Bronchoalveolar Lavage Cell Data in Amiodarone-Associated Pneumonitis*

Evaluation in 22 Patients


To assess the value of bronchoalveolar lavage (BAL) for diagnosis, understanding, and treatment of amiodarone-associated pneumonitis, we examined the results of BAL total and differential cell counts and phenotyping of lymphocytes in 22 patients with this lung disorder and in 33 normal subjects. Overall, the total cell count was found to be almost the same as that seen in control subjects; the macrophage population was significantly reduced, and the lymphocyte, neutrophil, and eosinophil populations were increased in absolute number and percentage. When results were analyzed individually, BAL data appeared to be distributed according to two patterns. In the first pattern, there was no abnormal lymphocytosis. In the second pattern a lymphocyte alveolitis was found in percentage and in absolute number. This lymphocyte alveolitis was present either alone or associated with neutrophil alveolitis or with eosinophil alveolitis. In the first pattern, despite the normal level of the lymphocyte population, the percentage of CD4 T-lymphocytes and the CD4:CD8 T-lymphocyte ratio were significantly lowered. In the second pattern the CD8 T-lymphocyte count was increased in absolute number and percentage, with a low CD4:CD8 ratio. In six patients relavaged two to four months after amiodarone withdrawal, there was a significant fall in alveolar lymphocytosis, but the progressive increase in the neutrophil population over time seemed to be associated with the seriousness and progression of the disease. Finally, these findings closely resembled those obtained in patients with hypersensitivity pneumonitis due to inhalation of organic dust and suggest that an underlying immunologic cell-mediated mechanism may play a role in this iatrogenic pulmonary disease.

(Chest 1991; 99:1177-82)

Over the past ten years, many investigators using BAL have reported a growing number of data related to alveolar cell disorders in patients with amiodarone-associated pneumonitis. Some of these results are in accordance, but others appear somewhat conflicting.† During the past five years, we have collected a series of 22 such cases in which BAL was performed. In an attempt to define the BAL cell profile in this frequent iatrogenic pulmonary disease, we analyzed cell data from a homogeneous and large series by using systematically the total and differential cell counts and lymphocyte phenotyping. Taken together, all of these results seem to provide indications for diagnosis and discussion of pathogenetic mechanisms; likewise, some of these findings may supply valuable information regarding follow-up and treatment of these patients.

Materials and Methods

Study Populations

Diagnosis of amiodarone-associated pneumonitis was established

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Manuscript received August 7; revision accepted October 12.

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in accordance with the following criteria: (1) Patients had been receiving amiodarone treatment on a long-term basis before the onset of lung disorders. (2) New bilateral infiltrates appeared on chest roentgenograms. (3) No other cause of interstitial pneumonitis was found. Congestive heart failure was excluded by clinical evaluation and persistence of infiltrates after forced diuresis. Detection by clinical history and examination and serologic tests of exposure to environmental agents was negative, and there was no simultaneous administration of other drugs known to be liable to induce lung disorders. No pathogens (bacteria, viruses, fungi, and parasites) were found after repeated clinical and biologic examinations (including serologic tests and cultures of blood and BAL fluid). Likewise, there was no evidence of systemic or malignant diseases. (4) After discontinuation of amiodarone treatment, resolution of pneumonitis was ascertained by clinical and radiologic examination and pulmonary function tests. (5) In three cases with a fatal outcome, no other cause of pneumonitis was evidenced at autopsy.

These patients (15 men and seven women) had an average age of 73 ± 7 years (range, 60 to 83 years). Fifteen patients were not current smokers (seven of them had stopped smoking more than ten years ago). Seven were smokers (mean, 45.6 ± 43.8 pack-years; range, 10 to 150 pack-years). At presentation, all patients were symptomatic and complained of weight loss, mild fever, fatigue, or exertional dyspnea; two had acute respiratory failure. In all patients the chest x-ray film showed bilateral interstitial or alveolar opacities (or both) in both lungs, along with slight pleural effusion in five. Pulmonary function tests showed decreased vital capacity (mean, 84.5 ± 20 percent of predicted value; range, 45 to 122 percent of predicted), and PaO2 (mean, 69 ± 15 mm Hg; range, 41 to 95 mm Hg). In two patients, marked hypoxia (<60 mm Hg) was present. Seventeen patients were followed over 12 to 24 months with regular clinical and radiologic examinations and pulmonary function tests. Six patients were repeatedly lavaged (13 BAL procedures) after drug withdrawal.
Thirty-three normal volunteers acted as control subjects, comparable to the patients as to sex and the proportion of smokers (24 nonsmokers; nine smokers [40 ± 10 packs-years]). Their mean age was 30 ± 7 years.

**Amiodarone Treatment**

All patients had been receiving a daily maintenance dose of amiodarone (200 mg) for a mean of 132.5 ± 109.7 weeks (range, 9 to 468 weeks); the mean cumulative dose of amiodarone was 193.1 ± 222.9 g (range, 9 to 985 g). After cessation of amiodarone, 16 patients were given steroids.

**Bronchoalveolar Lavage**

Bronchoalveolar lavage was performed by the usual technique. After fibroptic examination the bronchoscope (Olympus BF 10) was wedged into a subsegment of the right middle lobe (showing opacities), and a total of 200 ml of sterile warm saline solution was instilled in four aliquots of 50 ml each. The fluid was recovered by gentle aspiration in a siliconized flask kept at 4°C. The percentage of fluid recovered was always above 50 percent of the fluid instilled. The lavage fluid was filtered through sterile surgical gauze and resuspended, and the total cell count was determined on an aliquot of fluid using a hemocytometer. A differential cell count was done on cytocentrifuge preparations prepared from uncentrifuged lavage fluid and stained with May-Grunwald-Giemsa stain. No morphologic evaluation of alveolar cells was done, as this was beyond the purpose of our study.

**Phenotyping of Lymphocytes**

Monoclonal antibodies used to identify CD4 and CD8 cell surface antigens on lymphocytes were OK T4 (helper/inducer T-lymphocytes; Ortho Diagnostics) and OK T8 (suppressor/cytotoxic T-lymphocytes; Ortho). Cells obtained by BAL were washed twice in Hanks balanced salt solution, (Eurobio) and adjusted to a concentration of 2 × 10^6 cells per milliliters. For each assay, 100 μl of this cell suspension was placed into a 75 × 13 mm tube, and 10μl of the monoclonal antibody at the proper dilution was added. Cells were incubated at 4°C for 30 minutes and washed three times in phosphate-buffered saline solution (pH 7.4) containing 0.1 percent sodium azide (PBS-azide). Ten microliters of fluorescein-conjugated goat antihuman IgG (Dyntech) was added. Cells were incubated for 30 minutes at 4°C, washed three times in PBS-azide, and subsequently resuspended in RPMI-1640 (Eurobio); they were then examined using a fluorescence microscope (Olympus BH) equipped with phase-contrast optics. The percentage of fluorescein-labeled lymphocytes was calculated after counting a minimum of 200 lymphocytes per slide.

**Statistical Analysis**

Values from patients different from the 98 percent confidence interval of the mean were considered abnormal.

All results are expressed as the mean ± SD. Statistical comparisons between patients and controls were made using one-way analysis of variance and Student’s t-test for unpaired samples. Comparisons between values obtained in patients on admission and values observed after drug withdrawal were made using Student’s t-test for paired samples. Correlations were tested by linear regression.

**RESULTS**

Mean values of the number and proportion of cell types and T-lymphocyte subsets in our series are shown in Table 1. Whereas the total cell count in our patients was found to be close to that observed in normal subjects, there were striking differences in the cell types as compared to those from controls. The absolute number and the proportion of alveolar macrophages were diminished; this diminution could not be accounted for by a difference in the proportion of smokers between patients and controls; also, mean tobacco consumption was comparable in patients and controls; in addition, the reduction in the macrophage population was observed in both patients who were smokers and nonsmokers when compared to controls who were smokers and nonsmokers (data not shown).

By contrast, there was a sharp rise in values (in absolute number and percentage) of the other three types of cells. In addition, there was an imbalance in the number and percentage of T-lymphocyte subsets: CD4 and CD8 T-lymphocyte populations were in-

### Table 1—Number and Proportion of Cell Types and Lymphocyte Subsets and Ratio in BAL from 22 Patients and 33 Control Subjects*

<table>
<thead>
<tr>
<th>Data</th>
<th>Cell Count per Microliter (Range)</th>
<th>p Value</th>
<th>Cell Percentage (Range)</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Amiodarone</td>
<td>Controls</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total cells</td>
<td>264.9 ± 151.3 (75-460)</td>
<td>301.5 ± 254.3 (54-1085)</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Macrophages</td>
<td>132.2 ± 116.5 (19-601)</td>
<td>270.9 ± 237.9 (50-985)</td>
<td>0.02</td>
<td>51.6 ± 26.8 (9-94)</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>94.6 ± 87.4 (3-315)</td>
<td>21.5 ± 15.4 (3-20.8)</td>
<td>0.001</td>
<td>33.2 ± 20.9 (3.5-70)</td>
</tr>
<tr>
<td>CD4 lymphocytes</td>
<td>37.7 ± 39.2 (1.4-154.4)</td>
<td>11.3 ± 9.3 (1.9-51.6)</td>
<td>0.01</td>
<td>31.9 ± 15.5 (8-52)</td>
</tr>
<tr>
<td>CD8 lymphocytes</td>
<td>63.6 ± 67.8 (6.5-242.6)</td>
<td>8.8 ± 6.7 (1-30.5)</td>
<td>0.001</td>
<td>35.1 ± 16.5 (33-97)</td>
</tr>
<tr>
<td>CD4:CD8 ratio</td>
<td>0.56 ± 0.41 (0.14-1.6)</td>
<td>1.5 ± 0.7 (0.5-3.1)</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>Neutrophils</td>
<td>24.2 ± 36.6 (0-128.9)</td>
<td>5.2 ± 7.9 (0-31.2)</td>
<td>0.02</td>
<td>9.7 ± 12.3 (0-48)</td>
</tr>
<tr>
<td>Eosinophils</td>
<td>12.6 ± 17.4 (0.7-16)</td>
<td>3.6 ± 12 (0-60.8)</td>
<td>0.05</td>
<td>4.6 ± 5.8 (0-20)</td>
</tr>
</tbody>
</table>

*Table values are expressed as means ± SD.
increased, and this expansion was more pronounced in CD8 T-lymphocytes (about seven times as high as controls). Consequently, their ratio was markedly lowered. It is of interest to note that there was no relationship between these alveolar cell changes and the cumulative dose of amiodarone, x-ray features, and pulmonary function test results (ie, PaO₂; VC; FEV₁).

Finally, upon examination of the individual values of these 22 patients, they appeared to be distributed according to two patterns: 16 BAL fluids contained a predominant lymphocyte alveolitis (group 2), and six BAL fluids did not (group 1). The only difference between these two groups was the period of time elapsed from the first clinical complaint to the time of diagnosis or BAL: in group 1, this period of time was a mean of 6 ± 4.5 months long, and in group 2, it was 2 ± 1 months long (p<0.01).

**BAL without Lymphocyte Alveolitis (Group 1)**

In this group were three patients with a normal BAL and two patients whose BAL contained a pure eosinophil alveolitis; in the sixth, an isolated neutrophil alveolitis was seen. Interestingly, studied as a whole, the mean values for the total and differential cell counts in this group were not significantly different from those found in normal subjects except for the percentage of CD4 T-lymphocytes, which was low (32 ± 19.6 percent vs 53.7 ± 13.1 percent in controls; p<0.01); accordingly, the subset ratio was also found to be significantly low (0.64 ± 0.42 vs 1.50 ± 0.7 in controls; p<0.02) (Fig 1).

**BAL with Predominant Lymphocyte Alveolitis (Group 2)**

In these 16 BAL fluids, the overall results shown in Table 2 regarding total and differential counts were comparable to those observed in the whole series of 22 patients (Table 1). In ten patients whose T-lymphocyte subsets were evaluated (Fig 1), an important rise in CD4 and CD8 T-lymphocytes was found, more importantly in CD8 lymphocytes; consequently, their ratio was found to be low (0.67 ± 0.43).

On the other hand, individual analysis of these 16 BAL fluids with predominant lymphocyte alveolitis evidenced existence of three subgroups among them (Table 2): four BAL fluids contained pure lymphocyte...
Table 2—Absolute Number and Proportion of Different Types of Cells Recovered by BAL in 16 Patients with Predominant Lymphocyte Alveolitis

<table>
<thead>
<tr>
<th>Data</th>
<th>Lymphocyte Alveolitis (Pure and Mixed; n = 16)</th>
<th>Pure Lymphocyte Alveolitis (n = 4)</th>
<th>Lymphocyte and Neutrophil Alveolitis (n = 9)</th>
<th>Lymphocyte and Eosinophil Alveolitis (n = 3)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cells per Microliter</td>
<td>Percent</td>
<td>Cells per Microliter</td>
<td>Percent</td>
</tr>
<tr>
<td>Total cells</td>
<td>257.6±120.1 (85-481)</td>
<td>. . .</td>
<td>240.5±97.2 (125-305)</td>
<td>. . .</td>
</tr>
<tr>
<td>Macrophages</td>
<td>104.9±49.2† (19.3-193.2)</td>
<td>43±22.9† (9-84)</td>
<td>124.7±41.3 (97-186)</td>
<td>55.7±21.6 (37-84)</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>106.4±70.9† (17.5-250.1)</td>
<td>39.2±16.9† (14-70)</td>
<td>107.2±61.8 (17.5-157.2)</td>
<td>40.7±19.8 (14-60)</td>
</tr>
<tr>
<td>Neutrophils</td>
<td>30.7±41.7‡ (0-128.9)</td>
<td>11.8±13.8‡ (0-48)</td>
<td>4.0±4.6 (0-8.1)</td>
<td>1.5±1.7 (0-3)</td>
</tr>
<tr>
<td>Eosinophils</td>
<td>14.5±19.8‡ (0-71.6)</td>
<td>5.1±6.4‡ (0-20)</td>
<td>4.7±7.8 (0-16.2)</td>
<td>2±2.8 (0-6)</td>
</tr>
</tbody>
</table>

*Table values are expressed as mean ± SD and range (within parentheses).
†p<0.02 vs normal subjects.
‡p<0.001 vs normal subjects.
§p<0.01 vs normal subjects.

alveolitis (subgroup 2a); in nine BAL fluids, lymphocytosis was associated with neutrophilia (subgroup 2b); and in the remaining three BAL fluids (subgroup 2c), lymphocytosis and eosinophilia were present. It is of interest to point to the expansion of the lymphocyte population, which was nearly the same in the three subgroups, as were the levels (in absolute number and proportion) of CD4 and CD8 lymphocytes and the ratio in ten patients so evaluated (Fig 1). Furthermore, there were no significant differences in the lymphocyte subset percentages and ratio in these three subgroups when compared with those found in the six lavages without lymphocytosis mentioned previously (Fig 1).

Follow-Up after Amiodarone and Sequential BAL Results

Seventeen patients were followed by serial examinations over 6 to 12 months. Fourteen had a favorable outcome. Clinically, their symptoms disappeared within a few weeks; likewise, radiographically, opacities had cleared, except in one patient despite steroid treatment. At the same time, pulmonary function tests exhibited a statistically significant improvement in values of VC (p<0.01) and PaO2 (p<0.001) but not in those of Dco. Of these 14 patients, 9 were receiving steroid treatment, and 5 were not; in all cases, any relationship between their course and the initial type of alveolitis did not exist. Three patients died; it should be mentioned that they were receiving steroids, that they had a high percentage of neutrophils in the first BAL (20, 36, and 48 percent, respectively), that a progressive increase in BAL neutrophils had been observed through three sequential BALs in one of them, and that autopsy (n=3) showed a pulmonary fibrosis without any other cause of pneumonitis.

In six of the 14 patients with a favorable outcome, BAL procedures were performed repeatedly at the 12th to 26th weeks after discontinuation of amiodarone. In all of them, alveolar lymphocytosis fell significantly (p<0.01) in the second BAL, whether (n=4) or not (n=2) the patients were given steroids (Fig 2).

Discussion

Although almost any pattern could be seen, the most frequent abnormality in BAL changes was an expansion in the lymphocyte population. In looking at T-lymphocyte subpopulations, there was usually a reversal of the CD4:CD8 ratio. In addition, a certain number of abnormalities relate to the total cell count and accessory and inflammatory cell counts.

The BAL total cell count in this series was not significantly different from that in normal subjects. This is not surprising, as in many studies, this count...
has been found to be quite variable.3-15

The reduction of the alveolar macrophage population in our series was also displayed in almost all cases reported so far.10,13,15-19 This reduction, not related to tobacco use, would be connected with amiodarone administration. Other workers14,19 have demonstrated the presence of abnormal inclusions in BAL macrophages and speculated that this abnormality could account for their low number and for cell changes in their ability to initiate immune responses;13 however, in our patients, we found no correlation between the alveolar macrophage count and the cumulative dose of amiodarone, and the presence of phospholipid-filled macrophages was usually mentioned by the laboratory.

In our series the frequency of lymphocytosis-associated neutrophilia was 41 percent (9/22). These data are somewhat different from those of previous reports: in the latter, pure neutrophilia was detected in 30 percent of the cases; its frequency, if associated with lymphocytosis, was 25 percent.5,10,15,16,19,20,21 The meaning of this neutrophilia remains ambiguous. Intervention of smoking can be eliminated: there was no significant correlation between tobacco consumption and neutrophil expansion. When moderate at the first lavage, neutrophilia might be considered as reflecting an influx of inflammatory cells to the lung, along with lymphocyte population expansion. This mechanism has been demonstrated at its highest level in cases of pneumonitis induced by other drugs where a provocation test was performed.32 Such a magnitude of neutrophilia led some authors to believe it was connected with a toxic mechanism.3,5,10 In three of our patients, the initial high level of BAL neutrophils, its regular and progressive increase over time, and the fatal outcome could prompt one to consider the possible value of repeated BAL for prognostic assessment in such patients.

The most prominent feature in our series of BAL fluids was the expansion of the lymphocyte population, which is comparable to several previous series.13-15,20,25,33 This expansion was related to an increase in T-lymphocyte subsets, more pronounced in CD8 T-lymphocytes. These characteristics of alveolitis were the same in pure lymphocyte alveolitis as in lymphocytosis associated with neutrophilia or eosinophilia. Less frequently, this lymphocytosis was quite variable from one case to another16,19,26 or absent, as it was in some cases.5,7 As for the ratio, it was found to be low in our series, not only in patients with, but also in those without BAL lymphocytosis. This striking feature of alveolitis is worth mentioning, as it seems never to have been reported before.

Taken together, these data lead us to hypothesize a role for lymphocyte population abnormalities, according to the following facts observed. Lymphocytes, preeminently immunocompetent cells, are present in most of the BAL fluids from patients with amiodarone-associated pneumonitis. The mean rise in the lymphocyte population was unrelated either to the cumulative dose of amiodarone or to the duration of treatments, and its increase resolved when amiodarone was withdrawn. This BAL cell profile is very similar to that observed in hypersensitivity pneumonitis due to inhalation of organic dust,14 whose immunologic pathogenesis is well established.33 These arguments for a possible underlying immunologic mechanism do not preclude intervention of other pathophysiologic factors such as direct drug toxicity in the lung secondary to drug accumulation,34,35 the production of amiodarone-related autoantibodies,36 and a predisposing role of macrophage dysfunction due to their abnormal lipid load.13 As a matter of fact, a possible correlation between the type of alveolitis and the morphogenesis of lung disorders remains to be elucidated.

To conclude, in this series of 22 patients with amiodarone-associated alveolitis, one of the most important features is the presence of alveolar lymphocyte population expansion in absolute number and percentage; however, more striking because constant, even if alveolar lymphocytosis is lacking, is the inverted ratio of CD4 to CD8 populations due either to an increase in the CD8 lymphocyte number, higher than that in the CD4 subset, or to a decrease in the CD4 percentage. These findings could be of value as a hint for diagnosis and provide interesting data that should be taken into account when dealing with the still controversial pathogenesis of amiodarone-associated pulmonary disease.30

ACKNOWLEDGMENTS: We thank Ms. Els Cauverien for fruitful discussion and Ms. Martine Dahancourt for her secretarial assistance.

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