Mast Cells in Bronchoalveolar Lavage Fluid and in Transbronchial Biopsy Specimens of Patients with Farmer’s Lung Disease*

Alberto Pesci, M.D., F.C.C.P.; Giuseppina Bertorelli, M.D.; and Dario Olivieri, M.D., F.C.C.P.

Recently, an increased number of mast cells have been reported in bronchoalveolar lavage fluid (BAL) of patients with farmer’s lung disease. Some authors pointed out the pathogenetic importance of mast cells in farmer’s lung on the basis of their correlation with the activity of the disease, with the BAL lymphocyte counts, and with the markers of lung fibrosis. To determine whether BAL reflects the histologic aspects of the lung histologic features in patients with farmer’s lung disease, mast cells recovered from lavage fluid were compared with tissue sections from transbronchial lung biopsies in 15 patients. Mast cell counts in BAL and lung biopsy specimens were significantly correlated ($r = 0.85; p < 0.01$), while no other correlations between BAL inflammatory cells and tissue mast cells were found. In lung tissue, there were four times the increased number of mast cells in respect to the control group ($84.4 \pm 25.8$ vs $20.4 \pm 13.4$ mast cells per square millimeter); 83.2 percent of mast cells were found in the alveolar septa, 14.9 percent within alveoli, 0.7 percent among alveolar lining cells, and 1 percent along blood vessels. No mast cells were located within alveoli in controls. In BAL, only lymphocyte and mast cell counts ($56.4 \pm 18.6$ percent, $p < 0.001$; $3.9 \pm 1.5$ percent, $p < 0.001$, respectively) were significantly increased. Our data suggest that in farmer’s lung disease, BAL correctly samples the alveolitis. Mast cells, such as lymphocytes, seem to be primary inflammatory cells involved at the site of the disease activity.

(CHEST 1991; 100:1197-1202)

**BAL** = bronchoalveolar lavage; **FLD** = farmer’s lung disease

Mast cells have long been recognized as major effector cells of the allergic or type 1 hypersensitivity reaction by virtue of their high-affinity surface receptors for IgE. The wide distribution of mast cells throughout the human body, in general, and at mucosal surfaces, in particular, in atopic and nonatopic individuals alike, as well as the recognition of mast cell activation by nonimmunologic means, suggest that these cells also may be involved in nonatopic conditions.

Recently, some authors have reported an increased number of mast cells in bronchoalveolar lavage fluid (BAL) obtained from patients affected by farmer’s lung disease (FLD).1-4 The pathogenetic importance of mast cells in FLD is underlined by the observations made by Bjørner et al5 showing a very large number of mast cells in BAL correlating to the activity of the disease and to the lymphocyte counts.

However, in the absence of information from biopsy specimens, it is not clear whether mast cells in BAL of patients with FLD reflect the accumulation of these inflammatory cells within the alveolar structures. This is important because the intensity of the alveolitis seems to represent the crucial aspect in the evaluation and the management of a number of interstitial lung diseases.

In an attempt to clarify this, we studied the number and the topographic distribution of mast cells in peripheral lung tissue and in BAL of 15 patients affected by FLD.

**Material and Methods**

**Subjects**

Abnormal lung tissue from transbronchial biopsies and BALs were obtained in 15 patients (ten men; mean age, 45.8 ± 8.2 years) affected by FLD. The diagnosis was based on criteria usually reported, notably the following: (1) documented history of exposure to moldy hay; (2) a symptomatic acute episode with chill, fever, cough, and breathlessness 4 to 8 h after farm exposure to specific antigen; (3) radiologic features and/or functional pattern of interstitial lung disease; and (4) evidence of antibodies against *Mucor*-spora *faeni*. All patients had been exposed recently to the antigen and were still symptomatic at the time of bronchoscopy. None of them had been treated with corticosteroids before the initial investigation. They were lifetime nonsmokers and did not have any personal or family history of allergic and respiratory disease.

Normal lung tissue was obtained, as control, from transbronchial biopsies in unaffected area in four patients (two men; mean age, 49 ± 9.6 years; all smokers) with carcinoma of the lung. All these subjects underwent bronchoscopy for routine diagnostic purposes. The BAL control group comprised seven healthy nonatopic volunteers (four men; mean age, 41.1 ± 11.1 years; nonsmokers). Each individual gave informed and signed consent before undergoing the bronchoscopy procedure.

**Pulmonary Function Tests**

Lung volumes (vital capacity and forced expiratory volume in 1 s) were measured by a standard spirometer (Morgan). Diffusion capacity (carbon monoxide transfer factor) was measured by the single-breath carbon monoxide technique (Morgan). The values
obtained were expressed in percent of predicted normal values.

**Bronchoscopy Procedures**

Bronchoscopy was performed between 8 and 9 AM on each occasion. Subjects abstained from food for at least 12 hours before bronchoscopy. In all subjects, upper airways were anesthetized by 4 ml of lidocaine at 2 percent. No other anesthetic drug was given before or during bronchoscopy.

A flexible fiberoptic bronchoscope (Olympus, Japan) was wedged into a segment of the right middle lobe, and three 50-ml aliquots of sterile saline solution, warmed at 37°C, were infused. Fluid was gently aspirated immediately after each aliquot was introduced and collected in a sterile container.

Lung biopsy specimens from each subject were taken with elliptical forceps under fluoroscopic control from the area of greatest radiologic abnormality. At least two satisfactory specimens were obtained from at least two segments.

**Handling of BAL Fluid**

After recovery, BAL fluid was strained through a monolayer of surgical gauze to remove mucus. A sample was reserved for total cell count. The total cell number was counted using a Nageotte's chamber and results were expressed as cells × 10^9/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 (Labofuge AE, Heraeus, FRG) were stained by May-Grunwald-Giemsa method. The differential cell count of macrophages, lymphocytes, neutrophils, and eosinophils was made under light microscopy at ×1,000, by counting approximately 300 cells in random fields. Due to the difficulty in detecting mast cells using the May-Grunwald-Giemsa stain, they were counted in cell suspension after staining with Alcian blue-safranine. Mast cells were quantified by counting at least 1,000 cells.

**Handling of Tranbronchial Biopsy Specimens**

The biopsy specimens were immediately placed in periodate-lysine-paraformaldehyde fixative and embedded in glycol methacrylate (JB4 Servall Dupont Co. Newton) as reported. One-micron-thick plastic sections (LK 8 microtome, Sweden) were stained in 2 percent toluidine blue.

Metachromatic cell counts were performed by one observer (P.A.) using a mechanical stage, ×10 eye pieces, and ×100 immersion oil objective (D Plan Olympus, Japan), and for each case 200 high-power fields were counted. At least two biopsy specimens from each subject were evaluated in random fields. The compressed areas where the biopsy forceps grasped the tissue were avoided when evaluating the parenchyma for the presence of metachromatic cells. Results were converted to mean values for mast cells per square millimeter by multiplying by a factor of 126.98 which was calculated from the area of the high-power field. The metachromatic cells were further classified according to their localization: (1) in alveolar septa, (2) within the alveolar epithelial cell layer, (3) within alveolar lumina, and (4) in vascular walls.

Microphotographs were taken using a 35-mm microscope camera (PM-6, Olympus, Japan), and film (Kodak Ektachrome 50 Professional) was used with semiautomatic controlled exposure times (EMM-7, Olympus, Japan).

**Statistical Analysis**

Correlations were estimated by calculating simple linear regression coefficient (r). Results were expressed as mean ± SD; differences between mean values were compared using the two-tailed Student's t test; we accepted a p value <0.01 as indicating significance.

**Results**

On first admission to the hospital, all patients complained of cough, chills, fever, and dyspnea related to exposure to moldy hay. Their symptoms had lasted an average of two weeks before admission. Exposure was confirmed, in all patients, by precipitins against *M. faeni*. Patient 10 also had antibodies against *Tha-
Moactinomyces vulgaris. No precipitins against pigeon droppings, pigeon serum, Aspergillus fumigatus, Pullularia pullulans, or Cryptostroma corticalis were found. The patients had bilateral crepitant rales and chest roentgenograms consistent with diffuse interstitial and alveolar pulmonary infiltrates.

The measures of pulmonary function showed that the carbon monoxide transfer factor was uniformly altered, ranging from 53 percent to 79 percent of predicted value. The vital capacity was 84.7 percent (59 to 117) and FEV₁ was 74.5 percent (52 to 108) of predicted value.

Absolute numbers of inflammatory and immune cells recovered from BAL were $542 \pm 211.2 \times 10^3$ in FLD and $175.7 \pm 37.3 \times 10^3$ in the control group ($p<0.001$). The differential cell counts are reported in Table 1. The frequencies of lymphocytes (56.4 ± 18.6 percent of total cells) and mast cells (3.9 ± 1.5 percent of total cells) were significantly higher in patients with FLD than in control subjects. The neutrophil and eosinophil fractions were also higher, but the difference was not significant. No correlation was found among inflammatory cells in BAL. Frequently mast cells of patients with FLD showed few granules in their cytoplasm; this aspect was not observed in control subjects (Fig 1).

Mast cells stained well with toluidine blue after periodate-lysine-paraformaldehyde fixation and glycol methacrylate embedding. Cells that contained variable numbers of metachromatic granules in their cytoplasm were encountered on light microscopic study of

---

**Figure 1.** Light photomicrograph of the cell suspension from BAL of patient with FLD. Two large mast cells are shown; one is well granulated (right) and one is partially granulated (left) (Alcian blue-safranine, original magnification × 1,200).

**Figure 2.** Light photomicrograph of alveolar septa of patient with FLD. Two mast cells are shown (arrows); one of these cells (left) contains only few granules (plastic section [1 μm thick] toluidine blue, original magnification × 400).

**Figure 3.** Light photomicrograph of alveolar septum of patient with FLD. One mast cell (arrows) has an elongated cytoplasm that seems to extend into the underlying connective tissue. Both cells have few granules (plastic section [1 μm thick], toluidine blue, original magnification × 400).

**Figure 4.** Light photomicrograph of mast cell (arrow) that is located in alveolar lumen of patient with FLD (plastic section [1 μm thick], toluidine blue, original magnification × 400).
lungs from patients with FLD and control subjects. Relevant histologic features of transbronchial biopsy specimens in FLD are illustrated in Figures 2 through 4.

The results of histologic analysis of transbronchial biopsy specimens from patients with FLD and control subjects are reported in Table 2. In patients with FLD, an average of 84.45 ± 28.8 metachromatic cells was found per square millimeter of lung tissue section with a range of 54.6 to 170.15. Of the total number of metachromatic cells that were counted in the lung biopsy specimens, 83.2 percent were located in the alveolar septa (Fig 2) frequently close to capillaries (Fig 3), 14.9 percent within alveoli (Fig 4), 0.7 percent among unilayered or multilayered cuboidal alveolar lining cells, and 1 percent along blood vessels.

The tissue from control patients (Table 2) contained lower numbers of metachromatic cells with a range of 13.8 to 40.6 and an average of 20.43 ± 13.4 cells per square millimeter of tissue section; 98.5 percent of these cells were located in alveolar septa, 1.5 percent in relatively large vessels, and no metachromatic cells were found within alveoli. Unlike the partial mast cell degranulation evident in the patients with FLD, the mast cells found in the control subjects were fully granulated.

When correlations between BAL inflammatory cells and mast cells of transbronchial biopsy specimens of patients with FLD were examined, a relevant correlation was found only between the fraction of mast cells in BAL and mast cells in biopsy specimens (r = 0.88; p < 0.01) (Fig 5). No correlations were found between BAL biopsy specimen changes and results of pulmonary function tests.

**DISCUSSION**

Our data support the hypothesis that in FLD, BAL reflects lung histologic features. In fact, the frequencies of mast cells recovered by lavage fluid accurately overlapped those observed in lung tissue sections. Although in different amounts, the mast cells were increased both in BAL fluid and lung biopsy specimens of patients with FLD and some of them showed a partial degranulation.

It must be emphasized that the degree of mast cells in the BAL of patients with FLD is 20 times higher than that observed in the control group, while the frequency of mast cells in tissue sections is only four times higher. This could be explained by the fact that the biopsy in the control group included four smokers, and it is known that smoke can increase the number of lung mast cells.7 Another possibility is that the lavage overestimated the number of mast cells in lung biopsy specimens as demonstrated for lymphocytes in hypersensitivity pneumonitis.8

Our study allowed the evaluation of the topographic distribution of mast cells in peripheral lung tissue. As a result, it has been demonstrated that BAL correctly

---

**Table 2—Distribution of Mast Cells in Lungs of 15 Patients with Farmer's Lung Disease and Four Control Patients**

<table>
<thead>
<tr>
<th>Patient No./Age, yr/Sex</th>
<th>Smoke</th>
<th>No. of MC* per 2 sq mm</th>
<th>A septa</th>
<th>A lumen</th>
<th>V walls</th>
<th>E lining</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/55/M</td>
<td>No</td>
<td>103.61</td>
<td>71</td>
<td>26</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>2/63/F</td>
<td>No</td>
<td>95.23</td>
<td>83</td>
<td>13</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>3/51/M</td>
<td>No</td>
<td>170.15</td>
<td>75</td>
<td>24</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>4/45/M</td>
<td>No</td>
<td>101.58</td>
<td>86</td>
<td>12</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>5/40/M</td>
<td>No</td>
<td>57.14</td>
<td>88</td>
<td>12</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>6/47/F</td>
<td>No</td>
<td>68.06</td>
<td>80</td>
<td>18</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>7/48/M</td>
<td>No</td>
<td>94.60</td>
<td>86</td>
<td>11</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>8/54/F</td>
<td>No</td>
<td>89.52</td>
<td>87</td>
<td>12</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>9/54/M</td>
<td>No</td>
<td>79.36</td>
<td>89</td>
<td>11</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>10/35/M</td>
<td>No</td>
<td>77.45</td>
<td>76</td>
<td>22</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>11/42/F</td>
<td>No</td>
<td>58.41</td>
<td>81</td>
<td>17</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>12/29/M</td>
<td>No</td>
<td>54.60</td>
<td>87</td>
<td>11</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>13/35/M</td>
<td>No</td>
<td>63.49</td>
<td>85</td>
<td>11</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>14/45/M</td>
<td>No</td>
<td>66.66</td>
<td>90</td>
<td>10</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>15/39/M</td>
<td>No</td>
<td>86.98</td>
<td>85</td>
<td>14</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>84.45</td>
<td>83.2</td>
<td>14.9</td>
<td>1.0</td>
<td>0.7</td>
</tr>
<tr>
<td>SD</td>
<td></td>
<td>25.87</td>
<td>5.5</td>
<td>5.2</td>
<td>1.2</td>
<td>0.9</td>
</tr>
<tr>
<td>Control patients</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>16/46/M</td>
<td>Yes</td>
<td>40.63</td>
<td>98</td>
<td>0</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>17/49/F</td>
<td>Yes</td>
<td>14.60</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>18/62/F</td>
<td>Yes</td>
<td>12.69</td>
<td>99</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>19/39/M</td>
<td>Yes</td>
<td>13.81</td>
<td>97</td>
<td>0</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>20.43</td>
<td>98.5</td>
<td>0</td>
<td>1.5</td>
<td>0</td>
</tr>
<tr>
<td>SD</td>
<td></td>
<td>13.48</td>
<td>1.2</td>
<td>1.2</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*MC = mast cells; a septa = alveolar septa; A lumen = alveolar lumen; V walls = vascular walls; and E lining = epithelial lining.
samples not only cells in alveolar spaces, but also the mast cells that are present throughout the lung parenchyma. We can hypothesize that mast cells present at the epithelial surface of alveolar structures recovered by BAL are in continual equilibrium with the cellular populations present in the interstitium, and that BAL reflects the intensity of mast cell presence in the lung.

The increase of the mast cell number in the tissue of patients with FLD in the present study confirms and extends previous histologic and ultrastructural observations. In particular, our results compare favorably with the observation made by Heard et al., who showed high mean values of mast cells per square millimeter of tissue section in samples from open lung biopsies in two patients with hypersensitivity pneumonitis (91.0 and 101.9, respectively). Few and controversial data are available on the number of metachromatic cells in the alveolar structures of normal human lungs. The results, in fact, varied from 2.6 to 51.9 metachromatic cells per square millimeter. These discrepancies may be explained on the basis of different fixation (neutral formalin or Carnoy's), different embedding (paraffin or plastic), and different methodologies (light or electron microscopy) used in these studies.

In our patients, the vast majority of metachromatic cells were found in alveolar septa, frequently in close proximity to capillaries according to that observed in other lung diseases. We often found mast cells with few metachromatic granules both in BAL and in lung biopsy specimens of patients with FLD. This aspect is suggestive of their possible role in the functional changes that occur in this disease through a rapid release of chemical mediators. Ultrastructural alterations favoring this idea are reported by Kawanami et al. in patients affected by hypersensitivity pneumonitis. On the other hand, the absence of correlation between mast cells and other immune-inflammatory cells in BAL fluid indicates that mast cells could not play a critical role in the pathogenesis of this disease and that they may be a nonspecific phenomenon related to inflammation.

In control patients, we did not observe infiltration of epithelium by mast cells; conversely, the presence of metachromatic cells in the alveolar epithelial cell layer of patients with FLD was noted and was similar in several aspects to that described in other fibrotic interstitial lung diseases. Other structural observations support the concept that mast cells can migrate throughout the epithelial lining of alveoli. According to this, 15 percent of metachromatic cells were observed in alveolar lumina and this nicely fits with the finding of increased numbers of mast cells in BAL. We can hypothesize that factors such as immune complexes may attract mast cells to migrate into alveolar lumina.

No correlations were found between the cells in BAL and results of pulmonary function tests. This may signify that not a single type of cell but all cells together contribute to lung damage in acute FLD. Recent advances suggest that FLD develops as the result of a complex series of immunologically specific events, involving contemporaneously and/or sequentially several immune-inflammatory cells.

These data indicate that the high mast cell counts observed in BAL fluid in patients with FLD are generated from lung parenchyma to at least some extent.

ACKNOWLEDGMENT: The authors gratefully acknowledge Dr. G. F. Consigli and Dr. A. Casalini of the Endoscopy Unit of the Rasori Hospital of Parma.

REFERENCES
VI National Update on Fiberoptic Bronchoscopy

This one-day workshop will be held on December 8 at Hyderabad, India, at the Hotel Krishna Oberoi. For information, contact Medinova Diagnostic Services, Panjagutta Main Road, Hyderabad 500482, India.