ASSESSMENT OF LYMPHOCYTE SUBGROUPS IN CHRONIC BRUCELLOSIS BEFORE AND AFTER IMMUNOTHERAPY

D. Tahamzadeh¹, A. Massoud¹*, G. Samar² and B. Nikbin¹

¹) Department of Immunology, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran
²) Department of Infectious Diseases, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran

Abstract- Brucellosis is an infectious disease and it seems that it affects human immune system and can cause acute, subacute and chronic clinical features. Forty patients suffering from chronic brucellosis were studied for CD3⁺, CD4⁺, CD8⁺, CD19⁺, cells and CD4⁺/CD8⁺ ratio by flow cytometry before and after treatment with antibiotics and immunopotentiators. The results were compared with 15 healthy controls. The patients were divided into three groups: 1) antibiotic, 2) levomisole + antibiotic, and 3) treated with cimetidine + antibiotic. The results showed a significant decrease of B cells (CD19⁺), CD4⁺ T cells and CD4⁺/CD8⁺ ratio in comparison with normal subjects before treatment. In the first group, significant decrease of CD4⁺ T cells and CD4⁺/CD8⁺ ratio inversion were seen after immunotherapy. The 3rd group showed the best correction of phenotype quantity. In other words, significant increase of CD4⁺ T cells, CD19⁺ B cells, CD4⁺/CD8⁺ ratio and decrease of CD8⁺ T cells were seen with cimetidine immunotherapy.

Key words: Brucellosis, immunotherapy, cimetidine, levamisole, flow cytometry

INTRODUCTION

Brucellosis is a worldwide zoonotic infection and a problem of developing countries. Its course is closely related to the host cellular immunity (1). Different resistance of Brucella species depends on the cellular response mediated by T cells and macrophages and certain cytokines like IL-12, IFN-γ and TNF-α, in vitro (2-4). It is shown that brucellosis affects immune response (5,6). It is suggested that potentiation of cellular immune response can control the course of disease because it is observed that in acute cases, there is a normal response to polyclonal mitogens but in chronic cases there is not (7). Based on these observations, Printzis used levamisole and IFN-α in chronic brucellosis patients (8). Cimetidine is a H-2 antagonist which has shown immunoregulatory effects (9-13). In the present study CD3, CD4, CD8, CD19 blood lymphocytes, CD4⁺/CD8⁺ ratio in chronic human brucellosis patients, and the effect of levamisole and cimetidine as immunopotentiators were investigated by flow cytometry. Iran is one of the hyperendemic regions of brucellosis. According to the pathogenesis of this disease, it is suggested that immunopotentiators along with antibiotics can be a better therapy and reveal disease mechanism.

MATERIAL AND METHODS

Patients

Forty untreated chronic brucellosis patients (25 male, 15 female; mean age: 33.7±13.2 years) were diagnosed by history, duration of clinical symptoms (more than 1 year) and positive antiglobulin Coombs titers against Brucella antigen (5). The control group comprised of 15 normal subjects (8 male, 7 female,
Lymphocyte subgroups in chronic brucellosis

mean age: 25±3 years). Patients were randomly divided in three groups according to their therapeutic regimen:

1) 21 patients (12 male, 9 female) were treated with conventional antibiotics (doxycycline 200 mg/day and rifampin 600 mg/day) for 6 weeks.

2) 6 patients (5 male, 1 female) who took levamisole Hcl (150 mg/day) as immunotherapy in addition to conventional antibiotics for 6 weeks.

3) 12 patients (7 male, 5 female) who received cimetidine (600 mg/day,3 tablets) as immunotherapy along with conventional antibiotic therapy for 6 weeks.

Flow Cytometric Analysis

Blood samples were collected before and after treatment; 5 ml of whole blood with EDTA (1.5 mg/ml) was collected. Leukocyte counting was done by Coulter counter and absolute number of lymphocytes was estimated. Two milliliters of this blood was used for flow cytometric analysis. Samples were analyzed before 24 hours and were not stored in <4°C because low temperature causes CD4 and CD8 markers to decline. The method described by Jackson and Ormerod(14,15) was used. According to this method 100 µl of whole blood was suspended with specific monoclonal antibody in three separate tubes. After 20 minutes, red blood cells were separated by the lysing buffer and were washed by PBS (1%). Monoclonal antibodies included: Anti CD14, FITC/CD45. RPE (DAKO), Anti CD4. FITC/CD8, RPE (DAKO) and Anti CD3, FITC/CD19, RPE (DAKO). Analysis was done by FACs-can (Becton-Dickinson, Mountain view CA). The resulting percentages were used to estimate the absolute number of lymphocytes and were used for statistical analysis.

The results of flow cytometric analysis of patients before treatment were compared with those of the normal control group by Student t-test. After treatment, the effect of treatment was analyzed in each group by paired t-test.

RESULTS

The results of the lymphocyte subtype analysis of patients before treatment and normal subjects are shown in figure1, and table 1. There was not a significant difference between the percentage of T cells (CD3+) in chronic brucellosis patients and control subjects, but a significant B cell (CD19) difference was found (p<0.05). CD4+ T cells have a significant decrease in patients (p<0.001). No significant increase of CD8+ T cells was found (p<0.05).

These results showed significant decreases of CD4/CD8 ratio of patients (p<0.001).

Comparison of the lymphocyte subtypes of patients, before and after treatment, showed that in patients with antibiotic therapy, the difference in T cell (CD3+) percentage was not significant (p= 0.55, paired t-test). In this group, the mean percentage of B cells (CD19+) had a decrease but it was not significant (p= 0.59, paired t-test). Significant decrease in CD4+ T cells (p= 0.04, paired t-test) and nonsignificant decrease in CD8+ T cells (p= 0.87, paired t-test) were found. Mean CD4/CD8 ratio showed significant decline (p= 0.01, paired t-test). Results of mean percentages are summarized in figure 2 and table 2.
Table 1. The absolute number and percentage of lymphocyte subpopulations in patients and control group

<table>
<thead>
<tr>
<th></th>
<th>Lymphocytes/µl</th>
<th>CD3 (%)</th>
<th>CD19 (%)</th>
<th>CD4 (%)</th>
<th>CD8 (%)</th>
<th>CD4/CD8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>4750±1400</td>
<td>75.96±6.47</td>
<td>11.93±5.45</td>
<td>45.64±7.21</td>
<td>30.03±5.64</td>
<td>1.82±0.40</td>
</tr>
<tr>
<td>Patients</td>
<td>3492.52±1664.16</td>
<td>73.8±10.01</td>
<td>8.95±4.53</td>
<td>34.8±8.49</td>
<td>31.72±8.58</td>
<td>1.19±0.45</td>
</tr>
</tbody>
</table>

Table 2. The absolute number and percentage of lymphocyte subpopulations in patients before and after treatment with antibiotics

<table>
<thead>
<tr>
<th></th>
<th>Lymphocytes/µl</th>
<th>CD3 (%)</th>
<th>CD19 (%)</th>
<th>CD4 (%)</th>
<th>CD8 (%)</th>
<th>CD4/CD8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before</td>
<td>2602.52±808.16</td>
<td>74.38±8.62</td>
<td>10.09±3.17</td>
<td>36.71±7.64</td>
<td>31.38±9.05</td>
<td>1.29±0.48</td>
</tr>
<tr>
<td>After</td>
<td>2794.15±774.06</td>
<td>72.19±8.49</td>
<td>7.47±2.73</td>
<td>32.38±7.39</td>
<td>31.85±7.88</td>
<td>1.00±0.24</td>
</tr>
</tbody>
</table>

Fig. 3. Average percentage of lymphocytes in patients before and after treatment with antibiotics and levamisole

Table 3. The absolute number and percentage of lymphocyte subpopulations in patients before and after treatment with antibiotics and levamisole

<table>
<thead>
<tr>
<th></th>
<th>Lymphocytes/µl</th>
<th>CD3 (%)</th>
<th>CD19 (%)</th>
<th>CD4 (%)</th>
<th>CD8 (%)</th>
<th>CD4/CD8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before</td>
<td>2992.66±508.44</td>
<td>77.00±6.48</td>
<td>7.00±4.93</td>
<td>31.66±9.79</td>
<td>30.83±6.11</td>
<td>1.00±0.40</td>
</tr>
<tr>
<td>After</td>
<td>2313±393.66</td>
<td>58.66±5.98</td>
<td>11.00±4.51</td>
<td>37.66±10.28</td>
<td>27.16±6.49</td>
<td>1.30±0.10</td>
</tr>
</tbody>
</table>

In patients who were treated by levamisole and conventional antibiotics, a significant decline in T cells (CD3⁺) was found after treatment (p= 0.01, paired t-test, Figure 3, table3). The average percentage of B cells (CD19⁺) showed an increase but it was not significant (p= 0.05, paired t-test). The increase in CD4⁺ T cells after treatment was not significant (p=0.26, paired t-test). CD8⁺ T cells did not show a significant difference after treatment (p= 0.25, paired t-test) and the increased CD4/CD8 ratio was not statistically significant either (p= 0.19, paired t-test).

The third group comprised of patients treated with cimetidine and conventional antibiotics (Fig. 4, Table 4).

Fig. 4. Average percentage of lymphocytes and ratios in patients, before and after treatment with antibiotics and cimetidine
Lymphocyte subgroups in chronic brucellosis

Table 4. The absolute number and percentage of lymphocyte subpopulations in patients, before and after treatment with antibiotics and cimetidine

<table>
<thead>
<tr>
<th></th>
<th>Lymphocytes/µl</th>
<th>CD3 (%)</th>
<th>CD19 (%)</th>
<th>CD4 (%)</th>
<th>CD8 (%)</th>
<th>CD4/CD8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before treatment</td>
<td>3018.16±581.50</td>
<td>70.66±11.25</td>
<td>8.33±3.65</td>
<td>33.41±9.46</td>
<td>31.75±9.00</td>
<td>1.14±0.41</td>
</tr>
<tr>
<td>After treatment</td>
<td>2491.41±879.38</td>
<td>72.75±5.78</td>
<td>8.75±2.48</td>
<td>45.25±10.50</td>
<td>24.41±3.60</td>
<td>1.85±0.52</td>
</tr>
</tbody>
</table>

The increase in mean percentage of T cells (CD3⁺) and B cells (CD19⁺) was not statistically significant (p= 0.6, paired t-test) (p= 0.75, paired t-test). The increase in CD4⁺ T cells (p<0.05) was significant (p=0.002, paired t-test) and a significant increase in CD8⁺ T cells (p<0.05) was also seen (p=0.009, paired-test). Mean CD4/CD8 ratio after treatment showed a significant difference (p=0.002, paired t-test).

DISCUSSION

Gazapo (1990) et al. found alterations in the immunological index (CD4/CD8 ratio), a decrease in CD4⁺ T cells, and CD11b⁺ monocytes, and unchanged CD20⁺ B cells as brucellosis progresses to chronic stages. They suggested that with relapsing disease, CD4/CD8 ratio would be inverted (7). In 1996, Pourfathollah et al. found significant differences in chronic and subacute patients for CD8⁺ and CD4⁺ T cell percentages and CD4/CD8 ratios, which were not significant in acute brucellosis by immunohistochemical method (5). Moreno et al. (1998) reported a nonsignificant difference in chronic and subacute patients for CD8⁺ and CD4⁺ T cell percentages and CD4/CD8 ratios, which were not significant in acute brucellosis by immunohistochemical method (5).

In the present study, we did not find a significant change in the mean percentage of CD3⁺ T cells but the reduction of CD4⁺ T cells and CD4⁺/CD8⁺ ratio, and the increase in CD19⁺ B cells were significant. It seems that quantitative alterations in lymphocytes is due to a deviation in cytokines released from leukocytes induced by infection (2,18). It has been suggested that as the disease progresses to chronic stages, suppressor cells are induced to change the aspect of the response, causing a decrease in one subtype of lymphocyte or its function, and an increase in others, so a decline of cellular response and an increase of B cells will be seen. Therefore it is reasonable to use specific immunopotentiators along with conventional treatments to correct these deviations, and study the mechanism of disease and the immune responses involved in it. Printzis et al. (1994) reported an enhancing effect of α-IFN and levamisole on T cell blast formation, with recovery of clinical symptoms in chronic brucellosis patients (8). Pokrovski et al. (1992) showed an enhancing effect of Thymohexin on the DNA repair system and inhibition of DNA sensitivity to destruction by oxygen free radicals (19, 20). Gazapo et al. (1990) suggested that the immunological index (CD4/CD8 ratio) would be inverted as brucellosis evolves to the chronic state (7). The immunomodulatory effect of levamisole has been shown before (8,20). In our study, levamisole caused a nonsignificant increase in CD4⁺, CD3⁺ T cells and CD4/CD8 ratio. It is suggested that short-term (1 month) levamisole administration may have been responsible for this failure, which could be overcome by an increased duration of immunopotentiator therapy. The importance of the function of lymphocytes, rather than the quantity of subpopulations is also noteworthy. If the function is improved, quantities will subsequently recover. Cimetidine immunotherapy causes favorable results, presumably by interfering with the function of CD8⁺ T cells, and not by their quantity. The antagonistic effect of cimetidine on H2-receptor and inhibition of CD8⁺ T suppressor secretory compounds was proved by Sahasrabudhe and Schnaper (21,22). It seems that cimetidine corrects the cellular response by improving secretion of specific cytokines, and thus improving subtype quantities. In other words, inhibition of suppressor cytokine(s) by cimetidine causes an up-regulation of T helper cells and may cause a Th1-Th2 balance which improves cellular immune responses, and limits infection and symptoms related to cytokines. The
immunomodulatory effect of cimetidine on Th1 and Th2 deviations and balance is an important subject which should be further investigated.

Acknowledgements

We want to thank the personnel of Immunogenetic Laboratory, Tehran university of Medical Sciences, especially M. Naroinezhad and A. Danesh, for their great help in flow cytometry processing and Kakh Laboratory personnel, S. Rahnama, Dr. B. Vaziri, and Dr. Rayani for their help in data analysis and flow cytometry.

REFERENCES