Abnormal Peripheral Blood T-Lymphocyte Subsets in a Subgroup of Patients With COPD*

Won-Dong Kim, MD, FCCP; Woo-Sung Kim, MD; Younsuck Koh, MD; Sang-Do Lee, MD; Chae-Man Lim, MD; Dong-Soon Kim, MD; and Young-Joo Cho, MD

Background: Smoking may induce changes in T-lymphocyte subsets in peripheral blood. Abnormalities of T-lymphocyte subsets in peripheral blood and in BAL fluid, and increased CD8+ T lymphocytes in the airways have been reported in patients with COPD. These findings suggest that T-lymphocyte abnormalities might be involved in the pathogenesis of airflow limitation in people who smoke.

Design: Cross-sectional study.

Setting: Outpatient pulmonary department of a university hospital.

Methods: To investigate this hypothesis, peripheral blood T-lymphocyte subsets were measured by flow cytometry using specific monoclonal antibodies in 20 healthy nonsmokers, 20 healthy smokers, and 20 smokers with stable COPD. No significant differences in the peripheral blood T-lymphocyte subsets were observed among the three groups. Because a previous study showed peripheral blood T-lymphocyte abnormalities in the subgroup of nonsmoking patients with COPD, we wanted to investigate what factors determine the subgroup of COPD with abnormal T-lymphocyte subsets. We tried to measure the relationship between T-lymphocyte subsets and physiologic indexes of pulmonary function tests in patients with COPD. The proportion of CD8+ T lymphocytes significantly correlated with diffusing capacity of the lung for carbon monoxide (DLCO) and DLCO per unit of alveolar volume (DLCO/VA), and CD4+/CD8+ ratio correlated with DLCO/VA. Therefore, we attempted to classify the patients with COPD into two subgroups on the basis of DLCO/VA: 10 COPD patients with low DLCO/VA (< 80% predicted) and 10 patients with normal DLCO/VA (≥ 80% predicted).

Results: The normal DLCO/VA subgroup had a significantly higher proportion of CD8+ T lymphocytes and a lower CD4+/CD8+ ratio than the healthy smokers or the low DLCO/VA subgroup. Moreover, FEV1/FVC significantly correlated with the CD4+/CD8+ ratio only in the normal DLCO/VA subgroup.

Conclusion: These findings suggest that T-lymphocyte abnormalities might be involved in the pathogenesis of airflow limitation in a subgroup of patients with COPD, presumably with small airways disease, but not in all cases of COPD.

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Key words: CD8+ T lymphocyte; COPD; diffusing capacity; T-lymphocyte subsets

Abbreviations: DLCO = diffusing capacity of the lung for carbon monoxide; DLCO/VA = diffusing capacity of the lung for carbon monoxide per unit of alveolar volume; FRC = functional residual capacity; HLA = human leukocyte antigen; RV = residual volume; TLC = total lung capacity

The role of cigarette smoking in the etiology of COPD has been well established, yet the pathogenesis by which smoking leads to chronic airflow limitation is not fully understood. Only 15 to 20% of smokers1 have clinically manifested COPD develop,1 indicating the existence of differences in individual susceptibility to COPD among smokers. Understanding of the host characteristics that influence this susceptibility would be of great importance in elucidating the pathogenesis of this disorder.

Studies2,3 analyzing T-lymphocyte subsets in pe-

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peripheral blood have demonstrated a relative decrease in CD4+ T lymphocytes and increase of CD8+ T lymphocytes with a low CD4+/CD8+ ratio in heavy smokers. In addition, it has been reported that the percentage of CD8+ T lymphocytes in the peripheral blood is significantly higher in nonsmoking patients with COPD compared with nonsmoking, healthy control subjects, and a lower CD4+/CD8+ ratio is associated with a lower FEV1 in these patients with COPD.4

A histopathologic study5 on surgical specimens from smokers with COPD has demonstrated an increased number of CD8+ T lymphocytes in the peripheral airways, which are the sites responsible for chronic airflow limitation. These patients had not only an increased number of CD8+ T lymphocytes, but also an increased smooth-muscle mass in the peripheral airways, suggesting the presence of small airways remodeling in these patients with chronic airflow limitation. In this study, when all the smokers were considered together, the number of CD8+ T lymphocytes showed a significant negative correlation with FEV1.

These findings suggest that T-lymphocyte abnormalities might play a role in the pathogenesis of airflow limitation in smokers. A hypothesis was proposed that smoking is most likely to induce the development of persistent airflow obstruction in individuals who have inherited the tendency to a low peripheral blood CD4+/CD8+ ratio.6 However, a previous study4 showed that T-lymphocyte abnormalities in peripheral blood are not present in all patients with COPD, but only in the nonsmoking patients with COPD, although the nature of this subgroup of nonsmoking patients with COPD is not known. To test this hypothesis, and, if it is true only in a subgroup of patients with COPD, then to know in what subgroup of patients with COPD it is true, we measured the T-lymphocyte subsets in peripheral blood of healthy nonsmokers, healthy smokers, and smokers with COPD.

### Materials and Methods

#### Study Population

The study population consisted of 20 healthy nonsmokers, 20 healthy smokers without respiratory symptoms and airflow limitation, and 20 smokers with respiratory symptoms and airflow limitation (smokers with COPD). All subjects were men (mean ± SD age, 59.7 ± 7.7 years, 57.1 ± 5.2 years, and 65.5 ± 7.7 years for healthy nonsmokers, healthy smokers, and smokers with COPD, respectively; healthy nonsmokers or healthy smokers vs smokers with COPD, p < 0.05; Table 1). The mean smoking history was 29.8 ± 15.2 pack-years for the healthy smokers and 37.2 ± 10.8 pack-years for the smokers with COPD (p > 0.05).

None of the subjects were receiving oral or inhaled corticosteroids, or immunosuppressive treatment; reported any other serious illness or acute viral syndrome during the 2 months before the test; or had tuberculosis, parasite infestation, or hepatitis B surface antigenemia. The healthy nonsmokers and healthy smokers with normal chest radiographic and spirometry findings were selected from a population that had undergone an annual health checkup. The smokers with COPD had a history of cough with sputum production and/or dyspnea on most days of the month for at least 3 months a year during > 2 years before the study, and airflow limitation on spirometry (FEV1/FVC < 70% and FEV1 < 80% of predicted normal). These patients’ airflow limitation was irreversible based on a negative immediate response to inhalation of 200 μg of albuterol. Their pulmonary function was stable for several months under observation. Positive immediate bronchodilator response required two of the three following criteria: an increase of ≥ 15% in FEV1, an increase of ≥ 15% in FVC, or an increase of ≥ 25% in the mean forced expiratory flow during the middle half of the FVC. The study protocol was approved by the Institutional Review Board, and informed consent was obtained from all patients.

#### Pulmonary Function Study

Spirometric measurement was performed according to the recommendation of the American Thoracic Society7 with a spirometer (System 2100; SensorMedics; Yorba Linda, CA) in all subjects. All values are expressed as percentage of predicted using the formula of Cho and colleagues8 for normal healthy nonsmokers and healthy smokers with COPD and as expressed was percentage of predicted using the prediction formula of Goldman and Becklake9 for carbon monoxide (DLco) was measured by the single-breath

### Table 1—Baseline Characteristics of Subjects*

<table>
<thead>
<tr>
<th>Variables</th>
<th>Healthy Nonsmokers (n = 20)</th>
<th>Healthy Smokers (n = 20)</th>
<th>COPD (n = 20)</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>59.7 ± 7.7</td>
<td>57.1 ± 5.2</td>
<td>65.5 ± 7.7†</td>
<td>0.001</td>
</tr>
<tr>
<td>Smoking, pack-yr</td>
<td>0</td>
<td>29.5 ± 15.2</td>
<td>37.2 ± 10.8†</td>
<td></td>
</tr>
<tr>
<td>FVC1, % predicted</td>
<td>99.7 ± 17.4</td>
<td>96.2 ± 9.2</td>
<td>59.6 ± 13.1&lt; †</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>FEV1, % predicted</td>
<td>107.2 ± 18.3</td>
<td>97.5 ± 11.9</td>
<td>45.1 ± 14.9†</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>FEV1/FVC, %</td>
<td>78.2 ± 5.1</td>
<td>81.0 ± 4.2</td>
<td>52.2 ± 8.6†‡</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

*Data are presented as mean ± SD.
†p < 0.05 vs healthy nonsmokers or healthy smokers by Student-Neuman-Keuls test.
‡p > 0.05 vs healthy smokers by Student t test.
Analysis of Mononuclear Cell Subsets

Peripheral venous blood was drawn from all subjects after obtaining informed consent. Aliquots were submitted to the laboratory for CBC count and differential WBC count. Peripheral blood mononuclear cells were separated from heparinized venous blood by centrifugation on Ficoll-Hypaque (Pharmacia; Uppsala, Sweden) within 24 h. Expression of the mononuclear cell surface markers CD3, CD19, CD4, CD8, CD14, and human leucocyte antigen (HLA)-DR was assessed using specific fluorescein isothiocyanate-conjugated or phycoerythrin-conjugated mouse monoclonal antibodies (Becton Dickinson Immunocytometry Systems; Mountain View, CA). For cell surface marker staining, aliquots of 100 μL of cells of peripheral blood mononuclear cell suspension (1 × 10^6/mL) were incubated for 45 min at 4°C with the appropriate monoclonal antibodies. Fluorescein isothiocyanate-IgG1 and phycoerythrin-IgG2 murine isotype control reagents were used to evaluate the nonspecific binding. Two-color analysis was performed on a FACScan (Becton Dickinson) equipped with an argon laser tuned to 488 nm, and the data analysis was performed using the CONSORT 30 Data Management System (Becton Dickinson Immunocytometry Systems; San Jose, CA). Lymphocyte or monocyte populations were gated on the basis of forward and right-angle light scatter, with 10,000 events evaluated for each determination. Positively labeled cells were quantified as a percentage of the total lymphocyte population in the range of fluorescence intensity channels above a predefined threshold channel.

Statistical Analysis

The results are reported as mean ± SD. A one-way analysis of variance was used to evaluate significant differences among groups, and, when a significance was found, post hoc between-group analysis was performed with the Student-Newman-Keuls test. A nonparametric analysis of variance (Kruskal-Wallis test) and post hoc analysis with Dunn test were used for data that did not pass a normality test or equal variance test. Spearman rank correlation was used to determine how T-lymphocyte subsets correlated with physiologic indexes in patients with COPD. All statistical analyses were performed with software (Sigma Stat Statistical Analysis System, Version 1.0; Jandel Corporation; San Rafael, CA); p values < 0.05 were considered significant.

RESULTS

Pulmonary Function Tests

Healthy nonsmokers and healthy smokers were originally selected for their normal spirometry values (Table 1). Smokers with COPD had severe airflow limitation (FVC, 59.6 ± 13.1%; FEV₁, 45.1 ± 14.9%; FEV₁/FVC, 52.2 ± 8.6%), hyperinflation (total lung capacity [TLC], 138.8 ± 27.3% and functional residual capacity [FRC], 161.1 ± 43.5%), air trapping (residual volume [RV], 220.4 ± 57.7%), and decreased DLCO (72.5 ± 26.8%) and DLCO per unit of alveolar volume (DLCO/VA), 76.1 ± 28.3%.

T-Lymphocyte Subsets in Healthy Nonsmokers, Healthy Smokers, and Smokers With COPD

No differences in the proportions of peripheral blood T-lymphocyte subsets and CD4+/CD8+ ratio among the healthy nonsmokers, healthy smokers, and smokers with COPD were observed (Table 2).

Subclassification of Smokers With COPD

Although we found no difference in peripheral blood T-lymphocyte subsets between the three groups, we thought there is a possibility that abnormalities in T-lymphocyte subsets would be present in a subgroup of COPD as reported before. To investigate what factors determine this subgroup of COPD patients with abnormal T-lymphocyte subsets, we tried to measure the relationship between T-lymphocyte subsets and physiologic indexes obtained by pulmonary function tests in the group of smokers with COPD. Of many indexes, the proportion of CD8+ T lymphocytes significantly correlated with DLCO (r_s = 0.552, p = 0.012) and DLCO/VA (r_s = 0.660, p = 0.002), and the CD4+/CD8+ ratio correlated with DLCO/VA (r_s = 0.561, p = 0.010) by Spearman rank correlation test. Therefore, we attempted to classify the smokers with COPD into two sub-

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Table 2—Lymphocyte Subsets in Subjects

<table>
<thead>
<tr>
<th>Cell Surface Antigen</th>
<th>Healthy Nonsmokers (n = 20)</th>
<th>Healthy Smokers (n = 20)</th>
<th>COPD (n = 20)</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD3+, %</td>
<td>57.3 ± 6.8</td>
<td>58.7 ± 10.5</td>
<td>55.3 ± 9.6</td>
<td>0.508</td>
</tr>
<tr>
<td>CD4+, %</td>
<td>37.8 ± 5.8</td>
<td>37.2 ± 9.6</td>
<td>33.2 ± 9.4</td>
<td>0.195</td>
</tr>
<tr>
<td>CD8+, %</td>
<td>30.6 ± 5.5</td>
<td>31.6 ± 7.2</td>
<td>31.9 ± 7.3</td>
<td>0.813</td>
</tr>
<tr>
<td>CD4+/CD8+ ratio</td>
<td>1.28 ± 0.34</td>
<td>1.25 ± 0.45</td>
<td>1.14 ± 0.55</td>
<td>0.280</td>
</tr>
<tr>
<td>CD19+, %</td>
<td>6.2 ± 4.1</td>
<td>6.7 ± 2.5</td>
<td>6.2 ± 4.4</td>
<td>0.232</td>
</tr>
<tr>
<td>CD14+, %</td>
<td>17.9 ± 6.9</td>
<td>16.9 ± 5.8</td>
<td>21.9 ± 8.9</td>
<td>0.126</td>
</tr>
<tr>
<td>HLA-DR, %</td>
<td>27.4 ± 9.2</td>
<td>29.7 ± 6.5</td>
<td>30.7 ± 11.3</td>
<td>0.522</td>
</tr>
</tbody>
</table>

*Data are presented as mean ± SD.
The original purpose of this study was to investigate the difference in T-lymphocyte subsets between healthy smokers and smokers with COPD. Therefore, we compared the T-lymphocyte subsets among the healthy smokers, the low DLCO/VA group, and the normal DLCO/VA group. Characteristics and results of pulmonary function tests of the three study groups of healthy smokers, low DLCO/VA patients, and normal DLCO/VA patients are shown in Table 3. Low DLCO/VA patients had severe airflow limitation, hyperinflation, air trapping, and, as designed for the study, decreased DLCO. Normal DLCO/VA patients also had severe airflow limitation, hyperinflation, and air trapping, but normal DLCO. Low DLCO/VA patients had higher TLC, FRC, and RV than normal DLCO/VA patients (p < 0.05).

**T-Lymphocyte Subsets in Healthy Smokers, Low DLCO/VA Patients, and Normal DLCO/VA Patients**

Although absolute peripheral blood lymphocyte counts were not different among the three groups (p = 0.294), normal DLCO/VA patients had a higher proportion of CD8+ T lymphocytes (p < 0.05; Fig 1) and a lower CD4+/CD8+ ratio (p < 0.05; Fig 2) than the healthy smokers or low DLCO/VA patients (Table 4).

**Correlation Between FEV1/FVC and CD4+/CD8+ Ratio**

FEV1/FVC significantly correlated with the CD4+/CD8+ ratio in normal DLCO/VA patients (r = 0.644, p = 0.044; Fig 3), but not in the healthy smokers (r = 0.215, p = 0.362) or low DLCO/VA patients (r = 0.304, p = 0.393).

<table>
<thead>
<tr>
<th>Variables</th>
<th>Healthy Smokers (n = 20)</th>
<th>Low DLCO/VA (n = 10)</th>
<th>Normal DLCO/VA (n = 10)</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>57.1 ± 5.2</td>
<td>65.8 ± 5.3</td>
<td>65.1 ± 9.8</td>
<td>0.001</td>
</tr>
<tr>
<td>Smoking pack-yr</td>
<td>29.8 ± 15.2</td>
<td>33.5 ± 8.8</td>
<td>40.8 ± 11.7</td>
<td>0.106</td>
</tr>
<tr>
<td>FVC, % predicted</td>
<td>96.2 ± 9.2</td>
<td>56.1 ± 13.6</td>
<td>63.1 ± 12.3</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>FEV1, % predicted</td>
<td>97.8 ± 11.9</td>
<td>38.1 ± 13.7</td>
<td>52.0 ± 13.2</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>FEV1/FVC, %</td>
<td>81.0 ± 4.2</td>
<td>47.0 ± 6.0</td>
<td>57.3 ± 7.1</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>TLC, % predicted</td>
<td>153.4 ± 20.4</td>
<td>126.1 ± 25.6</td>
<td>136.3 ± 38.3</td>
<td></td>
</tr>
<tr>
<td>FRC, % predicted</td>
<td>189.5 ± 28.2</td>
<td>136.3 ± 38.3</td>
<td>183.5 ± 43.1</td>
<td></td>
</tr>
<tr>
<td>RV, % predicted</td>
<td>261.1 ± 45.4</td>
<td>83.3 ± 15.7</td>
<td>96.7 ± 15.5</td>
<td></td>
</tr>
<tr>
<td>DLCO, % predicted</td>
<td>44.3 ± 17.0</td>
<td>110.0 ± 10.0</td>
<td>110.0 ± 10.0</td>
<td></td>
</tr>
<tr>
<td>DLCO/VA, % predicted</td>
<td>54.1 ± 20.9</td>
<td>54.1 ± 20.9</td>
<td>54.1 ± 20.9</td>
<td></td>
</tr>
</tbody>
</table>

*Data are presented as mean ± SD.
†p > 0.05 vs low DLCO/VA by Dunn test.
‡p < 0.05 vs low DLCO/VA by Student t test.
significant correlation was found between FEV₁, the total T-lymphocyte and CD4 ratio in patients with COPD, but the percentage of CD8 lymphocytes was significantly higher in the non-smoking patients with COPD compared with the nonsmoking, healthy control subjects, and a lower CD4+/CD8+ ratio was associated with a lower FEV₁ in these patients with COPD. A study on BAL fluid has also shown that the CD4+/CD8+ ratio is significantly decreased in smokers with and without COPD, and the percentage of CD8+ T lymphocytes positively correlates with the smoking history expressed as pack-years.

A pathologic study on lymphocytic infiltration of central airway epithelium showed that CD4+ and CD8+ T lymphocytes are increased in patients with chronic bronchitis and mild airflow limitation. Another study on bronchial biopsies obtained from healthy nonsmokers and smokers with bronchitis has demonstrated an increased number of CD8+ T lymphocytes in the central airways of patients with COPD and a correlation between the number of CD8+ T lymphocytes and the degree of airflow limitation. In the peripheral airways of the lung, the only difference observed between the smokers without COPD and smokers with COPD was an increased number of CD8+ T lymphocytes and airway remodeling in smokers with COPD compared with smokers without COPD. CD8+ T lymphocytes were not only increased in these subjects, but also correlated with the degree of airflow limitation. Thus, it was concluded that smokers who have COPD develop have an increased number of CD8+ T lymphocytes in both central and peripheral airways, compared with smokers who do not have COPD develop, supporting the important role of these cells in the pathogenesis of chronic airflow limitation in smokers.

It was proposed that the pathologic effect of cigarette smoke would be greater in those individuals who already have a genetically determined low CD4+/CD8+ ratio, and that this could explain why only a proportion of smokers succumb to disease and have their lung function adversely affected by cigarette smoke. To investigate this hypothesis, our data were analyzed according to the presence or absence of airflow limitation in the smokers. Although we expected a difference in T-lymphocyte subsets between healthy smokers and smokers with airflow limitation, we could not find any difference. These findings of no difference in T-lymphocyte subsets between healthy smokers and smokers with COPD are not inconsistent with the previous report. However, this previous study had shown abnormal peripheral blood T-lymphocyte subsets in a subgroup of nonsmoking patients with COPD, although the nature of this subgroup is not known. In these nonsmoking patients with COPD, ex-smokers with a history of smoking cessation of > 1 year were included, and they may be more severely affected by the disease.

**FIGURE 2.** Peripheral blood CD4+/CD8+ ratios in the healthy smokers, low DLco/VA patients, and normal DLco/VA patients. Normal DLco/VA patients had a significantly lower CD4+/CD8+ ratio than healthy smokers (p < 0.05) or low DLco/VA patients (p < 0.05). Horizontal bars represent mean values.

**DISCUSSION**

Although we did not find any difference between the healthy nonsmokers, healthy smokers, and smokers with COPD in peripheral blood T-lymphocyte subsets, we detected an alteration in the peripheral blood T-lymphocyte subsets in normal DLco/VA patients compared with the healthy smokers or low DLco/VA patients, in that normal DLco/VA patients had a higher proportion of CD8+ T lymphocytes and a lower CD4+/CD8+ ratio. In addition, a significant correlation was found between FEV₁/FVC, an index of airflow limitation, and CD4+/CD8+ ratio in the normal DLco/VA group, but not in the healthy smokers or the low DLco/VA group.

Analysis of T-lymphocyte subsets in peripheral blood indicates that, in light-to-moderate smokers, the total T-lymphocyte and CD4+ T-lymphocyte populations increase, whereas in heavy smokers, CD4+ T lymphocytes decrease and both the percentage and total numbers of CD8+ T lymphocytes increase. de Jong et al showed that percentages of blood lymphocyte subsets are not significantly different between healthy control groups and patients with COPD. Also no significant difference was observed between smoking healthy control subjects and smokers with COPD, but the percentage of CD8+ T lymphocytes was significantly higher in the non-smoking patients with COPD compared with the
In addition, a significant correlation was found between normal D\textsubscript{LCO}/VA and CD4\textsuperscript{+}/CD8\textsuperscript{+} ratio in the normal D\textsubscript{LCO}/VA group, but not in the healthy smokers or low D\textsubscript{LCO}/VA group.

Chronic airflow limitation may be caused by either emphysema or irreversible obstructive changes in the peripheral airways.\textsuperscript{15} Whether these two pathologic processes are pathogenetically related or represent two separate responses to a common exposure has remained a subject of investigation. We have shown that smokers have different patterns of lung destruction develop, some with pure or predominant centrilobular emphysema associated with a higher degree of abnormalities in the small airways, and others with pure or predominant panlobular emphysema with a smaller degree of abnormalities of the small airways.\textsuperscript{16} We have also shown that, in centrilobular emphysema, flow limitation is primarily a function of abnormalities of the small airways, whereas in panlobular emphysema, flow limitations are accompanied by loss of elastic recoil pressure of the lung. This suggests that different pathogenetic mechanisms could be present in these two types of emphysema.

Although no currently available physiologic test provides an accurate diagnosis of emphysema, emphysema severity has been shown to correlate most closely with the D\textsubscript{LCO}.\textsuperscript{17,18} The high degree of airflow limitation found in the normal D\textsubscript{LCO}/VA group with no history or evidence of asthma may indicate the presence of a large amount of peripheral small airways disease without a sufficient degree of emphysema that would decrease the surface area for diffusion. In contrast, our smokers with airflow limitation who had very low D\textsubscript{LCO}, high RV, and high TLC would represent predominant emphysema, probably panlobular. Therefore, it is presumed that most, if not all, of the normal D\textsubscript{LCO}/VA patients might have peripheral small airways disease, and most of the low D\textsubscript{LCO}/VA patients might have panlobular emphysema.

Therefore, it was necessary to know the nature of this subgroup of patients with COPD to find out which smokers with COPD have abnormalities in T-lymphocyte subsets. To investigate what factors determine this subgroup, we measured the relationship between T-lymphocyte subsets and physiologic indexes of pulmonary function tests in the smokers with COPD. The percentage of CD8\textsuperscript{+} T lymphocytes significantly correlated with D\textsubscript{LCO} and D\textsubscript{LCO}/VA, and CD4\textsuperscript{+}/CD8\textsuperscript{+} ratio correlated with D\textsubscript{LCO}/VA. Therefore, we classified the smokers with COPD into groups of normal D\textsubscript{LCO}/VA and low D\textsubscript{LCO}/VA, and found that normal D\textsubscript{LCO}/VA patients had a higher proportion of CD8\textsuperscript{+} T lymphocytes and a lower CD4\textsuperscript{+}/CD8\textsuperscript{+} ratio. In addition, a significant correlation was found between FEV\textsubscript{1}/FVC and CD4\textsuperscript{+}/CD8\textsuperscript{+} ratio in the normal D\textsubscript{LCO}/VA group, but not in the healthy smokers or low D\textsubscript{LCO}/VA group.

Table 4—Lymphocyte Subsets in Healthy Smokers and Smokers With COPD\textsuperscript{*}

<table>
<thead>
<tr>
<th>Cell Surface Antigen</th>
<th>Healthy Smokers (n = 20)</th>
<th>Low D\textsubscript{LCO}/VA (n = 10)</th>
<th>Normal D\textsubscript{LCO}/VA (n = 10)</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD3\textsuperscript{+}, %</td>
<td>38.7 ± 10.6</td>
<td>52.4 ± 8.8</td>
<td>58.2 ± 10.0</td>
<td>0.265</td>
</tr>
<tr>
<td>CD4\textsuperscript{+}, %</td>
<td>37.2 ± 9.6</td>
<td>35.0 ± 9.1</td>
<td>30.7 ± 9.4</td>
<td>0.336</td>
</tr>
<tr>
<td>CD8\textsuperscript{+}, %</td>
<td>31.6 ± 7.2</td>
<td>26.8 ± 5.9</td>
<td>36.9 ± 4.61</td>
<td>0.004</td>
</tr>
<tr>
<td>CD4\textsuperscript{+}/CD8\textsuperscript{+} ratio</td>
<td>1.25 ± 0.45</td>
<td>1.42 ± 0.61</td>
<td>0.85 ± 0.331</td>
<td>0.026</td>
</tr>
<tr>
<td>CD19\textsuperscript{+}, %</td>
<td>6.7 ± 2.5</td>
<td>7.6 ± 5.9</td>
<td>4.8 ± 1.6</td>
<td>0.211</td>
</tr>
<tr>
<td>CD14\textsuperscript{+}, %</td>
<td>16.9 ± 5.8</td>
<td>21.9 ± 6.7</td>
<td>21.5 ± 11.0</td>
<td>0.137</td>
</tr>
<tr>
<td>HLA-DR, %</td>
<td>29.7 ± 6.5</td>
<td>31.2 ± 12.5</td>
<td>30.2 ± 10.6</td>
<td>0.922</td>
</tr>
</tbody>
</table>

*Data are presented as mean ± SD. †p > 0.05 vs healthy smokers by Student-Neuman-Keuls test. ‡p < 0.05 vs healthy smokers or low D\textsubscript{LCO}/VA by Student-Neuman-Keuls test.

![Figure 3](image-url)  
**Figure 3.** Significant correlation between FEV\textsubscript{1}/FVC and peripheral blood CD4\textsuperscript{+}/CD8\textsuperscript{+} ratio in patients with COPD with normal D\textsubscript{LCO}/VA.
To our knowledge, no study has ever observed T lymphocytes simultaneously in both peripheral blood and pathologic airways of the same smokers with COPD. It has been suggested that continuing migration of CD4+ T lymphocytes from blood to sites of disease activity in the lung could result in a decreased number of circulating blood CD4+ T lymphocytes and increased number of these T cells in the lungs of pulmonary sarcoidosis. However, previous reports have shown increased peripheral blood CD8+ T lymphocytes in heavy smokers, positive correlation of CD8+ T lymphocytes in BAL fluid with pack-year smoking history, and positive correlation of CD8+/CD3+ ratio of cells infiltrating small-airway submucosa with pack-years smoked in smokers; these findings suggest the possibility that increased CD8+ T lymphocytes in peripheral blood are associated with increased CD8+ T lymphocytes in the airways of smokers with COPD.

In this study and in a previous report, abnormalities in the peripheral blood T-lymphocyte subsets were not present in all smokers with COPD, but only in a subgroup. In addition, in the previous study, the increased scores of CD8+ T lymphocyte and muscle in the peripheral airways were not present in all patients with COPD. These patients with increased CD8+ T lymphocytes and airway remodeling in the peripheral airways are thought to represent patients with small airways disease. Thus, we suspected that the degree of association between abnormalities in peripheral blood T lymphocytes and development of airflow limitation in smokers could be different between the two subtypes of COPD. Then we reasoned that abnormalities in peripheral blood T-lymphocyte subsets would more likely be associated with COPD patients with small airways disease than with COPD patients with panlobular emphysema, who are known to have lower levels of small airways disease.

The subgroups of low DLco/Va and normal DLco/Va had similar mean ages, smoking histories, and degrees of airflow limitation. When the patients were analyzed as a single group of patients with COPD, no difference in T-lymphocyte subsets from the healthy nonsmokers or healthy smokers was apparent; however, when we classified them into two subgroups, a difference in T-lymphocyte subsets became apparent. This suggests that different pathogenetic mechanisms are involved in the subtypes of COPD, and that T-lymphocyte abnormalities might be involved in the pathogenesis of airflow limitation in a subgroup of COPD patients with normal DLco/Va, probably patients with small airways disease. The significance of the change in T-lymphocyte subsets in this subgroup is further supported by the significant correlation between FEV1/FVC and the CD4+CD8+ ratio.

It has been suspected that the pathogenesis of the two subtypes of COPD could be different morphologically and physiologically, but direct evidence has not yet been reported. The result of this study could be the first clue that may lead to substantial evidence of the different pathogenetic mechanisms of the two subgroups of COPD, small airways disease, and panlobular emphysema. These results might also have an important potential implication in the field of research and clinical practice. Presently, patients with COPD are regarded as a single group of patients with a similar physiologic abnormality of airflow limitation. However, if further study confirms the present finding and consequently different disease entities of COPD, a different approach should be applied not only to basic and clinical research, but also to the application of therapeutics such as corticosteroid in the two subtypes of COPD, and presumably different results would be obtained. In clinical practice, it has been difficult to make a differential diagnosis of the subtypes of COPD; however, in the future, high-resolution CT might have a role in solving this problem.

A limitation of this study was the small number of patients with COPD, and consequently too small a number of presumed subtypes of patients with COPD. Further study in a larger number of smokers with COPD is needed to confirm these results. In addition, further study investigating both T-lymphocyte subsets in peripheral blood and T lymphocytes in the lung tissues of the same smokers with COPD are necessary.

The finding of a previous study has suggested that the diagnosis of emphysema based on diffusing capacity could be erroneous, in that severe small airways disease may cause a spurious reduction in diffusing capacity as well as severe loss of lung elastic recoil. Therefore, we might suspect that our study group of COPD patients with low diffusing capacity might have been “contaminated” by cases with small airways disease. If so, it would be expected that the difference in T-lymphocyte subsets between the two groups would be obscured by the contamination of cases. However, in this study, there was a statistically significant difference in T-lymphocyte subsets between the two groups, suggesting that only a small number of cases, if at all, with small airways disease might have been falsely classified as the group with low diffusing capacity. Moreover, if there was no contamination of cases by more correct method of differential diagnosis of subtypes of COPD such as high-resolution CT, the difference in T-lymphocyte subsets would have been more prominent between the two groups.
In conclusion, we have found that the proportions of peripheral blood CD8+ T lymphocytes and the CD4+/CD8+ ratio in a subgroup of COPD patients with normal DLCO/VA were significantly different from those in healthy smokers or in a subgroup of COPD patients with low DLCO/VA, and the degree of airflow limitation significantly correlated with the CD4+/CD8+ ratio only in this subgroup. This suggests that T-lymphocyte abnormalities might be involved in the pathogenesis of airflow limitation in a subgroup of patients with COPD, presumably with small airways disease, but not in all patients with COPD.

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