Role of some T-lymphocyte subsets in assessment of treatment response in tuberculous patients

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Abstract Background: CD4, CD8 T cells, T-helper 1 (Th1) and T-helper 2 (Th2) have an important role in host defence in pulmonary tuberculosis. Aim of the work: Evaluate the role of some T-lymphocyte subsets in treatment failure of tuberculous patients. Subjects and methods: This study was carried out on 52 persons divided into 4 groups: group I control, group II active TB patients, group III Responders to treatment and group IV (non-responders). Bronchoalveolar lavage (BAL) and peripheral blood samples from patients and controls were examined for the frequency of CD4, CD8, Th1 and Th2 cells using flow cytometry. Results: Bronchoalveolar lavage CD4% was significantly increased in group III as compared to other groups, CD8% was significantly increased in group IV as compared to other groups, Th1% was significantly high in group III as compared to other groups. Blood CD4% was increased in group III as compared to other groups, CD8% was significantly increased in groups II and IV as compared to other groups. Th2% was significantly increased in group IV as compared to other groups.

Conclusion: CD4, CD8, Th1 and Th2 cells play a major role in immunology against TB infection and can be used as a marker for response to anti-tuberculous treatment.

Introduction

One-third of the world’s population was infected with Mycobacterium tuberculosis and it remains a major threat to public health [1] with an estimated 7.5 million cases each year (more than 95 percent in developing countries) [2]. Granuloma formation is an essential component of host immunity for controlling infection and dependent on activation of mycobacteria-reactive T lymphocytes [3], particularly CD4 and CD8 T cells [4,5]. CD4 cells help in antibody production and thus, they involved in class-II MHC-restricted responses but CD8 cells are cytotoxic cells helping in cell mediated responses
and lyse foreign cells expressing class I MHC molecules on their surface (class I restricted response) [6]. Granuloma formation, however, is a complex process that requires not only the activation of the lymphocytes, but also their recruitment with monocytes to the site of the infection, migration into the tissues, and juxtaposition around mycobacteria-infected macrophages [7]. This colocation facilitates the activation of bactericidal mechanisms in infected macrophages by T cell-derived cytokines [3]. One theory of immune regulation involves homeostasis between T-helper 1 (Th1) and T-helper 2 (Th2) activity. Th1 cells drive the type-1 pathway (cellular immunity) to fight viruses and other intracellular pathogens, eliminate cancerous cells and stimulate delayed-type hypersensitivity reactions. Th2 cells drive the type-2 pathway (humoral immunity) and up-regulate antibody production to fight extracellular organisms. Overactivation of either pattern can cause disease [8].

Tuberculosis needs new improved vaccine and immunodiagnostic. CD4 and CD8 cells play an important role in host defence to TB. Definition of the antigens recognized by these T cells is critical for improved understanding of the immunobiology of TB and for development of vaccines and diagnostics. Immunodominant antigens and epitopes have been defined using approaches targeting particular TB proteins or classes of proteins and by genome-wide discovery approaches. Antigens and epitopes recognized by CD4 and CD8+ cells show extensive breadth and diversity in TB [9].

**Aim of the work**

Evaluate the role of some T-lymphocyte subsets in treatment failure of tuberculous patients.

**Subjects and methods**

This study was carried out on 52 tuberculous persons in the Chest Department, Tanta University Hospitals from February 2012 to November 2014 and were divided into 4 groups: group I, included 10 non-smoker control persons (5 males, 5 females) and their mean age was 27.2 ± 1.3 years, group II included 17 non-smoker newly diagnosed active TB patients, before starting of anti-tuberculous treatment, (9 males, 8 females) and their mean age was 31.2 ± 2.3 years, group III included 15 non-smoker TB patients completed initial phase of anti-tuberculous treatment with sputum conversion to negative (9 males, 6 females) and their mean age was 30.2 ± 2.9 years (Responders), and group IV included 10 non-smoker TB patients completed initial phase of anti-tuberculous treatment and their sputum was still positive (6 males, 4 females) with the mean age 36.6 ± 4.7 years (non-responders). All tuberculous patients were collected from many Chest Hospitals and outpatient clinics. Informed consents were taken from all participants and the protocol of this work was discussed with them (Tables 1–8).

**Inclusion criteria**

TB patients should fulfill the following:

1. Clinical manifestation of TB.
2. Radiological manifestation of TB.

**Exclusion criteria**

1. HIV positive patients.
2. Auto-immune diseases.
3. Patients with Lymphoma, Leukaemia or blood diseases.
4. Patients under cortico-steroids or immune-suppressant agents.
5. D.M
6. Any contraindication to fiberoptic bronchoscopy.

All subjects were subjected to the following:

a. Full history and clinical examination.
b. Plain X-ray chest P.A view.
c. Routine investigations including ESR, complete blood picture and fasting and post-prandial blood sugar level.
d. Z.N stain examination of sputum 3 successive times.
e. HIV antibody assay by Elisa.
f. Bronchoscopy and broncho-alveolar lavage (BAL) Bronchoscopy was performed according to standard guidelines. Thirty minutes prior to the procedure patients received i.m atropine sulphate ampoule, 1 mg/ml (C.I.D company) and i.v 5 mg midazolam, 5 mg/ml (Sunny Pharmaceutical). Local anaesthesia of the oropharynx was achieved by Lidocaine spray 10% (Cairo Company) until gag reflexes subsided. Bronchoscopy was performed using Olympus BF type P10, Tokyo, Japan bronchoscope through which, Bupivacaine (Al-Debeiky Pharmaceutical) 5 mg/ml was instilled to keep the tracheo-bronchial tree anaesthetized, then, 120 ml of normal saline in aliquots of 20 ml was instilled into a subsegment of the right middle lobe. BAL fluid was then immediately aspirated by gentle hand suction into plastic tubes and kept at 4 °C [10].
g. Examination of blood and BAL for CD4, Th1, Th2 and CD8 cells percent

- Processing of BAL cells
  BAL samples were filtered through a two-layer sterile gauze into sterile plastic vials (Falcon, Oxnard, CA), centrifuged at 4 °C and 500g for 10 min. The supernatant was removed and cells were washed twice in (phosphate buffered saline) PBS. The total cell number was counted using a Neubauer haemocytometer (Brand, Wertheim, Germany). Differential cell counts were performed after Giemsa staining (Merck, Darmstadt, Germany) of cell smears with 1000 cells per slide counted [10].
- Isolation of peripheral blood mononuclear cells (PBMC)
  Venous blood was drawn into sterile plastic containers containing 0.2 ml EDTA (Sarstedt, Nümbrecht, Germany) prior to the bronchoscopy and was separated on a gradient of Ficoll with a density of 1.077 g/l for 20 min at 1330 g. The band of peripheral blood mononuclear cells (PBMC) at the interface was collected and washed twice [10].
Analysis of T lymphocyte subsets in PBMC from peripheral blood and BAL cells.

PBMC and BAL cells were incubated in the presence of fluorescein-conjugated MoAb against the surface markers CD4 & CD8 for 20 min at room temperature in the dark. Non-specific fluorescence was detected by incubating mouse IgG of the same isotype, but with irrelevant antigen specificity. After two washes with PBS the cells were analysed by flow cytometry (FACSCalibur; Becton Dickinson, Heidelberg, Germany) [10].

- Analysis of Th1 and Th2 in T lymphocytes in PBMC from peripheral blood and BAL cells.

PBMC and BAL cells were stimulated with $10^{-8}$ M PMA and $10^{-6}$ M Ca-Ionophore A23187 for 4 h and cytokine release was blocked by adding brefeldin A (10 ng/ml). Subsequently cells were washed twice with PBS/1% fetal calf serum (FCS)/0.1% NaN₃ and fixed in 4% paraformaldehyde in PBS pH 7.4 for 15 min on ice. Cells were then incubated for 30 min at room temperature with (phycoerythrin) PE-conjugated MoAbs specific for CD4. Following two washes in PBS/1%

**Table 1** Total leucocyte count, percent of lymphocytes and neutrophils in bronchoalveolar lavage fluid in the 4 groups.

<table>
<thead>
<tr>
<th></th>
<th>GI</th>
<th>GII</th>
<th>GIII</th>
<th>GIV</th>
<th>F test</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total leucocyte count</td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>430–610</td>
<td>950–1580</td>
<td>420–800</td>
<td>900–1350</td>
<td>70.334</td>
<td>0.001</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>515 ± 50.17</td>
<td>1185 ± 196.24</td>
<td>632 ± 119.83</td>
<td>1127 ± 133.57</td>
<td></td>
<td></td>
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<tr>
<td>Percent of lymphocytes</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>12.8–15.6</td>
<td>19.3–29</td>
<td>13.5–21.2</td>
<td>24.1–35.9</td>
<td>76.830</td>
<td>0.001</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>13.99 ± 1</td>
<td>22.99 ± 2.99</td>
<td>17.15 ± 2.03</td>
<td>30.05 ± 3.76</td>
<td></td>
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</tr>
<tr>
<td>Percent of neutrophils</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>1.6–3.7</td>
<td>2.4–6.4</td>
<td>2.1–3.9</td>
<td>3.5–6.1</td>
<td>14.392</td>
<td>0.001</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>2.83 ± 0.702</td>
<td>3.52 ± 1.11</td>
<td>3.12 ± 0.571</td>
<td>4.98 ± 0.748</td>
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<td></td>
</tr>
</tbody>
</table>

**Table 2** Percent of CD4, CD8, Th1 and Th2 cells in bronchoalveolar lavage fluid in the 4 groups.

<table>
<thead>
<tr>
<th></th>
<th>GI</th>
<th>GII</th>
<th>GIII</th>
<th>GIV</th>
<th>F test</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Percent of CD4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>47.8–56.2</td>
<td>32.1–38.9</td>
<td>60.9–78.4</td>
<td>23.7–31.9</td>
<td>63.208</td>
<td>0.001</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>52.62 ± 2.98</td>
<td>35.04 ± 1.81</td>
<td>70.56 ± 5.43</td>
<td>27.18 ± 2.28</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Percent of CD8</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>21.9–26.9</td>
<td>27.5–36.8</td>
<td>15.8–27.01</td>
<td>36.2–44.1</td>
<td>50.033</td>
<td>0.001</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>24.09 ± 1.46</td>
<td>31.99 ± 2.77</td>
<td>21.15 ± 3.61</td>
<td>40.21 ± 2.22</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Percent of Th1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>35.2–42.1</td>
<td>51.1–59.1</td>
<td>45.0–75.0</td>
<td>27.0–55.0</td>
<td>37.443</td>
<td>0.001</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>38.25 ± 1.96</td>
<td>55.15 ± 2.2</td>
<td>62.0 ± 9.63</td>
<td>37.1 ± 9.33</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Percent of Th2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>0.15–0.39</td>
<td>0.19 – 0.42</td>
<td>0.21 – 0.45</td>
<td>0.19 – 0.48</td>
<td>0.392</td>
<td>0.760</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>0.276 ± 0.09</td>
<td>0.254 ± 0.064</td>
<td>0.262 ± 0.0617</td>
<td>0.242 ± 0.084</td>
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</tr>
</tbody>
</table>
the cells were permeabilized by using 0.1% saponin/PBS. Thereafter, cells were incubated with (peridinin–chlorophyll) PerCp-conjugated anti-IL-4, & (fluorescence isothiocyanate) FITC-conjugated anti-IFN-γ, for 20 min at room temperature in the dark and then washed twice with 0.1% saponin/PBS. Finally, the cells were resuspended in PBS and analysed by flow cytometry. The percentage of positive cells (CD4+/IL-

**Table 3** Total leucocyte count and percent of lymphocytes in blood in the 4 groups.

<table>
<thead>
<tr>
<th></th>
<th>GI</th>
<th>GII</th>
<th>GIII</th>
<th>GIV</th>
<th>F test</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total leucocytic count Range</td>
<td>5500–8100</td>
<td>6500–9500</td>
<td>5800–8200</td>
<td>5900–9100</td>
<td>4.162</td>
<td>0.011</td>
</tr>
<tr>
<td></td>
<td>Mean ± SD</td>
<td>6990 ± 884.9</td>
<td>7914.3 ± 918.9</td>
<td>7050 ± 704.3</td>
<td>7890 ± 1054.6</td>
<td></td>
</tr>
<tr>
<td>Percent of lymphocytes Range</td>
<td>26.6–35.8</td>
<td>19.5–29.1</td>
<td>23.5–38.3</td>
<td>19.8–28.7</td>
<td>16.592</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>Mean ± SD</td>
<td>31.09 ± 3.18</td>
<td>24.19 ± 2.59</td>
<td>30.49 ± 4.37</td>
<td>23.57 ± 2.78</td>
<td></td>
</tr>
</tbody>
</table>

**Table 4** Percent of CD4, CD8, Th1 and Th2 in blood in the 4 groups.

<table>
<thead>
<tr>
<th></th>
<th>GI</th>
<th>GII</th>
<th>GIII</th>
<th>GIV</th>
<th>F test</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Percent of CD4 Range</td>
<td>31.1–41.4</td>
<td>21.7–34.2</td>
<td>32.8–44.9</td>
<td>12.9–22.</td>
<td>101.973</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>Mean ± SD</td>
<td>36.54 ± 3.58</td>
<td>27.96 ± 3.51</td>
<td>38.34 ± 2.7</td>
<td>18.03 ± 2.66</td>
<td></td>
</tr>
<tr>
<td>Percent of CD8 Range</td>
<td>26–34.5</td>
<td>29.4–39.2</td>
<td>17.4–26.9</td>
<td>30–42.8</td>
<td>68.359</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>Mean ± SD</td>
<td>30.2 ± 2.68</td>
<td>34.07 ± 2.92</td>
<td>21.86 ± 2.05</td>
<td>36.29 ± 3.94</td>
<td></td>
</tr>
<tr>
<td>Percent of Th1 Range</td>
<td>0.5–2.01</td>
<td>0.2–0.45</td>
<td>0.75–1.7</td>
<td>0.13–0.3</td>
<td>60.905</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>Mean ± SD</td>
<td>1.148 ± 0.448</td>
<td>0.308 ± 0.065</td>
<td>1.205 ± 0.229</td>
<td>0.203 ± 0.051</td>
<td></td>
</tr>
<tr>
<td>Percent of Th2 Range</td>
<td>8.2–14.8</td>
<td>43.4–57.8</td>
<td>8.6–31</td>
<td>50.9–70.2</td>
<td>41.634</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>Mean ± SD</td>
<td>11.13 ± 1.81</td>
<td>50.73 ± 3.91</td>
<td>14.16 ± 4.83</td>
<td>61.11 ± 5.31</td>
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</tbody>
</table>

**Table 5** CD4/CD8 ratio in BAL in the 4 groups.

<table>
<thead>
<tr>
<th>CD4/CD8 ratio</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Range</td>
<td>1.87–2.41</td>
<td>0.934–1.415</td>
<td>2.25–4.62</td>
<td>0.537–0.881</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>2.19 ± 0.165</td>
<td>1.10 ± 0.131</td>
<td>3.42 ± 0.575</td>
<td>0.679 ± 0.091</td>
</tr>
<tr>
<td>F test</td>
<td>66.171</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>P value</td>
<td>0.001</td>
<td></td>
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</table>

<table>
<thead>
<tr>
<th></th>
<th>I &amp; II</th>
<th>I &amp; III</th>
<th>I &amp; IV</th>
<th>II &amp; III</th>
<th>II &amp; IV</th>
<th>III &amp; IV</th>
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</thead>
<tbody>
<tr>
<td>I</td>
<td>0.001</td>
<td>0.001</td>
<td></td>
<td>0.001</td>
<td>0.005</td>
<td>0.001</td>
</tr>
<tr>
<td>II</td>
<td></td>
<td></td>
<td></td>
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</tbody>
</table>
4+ and CD4+/IFN-\(\gamma^{+}\)) was also determined using isotype-matched IgG antibodies as a control. The relative fluorescence intensity (RFI) was calculated as intensity of cytokine staining/intensity of isotype matched control antibody staining according to Nakamura [11].

**Results**

In BAL, CD4% was significantly decreased in group IV as compared to other groups and there was a significant decrease in group II as compared to groups I and III but group III was significantly increased as compared to group I. CD8% was significantly increased in group IV as compared to other groups and there was a significant increase in group II as compared to other groups and there was a significant increase in group I as compared to group II but there was an insignificant difference between groups II and IV. Th1% was significantly decreased in groups II and IV as compared to other groups and there was a significant decrease in group IV as compared to group II but group III was insignificantly increased as compared to group I. CD8% was significantly increased in groups II and IV as compared to other groups and there was a significant increase in group I as compared to group II but there was an insignificant difference between groups II and IV. Th1% was significantly decreased in groups II and IV as compared to other groups and there was an insignificant difference in group IV as compared to group II but there was a significant increase in group III as compared to group I and also, II and IV. Th2% was significantly higher in groups II and IV as compared to other groups and there was a significant increase in group IV as compared to group II but there was an insignificant difference between groups I and III.

In blood, CD4% was significantly decreased in groups II and IV as compared to other groups and there was a significant decrease in group IV as compared to group II but group III was insignificantly increased as compared to group I. CD8% was significantly increased in groups II and IV as compared to other groups and there was a significant increase in group I as compared to group II but there was an insignificant difference between groups II and IV. Th1% was significantly decreased in groups II and IV as compared to other groups but there was an insignificant difference between groups I and IV and also, II and IV. Th2% was significantly higher in groups II and IV as compared to other groups and there was a significant increase in group IV as compared to group II but there was an insignificant difference between groups I and III.

**Table 6** Th1/Th2 ratio in BAL in the 4 groups.

<table>
<thead>
<tr>
<th>TH1/TH2 ratio</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Range</td>
<td>105.53–254.67</td>
<td>130.95–291.58</td>
<td>144.44–347.62</td>
<td>60.42–261.90</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>153.72 ± 54.84</td>
<td>228.13 ± 48.69</td>
<td>247.26 ± 64.84</td>
<td>165.15 ± 56.05</td>
</tr>
<tr>
<td>F test</td>
<td>7.912</td>
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<td></td>
</tr>
<tr>
<td>P value</td>
<td>0.001</td>
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</table>

<table>
<thead>
<tr>
<th>I &amp; II</th>
<th>I &amp; III</th>
<th>I &amp; IV</th>
<th>II &amp; III</th>
<th>II &amp; IV</th>
<th>III &amp; IV</th>
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<tr>
<td>0.003</td>
<td>0.001</td>
<td>0.638</td>
<td>0.379</td>
<td>0.012</td>
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</tr>
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</table>

**Table 7** CD4/CD8 ratio in blood in the 4 groups.

<table>
<thead>
<tr>
<th>CD4/CD8 ratio</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Range</td>
<td>0.926–1.551</td>
<td>0.565–1.069</td>
<td>1.413–2.363</td>
<td>0.430–0.644</td>
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<tr>
<td>Mean ± SD</td>
<td>1.22 ± 0.182</td>
<td>0.826 ± 0.121</td>
<td>1.77 ± 0.230</td>
<td>0.497 ± 0.059</td>
</tr>
<tr>
<td>F test</td>
<td>1393.105</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P value</td>
<td>0.001</td>
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</table>

<table>
<thead>
<tr>
<th>I &amp; II</th>
<th>I &amp; III</th>
<th>I &amp; IV</th>
<th>II &amp; III</th>
<th>II &amp; IV</th>
<th>III &amp; IV</th>
</tr>
</thead>
<tbody>
<tr>
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<td>0.001</td>
<td>0.001</td>
<td>0.001</td>
<td>0.001</td>
<td>0.001</td>
</tr>
</tbody>
</table>

**Table 8** Th1/Th2 ratio in blood in the 4 groups.

<table>
<thead>
<tr>
<th>TH1/TH2 ratio</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Range</td>
<td>0.051–0.156</td>
<td>0.005–0.009</td>
<td>0.039–0.147</td>
<td>0.002–0.005</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>0.102 ± 0.035</td>
<td>0.006 ± 0.001</td>
<td>0.089 ± 0.021</td>
<td>0.003 ± 0.001</td>
</tr>
<tr>
<td>F test</td>
<td>87.242</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P value</td>
<td>0.001</td>
<td></td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>I &amp; II</th>
<th>I &amp; III</th>
<th>I &amp; IV</th>
<th>II &amp; III</th>
<th>II &amp; IV</th>
<th>III &amp; IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.001</td>
<td>0.108</td>
<td>0.001</td>
<td>0.001</td>
<td>0.738</td>
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as compared to groups II and IV but there was an insignificant difference between groups I, III and II and IV.

Discussion

This study found that CD4 cells increased in responders but decreased in active TB and patients with treatment failure in contrast with, CD8 cells decreased in responders but increased in active TB and non-responder CD4 cells are most important in the protective response against \( M. \) \textit{tuberculosis}. In humans, the pathogenesis of HIV infection has demonstrated that the loss of CD4 cells greatly increases susceptibility to both acute and re-activation TB [12]. The primary effect or function of CD4 cells is the production of IFN-\( \gamma \) and possibly other cytokines, sufficient to activate macrophages. In a murine model of chronic persistent \( M. \) \textit{tuberculosis} infection [13], CD4 cell depletion caused rapid re-activation of the infection. Apoptosis or lysis of infected cells by CD4 cells may also play a role in controlling infection [14]. CD8 cells are also capable of secreting cytokines and thus may play a role in regulating the balance of Th1 and Th2 cells in the lungs of patients with pulmonary TB. Yu et al., analysed CD4 and CD8 populations from patients with a rapid, slow, or intermediate regression of disease while receiving therapy and found that slow regression was associated with an increase in CD8 cells in the BAL [15]. Taha et al. found increased CD8 T cells in the BAL of patients with active TB, along with striking increases in the number of BAL cells expressing IFN-\( \gamma \) and IL-12 mRNA. These studies point to a potential role for CD8 cells in the immune response to TB [16]. \( M. \) \textit{tuberculosis}-infected macrophages appear to be diminished in their ability to present antigens to CD4 cells, which leads to persistent infection [17]. In contrast, Nyendak et al., concluded that mycobacterial tuberculosis specific CD8 cell response declines with anti-tuberculosis treatment but not CD4 and could be a surrogate marker of response to therapy [18]. An effective T cell response determines whether the infection resolves or develops into clinically evident disease. Consequently, there is great interest in determining which T cell subsets mediate anti-mycobacterial immunity, delineating their effector functions. On the other hand, many aspects remain unsolved in understanding why some individuals are protected from TB infection while others go on to develop disease. Several studies have demonstrated that CD4 T cells are involved in protection against TB, as supported by the evidence that CD4 T cell depletion is responsible for TB reactivation in HIV-infected individuals. There are many subsets of CD4T cells, such as Th1, Th2, Th17, and regulatory T cells and all these subsets co-operate or interfere with each other to control infection; the dominant subset may differ between active and latent TB infection cases. TB specific-CD4 Th1 cell response is considered to have a protective role for the ability to produce cytokines such as IFN-\( \gamma \) or TNF-\( \alpha \) that contribute to the recruitment and activation of innate immune cells, like monocytes and granulocytes. Thus, while other antigen specific T cells such as CD8 T cells, natural killer cells and CD1-restricted T cells can also produce IFN-\( \gamma \) during TB infection, they cannot compensate for the lack of CD4 T cells. Although the role of CD8 T cells in TB is less clear than CD4 T cells, they are generally considered to contribute to optimal immunity and protection. CD8 T cells possess a number of anti-microbial effector mechanisms that are less prominent or absent in CD4 Th1 and Th17 T cells and display a functional profile termed “multifunctional so it can be a better marker of protection in TB than CD4 T cells due to their release of cytokines or cytotoxic molecules, which cause apoptosis of target cells [19]. Tuberculous bacilli resides primarily in a vacuole within the macrophage, and thus, MHC class II presentation of mycobacterial antigens to CD4 T cells is an obvious outcome of infection. These cells are most important in the protective response against TB. Murine studies with antibody depletion of CD4 T cells [20] or the use of gene-disrupted mice have shown that the CD4 T cell subset is required for control of infection. The primary effector function of CD4 T cells is the production of IFN-\( \gamma \) and possibly other cytokines, sufficient to activate macrophages. In CD4 deficient mice, levels of IFN-\( \gamma \) were severely diminished very early in infection. NOS2 expression by macrophages was also delayed in the CD4 T cell deficient mice, but returned to wild type levels in conjunction with IFN \( \gamma \) expression. IFN-\( \gamma \) levels overall were similar in the lungs of CD4 T cell-depleted and control mice, due to IFN \( \gamma \) production by CD8 T cells. Moreover, there was no apparent change in macrophage NOS2 production or activity in the CD4 T cell-depleted mice. This indicated that there are IFN-\( \gamma \) and NOS2-independent, CD4 T cell-dependent mechanisms for control of TB. Therefore, other functions of CD4 T cells are likely to be important in the protective response and also for the future studies of new vaccine design against TB [21]. Rozot et al., Concluded that flow cytometry-based assay and the combination of two immunological measures; mycobacterial tuberculosis specific CD4 T-cells and the detection of mycobacterial tuberculosis specific CD8 T-cell responses, represents a powerful diagnostic tool to discriminate between active TB and latent infection [22]. Afzal concluded that CD4 and CD8 T cells percentages may help to find out the immune status of TB patients before and after the completion of treatment [23]. BAL Th1% increased in responder and active TB groups but decreased in non-responders, Hickman et al. suggested that macrophages and dendritic cells (DCs), albeit both being APC, respond differently following \( M. \) \textit{tuberculosis} infection. DCs only, support Th1 priming because of their unique ability to make IL-12 in response to \( M. \) \textit{tuberculosis} infection. Dendritic cells capture antigens of \( M. \) \textit{tuberculosis} and transport it to the lymph nodes for T cell priming and Th1 polarization [24]. Zhang et al. studied cytokine production in pleural fluid and found high levels of IL-12 after stimulation of pleural fluid cells with \( M. \) \textit{tuberculosis} [25]. Lin et al., observed that TB patients showed evidence of high IFN \( \gamma \) production and no IL-4 secretion by the lymphocytes in the lymph nodes [26]. Robinson et al. found increased levels of IFN-\( \gamma \) mRNA in situ in BAL cells from patients with active pulmonary TB [27]. Condos et al., found that patients with limited TB have an alveolar lymphocytosis in infected regions of the lung and these lymphocytes produce high levels of IFN-\( \gamma \) [28]. In the present work, Th1% decreased in blood of patients with active TB and also, with treatment failure but increased in responders, reversely, Th2% increased in active TB and treatment failure but decreased in responders, Lienhardt found that in Africa, tuberculosis is associated with low Th1 and high Th2 activity in vivo, whereas close exposure to tuberculosis is associated with a high Th1/Th2 ratio and patients with favourable outcome after treatment exhibit a higher Th1/Th2 ratio compared to patients with poor clinical outcome [29]. In mice infected with virulent strain of \( M. \)
Assessment of treatment response in tuberculous patients

In tuberculosis, initially Th1 like and later Th2 like response has been demonstrated [30]. Multiple studies concluded that patients with TB had a Th2-type response in their peripheral blood, as Huygen et al., reported that PBMC, when stimulated in vitro with PPD, release lower levels of IFN-γ and IL-2, as compared to tuberculin positive healthy subjects [31]. Vliek et al., reported reduced IFN-γ [32] also, Sanchez et al., found increased IL-4 secretion [33] and Sureau et al., concluded that there was increased number of IL-4 secreting cells in pulmonary TB [34], Shahemabadi et al., found that IL-12 production by monocytes in response to M. tuberculosis total sonicate antigens was significantly decreased in the active TB patients but IL-10 production showed a significant increase in comparison to healthy individuals [35]. The present study found that Th1% decreased in BAL and blood of patients with treatment failure, Sodhi et al. [36] have demonstrated that low levels of circulating IFN-γ in peripheral blood were associated with severe clinical TB and in patients with far advanced or cavitary disease, no Th1-type lymphocytosis is present. Cozmei et al., found that plasma and pleural fluid cytokine analysis at the outset of tuberculosis disease reflect the same Th1 response dominated by IFN-gamma. In opposition, very low IFN-gamma levels were recorded in neoplastic pleural fluids [37]. Both types of cytokines (Th1 and Th2) were secreted in response to in vitro PPD stimulation of PBMCs and had different evolutions in moderate and advanced TB. Thus, IFN-gamma and IL-4 production after 6 months-treatment decreased in moderate TB and increased in severe disease. Experimentally, Th1 polarization is readily transformed to Th2 dominance through depletion of intracellular glutathione, and vice versa. Mercury depletes glutathione and polarizes towards Th2 dominance. Several nutrients and hormones measurably influence Th1/Th2 balance, including plant sterols/sterolins, melatonin, probiotics, progesterone, and the minerals selenium and zinc. The long-chain omega-3 fatty acids EPA (eicosapentaenoic acid) and DHA (docosahexaenoic acid) significantly benefit diverse inflammatory and autoimmune conditions without any specific Th1/Th2 effect. Th1/Th2-based immunotherapies, e.g., T-cell receptor (TCR) peptides and interleukin-4 (IL-4)-injections, have produced mixed results to date [8]. It was concluded that CD4 and Th1 cells increased in responders to anti-tuberculosis treatment but CD8 cells increased in patients with treatment failure locally and in peripheral blood, also Th2 cells increased in non-responders only in peripheral blood. So, CD4, CD8, Th1 and Th2 cells play a major role in immunity against TB infection and can be used as a marker for response to anti-tuberculous treatment and future studies must be done towards new treatment used these cells.

Conflict of interest

We have no conflict of interest to declare.

References


