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Review

Pathogenesis and Management of Virus Infection-Induced Exacerbation of Senile Bronchial Asthma and Chronic Pulmonary Emphysema

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YAMAYA, M. *Pathogenesis and Management of Virus Infection-Induced Exacerbation of Senile Bronchial Asthma and Chronic Pulmonary Emphysema.* Tohoku J. Exp. Med., 2002, **197** (2), 67–80 —— The number of senile patients with therapy resistant bronchial asthma, chronic pulmonary emphysema increases due to the habit of smoking and increased number of older people, and these inflammatory pulmonary diseases are the leading causes of death worldwide. Rhinoviruses cause the majority of common colds, and provoke exacerbations of bronchial asthma and chronic pulmonary emphysema. Here, I review the pathogenesis and management of rhinovirus infection-induced exacerbation of senile bronchial asthma and chronic pulmonary emphysema. ———— rhinovirus; chronic pulmonary emphysema; bronchial asthma; heme oxygenase

The number of senile patients with therapy resistant bronchial asthma and chronic pulmonary emphysema (CPE) increases due to the habit of smoking and increased number of older people. However, the pathogenesis and management of senile therapy resistant inflammatory pulmonary diseases caused by airway virus infection and the etiology of CPE in Japanese population have not been fully studied or established.

We studied the pathogenesis of respiratory virus infection-induced exacerbation of bronchial asthma or CPE by using cultured human tracheal epithelial cells infected with rhinovirus. We also examined the inhibitory effects of dexamethasone and macrolide antibiotics bafilomycin A1 and erythromycin on rhinovirus (RV) infection and RV infection-induced production of various cytokines and intercellular adhesion molecule (ICAM)-1 and low density lipoprotein (LDL) receptor. Secondly, we studied whether the measurement of exhaled carbon monoxide (CO) concentrations, caused by heme oxygenase-1 up-regulation, is a useful noninvasive means of monitoring airway inflammation and controlling elderly patients with bronchial asthma. Finally, we examined whether microsatellite polymorphism in the

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heme oxygenase-1 gene promoter is associated with susceptibility to CPE caused by cigarette smoke in the Japanese population. Here I review the pathogenesis and management of rhinovirus infection-induced exacerbation of senile bronchial asthma and CPE.

Pathogenesis and prevention of airway rhinovirus infection

Clinical importance of rhinovirus infection. Rhinoviruses (RVs) cause the majority of common colds, which often provoke wheezing in patients with asthma (Nicholson et al. 1993), and they have also been associated with exacerbations of chronic bronchitis (Lambert and Stern 1972), chronic sinusitis (Turner et al. 1992) and otitis media (Arola et al. 1990). Prospective studies have indicated that asthma attacks are associated with a viral infection in as many as 20–50% of the cases (Minor et al. 1976). Studies using polymerase chain reaction (PCR)-based diagnostics have emphasized the importance of RVs by demonstrating that RVs are responsible for 80–85% and 45% of the asthma flairs in 9- to 11-yr-old children and adults, respectively, with RV being the most commonly implicated pathogen (Nicholson et al. 1993; Johnston et al. 1995).

Pathogenesis of rhinovirus infection in human subjects. Infection of respiratory viruses including RVs activates histamine release from basophils of peripheral blood (Chonmaitree et al. 1988) and the plasma histamine content (Calhoun et al. 1991) and kinin concentration in nasal lavage fluid (Naclerio et al. 1988) increases after RV infection. RV infection causes infiltration of neutrophils, lymphocytes, and eosinophils in nasal (Levandowski et al. 1988) and bronchial mucosa (Fraenkel et al. 1995). Furthermore, RV 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 (Lemanske et al. 1989).

Effects of rhinovirus infection on the lung cells and other cells. Subauste et al. (1995) demonstrated that RV14 infection induced the release of interleukin(IL)-6, IL-8 and tumor necrosis factor-α (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 rhinovirus infection may increase the susceptibility to rhinovirus infection. RV infection increases the production of eotaxin and RANTES, which activate eosinophils, in the bronchial epithelial cells (Schroth et al. 1999; Papadopoulos et al. 2001). We also demonstrated that RV14, a major type RV, and RV2, a minor type RV, can be infected to primary culture of human tracheal epithelial cells and submucosal glands through binding to the intercellular adhesion molecule (ICAM)-1 and low density lipoprotein (LDL) receptor, respectively, and produce proinflammatory cytokines, ICAM-1 and LDL receptor (Terajima et al. 1997; Yamaya et al. 1999; Suzuki et al. 2001a). Activation of transcription factor NF-$\varkappa$B is associated with the production of proinflammatory cytokines and ICAM-1 (Zhu et al. 1996; Papi and Johnston 1999; Suzuki et al. 2001b), and endogenous production of IL-1β is also associated with the ICAM-1 expression after RV infection (Terajima et al. 1997). Hydrogen peroxide increases the transepithelial influx of mannitol in the cultured human tracheal epithelial layers, and RV infection further increases the mannitol influx in the cells treated with IL-1β (Ohrui et al. 1998). These findings suggest that RV infection may affect the integrity of airway epithelial cells.

Infection of respiratory viruses including RVs activates histamine release from basophils of peripheral blood (Chonmaitree et al. 1988). Virus infection including RV increases histamine release in basophils stimulated with

anti-IgE and calcium ionophore (Ida et al. 1977; Busse et al. 1983). RV activates lymphocytes to induce interferon (IFN)-γ production through a monocyte-dependent mechanism (Gern et al. 1996). Furthermore, RV infection increases rabbit and human airway smooth muscle responsiveness to acetylcholine (Hakonarson et al. 1998).

These events may be associated with the airway inflammation and subsequent exacerbations of bronchial asthma.

Inhibition and treatment of rhinovirus infection. A variety of antiviral agents have been studied on the inhibition of rhinovirus infection or common colds, including vitamin C (Pauling 1971; Hemila 1997), zinc gluconate lozenges (Marshall 1988; Macknin et al. 1998), WIN compounds (Lewis et al. 1998; Hadfield et al. 1999), VLDL receptor fragments (Marlovits et al. 1998), soluble ICAM-1 (Greve et al. 1991; Huguenel et al. 1997; Turner et al. 1999), rhinovirus 3C protease inhibitors (Witherell 2000), a compond R77975 (Hayden et al. 1995), interferon α (IFN-α) (Farr et al. 1984) and erythromycin (Suzuki et al. 2002). However, the effect of vitamine C supplementation has been controversial (Hemila 1997). Likewise, a controlled trial revealed that zinc gluconate was not effective (Macknin et al. 1998), and the nasal toxicities of INF-α may impose some limitation for clinical usage. WIN 52084 and R77975, antiviral agents that inhibit viral structural dynamics (Hayden et al. 1995; Lewis et al. 1998), was not effective at all in reducing cold symptoms (Hayden et al. 1995). Soluble ICAM-1 inhibits the rhinovirus from adhering to the cells (Greve et al. 1991) and inhibits rhinovirus infection in chimpanzees (Huguenel et al. 1997). So far, soluble ICAM-1 is the only possible agent that may be useful in alleviating the symptoms of the common cold (Turner et al. 1999). On the other hand, other WIN compounds (Hadfield et al. 1999) and a rhinovirus proteinase enzyme inhibitor (Witherell 2000) are undergoing phase III clinical trials.

We showed that macrolide antibiotics inhibit infection by the major RV subgroup by reducing ICAM-1, a receptor for a major subgroup of RVs, and infection by both major and minor RV subgroups by blocking the RV RNA entry into the endosomes in the human tracheal epithelial cells (Suzuki et al. 2001b). Furthermore, erythromycin inhibited the RV infection-induced production of proinflammatory cytokines and ICAM-1 (Suzuki et al. 2002) (Fig. 1). We also showed that dexamethasone inhibits infection by the major RV subgroup by reducing ICAM-1 in the human tracheal epithelial cells (Suzuki et al. 2000). Furthermore, we showed that erythromycin therapy has beneficial effects on the prevention of exacerbations in patients with chronic obstructive pulmonary disease (COPD) (Suzuki et al. 2001c).

Exhaled carbon monoxide as an indicator of inflammatory respiratory disease

In order to monitor the control of inflammatory respiratory diseases including bronchial asthma, it would be desirable to monitor inflammation in the airways. Monitoring the symptoms may be misleading, since bronchodilators relieve symptoms without treating the underlying inflammatory process. Airway hyperresponsiveness has been used as a marker of airway inflammation, but the changes in airway responsiveness after glucocorticoid therapy are modest and even when asthma is optimally controlled the values often remain abnormal (Barnes 1990; Juniper et al. 1990). More direct measurements of airway inflammation include bronchial biopsies, bronchoalveolar lavage and induced sputum. Bronchial biopsies and bronchoalveolar lavage are invasive procedures and are clearly unsuitable for clinically monitoring the airway inflammation of asthma (Djukanovic et al. 1990). Induced sputum is useful for assessing airway inflammation of asthma, but not all patients are able to produce satisfactory samples. Furthermore, it may be sometimes diffi-

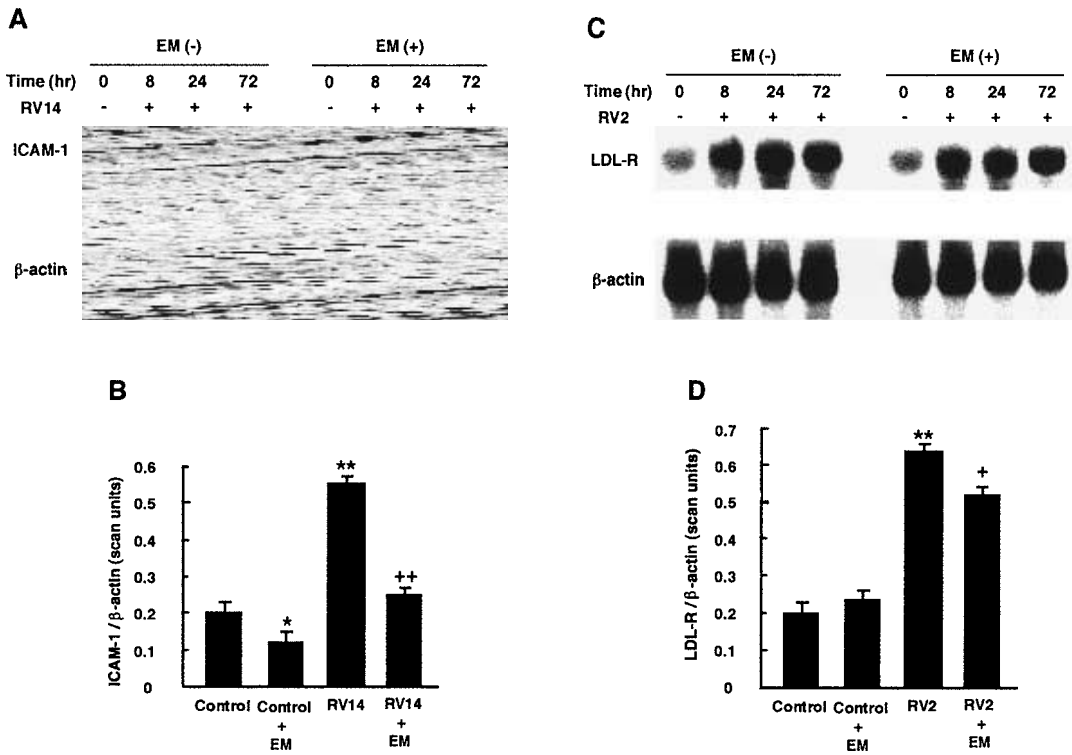

Fig. 1. A: Northern blot analysis for intercellular adhesion molecule (ICAM)-1 mRNA levels of human tracheal epithelial cells before (0), 8, 24, and 72 hours after RV14 infection in the absence or presence of erythromycin (EM; 10 μM). β-actin was used as a housekeeping gene. B: effects of erythromycin (EM; 10 μM) on the expression of ICAM-1 mRNA in human tracheal epithelial cells 3 days after RV14 or sham (control) infection. ICAM-1 mRNA was normalized to a constitutive expression of β-actin mRNA. Results are reported as means$\pm$S.E. from 7 samples. Significant differences from corresponding control values are indicated by $^{*}p<0.05$ and $^{**}p<0.01$. Significant differences from RV14 infection alone are indicated by $^{++}p<0.01$. C: Northern blot analysis for low density lipoprotein (LDL) receptor mRNA levels of human tracheal epithelial cells before (0), 8, 24, and 72 hours after RV14 infection in the absence or presence of erythromycin (EM; 10 μM). β-actin was used as a housekeeping gene. D: effects of erythromycin (EM; 10 μM) on the expression of the LDL receptor mRNA in human tracheal epithelial cells 3 days after RV14 or sham (control) infection. LDL receptor mRNA was normalized to a constitutive expression of β-actin mRNA. Results are reported as means$\pm$S.E. from 7 samples. Significant differences from corresponding control values are indicated by $^{**}p<0.01$. Significant differences from RV2 infection alone are indicated by $^{+}p<0.05$. To examine the effects of erythromycin on ICAM-1 and LDL receptor mRNA expression, the human tracheal epithelial cells were treated with erythromycin or a vehicle of erythromycin (ethanol, 0.1%) from 3 days before RV infection to the mRNA extraction after RV infection (Suzuki et al. 2002).

cult for older people to perform the measurement of peak flow (PEFR) and forced expiratory volume in one second (FEV_1) with maximum expiratory effort.

To monitor lung inflammation, non-invasive methods by measuring exhaled markers have been developed for patients with severe airflow limitations or in older patients, including exhaled nitric oxide (Nathan 1992; Alving et al. 1993; Kharitonov et al. 1994; Persson et al. 1994; Massaro et al. 1995), several volatile gases (ethane, pentane) (Olopade et al. 1997; Paredi et al. 1999), and several endogenous substances (inflammatory mediators, cytokines,

oxidants) (Baldwin et al. 1986). We also reported the use of exhaled carbon monoxide analysis in the monitoring of inflammatory respiratory diseases (Zayasu et al. 1997; Yamaya et al. 1998, 1999, 2001; Monma et al. 1999).

Source of exhaled carbon monoxide. There are three major sources of CO in exhaled air: endogenous production of CO through the degradation of heme by the heme oxygenase and through the non-heme-related release (lipid peroxidation, xenobiotics, bacteria), and exogenous CO. In the endogeneous production of CO, predominant CO is produced endogenously in many tissues of the body by the class of enzymes known collectively as heme oxygenase (HO) (Maines 1988), and approximately 15% of CO is produced by the degradation of myoglobin, catalase, NO syntheses, guanylyl cyclase, and cytochromes (Berk et al. 1974). Two forms of HO, HO-1 and HO-2, have been characterized. HO-1 is present in the pulmonary vascular endothelium (Otterbein et al. 1995), alveolar macrophages (Fukushima et al. 1995) and human airway epithelium (Yamada et al. 1999). HO-1 is induced by oxidative stress (Otterbein et al. 1995; Camhi et al. 1995), inflammatory cytokines (Cantoni et al. 1991; Lavrovsky et al. 1996), and NO (Kim et al. 1995) which are associated with the CO production in inflammatory pulmonary diseases as described below. In contrast, HO-2 is not inducible and is widely distributed throughout the body, with high concentrations in the brain (Maines 1988).

Measurement of exhaled CO. CO can be detected in exhaled air in smokers and nonsmokers (Jarvis et al. 1986). Exhaled CO was measured on a portable Bedfont EC50 analyzer (Bedfont Technical Instruments Ltd., Sittingbourne, UK) using the method described by Jarvis et al. (1986) in which subjects are asked to exhale fully, inhale deeply, and hold their breath for 20 seconds before exhaling rapidly into a disposable mouthpiece. This procedure was repeated three times, with 1 minutes of normal breathing between each repetition, and mean values were used for analysis. The exhaled CO concentration was determined by subtracting the background level from the observed reading as previously described (Jarvis et al. 1986; Zayasu et al. 1997; Yamaya et al. 1998). To avoid analysis with a value of exhaled CO concentration below 1.0 ppm, the background level was subtracted from the average value obtained from three sequential maneuvers in each patient. The exhaled CO concentration values were always above 1.0 ppm before subtracting the background level throughout the experiments (Zayasu et al. 1997; Yamaya et al. 1998). Prior to the start of the study, the analyzer was calibrated with a mixture of 50 ppm CO in air (Zayasu et al. 1997; Yamaya et al. 1998).

Increases in exhaled CO in inflammatory respiratory diseases and their mechanisms. Zayasu et al. (1997) firstly reported increases in CO levels in exhaled air in asthmatic subjects. They found that exhaled CO levels and eosinophil cell counts in sputum reduced after treatment with inhaled glucocorticoid in the patients. These findings suggest an elevation of exhaled CO in asthmatic patients that decreases with corticosteroid therapy. Likewise, we measured the time course changes in exhaled CO and peak expiratory flow rate (PEFR) in older patients with bronchial asthma before and after respiratory tract virus infection (Yamaya et al. 1999). We found that asthma exacerbations cause a fall in PEFR and a rise in exhaled CO in patients, and PEFR and exhaled CO levels were returned to baseline levels after oral glucocorticoids (Fig. 2). Increased CO in patients with asthma was also reported by Horváth et al. (1998, 2001) and others (Kharitonov 1999; Kharitonov and Barnes 2001). They observed increased HO-1 protein expression in airway macrophages and increased bilirubin levels in induced sputum in non-treated asthmatic

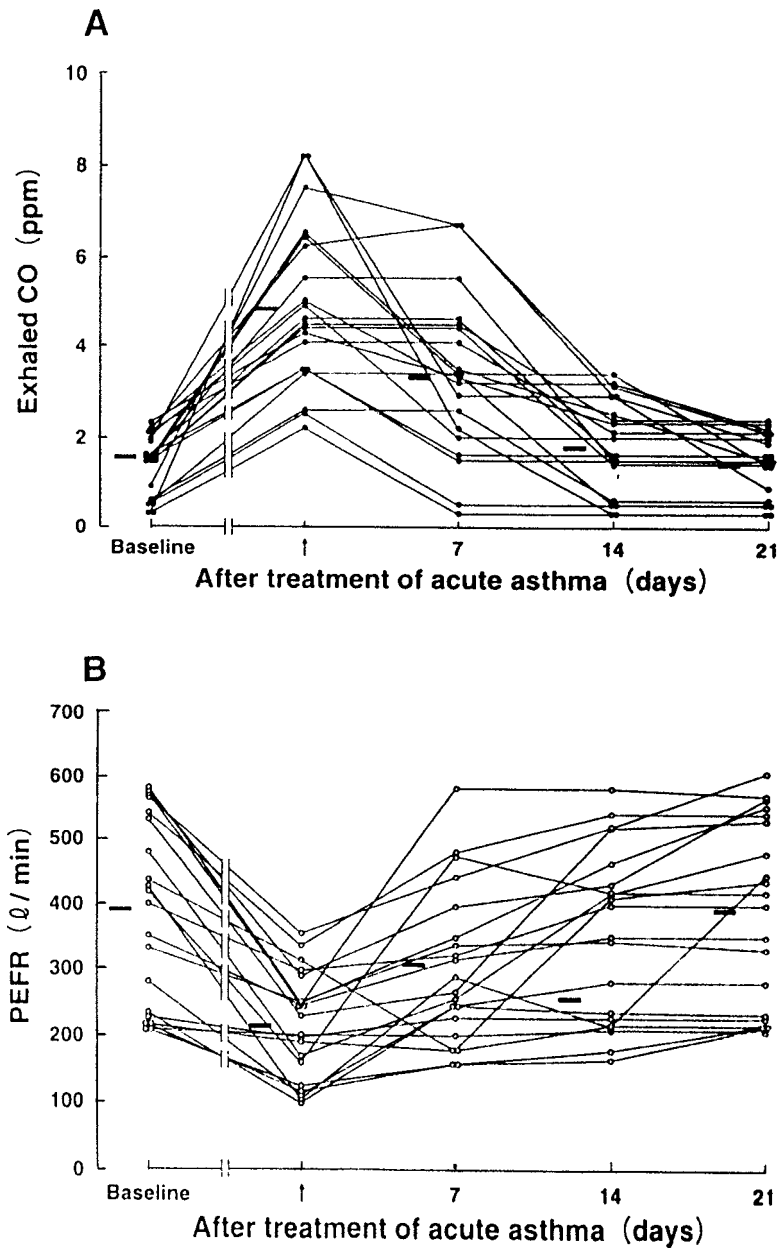

Fig. 2. Time course changes in exhaled carbon monoxide concentrations (A) and peak expiratory flow rate (PEFR; B) in asthmatic patients before acute asthma exacerbations (baseline) and after treatment with oral glucocorticoids. Ppm: parts per million. ↑: the start of treatment of acute asthma exacerbations with oral glucocorticoids. Horizontal bars indicate mean values of each time-point. Copyright remains with European respiratory Society Journal Ltd. (Yamaya et al. 1999a).

patients. Furthermore, we studied whether exhaled CO is related to severity of asthma (Yamaya et al. 2001). The exhaled CO in severe asthma was significantly higher than those of non-smoking control subjects. Exhaled CO in unstable severe asthmatics were significantly higher than those in stable severe asthmatics. However, exhaled CO in mild and moderate asthmatics did not differ significantly from those in non-smoking control subjects.

These findings suggest that exhaled CO may relate to the severity of asthma and exhaled CO may be a non-invasive useful means of monitoring airway inflammation in older patients with asthma.

Many cytokines are involved in the pathogenesis of asthmatic inflammation, including IL-1, IL-6, and TNF, which can upregulate HO-1 activity in animal and human tissues (Cantoni et al. 1991; Lavrovksy et al. 1996). Furthermore, asthmatic airways produce high levels of NO (Kharitonov et al. 1994; Persson et al. 1994; Massaro et al. 1995) and NO is shown to decrease cytochrome P450 and microsomal heme through increases in the activity of HO-1 (Kim et al. 1995). These factors may upregulate the HO-1 production in patients with bronchial asthma.

Exhaled CO elevates in the patients with inflammatory respiratory inflammation other than bronchial asthma, including patients with upper respiratory tract infection (Yamaya et al. 1998), allergic rhinitis (Monma et al. 1999), bronchiectasis (Horváth et al. 1998), cystic fibrosis (Paredi et al. 2000), and lower respiratory tract infection (Biernacki et al. 1998). Because upper respiratory tract infections are reported to induce increases in IL-1, IL-6 and TNF-α in nasal lavage fluid (Lau et al. 1996) as well as increases in exhaled NO (Kharitonov et al. 1995), these factors may be associated with increases CO production in viral respiratory tract infection. Likewise, a number of proinflammatory cytokines and chemokines such as IL-1, IL-5 and IL-6 (Ying et al. 1993; Sim et al. 1994, 1995) may be associated with the elevation of exhaled CO in patients with rhinitis. Histological examinations reveal neutrophil accumulation and infiltration in the lung in bacterial pneumonia (Stockley 1995). Increased levels of reactive oxygen species in neutrophils and bronchoalveolar lavage fluid were demonstrated in inflammatory lung diseases such as cystic fibrosis and adult respiratory distress syndrome (Brown and Kelly 1994; Chabot et al. 1998). Reactive oxygen species including superoxide anion and hydrogen peroxide up-regulate HO-1 production (Keyse et al. 1990; Yamada et al. 2000). Furthermore, the reactive oxygen species and proinflammatory cytokines are thought to increase exhaled CO in patients with bronchiectasis and cystic fibrosis. These factors may induce HO-1 production in bacterial infection of the lung, thereby resulting in increased CO production in the patients with cystic fibrosis and bronciectasis.

Genetic factors associated with development of the chronic pulmonary emphysema and COPD in Japanese people

Chronic obstructive pulmonary disease (COPD), which includes chronic pulmonary emphysema (CPE), chronic airway obstruction, and chronic bronchitis (Celli et al. 1995), is one of the leading causes of death worldwide, with an increasing prevalence and mortality. It is generally accepted that cigarette smoke is the most common identifiable risk factor for COPD. However, only 10–15% of smokers develop COPD (Thom 1989), and approximately 30% of smokers with a history of >60 pack years still have FEV_1: FVC ratios within the normal range (Kupperman and Riker 1973), suggesting the existence of a group susceptible to cigarette smoke.

There are two current established hypothesis in the pathogenesis of CPE. One is the endogenous protease/antiprotease theory (Janoff 1985; Snider 1992), which states that the loss of equilibrium between the level of degradative enzymes and their respective inhibitors damages the connective-tissue-matrix components of the lung (Hoidal and Niewoeher 1983). This theory could explain the mechanism of development of CPE in the α1-antitrypsin deficiency, and in animal models with excessive expressions of elastases and collagenases (Snider 1992). Laurell and Eriksson (1963) firstly reported five patients with α-1 globulin deficiency. Of the patients, three

patients had obstructive airway diseases with chronic bronchitis, bronchiectasis and CPE, and two patients did not have obstructive airway diseases. Eriksson (1964) suggested the relationship between the α1-antitrypsin deficiency and CPE in a large family study. Genetic factors associated with the α1-antitrypsin deficiency were then demonstrated by Yoshida et al. (1976, 1977), and by others (Owen and Carrell 1976; Garver et al. 1986) in patients with CPE. In Japanese patients with CPE, Seyama et al. (1991) demonstrated a new α1-antitrypsin deficient case. In contrast, the α1-antitrypsin deficiency is very rare in Japanese patients with CPE.

The other hypothesis is the oxidant/antioxidant theory, which postulates that an excess of oxidants and free radicals in the lung directly promotes cellular and tissue damage and is the major initiator of the disease process (Farber 1994). Furthermore, reactive oxygen species in cigarette smoke inhibit antiproteases, by oxidation of their active sites, and promote cell and tissue proteolysis in the lung (Ogushi et al. 1991). Direct and indirect oxidative injury caused by cigarette smoke may be a crucial mechanism for the process of CPE.

A recent report suggests the relation between the reduced activity of an antioxidant enzyme, microsomal epoxide hydrolase, and susceptibility to CPE (Smith and Harrison 1997). However, this gene polymorphism was not observed in the Japanese patients with CPE (Takeyabu et al. 2000), or was observed in only advanced COPD in the Japanese population (Yoshikawa et al. 2000). On the other hand, Ishii et al. (2000) demonstrated the glutathione *S*-transferase P1 (GSTP1) polymorphism in patients with COPD in the Japanese population. Heme oxygenase (HO) oxidatively degrades heme to biliverdin, which is subsequently reduced to bilirubin by biliverdin reductase (Maines 1988). HO-1, an inducible form of HO, plays a protective role as an antioxidant in the lung (Choi and Alam 1996; Otterbein et al. 1999). A (GT)n dinucleotide repeat in the 5′-flanking region of human HO-1 gene shows length polymorphism and modulates the level of gene transcription (Okinaga et al. 1996). To investigate the correlation between the length of the (GT)n repeat and susceptibility to the development of CPE, we screened the frequencies of alleles with varying numbers of (GT)n repeats in patients with CPE and smokers without CPE (Yamada et al. 2000). We found that the proportion of allele frequencies in the class with large size of a (GT)n repeat, as well as the proportion of genotypic frequencies in the group with the class with large size of a (GT)n repeat, was significantly higher in the patients with CPE (Table 1) (Yamada et al. 2000). Furthermore, H_2O_2 exposure up-regulated the transcriptional activity of the HO-1 promoter/luciferase fusion genes with small size of a (GT)n repeat. These findings suggest that large size of a (GT)n repeat in the HO-1 gene promoter may reduce HO-1 inducibility by reactive oxygen species in cigarette smoke, thereby resulting in the development of CPE in the Japanese population (Yamada et al. 2000).

TABLE 1. *Allelic frequencies at the polymorphic locus*

Allele	non-CPE (n=200)	CPE (n=202)	Odds ratio (95% CL)			
			vs. the Others	vs. Class S	vs. Class M	vs. Class L
Class L (30≦)	20 (10%)	42 (21%)	2.4 (1.3–4.1)[b]	2.9 (1.6–5.3)[d]	2.0 (1.1–3.6)[c]	1.0
Class M (25≦, <30)	88 (44%)	93 (46%)	1.1 (0.7–1.6)	1.5 (0.9–2.2)	1.0	
Class S (<25)	92 (46%)	67 (33%)	0.6 (0.4–0.9)[a]	1.0		

[a] $p<0.02$. [b] $p<0.004$. [c] $p<0.03$. [d] $p<0.001$.

(From reference by Yamaya et al. (2000) published by Chicago Press.)

Tumor necrosis factor-α (TNF-α) is a multifunctional cytokine. TNF-α could promote tracheal smooth muscle proliferation (Amrani et al. 1996), and alter smooth muscle function (Emala et al. 1997). The level of TNF-α is elevated in bronchoalveolar lavage fluid (Sun et al. 1998), bronchial biopsies (Mueller et al. 1996), and induced sputum (Keatings et al. 1996) of patients with COPD. TNF-α gene promoter polymorphism with alteration of TNF-α secretion was demonstrated in patients with chronic bronchitis in a male Taiwanese population (Hung et al. 1997) and in patients with COPD in Japanese smokers (Sakao et al. 2001). TNF-α gene promoter polymorphism may also be associated with the development of COPD in the Japanese population.

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