Induced Sputum in Patients With Newly Diagnosed Sarcoidosis*
Comparison With Bronchial Wash and BAL

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Objectives: Sarcoidosis is characterized by a diffuse alveolar inflammatory process, although bronchial airways are often involved. This study compares the cellular profiles of induced sputum (IS), bronchial washing (BW), and BAL in newly diagnosed sarcoidosis patients to those in control subjects, and examines whether inflammatory cell counts from IS are correlated with inflammatory cell counts from BW and BAL in sarcoidosis patients.

Patients and measurements: We recruited 15 untreated patients with stage I and II pulmonary sarcoidosis and 12 healthy volunteers. Sputum was induced with hypertonic saline solution in all individuals. Bronchoscopy was performed on a different occasion in all patients and in five control subjects.

Results: Mean lymphocyte counts in IS, BW, and BAL fluid from sarcoidosis patients were significantly higher than in control subjects (9.4% vs 3.8%, p < 0.05; 12.6% vs 3.9%, p < 0.05; 24.1% vs 2.6%, p < 0.05, respectively). Moreover, total cell count and percentage of epithelial cells in IS were significantly higher in sarcoidosis patients than in control subjects (p < 0.01 and p < 0.05, respectively). In sarcoidosis patients, comparison between different samples showed significantly higher percentages of macrophages in BW and BAL than in IS (p < 0.05 and p < 0.01, respectively), whereas the percentage of neutrophils was higher in IS compared with BW and BAL (p < 0.01 and p < 0.001, respectively). Finally, the percentage of lymphocytes in IS was significantly lower than that in BAL (p < 0.05) but not that in BW.

Conclusions: We demonstrated that, compared with IS in healthy control subjects, IS in untreated pulmonary sarcoidosis patients contains more total cells, lymphocytes, and epithelial cells. Although the relative proportion of inflammatory cells in the three samples differed, lymphocyte counts in IS were high. This finding suggests that IS could be used as a valuable alternative to more conventional invasive techniques in clinical assessment of pulmonary sarcoidosis patients.

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Key words: BAL; bronchoscopy; induced sputum; sarcoidosis

Abbreviations: BW = bronchial washing; IS = induced sputum

Sarcoidosis is a systemic granulomatous disease of unknown origin that often presents as an intrathoracic process. According to the World Association of Sarcoidosis and Other Granulomatous Disorders,¹ the diagnosis is based on radiologic findings and on histologic evidence of noncaseating epithelioid cell granulomas. The main pathophysiologic process of patients with pulmonary sarcoidosis is a macrophage–T lymphocyte alveolitis.² The analysis of cells recovered in BAL has been used extensively as an additional diagnostic tool because it reflects the inflammatory cell population of the interstitium.³ BAL in patients with pulmonary sarcoidosis demonstrates an increased number of lymphocytes, CD4+ lymphocytes, and activated macrophages.²⁻⁴ Although the diagnostic and prognostic value of the cell profile of BAL is controversial,⁴⁻⁶ this technique remains an invaluable research tool despite its invasiveness and its high cost.

The immune inflammatory process in patients with sarcoidosis is not compartmentalized within the...
alveolar walls, but also involves the bronchial airways. It has been demonstrated that endobronchial granulomas are often detectable by bronchial biopsy in patients with sarcoidosis at any disease stage; that cell cytology of bronchial brushing specimens shows the presence of epithelioid or giant cells; and that bronchial epithelium is extensively damaged in patients with active disease. Moreover, patients with sarcoidosis show different bronchial abnormalities by CT, have a progressive change in lung function parameters, and often demonstrate bronchial responsiveness to methacholine.

Sputum induction by hypertonic saline solution recently has been proposed and validated as a non-invasive method to study bronchial inflammation in patients with asthma. In addition, cell cytology of sputum has been compared with that found in bronchial washing (BW) and in BAL. In asthmatics, eosinophil count in sputum appears to be significantly related to that found in BW and BAL. Interestingly, the percentage of CD4+ lymphocytes in sputum has been found to correlate significantly with that found in BAL. Until now, to our knowledge, the potential use of induced sputum (IS) in the assessment of the pulmonary inflammatory process of sarcoidosis has never been examined. Additionally, the well-recognized cellular profile of more distal samples such as BW and BAL has never been compared with that of proximal samples such as BW and IS.

The aim of this study was to compare the cellular profile of IS, BW, and BAL in patients with newly diagnosed sarcoidosis to that observed in healthy subjects, and to examine whether or not inflammatory cell counts from IS are correlated with inflammatory cell counts from BW and BAL in patients with sarcoidosis.

Materials and Methods

Subjects

We enrolled 15 patients (8 women; age range, 25 to 76 years old; 2 ex-smokers) affected by sarcoidosis according to the definition of the World Association of Sarcoidosis and Other Granulomatous Disorders. Diagnosis was based on symptoms (cough and/or exertional dyspnea), standard chest radiography, CT scanning (lymphadenopathy and/or bilateral diffuse interstitial infiltrates), lung 67Ga scintigraphy (bilateral positive uptake), and laboratory test results (serum angiotensin-converting enzyme). According to chest radiography staging of sarcoidosis, 10 patients had stage I disease (lymphadenopathy alone) and 5 had stage II disease (parenchymal opacities). The diagnosis was confirmed by lung transbronchial biopsy specimen. None of the patients received oral or inhaled glucocorticoids or antibiotics during the 4 months preceding the study.

The control group included 12 healthy volunteers (8 women; age range, 21 to 70 years old) recruited among medical students and hospital staff. They were lifetime nonsmokers and did not experience any acute respiratory illness in the 4 weeks prior to the study. Each subject gave written informed consent. Study protocol was approved by the University Ethical Committee.

IS and Processing

Sputum was induced by the inhalation of hypertonic saline solution as previously described. All subjects inhaled 3% hypertonic saline solution four times for 5 min using an ultrasonic nebulizer (Heyer Orion 1; Carl Heyer GMBH; Bad End, Germany) with a mean volume output of 2.40 mL/min. Throughout the procedure, subjects were encouraged to cough and to expectorate into a plastic container. Three flow volume curves were performed before and after each inhalation, and the best FEV1 was recorded. Induction of sputum was stopped if the FEV1 value fell by at least 15% from baseline or if troublesome symptoms occurred.

The volume of the sputum sample was measured and an equal volume of dithiothreitol 0.1% was added and incubated at 37°C for 30 min. Ten microliters of the homogenized sample was used to determine the total cell count, and results were expressed as number of cells X 10⁶/mL. The remaining sputum was washed with phosphate-buffered saline solution and centrifuged at 2,000 rpm for a 5-min period. The supernatant was aspirated, and cell pellets were resuspended in saline solution (2,000 rpm for 10 min), cytocentrifuged at 600 rpm for 10 min, and stained with May-Grünwald-Giemsa. The percentage of macrophages, neutrophils, lymphocytes, eosinophils, and epithelial cells was counted on at least 400 cells, excluding squamous cells.

Bronchoscopy and Processing of BW and BAL

At least 2 days after sputum induction, subjects underwent bronchoscopy by flexible fiberoptic bronchoscope (Olympus 1T10; Olympus Optical Co Ltd; Tokyo, Japan). Subjects received IM atropine (0.5 mg). Then local anesthesia was performed by inhalation of an aerosol solution of 22 mL of 2% lidocaine followed by the sucking of a 20-ng tablet of tetracaine 15 min before bronchoscopy. BW and BAL were carried out as previously described. The bronchoscope was wedged into a segment of the right middle lobe, and three 50-mL aliquots of sterile saline solution, warmed at 37°C, were instilled into the subsegmental bronchus. Fluid was gently aspirated immediately after each aliquot was introduced, and collected in a sterile container. The first 50-mL aliquot of recovered fluid was labeled as BW. Another aliquot of recovered fluid was labeled BAL. During the bronchoscopy, oxygen saturation and ECG tracings were continuously monitored.

One aliquot was reserved for total cell number using a Nageotte’s chamber, and results were expressed as cells X 10⁷/mL. The remaining fluid was immediately centrifuged at 800 rpm for 10 min at 4°C. The cell pellet was washed twice with phosphate-buffered saline solution (without Ca++ and Mg++). Cytocentrifugates were stained by the May-Grünwald-Giemsa method. The differential cell count of macrophages, neutrophils, lymphocytes, eosinophils, and epithelial cells was made under a light microscope (magnification X 1,000) by counting at least 400 cells. Two of the authors (R.D. and C.L.) independently performed differential cell counts without any knowledge of subjects’ characteristics. Bronchoscopy was performed in all patients with sarcoidosis and in 5 of 12 normal subjects.

Statistical Analysis

Data were tested for normality by the Kolmogorov test and expressed as means ± SD. Differences between cell counts in IS,
BW, and BAL from patients with sarcoidosis and from normal subjects were analyzed by the unpaired Student’s t test. Comparisons between total and differential cell counts from IS, BW, and BAL in patients with sarcoidosis were made using analysis of variance and post hoc analysis with Tukey’s test. Correlations between different cells from different samples were examined by Pearson’s correlation coefficient. Probability values of \( p < 0.05 \) were accepted as significant.

**Results**

Characteristics of subjects are reported in Table 1. Patients with sarcoidosis were older than normal control subjects and had lower FVC, FEV₁, and forced expiratory flow (midexpiratory phase) values, but the differences were not statistically significant. All subjects tolerated sputum induction and bronchoscopy well, without experiencing adverse events. The sputum samples were adequate in all cases.

The total and differential cell counts in IS, BW, and BAL from sarcoidosis patients and normal subjects are reported in Table 2. Patients with sarcoidosis had a significantly greater number of total cells \( (p < 0.01) \), lymphocytes \( (p < 0.05) \), and epithelial cells \( (p < 0.05) \) in IS compared with normal subjects, whereas the number of macrophages was significantly lower \( (p < 0.05) \). In BW samples from sarcoidosis patients, the percentage of lymphocytes was significantly higher when compared with that in normal subjects \( (12.6\% \text{ vs } 3.9\%; \ p < 0.05; \text{ Table 2}) \). No other significant differences between the two subject groups were observed for any cell type. In BAL samples from patients with sarcoidosis, the percentage of lymphocytes \( (24.1\% \text{ vs } 2.6\%; \ p < 0.05) \) was significantly higher, and the percentage of macrophages significantly lower \( (p < 0.05) \) when compared with data from normal subjects (Table 2).

The comparison of the number of total cells recovered in IS, BW, and BAL from sarcoidosis patients demonstrated that sputum samples yielded higher cell counts compared with BW \( (p < 0.001) \) and BAL samples \( (p < 0.001) \). The differential cell counts of inflammatory cells in IS, BW, and BAL obtained from each sarcoidosis patient are reported in Figure 1. The percentage of macrophages in IS was significantly lower than that found in BW \( (p < 0.01) \) and BAL samples \( (p < 0.05) \). The percentage of neutrophils was significantly lower in BAL than in IS \( (p < 0.001) \) and BW \( (p < 0.01) \). Moreover, the percentage of lymphocytes was significantly lower in IS than in BAL \( (p < 0.05) \), but not lower than in BW. Eosinophils were found in very few samples.

No significant relationship among IS, BW, and BAL was found for total cells or percentage of any other inflammatory cell.

**Discussion**

In this study, we demonstrated that IS samples from newly diagnosed, untreated patients with pulmonary sarcoidosis contain significantly more total cells, lymphocytes, and epithelial cells when compared with IS samples recovered from healthy subjects. Moreover, the proportion of lymphocytes in BW and BAL samples was always higher in patients with sarcoidosis. However, there was no correlation among the lymphocyte counts in samples recovered with the three different techniques.

Several factors are known to markedly influence the diagnostic accuracy of differential cell count from BAL samples. Nevertheless, the assessment of lymphocyte counts and CD4/CD8 ratios in BAL samples is still in use and recommended in the clinical evaluation of patients with sarcoidosis. The number of lymphocytes in BAL from our patients with newly diagnosed sarcoidosis is comparable to that obtained in several other studies, ranging from 0.4 to 67.1%. Their mean value was significantly different from that found in healthy subjects (range, 0.6 to 77%).

Moreover, we found that the number of lymphocytes in BW and IS samples was greater in sarcoidosis patients than in healthy subjects. Surprisingly, cell count analysis of BW samples has received very little attention. Our results suggest that it is possible to recover a remarkable number of lymphocytes with this procedure. However, whether this better-tolerated procedure could be useful in the assessment of disease activity, especially in its advanced forms, has not yet been established.

To the best of our knowledge, this is the first time that differential cell counts of IS have been examined in patients with sarcoidosis. Cytologic examination of sputum was used in only one previous study, to count the number of epithelioid giant cells after appropriate staining. Our finding of an increased number of

**Table 1—Subject Characteristics**

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Sarcoidosis</th>
<th>Normal Subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>No.</td>
<td>15</td>
<td>12</td>
</tr>
<tr>
<td>Sex, M/F</td>
<td>7/8</td>
<td>4/8</td>
</tr>
<tr>
<td>Age, yr</td>
<td>45.4 ± 14</td>
<td>39.2 ± 16</td>
</tr>
<tr>
<td>FVC, % predicted</td>
<td>93.5 ± 20</td>
<td>104 ± 17</td>
</tr>
<tr>
<td>FEV₁, % predicted</td>
<td>95.1 ± 20</td>
<td>107 ± 17</td>
</tr>
<tr>
<td>FEF 25–75%, % predicted</td>
<td>82.4 ± 28</td>
<td>100 ± 15</td>
</tr>
<tr>
<td>KCO, % predicted</td>
<td>91.8 ± 6.0</td>
<td>—</td>
</tr>
</tbody>
</table>

*Values are expressed as mean ± SD. M = male; F = female; FEF 25–75% = forced expiratory flow, midexpiratory phase; KCO = carbon monoxide transfer coefficient.
lymphocytes in IS samples is of interest because sputum samples had greater cell concentrations than BW and BAL samples, and the percentage of lymphocytes ranged from 0 to 20.4%. Previous studies have reported that the percentages of lymphocytes in IS from nonselected asthmatics are usually 2 to 4%.14,17,18 Thus, percentages of lymphocytes in IS can probably permit assessments of T-lymphocyte subpopulations and CD4/CD8 ratio calculations in most patients with sarcoidosis. Interestingly, two recent studies have demonstrated that this was possible in asthmatics who showed rather low percentages of sputum lymphocytes.21 If sputum cytology in patients with sarcoidosis correlates with clinical and histologic findings, it could perhaps be employed in the diagnosis and follow-up of these patients. Also, we found evidence of an increase in the number of epithelial cells in addition to the increase in the lymphocyte count. This finding suggests that the inflammatory process in the airways of patients with sarcoidosis also involves the airway epithelium, as suggested by Laitinen and colleagues.9

Furthermore, we found that the differential cell counts in BAL and BW samples from patients with sarcoidosis were not statistically different, nor did recovered cells from either method correlate significantly. In addition, similar findings have been reported in asthmatics.14 These findings suggest that BW at least partially recovers cells from the alveolar compartment. This hypothesis is further supported by the lack of correlation between cell counts in BW and IS samples.

However, sputum induction primarily samples the

### Table 2—Cell Counts From IS, BW, and BAL Samples in Sarcoidosis Patients and Normal Subjects*

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>IS</th>
<th>Normal Subjects</th>
<th>BW</th>
<th>Normal Subjects</th>
<th>BAL</th>
<th>Normal Subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cell count, $\times 10^6$/mL</td>
<td>Sarcoi</td>
<td>dimosis (n = 15)</td>
<td>Normal Subjects (n = 12)</td>
<td>Sarcoi</td>
<td>dimosis (n = 15)</td>
<td>Normal Subjects (n = 5)</td>
</tr>
<tr>
<td>Macrophages, %</td>
<td>60.4 ± 9†</td>
<td>69 ± 9.8</td>
<td>77.7 ± 9</td>
<td>86.7 ± 7.4</td>
<td>74.1 ± 20†</td>
<td>95.3 ± 2.9</td>
</tr>
<tr>
<td>Neutrophils, %</td>
<td>16.3 ± 12</td>
<td>19.2 ± 12</td>
<td>5.5 ± 5</td>
<td>2.6 ± 3.6</td>
<td>1.2 ± 1.3</td>
<td>1.8 ± 1.3</td>
</tr>
<tr>
<td>Lymphocytes, %</td>
<td>9.4 ± 7†</td>
<td>3.8 ± 4.4</td>
<td>12.6 ± 8†</td>
<td>3.9 ± 3.0</td>
<td>24.1 ± 20†</td>
<td>2.6 ± 2</td>
</tr>
<tr>
<td>Eosinophils, %</td>
<td>0.2 ± 1</td>
<td>0.1 ± 0.2</td>
<td>0.3 ± 0.5</td>
<td>0.3 ± 0.6</td>
<td>0.8 ± 2</td>
<td>0.1 ± 0.3</td>
</tr>
<tr>
<td>Epithelial cells, %</td>
<td>13.6 ± 10.2†</td>
<td>6.5 ± 4.6</td>
<td>4.4 ± 2.7</td>
<td>6.3 ± 0.4</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

*Values are mean ± SD.
†p < 0.01.
‡p < 0.05.
more proximal airways. The mean percentage of neutrophils in IS samples was several times higher than in BW samples in both sarcoidosis patients and healthy subjects (Table 2). In addition, very few neutrophils were present in BAL. Moreover, the number of eosinophils in IS and BW is closely related to that in asthmatics. We also found that a high percentage of lymphocytes was present in IS samples from patients with untreated sarcoidosis. Whether lymphocytes in sputum from these patients come from the alveolar or bronchial compartments is unknown. Also, the dynamics of lymphocytes in the lung have not yet been definitely established.

Lymphocytes are the most represented cells in bronchial biopsy specimens from asthmatic patients, but very few lymphocytes are recovered in sputum or BAL fluid. In patients with sarcoidosis, lymphocytes from the lungs showed an increased motility, which might conceivably facilitate their accumulation in the epithelium. Also, the bronchial mucosa is often involved, whether or not endobronchial nodules are present. Thus, the bronchi may be a potential source of sputum lymphocytes.

In conclusion, we demonstrated that cell counts in IS from patients with newly diagnosed sarcoidosis do not reflect the inflammatory process of the distal airways. However, the cell concentration and the percentage of recovered lymphocytes in sputum are high. Thus, we would suggest that this inexpensive, less invasive, and easily repeated method could represent a valuable alternative to bronchoscopy both in research and in the clinical monitoring of patients with pulmonary sarcoidosis.

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