COPD and Macrolide

Mutsuo Yamaya,*1 Tomoko Suzuki,*1 Kota Ishizawa,*1 Takahiko Sasaki,*1 Hiroyasu Yasuda,*1 Daisuke Inoue,*1 Hiroshi Kubo,*1 Katsutoshi Nakayama,*1 Hidekazu Nishimura,*2 Kiyohisa Sekizawa*3

Abstract
Respiratory virus including rhinovirus (RV) induces exacerbations of chronic obstructive pulmonary disease (COPD). RV infection stimulates various cells in the airways such as epithelial cells, mast cells and eosinophils, and produces a variety of proinflammatory cytokines such as interleukin (IL)-6 and IL-8, mucin and chemical mediators including histamine. These factors may be associated with airway inflammation with leukocyte accumulation, mucus hypersecretion, airway smooth muscle contraction and subsequent COPD exacerbations with airway narrowing. Macrolide antibiotics bafilomycin A1 and erythromycin inhibit RV infection by reducing the expression of ICAM-1, the major RVs receptor, and by blocking RV entry. Furthermore, erythromycin reduced the frequency of common colds and COPD exacerbations. Erythromycin increases bactericidal activity of airway surface liquid in human airway epithelial cells through human beta-defensin production. Herein, we review the pathogenesis of RV infection-induced exacerbations of COPD. Furthermore, we describe the mechanisms of the inhibitory effects of erythromycin on COPD exacerbations.

Key words Macrolide, Rhinovirus, Cytokine, COPD, Defensin, Mucin

Clinical Importance of Rhinovirus Infection on COPD Exacerbation

Human rhinoviruses (RVs) are the most commonly implicated pathogens of common colds.1 The importance of RV infection in the exacerbation of bronchial asthma has been recognized.2,3 Respiratory virus infection, including RVs, influenza viruses, and respiratory syncytial viruses, is associated with the exacerbation of chronic obstructive pulmonary diseases (COPD).4 After the establishment of RT-PCR methods for RVs,5 the important role of RV infection on exacerbations of COPD has also been reported.4,5 In a report by Seemungal et al.,4 39% of 168 COPD exacerbations were associated with viral infection. RV was the most common respiratory virus detected, and was detected in 58% of viral infec-

Effects of Rhinovirus Infection on the Airway Epithelial Cells

In order to understand the mechanisms of airway inflammation after RV infection related to acute exacerbations of COPD and bronchial asthma, various studies have been performed on the production of pro-inflammatory substances, adhesion molecules and chemical mediators from the cells in the lung. RV infection increases the production of various pro-inflammatory substances...
including interleukin (IL)-1α, IL-1β, IL-6, IL-8, IL-11, tumor necrosis factor-α (TNF-α), RANTES, and granulocyte-macrophage colony-stimulating factor (GM-CSF) in the epithelial cells, primary cultures of epithelial cells or cell lines (Table 1). Subauste et al. 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. They suggested that inflammatory cytokines produced by RV infection may increase the susceptibility to RV infection. IL-6 induces antibody production in B cells as well as T cell activation and differentiation. IL-8 is a major chemotactic agent for neutrophils and stimulates neutrophils, causing enzyme release and production of reactive oxygen species. The number of neutrophils increases in the airway during the acute stage of a cold, and in the sputum in COPD. IL-8 increase in sputum of COPD patients at a stable condition and during exacerbations. Similarly, GM-CSF can prime both neutrophils and eosinophils for enhanced activation to chemical stimuli. RV infection increases the production of eotaxin and RANTES, which activates eosinophils, in the bronchial epithelial cells. IL-11 is suggested to have direct effects on bronchial hyperresponsiveness. We also demonstrated that primary cultures of human tracheal epithelial cells and submucosal gland cells can be infected with RV14, a major type RV, and RV2, a minor type RV, through binding to intercellular adhesion molecule (ICAM)-1 and low density lipoprotein (LDL) receptors, respectively, and produce pro-inflammatory cytokines, including IL-1α, IL-1β, IL-6, IL-8, TNF-α, and GM-CSF; and ICAM-1 and LDL receptor. Activation of the transcription factor, nuclear factor-kappa (NF-κ) B, is associated with the production of pro-inflammatory cytokines and ICAM-1, and the endogenous production of IL-1β is also associated with ICAM-1 expression after RV infection.

Thus, these proinflammatory cytokines may be partly associated with the accumulation of inflammatory leukocytes and with subsequent airway thickness and inflammation as reported by Hogg et al. The proinflammatory cytokines may relate to the upregulation of the inducible form of heme oxygenase (HO-1), and the increased concentrations of carboxyhemoglobin concentrations in patients with COPD at exacerbations.

The up-regulation of ICAM-1 could increase susceptibility to major group RVs and could lead cells adjacent to infected cells to infection when viruses are released from the cells originally infected. Inflammatory conditions such as asthma, smoking, ozone exposure, and production reactive oxygen species in COPD in which ICAM-1 expression is increased on respiratory epithelial surfaces, may cause a predisposition to RV infection by increasing the expression of the major group of RV receptors. The RV infection would enhance airway inflammation by recruiting neutrophils, and, potentially, other inflammatory cells, causing increased mediator release and exacerbation of COPD.

Furthermore, we demonstrated that hydrogen peroxide increases the transepithelial influx of

### Table 1 Effects of rhinovirus infection on the function of cells in airways

<table>
<thead>
<tr>
<th>Cell</th>
<th>Effects of rhinovirus infection</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epithelial cells</td>
<td>1. Cytokines production: IL-1, IL-6, IL-8, TNF-alpha, GM-CSF, RANTES</td>
<td>6–14, 51</td>
</tr>
<tr>
<td></td>
<td>2. ICAM-1 expression</td>
<td>9, 21</td>
</tr>
<tr>
<td></td>
<td>3. Mucin secretion</td>
<td>34, 35</td>
</tr>
<tr>
<td></td>
<td>4. Affected barrier function</td>
<td>32</td>
</tr>
<tr>
<td>Mast cells</td>
<td>1. Histamine release</td>
<td>45</td>
</tr>
<tr>
<td>Eosinophils</td>
<td>2. Cytokines production: IL-4, IL-6, IL-8</td>
<td>45</td>
</tr>
<tr>
<td>Smooth muscle</td>
<td>Contraction</td>
<td>53</td>
</tr>
</tbody>
</table>
manitotin in cultured human tracheal epithelial layers, and RV infection further increases the manitotin influx in cells treated with IL-1β.32 These findings suggest that RV infection may affect the integrity of airway epithelial cells, although RV infection does not induce airway epithelial cell damage33 as induced by influenza virus infection. Furthermore, RV infection induces mucus release, including MUC5AC, into the mucosal side of airway epithelial cells.34,35 Mucus hypersecretion36 and airway epithelial hyperpermeability may induce mucous hypersecretion and airway narrowing in COPD.36 Infection with Streptococcus pneumoniae after respiratory infection is associated with severity of illness and more frequent hospitalization. RV infection also increases the adherence of Streptococcus pneumoniae to human tracheal epithelial cells via increases in PAF receptors,37 suggesting that increased adherence of Streptococcus pneumoniae may be one of the reasons that pneumonia develops after RV infection.38

**Effects of Rhinovirus Infection on Mast Cells and Cells Other than Airway Epithelial Cells**

Cells other than lung epithelial cells also have been reported to produce pro-inflammatory substances and chemical mediators such as histamine, and to be associated with the exacerbation of COPD and asthma.16,39 Infection with respiratory viruses including RVs activates histamine release from the basophils of peripheral blood,40 and the plasma histamine content increases after RV infection.41 Virus infection including RV increases histamine release in basophils stimulated with anti-IgE and calcium ionophore after RV infection.42 Response to histamine increases in human small airway smooth muscle from COPD.43 Mast cells are major sources of histamine release in airways and are associated with the pathogenesis of bronchial asthma44 and COPD.16,39 We demonstrated that RV infection primes the production of IL-4, IL-6, IL-8, GM-CSF, and histamine in response to stimuli including IgE in both the human mast cell line and the human basophilic leukocyte cell line (Table 1).45

The number of macrophages increases in the airway epithelium in patients with COPD.25,39 and airway macrophages secrete TNF-α after RV infection.46 Increased levels of TNF-α in sputum may suggest the role of TNF-α in the pathogenesis of COPD.18,19 Similarly, eosinophil accumulation is observed in airway mucosa after experimental RV infection.47 Eosinophil granular proteins including eosinophil cationic protein (ECP) have also been detected in the nasal secretions of children with wheezing illness caused by RV infection,48 and sputum in asthmatic patients experimentally infected with RV type 16,49 and in patients with COPD.16,18 On the other hand, RV 16 did not induce superoxide production from peripheral blood eosinophils as shown by Handzel et al.50 Therefore, inflammatory mediators such as RANTES and GM-CSF47,48,49 released from cells including airway submucosal cells and mast cells may activate eosinophils after RV 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.51

RV stimulates lymphocytes to induce interferon (IFN)-γ production and T cell proliferation through eosinophil and monocyte activation.52 Experimental RV infection revealed the accumulation of lymphocytes and monocytes in the airway mucosa and submucosa.47 Activated lymphocytes may also be associated with the exacerbation of bronchial asthma and COPD.26 Furthermore, the direct effects of RV on airway smooth muscle contraction were demonstrated by Hakonarson et al.53

**Inhibition of Rhinovirus Infection by Bafilomycin**

In contrast to influenza virus, an effective vaccination for RVs has not been developed because there are more than 100 serotypes of RVs. Although a variety of antiviral agents has been studied on the inhibition of RV infection or common colds, soluble ICAM-1 is the only possible agent that may be useful in alleviating the symptoms of the common cold.54 On the other hand, other WIN compounds55 and a RV proteinase enzyme inhibitor56 are undergoing clinical trials. Two viral proteases designated 2A and 3C have been viewed as excellent targets for antiviral intervention for the picornavirus family including human RV.57

The vacuolar (H⁺)-ATPases (V-ATPases) are a
family of ATP-driven proton pumps responsible for the acidification of a variety of intracellular compartments in eukaryotic cells. V-ATPases provide the acidic environment required for the dissociation of internalized ligand-receptor complexes within endosomes. Furthermore, exposure of influenza virus to a low pH within endosomes by V-ATPases induces the formation of a fusion pore between the viral and endosomal membranes that permits entry of the viral RNA. The specific V-ATPases inhibitor bafilomycin blocks infection with influenza virus and RV in HeLa cells and Madin-Darby canine kidney (MDCK) cells, and inhibits the uncoating of RV type 2 and type 14 from late endosomes. However, the role of V-ATPases in RV infection of human airway epithelial cells, the primary target for respiratory viruses, has not been elucidated.

To examine the effects of bafilomycin A1 on RV infection in the airway epithelium, primary cultures of human tracheal epithelial cells were infected with RV14. Viral infection was confirmed by showing that viral RNA in the infected cells and viral titers of the supernatants and lysates from infected cells increased with time. RV14 infection upregulated the expression of mRNA of ICAM-1, the major RV receptor, on epithelial cells, and it increased the production of IL-1β, IL-6, IL-8 and TNF-α in supernatants. Treatment with bafilomycin A1 after viral infection reduced viral titers of RV14 in supernatants and cell lysates in association with the inhibition of cytokine production and ICAM-1 induction after viral infection. Furthermore, preincubation with bafilomycin A1 reduced ICAM-1 mRNA expression and cytokine production before RV14 infection, and reduced susceptibility to RV14 infection of epithelial cells. RV14 increased activated NF-κB in cultured human tracheal epithelial cells, and bafilomycin A1 reduced the activated NF-κB before and after RV14 infection. Bafilomycin A1 inhibited this acidification of intracellular pH and decreased the number of acidic endosomes in the epithelial cells. These results suggest that bafilomycin A1 may inhibit infection with RV14 by not only blocking the RV RNA entry in the endosomes but also reducing ICAM-1 expression in cultured human tracheal epithelial cells.

Inhibition of Rhinovirus Infection by Erythromycin

Macrolide antibiotics inhibit the production of ICAM-1, which plays a vital role in the accumulation of immune effector cells to sites of local infection.

![Fig. 1 Time course of replication of RV RNA from human tracheal epithelial cells after infections of either RV14 (A) or RV2 (B) in the presence of erythromycin (10 μM; blue columns) or ethanol (0.1%) as a vehicle of erythromycin (control; white columns) as detected by real-time quantitative amounts of RT-PCR. Results are expressed as relative amounts of RNA expression (%) compared with those of maximal RV RNA at day 3, and reported as means ± SE from 7 samples. Significant differences from treatment with a vehicle of erythromycin (control) at each time are indicated by *P<0.05, **P<0.01 and ***P<0.001. To examine the effects of erythromycin on viral RNA in the cells, the cells were treated with erythromycin or a vehicle of erythromycin from 3 days before RV infection to the RNA extraction after RV infection. The RNA extraction was performed at either 0, 8, 24, 72 or 120 h after RV infection. Reproduced with permission from Suzuki et al.20](image-url)
inflammation, and is also known as a receptor for a major subgroup of RVs such as RV14. Because glucocorticoid-induced reductions in ICAM-1 expression inhibit RV14 infection, macroline antibiotics may also inhibit RV14 infection. Furthermore, a macroline antibiotic bafilomycin A1 inhibits RV14 infection. However, the effects of erythromycin, a clinically used macrolide antibiotic, on RV infection have not been investigated.

To examine the effects of erythromycin on RV infection in the airway epithelium, primary cultures of human tracheal epithelial cells were infected with the RV major subgroup, RV14, and the minor subgroup, RV2. Infection was confirmed by increases in viral RNA of the infected cells and viral titers of the supernatants. RV14 upregulated the expression of the mRNA and protein of ICAM-1, the major RV receptor, and increased cytokine production. Erythromycin reduced the supernatant RV14 titters, RV14 RNA, susceptibility to RV14 infection, and the production of ICAM-1 and cytokines (Fig. 1). Erythromycin also reduced the supernatant RV2 titers, RV2 RNA, susceptibility to RV2 infection, and cytokine production, although the inhibitory effects of erythromycin on the expression of the LDL receptor, the minor RV receptor, were small. Erythromycin reduced NF-κB activation by RV14, and decreased the number of acidic endosomes in the epithelial cells. These results suggest that erythromycin inhibits infection by the major RV subgroup by reducing ICAM-1 and infection by both RV subgroups by blocking RV RNA entry into the endosomes. Erythromycin may also modulate airway inflammation by reducing the production of proinflammatory cytokines and ICAM-1 induced by RV infection.

Prevention of Common Colds and Exacerbations in COPD: Effects of Erythromycin

RV infection is associated with exacerbation of COPD, and erythromycin inhibits RV infection in airway epithelial cells. Low-dose, long-term erythromycin therapy has been reported to be effective in treating patients with diffuse panbronchiolitis (DPB) or bronchiectasis via mechanisms other than antibacterial activity. However, the inhibitory effects of erythromycin on common colds and exacerbations in COPD have not been studied.

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 on the study. The patients were randomly assigned to erythromycin therapy or to no active treatment in September 1997. The 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 and COPD exacerbations for 12 months was significantly lower in the erythromycin group than that in the control group (Table 2). 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

<table>
<thead>
<tr>
<th>Measures</th>
<th>Control group (n = 54)</th>
<th>Erythromycin group (n = 55)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total common colds, No.</td>
<td>245</td>
<td>67</td>
<td>0.0002</td>
</tr>
<tr>
<td>Total number of exacerbations, No.</td>
<td>64</td>
<td>14</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Total patients with exacerbations, No.</td>
<td>30</td>
<td>6</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Total patients with severe exacerbations, No.</td>
<td>10</td>
<td>0</td>
<td>0.0004</td>
</tr>
</tbody>
</table>

Reproduced with permission from Suzuki et al.69
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 of exacerbations of COPD because of the potential risk for the emergence of erythromycin-resistant pathogens.

**Increased Bactericidal Activity of Surface Liquid in Human Airway Epithelial Cells by Erythromycin**

As already described in this article, erythromycin reduces the frequency of common colds and exacerbations in COPD, and erythromycin inhibits RV infection, and reduces the production of ICAM-1 and proinflammatory cytokines before and after RV infection. To further investigate the mechanisms of the inhibition of COPD exacerbations, we studied the effects of erythromycin on the bactericidal activity of surface liquid in human airway epithelial cells.

Defensins, one of the most intensively studied classes of antimicrobial peptides, are identified in a wide distribution of animals including humans. It is suggested that the main function of defensins is to kill bacteria and fungi either on the surfaces of the epithelial cells or within phagolysosomes of phagocytes. Defensins are small cationic peptides containing arginine-rich 29–47 amino acids with three disulfide bonds, which can be divided into the α- and β-defensin subfamilies in human subjects. Of the β-defensins, airway epithelial cells produce human β-defensin (HBD)-1, HBD-2, and HBD-3. Recent studies have demonstrated that human airway epithelial cells produce sodium-sensitive antimicrobial peptides into the apical side of the surface liquid, suggesting a major role of HBDs in host defense against bacterial infections.

Macrolide antibiotics have clinical benefits in patients with DPB and in patients with cystic fibrosis (CF). Although many mechanisms have been proposed, the precise mechanisms are still uncertain. We examined the effects of erythromycin on the bactericidal activity of airway surface liquid (ASL) secreted by cultured human tracheal epithelial cells. ASL was collected by washing the surface of the cells with a sodium solution (40 mEq/L). Methicillin-resistant *Staphylococcus aureus* and *Pseudomonas aerugi-* nosa were incubated with airway surface liquid and the number of surviving bacteria was examined. The number of bacteria in ASL from the cells cultured in medium alone was significantly lower than that in the sodium solution. Furthermore, the number of bacteria in ASL from the cells treated with erythromycin was significantly lower than that in ASL from the cells treated with solvent alone. The production of messenger ribonucleic acid and protein of human beta-defensin-1 and human beta-defensin-2 were significantly increased by erythromycin. The bactericidal activity of ASL was observed at low concentrations (40 mEq/L) of sodium but not at higher concentrations (≥80 mEq/L). ASL did not contain significant amounts of antibiotics supplemented in the culture medium. Erythromycin at the levels in ASL and in the culture medium did not inhibit bacterial growth. These results suggest that erythromycin may increase the bactericidal activity of ASL in human airway epithelial cells through human beta-defensin.

<table>
<thead>
<tr>
<th>Target cell or site</th>
<th>Function</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neutrophil</td>
<td>Reduced intrapulmonary influx, Reduced chemotaxis</td>
<td>79</td>
</tr>
<tr>
<td>Airway epithelial cell</td>
<td>Reduced production of IL-6, IL-8 and ICAM-1</td>
<td>20, 66</td>
</tr>
<tr>
<td></td>
<td>Improvement of sputum mucosity</td>
<td>80</td>
</tr>
<tr>
<td></td>
<td>Inhibition of goblet cell hypersecretion</td>
<td>81</td>
</tr>
<tr>
<td></td>
<td>Increased production of human-beta defensin</td>
<td>76</td>
</tr>
<tr>
<td>Biofilm</td>
<td>Reduced biofilm formation</td>
<td>82</td>
</tr>
<tr>
<td>Pseudomonas</td>
<td>Direct anti-pseudomonal activity</td>
<td>83</td>
</tr>
<tr>
<td>Rhinovirus</td>
<td>Inhibition of rhinovirus infection</td>
<td>20</td>
</tr>
</tbody>
</table>
Macrolide antibiotics improve survival in patients with DPB,77 and have clinical benefits in patients with CF.78 Many effects of macrolides have been proposed (Table 3), including the effects on neutrophil function,79 reduced IL-8 production,80 improvement of sputum mucosity,81 with CF.

Biologic Functions of Erythromycin in Airways

Macrolide antibiotics improve survival in patients with DPB,77 and have clinical benefits in patients with CF.78 Many effects of macrolides have been proposed (Table 3), including the effects on neutrophil function,79 reduced IL-8 production,80 improvement of sputum mucosity,81 with CF.

Macrolide antibiotics improve survival in patients with DPB,77 and have clinical benefits in patients with CF.78 Many effects of macrolides have been proposed (Table 3), including the effects on neutrophil function,79 reduced IL-8 production,80 improvement of sputum mucosity,81 with CF.

Biologic Functions of Erythromycin in Airways

Macrolide antibiotics improve survival in patients with DPB,77 and have clinical benefits in patients with CF.78 Many effects of macrolides have been proposed (Table 3), including the effects on neutrophil function,79 reduced IL-8 production,80 improvement of sputum mucosity,81 with CF.

Macrolide antibiotics improve survival in patients with DPB,77 and have clinical benefits in patients with CF.78 Many effects of macrolides have been proposed (Table 3), including the effects on neutrophil function,79 reduced IL-8 production,80 improvement of sputum mucosity,81 with CF.

Biologic Functions of Erythromycin in Airways

Macrolide antibiotics improve survival in patients with DPB,77 and have clinical benefits in patients with CF.78 Many effects of macrolides have been proposed (Table 3), including the effects on neutrophil function,79 reduced IL-8 production,80 improvement of sputum mucosity,81 with CF.

Macrolide antibiotics improve survival in patients with DPB,77 and have clinical benefits in patients with CF.78 Many effects of macrolides have been proposed (Table 3), including the effects on neutrophil function,79 reduced IL-8 production,80 improvement of sputum mucosity,81 with CF.

Biologic Functions of Erythromycin in Airways

Macrolide antibiotics improve survival in patients with DPB,77 and have clinical benefits in patients with CF.78 Many effects of macrolides have been proposed (Table 3), including the effects on neutrophil function,79 reduced IL-8 production,80 improvement of sputum mucosity,81 with CF.

Biologic Functions of Erythromycin in Airways

Macrolide antibiotics improve survival in patients with DPB,77 and have clinical benefits in patients with CF.78 Many effects of macrolides have been proposed (Table 3), including the effects on neutrophil function,79 reduced IL-8 production,80 improvement of sputum mucosity,81 with CF.

Acknowledgements

The authors thank Mr. Grant Crittenden for reading the manuscript. This study was supported by a Grant-In-Aid for Scientific Research from the Ministry of Education, Science and Culture of the Japanese government to MY (16590732), and supported by Health and Labor Sciences Research Grants of Research on Measures for Intractable Disease from the Ministry of Health, Labor and Welfare of the Japanese government to MY (17243601).

References


30. Greve JM, Davis G, Meyer AM, et al. The major human rhinovi-


