CHARACTERISATION OF LOWER RESPIRATORY-TRACT INFLAMMATION IN PATIENTS WITH SMEAR-NEGATIVE PULMONARY TUBERCULOSIS

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ABSTRACT

Bronchoalveolar lavage (BAL) studies were carried out in 27 sputum smear negative but radiographic probable pulmonary tuberculosis patients to characterize the inflammatory and immune effector cells in the lower respiratory tract and in 17 normal subjects. The diagnosis of active pulmonary tuberculosis was confirmed by isolation of Mycobacterium tuberculosis in culture from sputum and/or lavage specimens. BAL was done first from radiologically normal lobe and then from radiologically abnormal lobe prior to treatment and the BAL fluids were analyzed separately. Two groups were identified based on lavage results. One group (macrophage predominant) had significantly elevated total cells and alveolar macrophages in both radiologically normal (p<0.01) and abnormal lobes (p<0.001) compared to normal controls. The other group (lymphocyte predominant) had expanded numbers of total cells (p<0.01), lymphocytes (p<0.01), neutrophils (p<0.05) and eosinophils (p<0.01) in radiologically abnormal lobe. The cell profile in radiologically normal lobe in lymphocyte predominant group was within normal limits. These results suggest that two distinct cell profiles, in one group an increase in alveolar macrophages and in the other an increase in lymphocytes can occur in the lower respiratory tract of patients with sputum smear negative, but radiographic probable pulmonary tuberculosis.

Introduction

Although treatment for pulmonary tuberculosis prevents the replication of mycobacteria and reduces the mycobacterial load thereby reducing the immune-mediated lung injury, the lung damage in tuberculosis results from host reaction rather than by direct bacterial effects (1). In conformity with this, it had been noticed that radiographic and pulmonary function abnormalities persist in a proportion of patients despite treatment with potent regimens (2-4). Thus, in order to “treat” all pulmonary tuberculosis patients successfully, we should be able to prevent the lung damage and subsequent fibrosis. However, the pathogenesis and mechanism of lung injury and fibrosis in pulmonary tuberculosis are not well understood to formulate modalities of treatment that may prevent fibrosis. Bronchoalveolar lavage (BAL) Studies have been found to be useful in better understanding of pathogenesis and development of fibrosis in various interstitial lung diseases (5). There are also reports of cellular compositions of BAL fluid in pulmonary tuberculosis (6-10). Bronchoalveolar lavage studies of tuberculosis patients may, therefore, help to assess the role of inflammatory and immune effector cells in lower respiratory tract (LRT) in mediating injury and fibrosis. A preliminary study was, therefore, planned to characterise the inflammatory and immune effector cells in the lower respiratory tract of patients with sputum smear negative, but radiographic probable pulmonary tuberculosis.

Materials and Methods

Study subjects

Sixty nine patients presenting with respiratory symptoms such as cough, fever, haemoptysis and loss of weight for less than four months, and at least four sputum smears negative for acid fast bacilli (AFB), but with radiographic appearances suggestive of pulmonary tuberculosis were evaluated by BAL during a four-year period. The duration of disease was calculated from the onset of symptoms as provided by the patient during initial interrogation by the chest physician (VKV). The diagnosis of tuberculosis was based on the demonstration of M. tuberculosis in culture in sputum...
or BAL fluid. None had received any anti tuberculosis treatment in the past. Skin tests were done by the intradermal Mantoux technique with one tuberculin unit purified protein derivative (PPD RT 23 with Tween 80) and were examined at 48 or 72 hours. Full-size postero-anterior chest radiographs were assessed by the International Labour Organisation (ILO) criteria (11). None of them was suffering from diabetes mellitus as assessed by urine examination for sugar or receiving immunosuppressive drugs.

Occupational history revealed shop workers (eight) plumber and turner (two), executive, rickshaw puller, labourer, milkman, driver, tinkering and gardening work (one each). Three were unemployed and seven were housewives. None had been exposed regularly to dusts / chemical: at occupation. Four patients gave a family / contact history of tuberculosis. Informed consent was obtained from each subject and the study was approved by the Institutional Ethical Committee.

Classification of BAL results.

If lymphocytes in BAL fluid are more than 22% (more than one SD above normal mean) and macrophages less than 75% (less than one SD below normal mean) from radiologically abnormal lobe, it was classified as lymphocyte predominant. If lymphocytes are less than 22% and macrophages more than 75%, it was classified as macrophage predominant. We had not done subtyping of lymphocytes.

Statistical analysis

The results are presented as mean ± SEM. All the mean differences between groups were compared using Mann-Whitney U test.

Results

Of the 69 patients assessed, 27 had bacteriological confirmation by sputum culture including 18 in whom BAL fluid cultures were also positive for M. tuberculosis. The mean age of these 27 subjects was 29.1 ± 1.63 Yrs (range 17 to 45 years) and the mean weight was 44.4 ± 1.03 Kg. (range 36.6 to 55.7 Kg.). There were 20 males and seven females. Eleven subjects were smokers. Four had smoked more than 10 beedis/cigarettes daily for more than five years. The mean duration of symptoms was 1.7±0.24 months (range seven days to four months) and the mean reaction to PPD was 19.9±1.3 mm. (range 10 to 40 mm.). All had radiographic abnormalities of category A (11) with exudative pattern confined to one of the upper zones and none had cavitary lesions. Based on the BAL results, two groups were identified. There were fourteen patients with predominance of macrophages (Group I) and 13 with predominance of lymphocytes (Group 2).

Group 1 (Macrophage Predominant)

There were nine males and five females in this group. The mean age was 29.1±2.2 Yrs (range 22 - 45 years) and six were smokers. There were no difference in lavage findings between smokers and non-smokers. The mean duration of symptoms was 1.5 ± 0.26 months (range seven days to three months) and the mean body weight was 43.9±1.4kg (range 36.6 to 52.0). The mean reaction to PPD in this group was 19.5±1.9 mm. (range 10 to 40 mm.). BAL results are shown in the Table.
TABLE
Inflammatory and Immune effector cells in the lower respiratory tract
(Mean ± SEM)

<table>
<thead>
<tr>
<th>Normal Subjects (n:17)</th>
<th>Macrophage Predominant</th>
<th>Lymphocyte Predominant</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RNS (n:14)</td>
<td>RAS (n:14)</td>
</tr>
<tr>
<td>Total cells, (x 10^6/dl)</td>
<td>33.6±7.0**</td>
<td>16.1±1.6</td>
</tr>
<tr>
<td>Macrophages %</td>
<td>83.5±1.5</td>
<td>89.1±2.2*</td>
</tr>
<tr>
<td>x 10^6/dl</td>
<td>13.4±1.4</td>
<td>23.8±3.8**</td>
</tr>
<tr>
<td>Lymphocytes, %</td>
<td>14.9±1.6</td>
<td>8.9±1.9*</td>
</tr>
<tr>
<td>x 10^6/dl</td>
<td>2.5±0.4</td>
<td>1.7±0.3</td>
</tr>
<tr>
<td>Neutrophils, %</td>
<td>0.7±0.2</td>
<td>1.0±0.3</td>
</tr>
<tr>
<td>x 10^6/dl</td>
<td>0.1±0.03</td>
<td>0.2±0.05</td>
</tr>
<tr>
<td>Eosinophils, %</td>
<td>0.9±0.3</td>
<td>1.0±0.3</td>
</tr>
<tr>
<td>x 10^6/dl</td>
<td>0.1±0.03</td>
<td>0.2±0.05</td>
</tr>
<tr>
<td>% Recovery</td>
<td>59.6±1.6</td>
<td>62.6±4.1</td>
</tr>
</tbody>
</table>

RNS: Radiologically normal site
RAS: Radiologically abnormal site
p values * < 0.05, ** <0.01, *** <0.001 as compared to normal subjects
£ <0.05, ££ <0.01, as compared radiologically normal site.

Total inflammatory cells and absolute (total) numbers of alveolar macrophages were significantly higher in both radiologically abnormal (p < 0.001) and normal (p < 0.01) lobes compared to normal subjects. Other types of cells were similar in both abnormal and normal lobes on comparison with normals. This was true whether all, or only non-smokers were considered. Because of a significant rise in macrophage percentages at both sites, there was a reciprocal significant reduction in lymphocyte percentages. However, total lymphocytes were normal at both sites. The fluid recovery was similar in normal subjects and patients in both lobes.

**Group 2 (Lymphocyte Predominant)**

Of the 13 patients in this group, two were females and five were smokers. BAL results between smokers and non-smokers were similar. The mean age was 29.0 ± 2.5 Yrs (range 17- 44 years). The mean duration of symptoms was 2.1 ± 0.5 months (range 15 days to four months) and the mean body weight was 45.1±1.5 kg (38.5 to 55.7). The mean reaction to PPD in these patients was 20.6±1.6 mm. (range 14 to 28 mm.). BAL results in Group 2 patients are given in Table. The total inflammatory cells (p<0.01), lymphocytes (p<0.01), neutrophils (p<0.05) and eosinophils (p<0.01) were significantly higher in radiologically abnormal site compared to normal subjects. This was true whether all individuals or only non-smokers were considered. There was also a significant increase in the proportion of lymphocytes, neutrophils and eosinophils and a significant reduction in proportion of alveolar macrophages at radiologically abnormal site. Total lymphocytes (p<0.05) and eosinophils (p<0.05) were also significantly higher at radiologically abnormal site in comparison with radiologically normal site. All types of cells were similar except for a suggestion of an increase in total inflammatory cells (p = 0.06) in radiologically normal site in comparison with normal subjects. Fluid recovery was similar in normal subjects and patients in this group.

**Discussion**

We have demonstrated in this study that two distinct cell profiles, one group with an increase in
alveolar macrophages and the other with an increase in lymphocytes can occur in the tower respiratory tract of patients with sputum smear-negative but radiographic probable, pulmonary tuberculosis. We had earlier reported that a third type of cell profile with an increase in granulocytes (neutrophils and eosinophils) can also occur in smear negative pulmonary tuberculosis (14). Since the age, smoking habit, fluid recovery during BAL, the reaction to PPD, the nutritional status as assessed by body weight and the radiological abnormalities were similar in both groups, the reason for the observed difference in cell profile between the two groups could not be explained, except for a suggestion that the duration of illness (1.5±0.26 vs 2.1±0.5 months; is longer in lymphocyte predominant group. Neutrophils are the first cells to appear in tuberculosis exudate (15) and the BAL findings of granulocyte accumulation in tuberculosis patients (14) may thus represent the earliest response after inhalation of Mycobacterium tuberculosis into an alveolus. The demonstration of abnormally elevated macrophages in the radiologically normal and abnormal lobes of pulmonary tuberculosis patients in this study may represent recruitment of monocytes to the lungs (16) from the circulating pool of peripheral blood monocytes (17) by the chemotactins released by neutrophils (18). Expanded numbers of alveolar macrophages alone in lower respiratory tract had been described in early stages of pneumoconioses (19,20) and subacute phase of toxic gas exposure (12). Macrophage activation is characterised by spontaneous release of reactive oxygen species and a number of a microbialid enzymes by these cells (21-23). We had reported previously that alveolar macrophages from tuberculous patients were activated as evidenced by spontaneous release of hydrogen peroxide (H₂O₂) by these cells (24).

Lymphocytes which are building blocks of granuloma are recruited to the site of lesion and activated by interleukin-1 and gamma-interferon released by activated macrophages (25). Thus lymphocytosis at radiologically abnormal but not in radiologically normal lobe in this study may represent the localisation of granuloma at the site of lesion. Other cell types such as neutrophils and eosinophils also contribute to granuloma formation (16) and in conformity with this, there is a significant elevation of neutrophils and eosinophils in radiologically abnormal lobe in this study as well.

Previous workers had demonstrated BAL lymphocytosis in sputum smear positive pulmonary tuberculous patient (6-8). It had also been observed that lymphocytosis occurred in active tuberculous lesion, but not in non-affected parts of the lungs (8), as seen in this study. In a study of sputum smear negative pulmonary tuberculosis, lymphocytosis was, observed in 55% of patients at local site (9) and this was similar to the observation of lymphocytosis in 48% (13 of 27) of patients in our series. Generalised lymphocytosis in lung has been described in other granulomatous lung diseases such as sarcoidosis, hypersensitivity pneumonitis and berylliosis (5). The main type of lymphocytes in tuberculous granuloma is T cells (6,26) and an increase in CD4+ T cells is also demonstrated in tuberculous pleural effusion (27). Activated T cells are capable of spontaneously releasing lymphokines such as interleukin-2, gamma interferon and tumor necrosis factor and play an important role in immune response in tuberculo sis (16,25). The demonstration of expanded numbers of alveolar macrophages in both radiologically normal and abnormal lobes of patients with shorter duration of symptoms may suggest the possibility that monocyte/macrophage recruitment to the lung precedes lymphocyte recruitment and granuloma formation at the site of lesion following tuberculous infection. This may further be suggested by the fact that abnormal accumulation of lymphocytes and granulocytes were seen only in radiographically abnormal lung segments of patients with longer duration of symptoms. However, there was no significant difference in duration of symptoms between the two groups for a definite conclusion.

A dissociation of cell mediated immunity (CMI) and delayed type hypersensitivity (DTH) was described in tuberculosis and it was suggested that cellular types of immune responses were involved in both (28). CMI was defined as a beneficial host response and DTH as the immunological reaction that caused caseous necrosis (23). Although tuberculin-positive hosts with good CMI and tuberculin-positive hosts with poor CMI could arrest bacillary growth, the host with good CMI might recover whereas the host with poor CMI might suffer excessive tissue destruction (23). All patients in Our study had strong tuberculin reaction (> 10 mm) and the observation of two types of cell profile may, therefore, suggest the possibility that one group may have tuberculin-reactive good CMI and the other tuberculin-reactive poor CMI. It had also been
Vijayan et al: BAL in smear negative tuberculosis suggested that two mechanisms of cell mediated responses referred to as the Listeria-type and the Koch- type could occur in infections with mycobacteria and the Listeria-type is thought to provide protective immunity (29,30). It had also been described previously that healing could occur in a proportion of tuberculous patients without treatment (31, 32) which might be due to the development of good CMI in some of these patients. The finding of generalised recruitment of macrophages to the lung in one group may, therefore, suggest the inability of the host to localise the immune response as served in lymphocyte predominant group. We have recently reported that lymphocytic alveolitis in tuberculosis patients is associated with increased bactericidal activity against Staphylococcus aureus, whereas no lymphocytic alveolitis is associated with reduced activity (33). Ethical considerations will not permit the follow up without treatment to know the fate of these patients. However; Immunological studies of the cytokines (34,35) released from lung immune and inflammatory cells to assess the immunologic competence of the host may aid in understanding the pathogenesis of tissue destruction and healing in pulmonary tuberculosis.

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