Airway Inflammation in COPD Assessed by Sputum Levels of Interleukin-8

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Study objective: To assess the characteristics of airway inflammation in patients with COPD.

Methods: We measured the sputum concentration of interleukin-8 (IL-8), a chemokine involved in the migration and activation of neutrophils and eosinophils. We also measured myeloperoxidase (MPO) as a parameter of neutrophil activity and eosinophil cationic protein (ECP) as a parameter of eosinophil activity. Spontaneous sputum samples were obtained from 33 patients with stable COPD and 30 patients with asthma. Induced sputum samples were obtained from 12 normal control subjects.

Results: The sputum concentration of IL-8 was significantly higher in the patients with COPD than in the patients with asthma or in the control subjects (p<0.0001). Concentrations of MPO and ECP were significantly higher in the patients with COPD than in the control subjects but did not differ significantly between the patients with COPD and those with asthma. In the patients with COPD, the sputum concentration of IL-8 was significantly correlated with the concentration of MPO (r=0.55, p<0.001) and of ECP (r=0.53, p<0.01). The sputum concentration of IL-8 was negatively correlated with FEV₁/FVC (r=−0.78, p<0.0001) in the COPD group.

Conclusions: Results suggest the activation of both neutrophils and eosinophils in the airways of patients with COPD. It appears that IL-8 plays a primary role in this activation. The sputum concentration of IL-8 appeared to be closely associated with the degree of airflow obstruction in patients with COPD and may serve as a marker in evaluating the severity of airway inflammation, which is a risk factor for COPD.

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Key words: airway inflammation; chronic obstructive pulmonary disease; eosinophil cationic protein; interleukin-8; myeloperoxidase; sputum

Abbreviations: ECP=eosinophil cationic protein; ELISA=enzyme-linked immunosorbent assay; IL-8=interleukin-8; MPO=myeloperoxidase

Airway inflammation is the main pathophysiologic feature of patients with bronchial asthma. However, airway inflammation in patients with COPD is not well understood. Inflammatory lesions in the airway wall are thought to be involved in airflow obstruction, the main characteristic of COPD, and the degree of airway inflammation seems to be associated with the prognosis of patients with COPD. Our purpose was to study the characteristics of airway inflammation in COPD patients with a noninvasive technique by analysis of sputum and to assess whether the levels of inflammatory markers in sputum correlated with the degree of airflow obstruction.

Studies using bronchial lavage fluid or bronchial biopsy specimens suggest important roles of the eosinophils in airway inflammation in asthma and of the neutrophils in airway inflammation in COPD. Interleukin-8 (IL-8), which is involved in neutrophil chemotaxis and activation, is also produced by airway epithelial cells. Recent in vitro studies have suggested the chemotaxis and activation of eosinophils by IL-8. It can therefore be speculated that IL-8 may recruit neutrophils and eosinophils into the airway and activate them in patients with COPD or asthma.

In the present study, we measured the concentrations of IL-8, myeloperoxidase (MPO) as a parameter of neutrophil activation, and eosinophil cationic protein (ECP) as a parameter of eosinophil activation in the supernatant of sputum. Their concentrations were compared in spontaneous sputum samples from patients with COPD and from patients with asthma and in induced sputum samples ob-
tained from normal control subjects. Possible associations among sputum IL-8, MPO, and ECP concentrations, smoking habits, and pulmonary function were evaluated.

**Materials and Methods**

**Subjects**

The subjects consisted of 33 patients with COPD and 30 with asthma seen at the outpatient clinic of Nara Medical University Hospital, and 12 normal volunteers. Their characteristics are listed in Table 1. Informed consent was obtained from all subjects, and the study was approved by the ethics committee of our university. The criteria for diagnosis of COPD or asthma were based on the standards of the American Thoracic Society. In the patients with COPD, their FEV$_1$ was <80% of predicted values, FEV$_1$/FVC ratio was <70%, and reversibility in FEV$_1$ was <10% after inhalation of 200 µg fenoterol. All patients in this group had a history of smoking (11 current smokers and 22 ex-smokers), and they were not receiving oral or inhaled steroid therapy. The patients with asthma had a history of wheezing and a reversible airflow obstruction as judged by an improvement of >15% in FEV$_1$ after inhalation of 200 µg fenoterol or by airway hyperresponsiveness to methacholine. This group consisted of 5 current smokers, 7 ex-smokers, and 18 nonsmokers. Nineteen of this group were receiving inhaled corticosteroid (daily dose, approximately 400 to 500 µg) and none of this group was receiving oral steroid therapy. All patients in both groups were in clinically stable condition, without symptoms of infection. The normal control group consisted of five current smokers and seven nonsmokers.

**Collection of Sputum**

In the patients with COPD or asthma, the sample consisted a small amount of sputum that was spontaneously discharged when they got up in the early morning. In the normal control subjects, sputum was induced by the inhalation of 3% hypertonic saline solution from the reservoir of an ultrasonic nebulizer (NE-U12; Omron; Tokyo, Japan) for 20 min. The mean volume of the collected sputum was 2.8 mL (range, 1.5 to 5.4 mL) in the patients with COPD, 2.5 mL (range, 1.3 to 5.0 mL) in the patients with asthma, and 1.8 mL (range, 1.3 to 3.2 mL) in the normal control subjects. None of the collected sputum was purulent in appearance.

**Separation of Sputum Supernatant**

The collected sample was transferred to a Petri dish. One milliliter of the portion that macroscopically appeared free of salivary contamination was placed in a centrifuge tube with 3 mL of phosphate-buffered saline solution, vortexed briefly for 1 min using a mixer, and centrifuged at 5,000 g for 20 min at 4°C. The supernatant was stored at −80°C until analyzed.

**Measurement of IL-8 in Sputum Supernatant**

The sputum supernatant was thawed and further diluted 10 to 50 times with phosphate-buffered saline solution. The IL-8 concentration in the sputum supernatant was measured by enzyme-linked immunosorbent assay (ELISA; Human IL-8 ELISA kit; Toray Industries Inc; Tokyo, Japan). Briefly, each sample was incubated in microtiter wells coated with an affinity-purified polyclonal antibody to human IL-8. After washing, a horseradish peroxidase-labeled antihuman IL-8 monoclonal antibody was added and the plates were incubated. The amount of IL-8 was measured spectrophotometrically after adding tetramethylbenzidine as enzyme substrate. Recombinant IL-8 was used as a standard. The interassay coefficient of variation was <10% and the lower limit of detection was 3 pg/mL. The recovery of IL-8 in sputum supernatant, tested on five samples by adding known amounts of IL-8 to the sputum supernatant, was >90%.

**Measurement of MPO and ECP in Sputum Supernatant**

The MPO concentration in the sputum supernatant was measured by ELISA (MPO-EIA kit; Bioxytech; Marne, France) using highly purified MPO as a standard. The ECP concentration was measured by a double-antibody radioimmunoassay (ECP-RIA kit; Pharmacia; Uppsala, Sweden). ECP in the samples competed with a fixed amount of $^{125}$I-labeled ECP for the binding sites of a specific polyclonal rabbit antibody. The ECP standards are calibrated against pure ECP prepared according to the method of Peterson et al. Both methods showed the interassay coefficient of variation was lower than 10%.

**Sputum Cell Counts**

Total and differential cell counts were performed as described by Gibson et al from the samples of 10 patients with COPD and 10 with asthma. There was no difference in total cell count between the groups with COPD and asthma (mean, 2.7×10$^6$/mL, 2.5×10$^6$/mL, respectively). In regard to differential cell count stained with May-Grumwald-Giemsa, the proportion of neutrophils was higher in the group with COPD than in the group with asthma (mean, 70.1%, 43.4%, respectively) and the proportion of eosinophils was lower in the group with COPD than in the group with asthma (mean, 1.6%, 4.9%, respectively). However these data were excluded from the present study because some of the cells had degenerated and therefore accurate counts of neutrophils and eosinophils were difficult to obtain.

**Pulmonary Function Tests**

Pulmonary function tests were performed by the standard method using a spirometer (FUDAC50; Fukuda Denshi; Tokyo,

<table>
<thead>
<tr>
<th>Table 1—Characteristics of the Subjects*</th>
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<tr>
<td><strong>Group</strong></td>
</tr>
<tr>
<td>COPD</td>
</tr>
<tr>
<td>Asthma</td>
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<tr>
<td>Control Subjects</td>
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*Data are expressed as means±SEM (range). ND=not done.
Statistical Analysis

Results are expressed as the mean±SEM. Statistical analysis was performed by the Mann-Whitney U test and Spearman’s rank correlation coefficient. A level of p<0.05 was considered to be statistically significant.

RESULTS

Concentration of IL-8 in Sputum Supernatant

The sputum concentration of IL-8 was significantly higher in the group with COPD than in the group with asthma or in the normal control group. The group with asthma had a significantly higher IL-8 concentration in sputum than the normal control group (COPD: 21.0±1.8 ng/mL [range, 2.8 to 35.2 ng/mL; median, 21.4 ng/mL]; asthma: 8.7±1.4 ng/mL [range, 0.8 to 27.8 ng/mL; median, 5.7 ng/mL]; control: 3.3±0.7 ng/mL [range, 0.8 to 7.2 ng/mL; median, 2.4 ng/mL]; Fig 1).

Concentration of MPO in Sputum Supernatant

The sputum concentration of MPO was significantly higher in the group with COPD and in the group with asthma than in the normal control group. No difference was observed between the two groups with respiratory disease (COPD: 83.9±4.9 ng/mL [range, 14.7 to 147.9 ng/mL; median, 79.7 ng/mL]; asthma: 83.3±8.7 ng/mL [range, 18.0 to 245.0 ng/mL; median, 70.6 ng/mL]; control: 42.6±5.7 ng/mL [range, 16.0 to 72.0 ng/mL; median, 40.0 ng/mL]; Fig 2).

Concentration of ECP in Sputum Supernatant

The sputum concentration of ECP was significantly higher in the group with COPD and in the group with asthma than in the control group. No significant difference was observed between the groups with COPD and asthma (COPD: 609.7±103.2 ng/mL [range, 0 to 2,144.0 ng/mL; median, 325.8 ng/mL]; asthma: 718.7±146.6 ng/mL [range, 12.8 to 3,084.0 ng/mL; median, 295.6 ng/mL]; control: 69.5±24.2 ng/mL [range, 0 to 240.0 ng/mL; median, 40.1 ng/mL]; Fig 3).

Relationship Between IL-8 and MPO or ECP Concentrations in Sputum

In the group with COPD, the concentration of IL-8 was significantly correlated with MPO (r=0.55, p<0.001) and with ECP (r=0.53, p<0.01). No correlation was observed in the group with asthma.

Relationship Between IL-8 in Sputum and Smoking Status

In the group with COPD, the sputum concentration of IL-8 did not significantly differ between the current smokers and the ex-smokers. In the control group, the sputum concentration of IL-8 did not differ significantly between the current smokers and

![Graph of IL-8 concentration in sputum](image1)

![Graph of MPO concentration in sputum](image2)

Figure 1. The concentration of IL-8 in sputum in patients with COPD and asthma and healthy control subjects.

Figure 2. The concentration of MPO in sputum in patients with COPD and asthma and healthy control subjects. NS=not significant.
the nonsmokers. In the group with asthma, the sputum concentration of IL-8 was significantly higher in the smokers than in the nonsmokers (Table 2). No association was observed between MPO or ECP and smoking habit.

**Relationship Between IL-8 and Pulmonary Function**

In the group with COPD, the sputum concentration of IL-8 was negatively correlated with FEV$_1$ (percent predicted) ($r=-0.60$, $p<0.01$) and with FEV$_1$/FVC ($r=-0.78$, $p<0.0001$; Fig 4). No association was observed between MPO or ECP and any parameter of pulmonary function. In the group with asthma, no correlation was obtained.

**Table 2—Sputum Concentration of IL-8 According to Smoking Habits in Patients With COPD and Asthma and Healthy Control Subjects**

<table>
<thead>
<tr>
<th></th>
<th>No.</th>
<th>IL-8, ng/mL</th>
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<tbody>
<tr>
<td><strong>COPD</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Current smoker</td>
<td>11</td>
<td>18.8±3.1</td>
</tr>
<tr>
<td>Ex-smoker</td>
<td>22</td>
<td>23.3±2.1</td>
</tr>
<tr>
<td><strong>Asthma</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Current smoker</td>
<td>5</td>
<td>13.1±2.8*</td>
</tr>
<tr>
<td>Ex-smoker</td>
<td>7</td>
<td>12.4±3.3*</td>
</tr>
<tr>
<td>Nonsmoker</td>
<td>18</td>
<td>5.3±1.0</td>
</tr>
<tr>
<td><strong>Control subjects</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Current smoker</td>
<td>5</td>
<td>4.5±1.3</td>
</tr>
<tr>
<td>Nonsmoker</td>
<td>7</td>
<td>2.8±0.7</td>
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</table>

*p<0.05 vs nonsmokers in the group with asthma.

**DISCUSSION**

Airway inflammation, a cardinal pathophysiologic feature in patients with asthma and also COPD, is usually evaluated using specimens obtained by bronchial lavage or bronchial biopsy. However, these methods are invasive and difficult to perform repeatedly or to use in patients with severe conditions. More recently, studies have been performed using sputum induced by the inhalation of hypertonic saline solution. Although this method appears to be useful, it is not physiologic and its safety has not been established. The use of hypertonic saline solution may lead to an excess number of neutrophils in induced sputum. Also, while the inflammation in COPD and asthma is mainly present in the peripheral airway rather than the central airway, only the thicker central airway may be evaluated using specimens of induced sputum. Patients with COPD or asthma often discharge a small amount of sputum in the early morning, even though they may be unaware of it. Sputum that is spontaneously expressed in the early morning had been transported from the peripheral to the central airway during the night, so that it reflects a more extensive area of the airway. We therefore used samples of the sputum that was spontaneously expressed when the patients arose early in the morning. This method is safe and readily available, but the analysis of spontaneous sputum can be problematic. Degeneration in the cells is marked, and accurate counts of neutrophils and eosinophils are difficult to obtain. Therefore, we also measured MPO as an indirect marker of neutrophil activity and ECP as an indirect marker of eosinophil activity.

IL-8 is a strong factor in neutrophil chemotaxis and activation that also is produced by airway epithelial cells. Production of IL-8 indicates the che-
motaxis and activation of the neutrophils and chemotaxis of the T lymphocytes in the airway. Recent studies suggest the chemotaxis and activation of eosinophils by IL-8. In the present study, we observed a higher sputum concentration of IL-8 in the patients with COPD than in those with asthma or in normal control subjects. These results are consistent with those of Nocker et al. and with those of Keatings et al. in induced sputum samples. In patients with COPD, IL-8 may be produced in airway epithelial cells to produce chemotaxis of the neutrophils and eosinophils in the airway. In particular, neutrophils release neutrophil elastase, which induces the expression of the IL-8 gene in airway epithelial cells, resulting in a further secretion of IL-8. In this "inflammatory cycle" in the airway, IL-8 appears to play the primary role.

Richman-Eisenstat et al. reported a higher concentration of IL-8 in patients with cystic fibrosis, bronchiectasis, and chronic bronchitis than in normal control subjects, suggesting neutrophil chemotaxis in the airway by IL-8 in these patients. Taranneh et al. found a high sputum IL-8 concentration, which was correlated with the clinical condition, in children with cystic fibrosis. These disorders are characterized by purulent sputum associated with infection. We observed a high concentration of IL-8 even in sputum that was macroscopically nonpurulent obtained from the patients with COPD or asthma. These results suggest that IL-8 is closely involved in airway inflammation that is associated not only with infection but also with other pathophysiological mechanisms.

Previous studies using bronchial lavage fluid or bronchial biopsy specimens suggest a central role of eosinophils in airway inflammation in asthma and of neutrophils in COPD. We therefore expected a high MPO concentration in our patients with COPD and a high ECP concentration in those with asthma. Although the concentrations of both MPO and ECP were higher in the patients with COPD and asthma than in the control subjects, they did not differ between the patients with COPD and those with asthma. Linden et al. reported increased concentrations of both MPO and ECP in BAL fluid from patients with asthma and COPD. These findings suggest that activation of the neutrophils and eosinophils occurs in the airway of patients with COPD or asthma. There may be no difference between COPD and asthma in terms of the inflammation associated with neutrophils or eosinophils. It is possible that factors other than IL-8, such as IL-5, also cause chemotaxis and activation of eosinophils in asthma. However, in our patients with COPD, the concentration of IL-8 was significantly correlated with those of MPO and ECP, indicating that IL-8 was the main mediator. These results are consistent with the recent study by Riise et al. who used bronchial lavage in chronic bronchitis, and support an in vitro report showing the chemotaxis and activation of neutrophils and eosinophils by IL-8.

It is generally accepted that smoking induces airway inflammation. An increase in the number of neutrophils in the BAL fluid or in the sputum of smokers has been reported. One study reported a higher concentration of IL-8 in induced sputum in normal smokers than in normal nonsmokers, while another study showed no significant difference in concentration of IL-8 in the BAL fluid of normal smokers vs nonsmokers. In our study, the concentration of IL-8 differed according to the presence or absence of smoking in the patients with asthma. This finding suggests that smoking increases the concentration of IL-8 in sputum, activating the "inflammatory cycle" in the airway. However, the concentration of IL-8 did not differ significantly between the current smokers and ex-smokers with COPD or asthma. This suggests that an abstention from smoking does not alter the activation of the inflammatory cycle.

We observed a significant negative correlation between the sputum concentration of IL-8 and the FEV/FVC ratio in the patients with COPD. This suggests a close association between the sputum concentration of IL-8 and the severity of airflow obstruction in COPD. In this disorder, airway inflammation involves the central as well as peripheral airway. Airflow obstruction, a characteristic of COPD, is thought to be due to inflammatory lesions in the airway. In patients with COPD, the IL-8 produced in the airway epithelial cells may activate the inflammatory cycle, causing a thickening of the airway wall and a narrowing of the lumen, thereby resulting in airflow obstruction.

In conclusion, both neutrophils and eosinophils were locally activated in COPD. Such activation was mainly associated with IL-8. The sputum concentration of IL-8 was closely related to the severity of airflow obstruction. The measurement of sputum IL-8 may perhaps be useful in evaluating the severity of airway inflammation.

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