To obtain information on the cellular reactions to Mycobacterium (M) tuberculosis in the lungs, we analyzed the cells in bronchoalveolar lavage (BAL) fluid from pulmonary lesions in comparison with those in BAL fluid from nonaffectected regions of the lungs, and control lungs, and in peripheral blood of patients with tuberculosis. Neutrophils and lymphocytes were increased in number in BAL fluid from affected lesions of the lungs of patients with miliary tuberculosis and patients with active pulmonary tuberculosis compared with those in BAL fluid from control patients, but the number of alveolar macrophages was decreased in BAL fluid from tuberculous lesions. However, the numbers of these cells were not changed in the BAL fluid from nonaffectected regions of the lungs of patients with active or inactive pulmonary tuberculosis. The numbers of lymphocytes were decreased and the numbers of monocytes were increased in peripheral blood from patients with miliary tuberculosis and with active tuberculosis, indicating inverse changes in the numbers of lymphocytes and monocytes in the peripheral blood to those in the BAL fluid of patients with tuberculosis. These results indicate characteristic redistributions of immune or inflammatory cells in response to infection with M tuberculosis and suggest that these changes are important for understanding the pathophysiology of pulmonary tuberculosis.

**Materials and Methods**

**Subjects**

Three patients who developed fever and cough and had abnormal shadows on chest roentgenograms were diagnosed as having miliary tuberculosis by identification of M tuberculosis in their fluid or sputum on repeated examinations or by histologic findings in transbronchial biopsy specimens of the lung (Table 1). Their average age was 33.6 ± 26.2 years (all data are presented as mean ± SD); one was male and one was a smoker. Seventeen patients who had cough with sputum and whose roentgenograms showed infiltration were diagnosed as having active pulmonary tuberculosis by bacterial analysis of sputa or BAL fluids or by histologic examination of biopsy specimens. Their mean age was 51.8 ± 16.6 years; nine of them were male and nine of them were smokers. One patient with miliary tuberculosis and one patient with active pulmonary tuberculosis gave negative results in a skin test with purified protein derivative (PPD). Ten patients who had histories of pulmonary tuberculosis and showed abnormal shadows indicative of inactive tuberculous lesions on chest roentgenograms were diagnosed as having inactive pulmonary tuberculosis. They had no abnormal symptoms, and no detectable M tuberculosis in their sputa or BAL fluids. Their mean age was 55.5 ± 10.5 years; nine of them were male and eight of them were smokers. Twelve patients with lung cancer localized in one lung by chest roentgenograms were used as age-matched controls. Their mean age was 53.8 ± 10.8 years; 11 were male and five were smokers.

Before the study, informed consent was obtained from all patients after a full explanation of the procedures involved.

**BAL**

BAL was performed by a fiberoptic bronchoscope (Olympus Type BF-1T, Olympus Co, Tokyo, Japan) as described previously before treatment of pulmonary tuberculosis. The bronchoscope was introduced into the subsegmental bronchus of the lesion to obtain information about bacteria, cells, and soluble factors for diagnosis of the pulmonary disease of the patient. BAL was also performed in a subsegmental bronchus (B+ or B++ of a nonaffectected region of...
Table 1—Subjects

<table>
<thead>
<tr>
<th>Patient Groups</th>
<th>No.</th>
<th>Male</th>
<th>Smoker</th>
<th>Age, yr±</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients with miliary tuberculosis</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>33.6±26.2</td>
</tr>
<tr>
<td>Patients with active pulmonary tuberculosis</td>
<td>17</td>
<td>9</td>
<td>9</td>
<td>51.8±16.5</td>
</tr>
<tr>
<td>Patients with inactive pulmonary tuberculosis</td>
<td>10</td>
<td>9</td>
<td>8</td>
<td>55.5±10.5</td>
</tr>
<tr>
<td>Control patients</td>
<td>12</td>
<td>11</td>
<td>5</td>
<td>53.8±10.8</td>
</tr>
</tbody>
</table>

*Data are mean ± SD.

the lungs of patients with active or inactive pulmonary tuberculosis or patients with lung cancer as controls. Briefly, the tip of the bronchoscope was wedged into the subsegmental bronchus and a volume of 50 ml of sterilized saline solution was instilled through the bronchoscope. The patients inspired deeply and then the lavage fluid was aspirated gently with a syringe. This procedure was repeated three times and samples of BAL fluid were combined. The BAL fluid was filtered through three layers of gauze and then cells were collected by centrifugation at 250 x gravity for 10 min. The cells were resuspended in phosphate-buffered saline solution and counted. For cell analysis, about 1 x 10^6 cells were collected on a glass slide by centrifugation (Cytospin 2, Shandon Southern Products, Manchester, United Kingdom).

Cell Analysis of BAL Fluid and Peripheral Blood

Cell viability was more than 95 percent as measured by the trypan blue dye exclusion test. Total cell numbers in the BAL fluid and in peripheral blood, obtained just before BAL, were counted in a hemocytometer and differential cell counts were made by microscopic examination after staining the cells on the glass slides with May-Giemsa and nonspecific esterase stains. Results were expressed as numbers per volume of lavage fluid or peripheral blood.

CD4+ cells and CD8+ cells in BAL fluid were counted by two-color flow cytomtery (EPICS V, Coulter Electronics, Hialeah, Fla) after incubation of the cells with fluorescein isothiocyanate (FITC)-conjugated OKT4 and OKT8 (Ortho Diagnostic System Inc, Raritan, NJ).

Measurement of Albumin and Immunoglobulin G in BAL Fluid and Serum

The contents of albumin and immunoglobulin (Ig) G in BAL fluid and serum were measured by nephelometric immunosassay* using a system (Nephelotik System, Daiichi Kagaku, Kyoto, Japan) consisting of a nephelometer and a calculator and antibodies to human albumin and IgG (Behringwerke, Marburg, Germany). For the measurements of albumin and IgG in BAL fluid, 1 ml of antialbumin antiserum and anti-IgG antiserum, respectively, diluted 1:100 with 0.85 percent NaCl solution containing 4 percent polyethylene glycol and 25 µl of BAL fluid were mixed gently and incubated for 60 min at room temperature. Then the light scattering of the mixture was measured in the nephelometer. The contents of albumin and IgG were calculated from standard curves.

RESULTS

Differential Cell Counts in BAL Fluid

The recovery of infused saline solution in BAL fluid ranged from 51.7 to 60.5 percent and did not differ significantly in affected and nonaffected regions of the lung of patients with active pulmonary tuberculosis. Figure 1 shows the differential cell counts in BAL fluid obtained from different groups of patients with tuberculosis. The total cell numbers in BAL fluid from these groups were not significantly different from those in BAL fluid from control patients (p>0.05).

The numbers of alveolar macrophages per aliquot volume of BAL fluid obtained from patients with miliary tuberculosis (10.1±3.6 X 10^5/ml) and from affected lesions of patients with active pulmonary tuberculosis (15.9±4.9 X 10^5/ml) were less than those obtained from nonaffected regions of the lungs of patients with active or inactive pulmonary tuberculosis (25.2±2.4 X 10^5/ml or 30.5±4.6 X 10^5/ml) (p<0.01) and controls (nonsmokers, 25.6±2.4 X 10^5/ml; smokers, 38.5±5.4 X 10^5/ml) (p<0.01). There was no significant difference in the alveolar macrophage number between nonsmokers and smokers of patients with miliary tuberculosis and patients with pulmonary tuberculosis. On the other hand, the numbers of

Figure 1. Differential cell counts in bronchoalveolar lavage fluid from patients with miliary tuberculosis (Miliary Tbc), active pulmonary tuberculosis (Active pulmonary Tbc), and inactive pulmonary tuberculosis (Inactive pulmonary Tbc), and control patients. Asterisks indicate significant differences from values in control nonsmokers (one asterisk, p<0.05; two asterisks, p<0.01; Student's t test).

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lymphocytes were increased in BAL fluid from the lungs of patients with miliary tuberculosis (11.2 ± 3.7 × 10⁶/ml) and affected regions of patients with active pulmonary tuberculosis (13.4 ± 6.8 × 10⁶/ml) compared with those in BAL fluid from controls (nonsmokers, 2.8 ± 1.4 × 10⁶/ml; smokers, 0.9 ± 0.3 × 10⁶/ml, p<0.05) and from nonaffected regions of patients with active pulmonary tuberculosis (3.6 ± 2.2 × 10⁶/ml) (p<0.01). The numbers of neutrophils and eosinophils were more in BAL fluid obtained from tuberculous lesions of patients with active pulmonary tuberculosis (neutrophils, 4.9 ± 2.2 × 10⁶/ml; eosinophils, 1.6 ± 0.6 × 10⁶/ml) than in those from control nonsmoker patients (neutrophils, 0.3 ± 0.2 × 10⁶/ml; eosinophils, 0.1 ± 0.1 × 10⁶/ml) (both p<0.01). The numbers of neutrophils and eosinophils in BAL fluid from patients with miliary tuberculosis (neutrophils, 0.4 ± 0.1 × 10⁶/ml; eosinophils, 0.2 ± 0.2 × 10⁶/ml) were not different from those in BAL fluid from control nonsmoker patients (p>0.05).

Analysis of T-cell subpopulation in BAL fluid from patients with active pulmonary tuberculosis shows that percentages of CD4+ T cells and CD8+ T cells were not different from those from control patients, but the ratio of CD4+ T cells/CD8+ T cells was increased in BAL fluid from the site of tuberculosis (Table 2).

### Differential Cell Counts in Peripheral Blood

There were no significant differences in the total cell numbers in peripheral blood of patients with miliary tuberculosis (6,566 ± 2,824/μl), active and inactive pulmonary tuberculosis (5,860 ± 1,337/μl and 5,730 ± 906/μl), and control patients (nonsmokers, 5,310 ± 574/μl; smokers, 6,160 ± 1,113/μl). However, the numbers of neutrophils and monocytes was increased in peripheral blood of patients with miliary tuberculosis (neutrophils, 4,878 ± 414/μl, p<0.001; monocytes, 735 ± 452/μl, p<0.01) and active pulmonary tuberculosis (neutrophils, 3,572 ± 669/μl, p<0.001; monocytes, 341 ± 159/μl, p<0.01) compared with those in peripheral blood of control nonsmoker patients (neutrophils, 2,793 ± 433/μl; monocytes, 103 ± 92/μl), and the numbers of lymphocytes in peripheral blood from patients with miliary tuberculosis (828 ± 190/μl) and active pulmonary tuberculosis (1,690 ± 371/μl) were decreased significantly (p<0.05) (Fig 2). Two patients with tuberculosis with a low response to PPD (negative skin test) also showed markedly decreased numbers of lymphocytes in their peripheral blood (735 and 430/μl).

**Contents of Albumin and Immunoglobulin G**

The albumin contents of BAL fluid from patients...
Table 3—Contents of Albumin and Immunoglobulin G*

<table>
<thead>
<tr>
<th>Bronchoalveolar lavage fluid</th>
<th>N</th>
<th>Albumin (mg/ml)</th>
<th>IgG (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients with tuberculosis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Miliary tuberculosis</td>
<td>3</td>
<td>6.72±2.45†</td>
<td>7.54±3.58†</td>
</tr>
<tr>
<td>Active pulmonary tuberculosis</td>
<td>4</td>
<td>10.80±3.70‡</td>
<td>5.46±3.22‡</td>
</tr>
<tr>
<td>Affect ed regions</td>
<td>6</td>
<td>2.72±1.49</td>
<td>1.56±0.90</td>
</tr>
<tr>
<td>Nonaffected regions</td>
<td>2</td>
<td>6.08±1.08</td>
<td>0.54±0.11</td>
</tr>
<tr>
<td>Inactive pulmonary tuberculosis</td>
<td>7</td>
<td>3,500±300</td>
<td>1,899±384</td>
</tr>
<tr>
<td>Control patients</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nonsmokers</td>
<td>6</td>
<td>3.02±1.62</td>
<td>1.43±0.78</td>
</tr>
<tr>
<td>Smokers</td>
<td>5</td>
<td>3.41±1.77</td>
<td>1.89±0.83</td>
</tr>
<tr>
<td>Peripheral blood</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patients with tuberculosis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Miliary tuberculosis</td>
<td>3</td>
<td>3,800±300</td>
<td>2,252±660†</td>
</tr>
<tr>
<td>Active pulmonary tuberculosis</td>
<td>10</td>
<td>3,600±300</td>
<td>1,764±319</td>
</tr>
<tr>
<td>Inactive pulmonary tuberculosis</td>
<td>7</td>
<td>3,500±300</td>
<td>1,899±384</td>
</tr>
<tr>
<td>Control patients</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nonsmokers</td>
<td>6</td>
<td>3,900±300</td>
<td>1,449±236</td>
</tr>
<tr>
<td>Smokers</td>
<td>5</td>
<td>4,000±300</td>
<td>1,359±254</td>
</tr>
</tbody>
</table>

*Data are mean ± SD. IgG = immunoglobulin G.
†p<0.05.
‡p<0.01, vs value for control nonsmokers (Student’s t test).

with miliary tuberculosis and lesions of active pulmonary tuberculosis were higher than those of BAL fluids from nona dapted regions of patients with active or inactive pulmonary tuberculosis, which were the same as those of BAL fluid from control patients (Table 3). The IgG contents of the BAL fluids obtained from patients with miliary tuberculosis and from lesions in patients with active pulmonary tuberculosis were also higher than those in BAL fluid from nonaffected regions of patients with pulmonary tuberculosis or control patients (Table 3). The IgG content of peripheral blood was higher in patients with miliary tuberculosis than in control patients, but there were no significant differences in the contents of albumin and IgG in the peripheral blood of patients with tuberculosis and control patients (Table 3).

**DISCUSSION**

In this study, we observed a characteristic change in cell distributions in pulmonary lesions and peripheral blood of patients with pulmonary tuberculosis.

Lymphocyte accumulation in the airway was seen in various types of pulmonary diseases, such as sarcoidosis, hypersensitivity pneumonitis, and berylliosis. 10-12 Lymphocytosis in BAL fluid from patients with pulmonary tuberculosis was also reported. 4-7 And our data showed that lymphocytosis in BAL fluid was seen in active tuberculosis lesions but not in nona dapted parts of the lungs, indicating that lymphocyte accumulation is one of important cellular responses in the pulmonary lesions infected with *M tuberculosis*. Among lymphocytes, T cells are known to be the major cell population, 4,6,13,14 and CD4+ T cells are also reported to be increased in the tuberculous pleural fluid. 15,16 In this study, percentage of CD4+ T cells in total lymphocytes in BAL fluid from patients with tuberculosis was not different from that of control patients, but the ratio of CD4+ T cells to CD8+ T cells was increased in BAL fluid from tuberculous lesions of the lung. T cells, including CD4+CD29+ T cells and γδ+ T cells, are thought to play important roles in generating a local immune response by recognition of bacteria. 15,17,18 Additionally, the lymphocytes from tuberculous lesions are capable of releasing a variety of lymphokines, such as interleukin 2 (IL-2), interferon-γ, and tumor necrosis factor, and they are thought to relate to lymphocyte proliferation in the lesions. 14,15 The specific lymphocyte accumulation in the active pulmonary lesions compared with those in inactive lesions or nona dapted regions of the lung indicates that lymphocytosis in the lesions may be the result of, at least in part, the proliferation of lymphocytes in the lesions locally by *M tuberculosis*.

Another important cause of lymphocyte accumulation in the lesions may be movement of lymphocytes from peripheral blood to local area of infection. The lymphopenia observed in the peripheral blood of patients with miliary tuberculosis and patients with active pulmonary tuberculosis, which is the opposite phenomenon of increased lymphocytes in BAL fluid, might have resulted from migration of lymphocytes into the lesions from the peripheral blood. This finding is consistent with reports that lymphocyte accumulation in the lesions of patients with tuberculosis resulted in decrease in the lymphocyte number in the peripheral blood. 15,19 Also, lymphocyte chemotactic factors may be involved in the lymphocyte movement. 20,21 Lymphopenia in peripheral circulation was thought to decrease in the cellular immune response in nona dapted organs such as the skin. 13 In this study, two patients with tuberculosis with lymphocytosis in BAL fluid associated with significant lymphopenia in peripheral blood showed negative reactions in a skin test to PPD. However, some patients with lymphopenia gave a positive reaction to the PPD skin test, indicating that the negative reaction is due not only to lymphopenia but also to lymphopenia in combination with other immune factors. In this regard, the finding of increased numbers of monocytes in peripheral blood of patients with active pulmonary tuberculosis, which is consistent with previous reports, 19,22,23 may play an important role in suppression of cellular immune response in the peripheral organs. Monocytes from patients with pulmonary tuberculosis are known to be capable of suppressing tubulin-induced immune reactions, such as IL-2 production and T-cell proliferation in vitro. 24-26 so increase in their number may amplify a suppressor function on cellular immune

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responses in peripheral organs and may result in modulation of the skin reaction to antigen.

We observed decrease of alveolar macrophage number in the lower respiratory tract of patients with miliary tuberculosis and in lesions of active pulmonary tuberculosis in contrast to the increased number of monocytes in the peripheral blood. The number of alveolar macrophages was increased by smoking as shown in control patients, but there was no significant effect of smoking on the number of alveolar macrophages in these patients. Alveolar macrophages are believed to be derived from peripheral monocytes, so these inverse changes in numbers of alveolar macrophages in BAL fluid and monocytes in peripheral blood may be the result of movement of monocytes from peripheral blood to the site of infection as well as decreased migration of monocytes/macrophages from the lesions to the surface of alveolar or respiratory tract. Also, an increase in the circulating pool of monocytes is thought to be a major source of monocytes in tuberculous lesions. In this point of view, factors that regulate the monocyte/macrophage migration may play an important role in the distribution of monocytes/macrophages. Monocytes are known to initiate the immune response within the lung by the ability to confer antigen presentation to lymphocytes; monocytes/macrophages, in cooperation with lymphocytes, may play a central role in the cellular immune response in the tuberculous lesions and in the formation of tuberculous granulomas which are composed of monocytes (epithelioid cells) and lymphocytes. Therefore, changes in the numbers of monocytes and lymphocytes seem to be critical in the formation of tuberculous lesions.

We also reported the neutrophil accumulation in the affected lesions of pulmonary tuberculosis. This corresponds with previous reports, too. Increased numbers of neutrophils in affected lesions, but not in nonaffected regions of the lungs of patients with active pulmonary tuberculosis, suggest that neutrophils are attracted to active tuberculous lesions such as those in inflammatory or exudative stages. The increased contents of albumin and IgG in the BAL fluid from active lesions, which are known to be derived from peripheral blood, also suggest increased vascular permeability in the active sites of the disease. The accumulation of neutrophils in the lesion suggests that some chemotactic factors are produced in the lesion. In this regard, it is noteworthy that alveolar macrophages are known to play an important role in neutrophil accumulation in the lung infected with bacteria by releasing neutrophil chemotactic factors such as IL-8 after bacterial phagocytosis. So alveolar macrophages may contribute to the neutrophil accumulation in the lower respiratory tract of tuberculous lesions by releasing neutrophil chemotactic factors. On the other hand, neutrophils in the tuberculous lesions have been shown to increase monocyte accumulation in lesions by releasing a monocyte chemotactic factor and to enhance the activation of alveolar macrophages. Therefore, they are thought to play a role in the pathophysiology of certain kinds of pulmonary tuberculosis in cooperation with other inflammatory or immune cells.

In summary, from cellular analysis of BAL fluid from patients with tuberculosis, we conclude that monocytes, alveolar macrophages, lymphocytes, and neutrophils are redistributed in response to infection with M. tuberculosis and play important roles in the pathophysiology of tuberculosis.

REFERENCES