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Immunophenotypic characterisation of peripheral T lymphocytes in pulmonary tuberculosis

Al Majid FM, Abba AA

ABSTRACT

Background: The cellular immune response plays an important role in determining the outcome of infection and disease in *Mycobacterium tuberculosis*. Many studies of these disease interactions yield contradictory results. Aim: This study aims at determining the changes that take place in the subpopulations of T lymphocytes in the blood of patients with pulmonary tuberculosis (TB). Settings and Design: This cross-sectional study was done at King Khalid University Hospital, Riyadh, Saudi Arabia. Materials and Methods: Flow cytometry was used to determine the absolute numbers and percentages of T CD3, T CD4, T CD8, T CD19 and natural killer (NK) T cells in 54 patients with active pulmonary TB before the commencement of treatment and in 25 healthy PPD negative volunteers. Statistical Analysis: Statistical Package for Social Sciences (version 11.5) was used for analysis. Results: There were significant differences in the values of CD3, CD4 and NK T cells among the groups. The numbers of CD3 and CD4 cells were lower in subjects than in controls [1091.9 ± 321.4 vs. 1364.6 ± 251.2; *P* < 0.001 and 639.8 ± 285 vs. 822 ± 189.9; *P* < 0.004, respectively] while numbers of NK T cells were much higher in patients than in controls (410.7 ± 286 vs. 182.3 ± 140; *P* < 0.001). The numbers of CD8 cells were not significantly changed with disease (609 ± 233.5 in subjects and 613.4 ± 170.3 in controls *P* = 0.761). Conclusion: There are significant changes in the cellular immune response particularly affecting the CD3, CD4 and NK T cells with the development of pulmonary TB. Therefore, further studies of these changes may have important implications on the development of diagnostic tools, vaccines and treatment modalities.

KEY WORDS: Immunophenotyping, pulmonary, tuberculosis, T lymphocytes
These cells promote protection by the rapid production of IFN-γ and IL-4.[14]

Since these cells facilitate the containment of infection, it is postulated that there will be changes in the immunophenotypic characterisation of the T lymphocytes and their subpopulations in patients with active TB. Therefore, this study aims at determining whether there could be a change in the T lymphocytes and its subpopulations in patients who have active pulmonary TB.

Materials and Methods

Subjects

Fifty-four adult Saudi patients diagnosed with pulmonary TB and 25 healthy volunteers were included in the study conducted at King Khalid University Hospital Riyadh between July 2005 and June 2006. The mean age of the patients was 37.27 (SD ±17.13) with a range of 13-90 years. The mean age of the control population was 36.24 (SD ±6.34) with a range of 20-45 years. There are 32 (59.26%) and 15 (60%) males among the patient group and the controls, respectively. In both age and gender, there was no significant difference between the groups. The extent of disease was evaluated by the number of zones involved in a posteroanterior radiograph of the chest. A total of 18 (33.3%), 20 (37.0%), 5 (9.25%), 10 (18.5%) and 1 (1.85%) patients have involvement of 1, 2, 3, 4 and 6 zones, respectively.

All patients had symptoms of TB for at least 4 weeks and were positive for M. tuberculosis based on direct smear and/or culture of sputum. All controls had negative reaction to Mantoux test while all subjects had a positive reaction ranging from 4 to 35 mm with a mean of 17.0± 5.54 mm using five tuberculin units of purified protein derivative (PPD). Both groups (controls and patients) tested negative for the HIV and had negative relevant tests for atopic and common helminthic/parasitic infections. None of the study subjects was on immunosuppressant medications. Approval for the study was obtained from the Ethical Committee of the Centre for Medical Research of King Saud University and informed consent was obtained from individual subjects and controls.

Immunophenotypic analysis

Blood samples were collected in EDTA tubes and analyzed within 6 h of storage at room temperature. The monoclonal antibodies used for this study which include mouse anti-human CD3 fluorescein isothiocyanate (FITC), CD19 phycoerythrin (PE), CD4 FITC, CD4 PE, NK Cells PE and HLA-DR PE were obtained from Becton Dickinson Immunochemistry Systems (San Jose, CA, USA). Appropriate immunoglobulin G1 and Ig Ga fluorochrome-conjugated antibodies used as isotype controls were also obtained from the same company. Acquisition was performed on a FACS Calibur Flow Cytometer (Becton Dickinson, San Jose, CA, USA) and 3 × 10⁴ events were collected for each sample. Analysis was performed using CELLQuest (Becton Dickinson, San Jose, CA, USA) on list mode data and the lymphocyte gate was defined forward/side scatter characteristics. For two-colour analysis, FL1/FL2 contour plots were employed to determine the level of autofluorescence and nonspecific binding.

One hundred microlitres of whole blood was mixed with 20 µL of monoclonal antibodies and incubated for 15 min. Haemolysis was performed using 2 mL of commercial solution for haemolysis (FACS Lysing Solution, Becton Dickinson). After 10 min of incubation, the tubes were centrifuged to remove lysed red blood cells and washed twice with cell wash (0.015 M, pH 7.4). Peripheral blood leucocytes (PBL) were identified using monoclonal antibodies as shown in Table 1. Subsets of PBL were assayed immediately by a three-colour flow cytometry. Absolute cell count was computed from the lymphocyte percentage of the differential white cell count obtained using standard laboratory procedures. The absolute count for each subset was calculated using this formula: % CD100 x WBC (µL)/100 x % lymphocytes.

Statistical analysis

Data were analyzed using Statistical Package for Social Sciences (version 11.5). Mean and SD were used to summarise continuous variables while percentages were used for categorical variables. Mann-Whitney U-test was used to investigate statistical significance between groups. The significance considered was P < 0.05.

Results

Table 2 gives the results of the absolute values of CD3, CD4, CD8, CD4/CD8 ratio, CD19, NK T cells and HLA-DR among the patients with pulmonary TB and the controls. The absolute values of CD3 and CD4 are significantly lower among the patients compared to the control group (P = 0.001 and 0.004, respectively). Although there is a difference between the mean absolute count of CD8 between the patients and the control, this portrays no statistical significance. The ratio between CD4 and CD8 cells is significantly lower among the patients compared to the control (P = 0.007). The number of NK T cells in the peripheral blood is significantly higher in the patients than in controls (P < 0.001). The HLA-DR is the same in the two groups. Table 3 details the comparison of the mean of the two groups showing similar significant differences as demonstrated by the absolute figures. The degree of lung involvement did not correlate with the changes in immunological parameters.

Discussion

This study was undertaken to determine the changes in the mean and absolute numbers of peripheral lymphocytes in...
Table 2: Comparison of absolute values of cell types between patients with pulmonary tuberculosis and controls

<table>
<thead>
<tr>
<th>Cell type</th>
<th>Patients</th>
<th>Controls</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD3</td>
<td>1091.9 ± 321.4</td>
<td>1364.6 ± 251.2</td>
<td>0.001</td>
</tr>
<tr>
<td>CD4</td>
<td>639.8 ± 285.0</td>
<td>822.0 ± 189.9</td>
<td>0.004</td>
</tr>
<tr>
<td>CD8</td>
<td>609.0 ± 233.5</td>
<td>613.4 ± 170.3</td>
<td>0.761</td>
</tr>
<tr>
<td>CD4/CD8</td>
<td>1.1 ± 0.43</td>
<td>1.4 ± 0.4</td>
<td>0.007</td>
</tr>
<tr>
<td>CD19</td>
<td>192.3 ± 132.8</td>
<td>212.1 ± 70.34</td>
<td>0.056</td>
</tr>
<tr>
<td>NK T</td>
<td>410.7 ± 286</td>
<td>182.3 ± 140</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HLA-DR</td>
<td>333.7 ± 197.0</td>
<td>254.9 ± 100.4</td>
<td>0.111</td>
</tr>
</tbody>
</table>

Table 3: Comparison between the mean of subtypes of lymphocytes in patients with pulmonary tuberculosis and controls

<table>
<thead>
<tr>
<th>Cell type</th>
<th>Patients, N = 54</th>
<th>Controls, N = 25</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD3</td>
<td>65.0 ± 12.6</td>
<td>76.3 ± 5.7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CD4</td>
<td>37.7 ± 10.4</td>
<td>44.1 ± 5.4</td>
<td>0.001</td>
</tr>
<tr>
<td>CD8</td>
<td>36.1 ± 9.4</td>
<td>33.3 ± 7.6</td>
<td>0.249</td>
</tr>
<tr>
<td>CD19</td>
<td>10.4 ± 5.0</td>
<td>11.1 ± 2.5</td>
<td>0.418</td>
</tr>
<tr>
<td>NK T</td>
<td>22.7 ± 10.6</td>
<td>9.9 ± 5.6</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>HLA-DR</td>
<td>19.5 ± 10.6</td>
<td>12.9 ± 5.0</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Adult patients with pulmonary TB. Earlier studies have clearly demonstrated significant variations in the percentages and absolute numbers of lymphocytes and their subsets among different races, genders and age groups.[15-17] Our control population, composed of adult Saudi of mixed gender, had absolute and percent lymphocytes subpopulations comparable to earlier figures defined for the study population.[16,18] The study shows a clear reduction in the absolute numbers and percentages of CD3 and CD4 in patients with pulmonary TB. This is reflected in the lower CD4/CD8 ratio as there is no significant difference in the CD8. The NK T cells are also significantly higher. These alterations in the cell-mediated immunity are in consonance with observations presented by other workers.[19-21] The CD4 T cells appear to exert their immunological effect through cytotoxicity against infected target cells partly by production of T cell interferon gamma (IFN-γ) and also by intern activation of the M. tuberculosis-infected macrophage which then kills the bacilli.[22-24] The reduction in CD4 has earlier been noted[25,26] and is believed to be a result of pooling or homing of these subset of cells in the lungs.[27] Lymphocyte homing has been described in other tissues including lymph nodes[28] and pleura.[29] Our study suggests that it is principally the CD4 T cells that are homed. Tsao et al. have shown that this shift is related to the severity of pulmonary TB and is inversely related to the CD8 in the peripheral blood and bronchoalveolar lavage fluid.[30]

Another lymphocyte subset that is important in the immunity against TB is the CD8. Similar to CD4 T lymphocytes, IFN-γ production seems to be the key marker for the ability of CD8 lymphocytes to exert their cytotoxic activity in mice and humans.[31,32] Studies in animal model have shown that deficiency of CD8 T cells can result in susceptibility to TB.[33] Our study failed to demonstrate any significant difference in the absolute count and the percentages of CD8 between the patients and control. So in this respect we differ from other studies. This difference may be related to the differences in the methods of assay. Different populations have also been shown to have differences in counts.[16,34] Rodrigues et al. noted markedly decreased CD8 in patients with active TB which recovered after treatment.[26] They assessed the cellular activation of this population of lymphocytes by measuring the percentage of cells expressing the ectoenzyme CD38. A higher percentage of cells expressing the CD38 molecule were observed in patients with active TB which returned to control levels after therapy. So although, absolute numbers were reduced, they were at a higher level of cellular activity. Other workers have, however, noted higher numbers of CD8 which reverted to normal levels after treatment.[35,36]

The Natural killer cells constitute a distinct subpopulation of lymphoid cells defined as CD5-+CD16+ and/or CD56+.[37] Most NK T cells in circulation are CD16+/CD56+ and are able to lyse certain target cells without the need for prior sensitisation. They are able to exert their effect before the immunity triggered by T cells comes into play and therefore of utmost importance in the initial fight against certain obligatory intracellular pathogens including M. tuberculosis. They are able to recognise the antigen independent of the αβ T cells.[38] In this study, there is a significant higher number and percentage of NK T cells in the patients compared to the controls. Our results corroborate earlier findings[34,38-40] which indicate that input from intracellular organisms may induce expansion of NK T cells through the production of IFN-γ. The role of NK cells in mycobacterial infections is not clear, but it has been demonstrated that the activation of the innate immune response in the beginning of the infection through recruiting of pre-NK cells, could be a linkage between innate and acquired immune response.[41] The NK cells can be separated into NKbright and NKdim. The latter, which make up 90% of the NK cells,
present a higher cytotoxic activity than the former.\[42\] Barcelos et al.\[35\] have shown a larger population of NKdim at the beginning of the treatment and NKbright at the end. They suggest that the cytolytic potential in these cells could be due to a higher specific activity of NKdim cells, trying to control M. tuberculosis.

Our study has not revealed any significant difference in the CD19 subpopulation underlying the small role played by the humoral system in the immunity of TB disease. Barcelos et al.\[35\] and Dubaniewicz et al.\[40\] have similar data in untreated patients. However, after treatment these cells increased significantly. It is probable that the increase is due to the endogenous booster that caused an increased reaction to Mycobacterium's soluble antigen released by the action of the chemotherapeutic agents. These findings are buttressed by significant increase in IgG class antibody levels after healing.\[43\] Caplin et al. have also shown higher titres of IgG2 antibody to the whole killed M. tuberculosis and to the M134 epitope shared by many species of mycobacteria.\[44\] In consonance with our study, this latter group of workers also found that the severity of TB, defined radiologically, was not related to current features of cell-mediated immunity.

In conclusion, this study demonstrates that there are changes in the T-lymphocyte number and distribution in patients suffering from pulmonary TB. The identification of these changes is a first step. In addition, there is a need to further evaluate the mechanisms leading to these changes so as to understand the pathogenesis and prognostic markers of the disease and to develop immunomodulatory modalities of therapy.

References


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