

Fig. 4 (a,c) Release of histamine into the supernatants of HMC-1 cells in the presence of (a) phorbol myristate acetate (PMA) plus A23187, (c) IgE plus anti-IgE (IgE + a-IgE) or vehicle after RV14 or sham infection (control). (b,d) Release of histamine into the supernatants of KU812 cells in the presence 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 ± SEM from seven samples. $^{**}P < 0.01$, $^{***}P < 0.001$ compared with RV14 infection alone (control); $^{\dagger\dagger}P < 0.05$, $^{\dagger\dagger\dagger}P < 0.01$ 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*.[37]

patients infected experimentally with RV16.[44] Increases in ECP levels and the percentage of eosinophils in sputum correlated with airway hyperresponsiveness.[44] However, RV16 did not induce superoxide production from peripheral blood eosinophils, as shown by Handzel *et al*.[45] Therefore, inflammatory mediators such as RANTES and GM-CSF[18,19,21,37] 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.[46]

Rhinovirus stimulates lymphocytes to induce interferon (IFN)-γ production and T cell proliferation through the activation of eosinophils[45] and monocytes.[47] Experimental rhinovirus infection revealed the accumulation of lymphocytes and monocytes in the airway mucosa and submucosa.[11] 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*.[48] 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.[48]

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,[49,50] zinc gluconate lozenges,[51,52] WIN compounds,[53,54] very low-density lipoprotein receptor fragments, soluble ICAM-1,[29,55,56] rhinovirus 3C protease inhibitors,[57] the compound R77975,[58] IFN-α,[59] dexamethasone,[60] macrolide antibiotics bafilomycin[28] and erythromycin.[26,61] However, the effect of vitamin C supplementation has been controversial.[50] Similarly, controlled trials have revealed that zinc gluconate is not effective[52] 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,[53,58] were not effective at all in reducing cold symptoms.[58] Soluble ICAM-1 inhibits the rhinovirus from adhering to the cells[29] and inhibits rhinovirus infection in chimpanzees.[55] So far, soluble ICAM-1 is the only possible agent that may be useful in alleviating the symptoms of the common cold.[56] However, other WIN compounds[54] and a rhinovirus proteinase enzyme inhibitor[57] 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.[62]

The specific vacuolar H^+-ATPase inhibitor and macrolide antibiotic bafilomycin A_1 blocks the infection of influenza virus and rhinovirus in HeLa cells and Mardin-Darby canine kidney (MDCK) cells[63,64] and uncoating of rhinovirus type 2 and type 14 (RV14) from late endosomes.[65,66] We demonstrated the inhibitory effects of bafilomycin A_1 on rhinovirus infection in human tracheal epithelial cells.[28] Bafilomycin A_1 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 A_1 reduced the susceptibility of epithelial cells to RV14 infection. Infection with RV14 increased activated NF-κB in the cells and bafilomycin A_1 reduced the activated NF-κB. Bafilomycin A_1 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).[26] Erythromycin also reduces the supernatant RV2 titers, 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 A_1 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.[26,28]

We also showed that dexamethasone inhibits infection by the major rhinovirus subgroup by reducing ICAM-1 in human tracheal epithelial cells.[60] Dexamethasone also reduced the production of cytokines in epithelial cells.[60]

Furthermore, we showed that erythromycin therapy has beneficial effects on the prevention of the common

cold and exacerbations in patients with chronic obstructive pulmonary disease (COPD).[61] 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.[67]

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.[61] 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,[31] whereas rhinovirus infection has been reported not to induce epithelial damage,[32] such as epithelial detachment by influenza virus. 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.[17–25] These mediators may induce the accumulation of inflammatory cells, such as neutrophils, eosinophils and lymphocytes, in the airway mucosa and submucosa[11] and may activate these cells to release reactive oxygen species and elastase,[1] thereby resulting in airway epithelial cell damage.[68] Direct and indirect airway epithelial damage and airway inflammation may be associated with airway hypersensitivity after rhinovirus infection.[8–10]

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,[37] 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[69] and neutrophil elastase is suggested to induce mucus production from airway submucosal glands.[70] Increased permeability in airway epithelial cells may also be also associated with airway hypersecretion. Rhinovirus infection directly stimulates airway smooth muscle contraction.[48] 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

1 Nicholson KG, Kent J, Ireland DC. Respiratory viruses and exacerbations of asthma in adults. *BMJ* 1993; **307**: 982–6.

2 Minor TE, Dick EC, Baker JW, Quellette JJ, Cohen M, Reed CE. Rhinovirus and infuenza type A infections as precipitants of asthma. *Am. Rev. Respir. Dis.* 1976; **113**: 149–53.

3 Johnston SL, Pattemore PK, Sanderson G *et al*. Community study of role of viral infections in exacerbations of asthma in 9–11 year old children. *BMJ* 1995; **310**: 1225–9.

4 Pattemore PK, Johnston SL, Bardin PG. Viruses as precipitants of asthma symptoms. I. Epidemiology. *Clin. Exp. Allergy* 1992; **22**: 325–36.

5 Gama RE, Horsnell PR, Hughes PJ *et al*. Amplification of rhinovirus specific nucleic acids from clinical samples using the polymerase chain reaction. *J. Med. Virol.* 1989; **28**: 73–7.

6 Johnston SL, Pattemore PK, Sanderson G *et al*. The relationship between upper respiratory infections and hospital admissions for asthma: A time trend analysis. *Am. J. Respir. Crit. Care Med.* 1996; **154**: 645–60.

7 Minor TE, Baker JW, Dick EC *et al*. Greater frequency of viral respiratory infections in asthmatic children as compared with their nonasthmatic siblings. *J. Pediatr*. 1974; **85**: 472–7.

8 Lemanske Jr RF, Dick EC, Swenson CA, Vrtis RF, Busse WW. Rhinovirus upper respiratory infection increases airway hyperreactivity and late asthmatic reactions. *J. Clin. Invest*. 1989; **83**: 1–10.

9 Cheung D, Dick EC, Timmers MC, De Klerk EPA, Spaan WJM, Sterk PJ. Rhinovirus inhalation causes long-lasting excessive airway narrowing in response to methacholine in asthmatic subjects *in vivo*. *Am. J. Respir. Crit. Care Med*. 1995; **152**: 1490–6.

10 Gern JE, Calhoun WJ, Swenson C, Shen G, Busse WW. Rhinovirus infection preferentially increases lower airway responsiveness in allergic subjects. *Am. J. Respir. Crit. Care Med*. 1997; **155**: 1872–6.

11 Fraenkel DJ, Bardin PG, Sanderson G, Lampe F, Johnston SL, Holgate ST. Lower airways inflammation during rhinovirus colds in normal and in asthmatic subjects. *Am. J. Respir. Crit. Care Med*. 1995; **151**: 879–86.

12 Yamaya M, Sekizawa K, Ishizuka S, Monma M, Sasaki H. Exhaled carbon monoxide levels during treatment of acute asthma. *Eur. Respir. J*. 1999; **13**: 757–60.

13 Zayasu K, Sekizawa K, Okinaga S, Yamaya M, Ohrui T, Sasaki H. Increased carbon monoxide in exhaled air of asthmatic patients. *Am. J. Respir. Crit. Care Med*. 1997; **156**: 1140–3.

14 Chonmaitree T, Lett-Brown MA, Tsong Y, Goldman AS, Baron S. Role of interferon in leukocyte histamine release caused by common respiratory viruses. *J. Infect. Dis*. 1988; **157**: 127–32.

15 Naclerio RM, Proud D, Lichtenstein LM *et al*. Kinins are generated during experimental rhinovirus colds. *J. Infect. Dis*. 1988; **157**: 133–42.

16 Calhoun WJ, Swenson CA, Dick EC, Schwartz LB, Lemanske Jr RF, Busse WW. Experimental rhinovirus 16 infection potentiates histamine release after antigen bronchoprovocation in allergic subjects. *Am. Rev. Respir. Dis*. 1991; **144**: 1267–73.

17 Subauste MC, Jacoby DB, Richards SM, Proud D. Infection of a human respiratory epithelial cell line with rhinovirus. Induction of cytokine release and modulation of susceptibility to infection by cytokine exposure. *J. Clin. Invest*. 1995; **96**: 549–57.

18 Papadopoulos NG, Papi A, Meyer J *et al*. Rhinovirus infection up-regulates eotaxin and eotaxin-2 expression in bronchial epithelial cells. *Clin. Exp. Allergy* 2001; **31**: 1060–6.

19 Schroth MK, Grimm E, Frindt P *et al*. Rhinovirus replication causes RANTES production in primary bronchial epithelial cells. *Am. J. Respir. Cell Mol. Biol*. 1999; **20**: 1220–8.

20 Terajima M, Yamaya M, Sekizawa K *et al*. Rhinovirus infection of primary cultures of human tracheal epithelium: Role of ICAM-1 and IL-1β. *Am. J. Physiol*. 1997; **273**: L749–59.

21 Yamaya M, Sekizawa K, Suzuki T *et al*. Infection of human respiratory submucosal glands with rhinovirus: Effects on cytokine and ICAM-1 production. *Am. J. Physiol*. 1999; **277**: L362–71.

22 Suzuki T, Yamaya M, Kamanaka M *et al*. Type 2 rhinovirus infection of cultured human tracheal epithelial cells: Role of LDL receptor. *Am. J. Physiol*. 2001; **280**: L409–20.

23 Zhu Z, Tang W, Ray A *et al*. Rhinovirus stimulation of interleukin-6 *in vivo* and *in vitro*. Evidence for nuclear factor κB-dependent transcriptional activation. *J. Clin. Invest*. 1996; **97**: 421–30.

24 Einarsson O, Geba GP, Zhu Z, Landry M, Elias JA. Interleukin-11 stimulation *in vivo* and *in vitro* by respiratory viruses and induction of airways hyperresponsiveness. *J. Clin. Invest*. 1996; **97**: 915–24.

25 Johnston JL, Papi A, Bates PJ, Mastronarde JG, Monick MM, Hunninghake GW. Low grade rhinovirus infection induces a prolonged release of IL-8 in pulmonary epithelium. *J. Immunol*. 1998; **160**: 6172–81.

26 Suzuki T, Yamaya M, Sekizawa K *et al*. Erythromycin inhibits rhinovirus infection in cultured human tracheal epithelial cells. *Am. J. Respir. Crit. Care Med*. 2002; **165**: 1113–18.

27 Papi A, Johnston SL. Rhinovirus infection induces expression of its own receptor intercellular adhesion molecule 1 (ICAM-1) via increased NF-kappa B-mediated transcription. *J. Biol. Chem*. 1999; **274**: 9707–20.

28 Suzuki T, Yamaya M, Sekizawa K *et al*. Bafilomycin A1 inhibits rhinovirus infection in human airway epithelium: Effects on endosome and ICAM-1. *Am. J. Physiol*. 2001; **280**: L1115–27.

29 Greve JM, Davis G, Meyer AM *et al*. The major human rhinovirus receptor is ICAM-1. *Cell* 1989; **56**: 839–47.

30 Wegner CD, Gundel RH, Relly P, Haynes N, Letts LG, Rothlein R Intercellular adhesion molecule-1 (ICAM-1) in the pathogenesis of asthma. *Science* 1990; **247**: 456–9.

31 Ohrui T, Yamaya M, Sekizawa K *et al*. Effects of rhinovirus infection on hydrogen peroxide-induced alterations of barrier function in the cultured human tracheal epithelium. *Am. J. Respir. Crit. Care Med*. 1998; **158**: 241–8.

32 Winther B, Gwaltney JM, Hendley JO. Respiratory virus infection of monolayer cultures of human nasal epithelial cells. *Am. Rev. Respir. Dis*. 1990; **141**: 839–45.

33 Ishizuka S, Yamaya M, Suzuki T *et al*. Effects of rhinovirus infection on the adherence of *Streptococcus pneumoniae* to cultured human airway epithelial cells. *J. Infect. Dis*. 2003; **188**: 1928–39.

34 Juven T, Mertsola J, Waris M *et al*. Etiology of community-acquired pneumonia in 254 hospitalized children. *Pediatr. Infect. Dis. J*. 2000; **19**: 293–8.

35 Ida S, Hooks JJ, Siraganian RP, Notkins AL. Enhancement of IgE-mediated histamine release from human basophils by viruses: Role of interferon. *J. Exp. Med*. 1977; **145**: 892–905.

36 Galli SJ. New concepts about the mast cell. *N. Engl. J. Med*. 1993; **328**: 257–65.

37 Hosoda M, Yamaya M, Suzuki T *et al*. Effects of rhinovirus infection on histamine and cytokine production by cell lines from human mast cells and basophils. *J. Immunol*. 2002; **169**: 1482–91.

38 Gern JEEC, Dick WM, Lee EC *et al*. Rhinovirus enters but does not replicate inside monocytes and airway macrophages. *J. Immunol*. 1996; **156**: 621–7.

39 Balfour-Lynn IM, Valman HB, Wellings R, Webster ADB, Taylor GW, Silverman M. Tumor necrosis factor-alpha and leukotriene E_4 production in wheezy infants. *Clin. Exp. Allergy* 1994; **24**: 121–6.

40 Anticevich SZ, Hughes JM, Black JL, Armour CL. Induction of human airway hyperresponsiveness by tumor necrosis factor-alpha. *Eur. J. Pharmacol*. 1995; **284**: 221–5.

41 Turner RB. The role of neutrophils in the pathogenesis of rhinovirus infections. *Prdiatr. Infect. Dis*. 1990; **9**: 832–5.

42 Teran LM, Johnston SL, Schroder JM, Church MK, Holgate ST. Role of nasal interleukin-8 in neutrophil recruitment and activation in children with virus-induced asthma. *Am. J. Respir. Crit. Care Med*. 1997; **155**: 1362–6.

43 Heymann PW, Rakes GP, Hogan AD, Ingram JM, Hoover GE, Platts-Mills TA. Assessment of eosinophils, viruses and IgE antibody in wheezing infants and children. *Int. Arch. Allergy Immunol*. 1995; **107**: 380–2.

44 Grunberg K, Smits HH, Timmers MC *et al*. Experimental rhinovirus 16 infection. Effects on cell differentials and soluble markers in sputum in asthmatic subjects. *Am. J. Respir. Crit. Care Med*. 1997; **156**: 609–16.

45 Handzel ZT, Busse WW, Sedgwick JB *et al*. Eosinophils bind rhinovirus and activate virus-specific T cells. *J. Immunol*. 1998; **160**: 1279–84.

46 Furukawa E, Ohrui T, Yamaya M *et al*. Human airway submucosal glands augment eosinophil chemotaxis during rhinovirus infection. *Clin. Exp. Allergy* 2004 (in press).

47 Gern JE, Vrtis R, Kelly EAB, Dick EC, Busse WW. Rhinovirus produces nonspecific activation of lymphocytes through a monocyte-dependent mechanism. *J. Immunol*. 1996; **157**: 1605–12.

48 Hakonarson H, Maskeri N, Carter C, Hodinka RL, Campbell D, Grunstein MM. Mechanism of rhinovirus-induced changes in airway smooth muscle responsiveness. *J. Clin. Invest*. 1998; **102**: 1732–41.

49 Pauling L. The significance of the evidence about ascorbic acid and the common cold. *Proc. Natl Acad. Sci. USA* 1971; **68**: 2678–81.

50 Hemila H. Vitamin C supplementation and the common cold: Was Linus Pauling right or wrong? *Int. J. Vitam. Nutr. Res*. 1997; **67**: 329–35.

51 Marshall S. Zinc gluconate and the common cold: Review of randomized controlled trial. *Can. Fam. Physician* 1988; **44**: 1037–42.

52 Macknin ML, Piedmonte M, Calendine C, Janosky J, Wald E. Zinc gluconate lozenges for treating the common cold in children: A randomized controlled trial. *JAMA* 1998; **279**: 1962–7.

53 Lewis JK, Bothner B, Smith TJ, Siuzdak G. Antiviral agent blocks breathing of the common cold virus. *Proc. Natl Acad. Sci. USA* 1998; **95**: 6774–8.

54 Hadfield AT, Diana GD, Rossmann MG. Analysis of three structurally related antivial compounds in complex with human rhinovirus 16. *Proc. Natl Acad. Sci. USA* 1999; **96**: 14 730–5.

55 Huguenel ED, Cohn D, Dockrum DP *et al*. Prevention of rhinovirus infection in chimpanzees by soluble intercellular adhesion molecule-1. *Am. J. Respir. Crit. Care Med*. 1997; **155**: 1206–10.

56 Turner RB, Wecker MT, Pohl G *et al*. Efficacy of tremacamra, a soluble intercellular adhesion molecule 1, for experimental rhinovirus infection: A randomized clinical trial. *JAMA* 1999; **281**: 1797–804.

57 Witherell G. AG-7088 Pfizer. *Curr. Opin. Invest. Drugs* 2000; **1**: 297–302.

58 Hayden FG, Hipskind GJ, Woerner DH *et al*. Intranasal pirodavir (R77,975) treatment of rhinovirus colds. *Antimicrob. Agents Chemother*. 1995; **39**: 290–4.

59 Faa BM, Gwaltney Jr JM, Adams KF, Hayden FG. Intranasal interferon-alpha 2 for prevention of natural rhinovirus colds. *Antimicrob. Agents Chemother*. 1984; **26**: 31–4.

60 Suzuki T, Yamaya M, Sekizawa K *et al*. Effects of dexamethasone on rhinovirus infection in cultured human tracheal epithelial cells. *Am. J. Physiol*. 2000; **278**: L560–71.

61 Suzuki T, Yanai M, Yamaya M *et al*. Erythromycin and common cold in COPD. *Chest* 2001; **120**: 730–3.

62 Wang QM. Protease inhibitors as potential antiviral agents for the treatment of picornaviral infections. *Prog. Drug Res*. 2001; **Spec. No**.: 229–53.

63 Ochiai H, Sakai S, Hirabayashi T, Shimizu Y, Terasawa K. Inhibitory effect of bafilomycin A_1, a specific inhibitor of vacuolar-type proton pump, on growth of influenza A and B viruses in MDCK cells. *Antiviral Res*. 1995; **27**: 425–30.

64 Pérez L, Carrasco L. Entry of poliovirus into cells does not require a low-pH step. *J. Virol*. 1993; **67**: 4543–8.

65 Bayer N, Prchla E, Schwab M, Baas D, Fuchs R. Human rhinovirus HRV14 uncoats from early endosomes in the presence of bafilomycin. *FEBS Lett*. 1999; **463**: 175–8.

66 Prchla E, Kuechler E, Blaas D, Fuchs R. Uncoating of human rhinovirus serotype 2 from late endosomes. *J. Virol*. 1994; **68**: 3713–23.

67 Kudoh S. Erythromycin treatment in diffuse panbronchiolitis. *Curr. Opin. Pulmon. Med*. 1998; **4**: 116–21.

68 Nakajoh M, Fukushima T, Suzuki T *et al*. Retinoic acid inhibits elastase-induced injury in human lung epithelial cell lines. *Am. J. Respir. Cell. Mol. Biol*. 2003; **28**: 296–304.

69 Hosoda M, Yamaya M, Yamamoto T, Sasaki H. The effects of rhinovirus infection on the mucin production in primary cultures of human tracheal epithelial cells. *Eur. Respir. J*. 2000; **16**: S506 (Abstract).

70 Schuster A, Fahy JV, Ueki I, Nadel JA. Cystic fibrosis sputum induces a secretory response from gland serous cells that can be prevented by neutrophil protease inhibitors. *Eur. Respir. J*. 1995; **8**: 10–14.