Lymphocyte and lymphocyte subset numbers in blood and in bronchoalveolar lavage and pleural fluid in various forms of human pulmonary tuberculosis at presentation and during recovery

G M Ainslie, J A Solomon, E D Bateman

Abstract

Background Lymphocytes have a central role in human defences against mycobacteria. A study was designed to assess the relation between lymphocyte responses and clinical pattern of disease, nutrition and recovery during treatment in patients with tuberculosis.

Methods Lymphocyte numbers and subsets (on the basis of CD3, CD4, and CD8 monoclonal antibodies) were measured in peripheral blood and, where appropriate, bronchoalveolar lavage or pleural fluid of patients with different forms of pulmonary tuberculosis. Eleven had localised pulmonary tuberculosis, 18 miliary tuberculosis and seven a tuberculous pleural effusion.

Results CD4 lymphocytes were found in greatly increased numbers in pleural fluid and were relatively depleted in the blood. Lymphocyte numbers in bronchoalveolar lavage fluid varied widely in localised pulmonary and miliary tuberculosis but were highest in pleural fluid from patients with miliary tuberculosis. This was due to an increase in CD8 lymphocytes, which were also increased in the blood. Lymphocyte numbers bore no relation to nutrition, symptom duration, or radiographic profusion scores. In miliary tuberculosis the time taken for the chest radiograph to clear (mean (SD) 17±6 (7±8) weeks) correlated with lymphocyte numbers in lavage fluid, especially CD8 cells (r = 0.74), but not with the patients' age or nutrition. After 8 weeks' treatment, total and CD4 lymphocyte numbers in lavage fluid showed a substantial increase.

Conclusion The association of CD8 cells with delayed recovery is compatible with suppression of the antimycobacterial action of macrophages. The switch to predominance of CD4 cells in lavage fluid during successful treatment supports the view that they may have a role in eliminating mycobacteria.

The variable manifestations and natural history of pulmonary tuberculosis are determined by host factors, including cell mediated immunity, rather than differences in virulence of different strains of Mycobacterium tuberculosis. The immune response to M tuberculosis involves subpopulations of specifically sensitised CD4 helper/inducer or cytolytic T lymphocytes. Most reports of human disease are based on findings in blood and pleural fluid. In advanced or disseminated tuberculosis circulating T cells, especially CD4 cells, are reduced in number and CD8 T cells are increased relatively. In tuberculous pleural effusions CD4 functionally helper T cells are increased in the pleural fluid compared with blood. There have been few reports of lymphocytes in infected lungs. Options for studying these include examination of pathology specimens and cells recovered by bronchoalveolar lavage. Previous studies of lymphocytes in lavage fluid in tuberculosis have shown increased numbers of lymphocytes in local and miliary tuberculosis.

We have compared lymphocyte numbers and subsets in the lungs, blood and, where possible, the pleura of patients with different forms of pulmonary tuberculosis (local, miliary and pleural) and assessed the serial changes that occurred during successful treatment.

Methods

STUDY POPULATION

Approval for the study was obtained from the Medical Faculty Research and Ethics Committee. All patients gave informed written consent for participation in the study. The study groups comprised seven patients with tuberculosis pleurisy, 11 with localised pulmonary tuberculosis (tuberculosis confined to one lobe or segment) and 18 with miliary tuberculosis. All patients were black or of mixed race. In each case direct sputum examination failed to show acid and alcohol fast bacilli and bronchoscopy and/or thoracentesis were required to confirm active tuberculosis. The clinical assessment included measurement of body mass, height, triceps skinfold thickness, midarm circumference and serum albumin; chest radiography; a full blood count; and a Mantoux skin test employing 5 TU purified protein derivative (PPD). Chest radiographs were reviewed and the size, profusion and distribution of lung changes assessed according to the UICC/ILO standard radiographs for...
Lymphocyte and lymphocyte subset numbers in blood and in bronchoalveolar lavage and pleural fluid in tuberculosis

Table 1 Mean (SD) demographic and nutritional data at presentation of patients with pulmonary and pleural tuberculosis

<table>
<thead>
<tr>
<th></th>
<th>Pleural tuberculosis (n = 7)</th>
<th>Local tuberculosis (n = 11)</th>
<th>Miliary tuberculosis (n = 18)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>31.9 (15.1)</td>
<td>37.4 (17.9)</td>
<td>36.2 (17.5)</td>
</tr>
<tr>
<td>Sex (M : F)</td>
<td>6 : 1</td>
<td>7 : 4</td>
<td>7 : 11</td>
</tr>
<tr>
<td>Smokers</td>
<td>3</td>
<td>8</td>
<td>10</td>
</tr>
<tr>
<td>Duration of symptoms (weeks)</td>
<td>1 (2)</td>
<td>2 (3.4)</td>
<td>2.2 (2.03)</td>
</tr>
<tr>
<td>Body mass (% ideal)†</td>
<td>91.7 (22.6)</td>
<td>96.3 (8.8)</td>
<td>95.1 (17.8)</td>
</tr>
<tr>
<td>Midarm circumference (%) of predicted†</td>
<td>19.5 (27.9)</td>
<td>22.8 (22.2)</td>
<td>151 (21.5)</td>
</tr>
<tr>
<td>Triceps skinfold thickness (% of predicted)§</td>
<td>31 (18.8)</td>
<td>24 (26.2)</td>
<td>16.8 (26.2)</td>
</tr>
<tr>
<td>Serum albumin (g/l)$</td>
<td>36.7 (5.9)</td>
<td>37.7 (6.1)</td>
<td>30.3 (4.8)*</td>
</tr>
<tr>
<td>Positive Mantoux reactivity</td>
<td>5</td>
<td>11</td>
<td>8</td>
</tr>
</tbody>
</table>

*p values (Mann-Whitney U test) versus local tuberculosis, p < 0.05. †Frisancho, 1974. §Normal values: albumin 35-50 g/l.

duration of symptoms were similar. Four patients with miliary tuberculosis and two each with localised pulmonary disease and tuberculous pleural effusions had a regular heavy intake of alcohol. Body mass was above the 90th centile in all groups but midarm circumference and triceps skinfold thickness were markedly reduced (below 24th centile). Serum albumin was significantly reduced in patients with miliary tuberculosis compared with those with localised pulmonary tuberculosis. More patients with miliary disease displayed anergy to PPD.

CELL COUNTS IN BLOOD AND LAVAGE AND PLEURAL FLUID (table 2)

Peripheral blood lymphopenia (less than 1.5 x 10^9/l) was present in six of seven patients with pleural disease, nine of 18 with miliary disease and seven of 11 with localised pulmonary tuberculosis. In the groups as a whole blood lymphocyte numbers were only reduced significantly in those with pleural disease (1.06 (0.46) x 10^9/l, p < 0.05). The total cell count and lymphocyte yield in lavage fluid, whether expressed as total lymphocytes, percentage of lymphocytes or lymphocytes/ml lavage fluid, were significantly increased in miliary tuberculosis. Lymphocyte numbers expressed per ml lavage fluid were elevated in both affected and unaffected lobes in localised pulmonary tuberculosis with a trend towards more lymphocytes in the affected than the unaffected lobe. A modest increase in the percentage of neutrophils was seen in the affected lobe of localised pulmonary tuberculosis but the proportion of eosinophils was normal in all categories of disease. More than 90% of cells in pleural fluid were lymphocytes (lymphocyte number 1234 (1196) x 10^3/ml).

Lymphocyte numbers in lavage fluid of patients with miliary tuberculosis bore a weak positive relationship to patient's age (r = 0.544; p < 0.05) but were unrelated to alcohol intake, duration of symptoms, chest radiographic profusion, measures of nutrition, serum albumin or peripheral blood lymphocyte numbers.

T LYMPHOCYTE SUBSETS (figure 1)

Peripheral blood CD3 cells (T lymphocytes) were present in similar proportions in control and disease categories (mean (SD) percentages of CD3 lymphocytes in peripheral blood were 68.6 (10.6) in localised pulmonary tuberculosis, 73.9 (1.1) in miliary tuberculosis and 71.8 (11) in pleural tuberculosis versus 68.4 (9.8) in control subjects). The ratio of CD4 to CD8 cells, however, was significantly lower in peripheral blood from patients (mean (SD) of CD4 : 8 was 1.6 (0.58) in miliary disease, 1.76 (0.88) in localised pulmonary tuberculosis and 1.21 (0.43) in pleural disease) than in blood from normal subjects (2.2 (0.8), p < 0.05). A similar but more pronounced trend was present in lavage fluid, especially in miliary disease (mean (SD) of CD4 : 8 lymphocytes in lavage fluid was 0.86 (0.43) in miliary tuberculosis, 1.23 (0.28) in localised pulmonary tuberculosis affected lobe, and 1.02 (0.23) in the unaffected lobe versus 1.98 (0.36) in normal subjects, p < 0.01). The low CD4 : 8 ratio in blood was due to an absolute decrease of CD4 cells (means of absolute numbers of CD4/CD8 lymphocytes x 10^3 in blood: 629/451 in miliary tuberculosis, 692/454 in localised pulmonary tuberculosis and 406/355 in tuberculous effusions versus 771/453 in control subjects). In the lavage fluid

Table 2 Mean (SD) numbers and proportions of cells in peripheral blood and bronchoalveolar lavage fluid

<table>
<thead>
<tr>
<th></th>
<th>Bronchoalveolar lavage fluid</th>
<th>Macrophages</th>
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<tbody>
<tr>
<td></td>
<td>Total cell yield (x 10^6/ml)</td>
<td>Neutrophils (%)</td>
</tr>
<tr>
<td></td>
<td>Total lymphocytes (x 10⁶)</td>
<td>(%)</td>
</tr>
<tr>
<td></td>
<td>Lymphocytes (%)</td>
<td>Eosinophils (%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Control (n = 8)</td>
<td>1.79 (0.57)</td>
<td>2.3 (0.6)</td>
</tr>
<tr>
<td>Local tuberculosis (n = 11)</td>
<td>1.67 (0.66)</td>
<td>3.3 (1.7)</td>
</tr>
<tr>
<td>Affected lobe</td>
<td>19 (2.19)</td>
<td>5.5 (2.9)</td>
</tr>
<tr>
<td>Unaffected lobe</td>
<td>9.9 (12.9)</td>
<td>0.8 (0.9)</td>
</tr>
<tr>
<td>Military tuberculosis (n = 18)</td>
<td>1.48 (0.76)</td>
<td>2.3 (0.3)</td>
</tr>
</tbody>
</table>

p values (versus controls): *p < 0.05, †p < 0.01.
T lymphocyte OKT4:8 (CD4:8) ratios in peripheral blood, bronchoalveolar lavage fluid and pleural fluid in patients with different forms of pulmonary tuberculosis. Bar values represent mean (standard deviation).
The changes in pleural tuberculosis, namely mild peripheral blood lymphopenia and a low CD4 : 8 ratio, in association with a marked pleural lymphocytosis, have been described previously.\textsuperscript{5,6} An interesting finding in the current study is the differing responses in the pleura and lung. In one patient with both miliary tuberculosis and a pleural effusion, the pleural fluid lymphocyte CD4 : 8 ratio was 4 : 3 (representing the expected excess of CD4 cells) while a marked CD8 lymphocyte excess (CD4 : 8 = 1 : 1) was noted in the lavage fluid. The reason for this difference in T cell subsets in the different compartments is not clear. It is possible that there are differences in the accessory cell function of pleural macrophages\textsuperscript{20} or that a brief and spontaneously remitting CD8 phase occurs during the early stage of pleural infection, before the CD4 cell proliferation that is associated with marked pleural exudates, and in the lung in miliary disease during successful treatment.

It is relevant to ask what factors determine the intensity of lymphocyte response in local and miliary tuberculosis. In the latter, no relationship with profusion of chest radiographic abnormality, duration of symptoms and measures of nutrition was observed. Dhand \textit{et al}.\textsuperscript{13} have shown a relative increase in total cell and lymphocyte counts in poorly nourished patients with local disease, especially when extensive. Increased T suppressor cells and a lower T helper to suppressor ratio have been observed in malnutrition\textsuperscript{21,22} and with advanced age.\textsuperscript{9} This shift, however, is usually associated with reduced T lymphocyte numbers. A heavier load of infecting organisms is an alternative explanation and, as this was not measured in our study, remains a possibility. A switch to functionally suppressive T cell proliferation with increased dose of mycobacteria has been observed in experimental animals, but total cell numbers did not increase greatly.\textsuperscript{8}

Previous studies looking at the pattern of lymphocyte responses during treatment have been confined to peripheral blood. Our patients with sputum negative localised pulmonary and pleural disease showed a lymphopenia at presentation, which resolved on treatment, as was seen with Onwubalili and Scott with sputum positive localised pulmonary tuberculosis.\textsuperscript{23} The CD4 : 8 ratio did not rise significantly in our patients, unlike the sputum positive and sputum negative patients of Singhal \textit{et al}.\textsuperscript{14} Our patients with miliary tuberculosis showed a rise in the blood CD4 : 8 ratio during treatment, as did the patients of Bhatnagar \textit{et al}.\textsuperscript{9} Their lymphopenia, however, did not resolve within the first 12 weeks.

This is the first study to assess the changes in cellular response in the lungs of patients with miliary tuberculosis during treatment, and to relate them to radiographic resolution. There was an inverse relationship between rate of recovery of miliary tuberculosis and total lymphocyte numbers and CD8 cells in lavage fluid at presentation. The differing rates of recovery were not accounted for by different profusion of abnormality on chest radiography or the patients' nutrition. It is not clear whether the increased CD8 cells form an essential part of the delayed recovery or whether they merely reflect more severe infection.

It is important to establish the functional role of the CD8 cells observed in our study. Effective host defence against mycobacteria involves collaboration between mononuclear phagocytes and lymphocytes, the final pathway being inhibition of mycobacterial replication within macrophages.\textsuperscript{24} Lymphokines from specifically sensitised clones of CD4 helper lymphocytes have been thought to be central to this process.\textsuperscript{25,27} The CD4 lymphocyte response is potentially tissue damaging as lymphokines are produced which prime macrophages for massive release of tumour necrosis factor when mycobacteria are ingested.\textsuperscript{25} If the CD8 cells shown in this study have suppressor function, they may serve to downregulate the proliferation of CD4 cells and potentially damaging host responses. This might be done at the expense of delaying clearance of the organism. Functionally suppressive lymphocytes have been identified in human tuberculous infections\textsuperscript{25,26} and experimental models.\textsuperscript{28,29} As yet there is no evidence for CD8 cytotoxic T cells in human tuberculosis.\textsuperscript{30,31}

There was a substantial increase in lymphocyte numbers in lavage fluid after eight weeks of treatment despite an improvement in the chest radiographs. This increasing lymphocytosis was associated with an impressive switch from CD8 to CD4 dominance. It is not clear what factors determine this change. It might follow as a consequence of diminishing bacterial load following antimycobacterial drug treatment. These observations in human pulmonary tuberculosis provide support for in vitro data emphasising the central role of CD4 cells in enhancing antimycobacterial responses mediated by macrophages.\textsuperscript{27,29} The CD4 lymphocytes found in tuberculous pleural effusions have previously been shown to have helper function.\textsuperscript{6,7,32} Further studies of the functional characteristics of both early CD8 and later CD4 cells in human pulmonary tuberculosis, particularly those associated with high intensity lung lymphocyte responses, are necessary.

In summary, we have established that the intensity of the lymphocyte responses in lung infected with \textit{M tuberculosis} varies widely in individual patients.

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4 Ottenhoff TH, Ab BK, van Embden JD, Thole JE, Kiessling R. The recombinant 65-kD heat shock protein of \textit{Mycobacterium bovis} Bacillus Calmette-Guérin/M tuberc-
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