The Characterization of Bronchoalveolar Lavage Fluid in Very Elderly Patients With Cerebrovascular Disease

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Background. Elderly patients with cerebrovascular disease have a high frequency of pneumonia due to impaired immune function and the occurrence of micro-aspiration.

Methods. We performed bronchoalveolar lavage in 11 very elderly subjects with cerebrovascular disease and 9 healthy volunteers to investigate whether there were changes of local immunity in the lungs of the elderly subjects. The total cell count, the cell characteristics, and the lymphocyte subsets in bronchoalveolar lavage fluid were compared between the two groups.

Results. A significant increase in the total cell count as well as in the number of neutrophils and CD8+ T cells was observed in the elderly group. In addition, the mean CD4/CD8 lymphocyte ratio was lower in the elderly patients than in the healthy volunteers.

Conclusions. These observations suggest that silent micro-aspiration occurs in many elderly individuals with cerebrovascular disease and that pulmonary defenses decrease with age.

The onset of cerebrovascular disease increases in elderly individuals, and such patients are more likely to become bedridden and to be immunosuppressed. Some previous studies have shown that elderly patients with cerebrovascular disease have depression of the swallowing and cough reflexes (1,2) and suffer from micro-aspiration (3). Thus, elderly patients with cerebrovascular disease may have increased risk factors for pneumonia due to impaired immune function or the existence of micro-aspiration.

Many studies have revealed that immune function in elderly persons changes as part of the aging process. However, there has been little investigation of immunological change in the lungs of elderly subjects (4–8), and no study has assessed pulmonary immunity in very elderly patients with cerebrovascular disease.

In the present study, we assessed the bronchoalveolar lavage fluid (BALF) in elderly patients who had cerebrovascular disease and compared the results with the findings obtained in healthy subjects.

METHODS

Subjects

The subjects were 11 very elderly patients with cerebrovascular disease (1 man and 10 women with a mean age of 90 years, all nonsmokers; see Table 1) and 9 healthy volunteers (7 men and 2 women with a mean age of 43 years, all nonsmokers). The patients had been admitted to a geriatric hospital because of difficulty in managing at home. Four of them had previous cerebral infarction, six had multiple lacunar infarcts, and one had Alzheimer’s disease. All patients had been bedridden for periods of 2 months to 9 years.

None of the subjects had a history of bronchial asthma, allergic rhinitis, or other allergic diseases. All of the healthy volunteers had normal pulmonary spirometry findings. None of the elderly patients or healthy volunteers was being treated with corticosteroids or other immunosuppressive therapy during the course of the study, and none had suffered from a pulmonary infection within 1 month prior to bronchofiberscopy and bronchoalveolar lavage (BAL). The study conformed to the Declaration of Helsinki and was approved by the institutional Ethics Committee. Written informed consent was obtained from each subject.

BAL and Flow Cytometry

Transoral bronchofiberscopy was performed (Olympus BF, Type p-8; Olympus, Tokyo, Japan) after local anesthesia of the upper airway with 4% lidocaine. Following observation of the airways, the tip of the fiberscope was wedged into the subsegmental bronchus of the right middle lobe, after which 150 ml of normal saline was instilled in 50-ml aliquots through the fiberscope and aspirated under low suction using a sterile syringe after each instillation. The BALF was filtered through two sheets of sterile gauze and centrifuged at 400 rpm for 3 minutes to prepare cells (Cytospin 2; Shandon, Sewickley, PA). The cell pellets were later stained by the May-Giemsa method, and differential counting was
performed on 500 cells under a light microscope. The remaining BALF cells were resuspended in RPMI-1640 medium (Gibco, Paisley, UK) supplemented with 10% heat-inactivated fetal calf serum, washed twice in phosphate-buffered saline (PBS), and finally adjusted to a concentration of 10^6/ml. A total of 175 μl of the BALF cell suspension was placed into a polystyrene tube, and 4 μl of a monoclonal antibody was added. Fluorescein-isothiocyanate (FITC)-conjugated anti-CD3 (Leu-4) and anti-CD4 (Leu-3a) antibodies and a phycoerythrin (PE)-conjugated anti-CD8 (Leu-2a) antibody were purchased from Becton Dickinson (Mountain View, CA). FITC-conjugated anti-CD29 (4B4) antibody and PE-conjugated anti-CD56 (NKH-1) antibody were purchased from Coulter Immunology (Hialeah, FL). For antibody staining, cells were incubated for 30 minutes in the dark and then washed twice in cold PBS containing 0.1% sodium azide. Next, the cells were resuspended in cold PBS containing 0.5% paraformaldehyde and were analyzed using a flow cytometer. Data on 10,000 to 20,000 events were stored in list mode files. A cell gate for lymphocytes was established on the basis of forward and side light scatter, and a computer system was used for data analysis.

All results are expressed as the mean ± SD. Analysis of variance or Scheffe’s test was used for comparison of median values between the groups. A p value of <.05 was considered significant.

### Table 1. Patient Characteristics

<table>
<thead>
<tr>
<th>Patient Number</th>
<th>Sex (M/F)</th>
<th>Disease</th>
<th>Nutrition</th>
<th>Duration</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>F</td>
<td>Multiple lacunar infarction</td>
<td>Oral</td>
<td>3 mo</td>
</tr>
<tr>
<td>2</td>
<td>M</td>
<td>Multiple lacunar infarction</td>
<td>Oral</td>
<td>6 y</td>
</tr>
<tr>
<td>3</td>
<td>F</td>
<td>Multiple lacunar infarction</td>
<td>Oral</td>
<td>9 mo</td>
</tr>
<tr>
<td>4</td>
<td>F</td>
<td>Alzheimer’s disease</td>
<td>Oral</td>
<td>4 mo</td>
</tr>
<tr>
<td>5</td>
<td>F</td>
<td>Previous cerebral infarction (right MCA)</td>
<td>Tube feeding</td>
<td>8 y</td>
</tr>
<tr>
<td>6</td>
<td>F</td>
<td>Multiple lacunar infarction</td>
<td>Oral</td>
<td>2 mo</td>
</tr>
<tr>
<td>7</td>
<td>F</td>
<td>Previous cerebral infarction (right MCA, left PCA)</td>
<td>Tube feeding</td>
<td>5 mo</td>
</tr>
<tr>
<td>8</td>
<td>F</td>
<td>Previous cerebral infarction (right hemisphere)</td>
<td>Tube feeding</td>
<td>8 mo</td>
</tr>
<tr>
<td>9</td>
<td>F</td>
<td>Previous cerebral infarction (left MCA)</td>
<td>Tube feeding</td>
<td>6 mo</td>
</tr>
<tr>
<td>10</td>
<td>F</td>
<td>Multiple lacunar infarction</td>
<td>IVH</td>
<td>2 mo</td>
</tr>
<tr>
<td>11</td>
<td>F</td>
<td>Multiple lacunar infarction</td>
<td>IVH</td>
<td>9 y</td>
</tr>
</tbody>
</table>

Note: MCA = middle cerebral artery; PCA = posterior cerebral artery; IVH = intravenous high calorie infusion.

### Results

#### Characteristics of BALF Cells

The total cell count and the differentiated count of BALF cells are shown in Table 2. The total cell count was significantly higher in the very elderly patients than in the healthy volunteers. There were no significant differences in the percentages of macrophages or lymphocytes between the two groups, but the mean absolute number of macrophages and lymphocytes was higher in the elderly patients. The concentration and percentage of neutrophils were also slightly higher in BALF from the elderly patients compared with BALF from the healthy volunteers, and the percentage of eosinophils was slightly lower in the elderly group.

#### Two-Color Flow Cytometry

T-cell subsets in BALF are shown in Table 3. The percentage of CD3^+ T cells and CD4^+ T cells did not differ between the very elderly patients and the healthy volunteers, but the percentage of CD8^+ T cells was higher in the elderly group. The mean absolute number of CD4^+ T cells was also higher in the elderly patients than in the healthy volunteers, as was the mean absolute number of CD8^+ T cells. However, the CD4/CD8 ratio was lower in the elderly patients than in the healthy volunteers. There were no significant differences between the two groups in the percentage and the mean absolute number of CD3^-CD56^- cells (natural killer cells) or CD4^+CD29^+ cells (memory cells).

#### Discussion

Morphological and physiological studies on the aging of the human lungs have been reported by many researchers. Aging is characterized by loss of elastic recoil (9), loss of elastin fibers (10), and dilatation of the air spaces (11), and the chief physiological abnormality associated with aging is the loss of elastin fibers (10), and dilatation of the air spaces (11), and the chief physiological abnormality associated with aging is decreased diffusing capacity (12). Examination of the BALF is a useful method for investigating inflammatory cells, proteins, and cytokines in many pulmonary disorders. However, few studies have assessed the cell characteristics and immunological state of the lower airways using BAL in normal elderly subjects (4–8), and there has been no assessment of pulmonary immunity in elderly subjects with cerebrovascular disease, especially among bedridden patients.

In the present study, we performed BAL in very elderly subjects with cerebrovascular disease to investigate age-related immunological changes of the lungs. We demonstrated that the total cell count as well as the absolute and relative neutrophil count were increased in BALF from the very elderly subjects with cerebrovascular disease.
elderly subjects compared with BALF from younger volunteers. Wallace and colleagues (5) described an increase of alveolar macrophages in the lungs of nonsmokers with age in a study using tissue sections. In addition, Meyer and colleagues (6,7) and Thompson and colleagues (4) reported an increase of neutrophils in the BALF of elderly individuals. These researchers concluded that low-grade inflammation exists asymptomatically in the lower respiratory tract of normal elderly individuals. Although these studies were similar to our study, the mean age of the elderly subjects was slightly higher in the present investigation (all subjects were over 80 years old) compared with the other studies, and all of the patients were bedridden and had cerebrovascular disease. Generally, elderly patients with cerebrovascular disease have depression of the swallowing and cough reflexes and may suffer from a high frequency of aspiration pneumonia. Therefore, it may be considered that, in elderly persons, an increase of neutrophils in the BALF suggests continuous inflammation of the lower respiratory tract due to silent microaspiration.

We also found a high percentage of CD8+ T cells and a decreased CD4/CD8 ratio in BALF from the elderly group compared with BALF from the healthy volunteers. On the other hand, Meyer and Soergel (8) described an increase of CD4+ T lymphocytes and an increase in the CD4/CD8 ratio in BALF from older subjects. Some researchers have reported a decrease in the absolute number of peripheral blood lymphocytes and CD3+ T lymphocytes, including both CD4+ and CD8+ subsets (13,14). In addition, the percentage and absolute number of CD3+HLA-DR+ cells, CD4+CD29+ cells, and natural killer cells have been shown to increase with age (14–16). However, another investigation revealed that the percentage of high CD8+ T lymphocytes and low CD4+ T lymphocytes was associated with mortality in very elderly individuals (17). In the present study, our findings about CD4+ and CD8+ T-lymphocyte subsets in the BALF of elderly individuals did not concur with the results of Meyer and coworkers (6,8). This discrepancy of BALF findings may be due to differences in the mean age of the subjects in elderly group (82–97 years vs 65–78 years).

It is well known that smoking may cause inflammatory changes of the airways and that it can influence lung function (18,19). Furthermore, the percentage and absolute number of CD8+ T lymphocytes are increased in chronic obstructive pulmonary disease (COPD), and the increase of CD8+ T cells shows a significant association with the decline of lung function (20). The BALF of the elderly individuals showed some overlap with the features of peripheral airway disease associated with smoking or COPD. Therefore, it is possible that irritants such as air pollution may cause chronic inflammation in the lower respiratory tract of elderly individuals and that these inflammatory changes may increase with aging.

In summary, we investigated the characteristics of BALF in very elderly subjects with cerebrovascular disease, and we demonstrated an increased percentage of neutrophils, an increased number and percentage of CD8+ T cells, and a decrease of the CD4/CD8 ratio when compared with younger volunteers. It is unclear whether these BALF data from our elderly subjects suggest all alterations of the lungs related to aging. However, our findings suggest that subjects with cerebrovascular disease may have continuous inflammation of the lower respiratory tract due to silent micro-aspiration. There may also be a possibility that host pulmonary defenses vary with the aging process.

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