Lymphocyte Subpopulations in Patients With Acute Brucellosis

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Abstract: The aim of this work was to evaluate changes in lymphocyte subpopulations, especially helper and cytotoxic T cells, in acute brucellosis patients undergoing treatment. Forty-three acute brucellosis patients were included in the study. Twenty healthy subjects served as controls. Total lymphocytes and the CD3⁺, CD4⁺, CD8⁺, CD19⁺ and CD (16⁺56)⁺ subpopulations were counted by two-color flow cytometric analysis. The CD4⁺ counts in patients before and after treatment were not statistically different (p = 0.7), but healthy subjects had significantly more of these cells (p = 0.001 and p = 0.001 compared to pre- and post-treatment patients, respectively). The CD8⁺ counts in acute brucellosis patients decreased after treatment (p = 0.004), but remained higher in both pre- and post-treatment samples than in healthy subjects (p = 0.001 and p = 0.01 respectively). Neither the total leukocyte counts nor the numbers of cells in any subpopulation correlated with blood culture results (positive or negative). No statistically significant differences in the patients’ CD4⁺ T cell counts were observed between the pre- and post-treatment periods, and the count was higher in healthy subjects. Counts of CD8⁺ T cells increased in acute brucellosis patients, and although they decreased after treatment, they remained higher than in the controls. In view of this increase, it was concluded that CD8⁺ T cells could be the major component in immunity against brucellosis.

Key Words: lymphocyte subpopulations, brucellosis, treatment.

Introduction

Brucellosis is one of the most important zoonoses affecting human welfare and livestock health worldwide. The disease is caused by bacteria of the genus Brucellae, which comprises several species differing in host affinity and virulence (1).

Cellular immune responses are a critical part of the host’s defense against intracellular bacterial infections (2). The response against Brucellae spp. involves the whole gamut of the immune system from innate to adaptive immunity. In brucellosis, the different arms of the immune system, namely antigen-presenting cells, NK cells, CD4⁺ and CD8⁺ T cells and B cells, act together to provide a coordinated response (3). However, the primary mechanism of control involves cell-mediated immunity rather than antibodies, although some immunity against re-infection is provided by serum immunoglobulins (3-5). T-cell counting is the standard method for evaluating the cellular immune response against intracellular pathogens. Previous studies on murine models have shown that cytokine-secreting CD4⁺ T cells are the fundamental components of immunity against Brucella. Nevertheless, it is known that CD8⁺ T cells are very important in protective immunity (4).

Our aim in this study was to evaluate lymphocyte subpopulations in acute brucellosis patients during the pre- and post-treatment periods. We also investigated whether changes in the numbers of these cells correlated with blood culture results.

Material and Methods

The forty-three patients included in the study were admitted to our hospital with fever and musculoskeletal system dysfunction evidenced by generalized aches and pains and associated with fatigue, prostration and mental depression. Clinical and laboratory findings supported a diagnosis of brucellosis. The control subjects were 20
healthy persons (11 men and 9 women) with no history of Brucella infection. The ages of the patients and controls were 18-64 years (36.42 ± 14.5 years) and 19-60 years (36.6 ± 12.8 years) respectively.

The controls had negative STA and 2-ME test results, showed an erythrocyte sedimentation rate (ESR) within normal limits and had no complaints. Exclusion criteria for the healthy control subjects included acute/chronic diseases, smoking, alcohol consumption, medication, pregnancy, abnormalities in renal and liver function tests, and other bacterial infections.

Brucellosis was diagnosed on the basis of clinical, serological, bacteriological and epidemiological data. The diagnostic criteria were: isolation of a Brucella species from blood culture (BACTEC 9050, Becton-Dickinson Diagnostic Instrument System, Sparks, USA) or a single Brucella titre of ≥ 1/160 (by a standard tube agglutination test or Coombs), confirmed by a 2-mercaptoethanol (2-ME) test titre of ≥ 1/160, in association with compatible clinical findings. A 2-ME test ≥ 1/160 suggested ongoing infection. Using the BACTEC system, Brucellae were cultured and identified. Brucella species were isolated from the blood cultures in 21 cases (48.8%). The bacteria were biotyped by H₂S production, urease activity, and a dye sensitivity test (20—40 µg/ml basic fuchsine and growth in thionine). All the Brucellae were identified as B. melitensis. Disease activity was defined by the presence of typical signs and symptoms and a 2-ME titre ≥ 1/160. The patients’ data were recorded on prepared forms.

Questionnaires concerning symptoms were completed by the specialist. Ethical consent was granted by the Local Ethics Committee. All participants were informed about the purposes of the study. Before treatment, blood samples (5 ml) were drawn from all consenting patients and healthy subjects, treated with EDTA and subjected to flow cytometry to evaluate the lymphocyte subpopulations. After isolation of the peripheral mononuclear cells, a direct immunofluorescence method was used. Two-color flow-cytometric analysis was performed on a FACScan flow cytometer (Becton Dickinson, San Jose, CA) using a Becton Dickinson Simultest IMK-Lymphocyte kit: CD45/CD14, isotype control, CD3/CD19, CD3/CD4, CD3/CD8, and CD3/CD16+56. All lymphocyte subpopulations were measured in all blood samples by the same method. The cells were fixed with 1% paraformaldehyde and a total of 10,000 total events was recorded using CellQuest software (Becton Dickinson). Each lymphocyte subpopulation count was expressed as a percentage of the total number of lymphocytes. Criteria of quality included a greater than 95% total lymphocyte frequency in the analysis gate and a homogenous CD45⁻ population.

Therapy, using one combination of doxycycline plus rifampin for 45 days, was initiated as soon as the diagnosis of brucellosis was established. After treatment, the lymphocyte subpopulations of all the patients were evaluated again as described above. Responses to treatment were indicated by (a) improvements in clinical findings and (b) 2-ME tests with titres ≤ 1/80. Non-response to the treatment was identified by ongoing complaints such as fever, myalgia and other constitutional symptoms, and by a 2-ME titre ≥ 1/160.

The data were expressed as arithmetic means with standard deviations. A Mann-Whitney U test for between-groups, a Wilcoxon Signed Rank Test for in-groups, and Spearman’s correlation analysis, were all performed using the SPSS 12.0 packet software. P < 0.05 was considered statistically significant.

Results

Forty-three patients were included in the study. The mean ages of the patients and control subjects were 36.42 ± 14.5 years (range 18-64) and 36.6 ± 12.8 (range 19-60) respectively. The patient group consisted of 19 men and 24 women, and the control subjects comprised 11 men and 9 women.

The most common mode of disease transmission was consumption of un-pasteurized cheese and uncooked meat (51.2%). Symptoms were first noticed between 5 and 75 days after infection (mean ± SD = 24.1 ± 17.3). Thirty-six patients had fever on admission (83.7%). The most common complaints were weakness (95.3%), arthralgia (88.4%), fever (83.7%), sweating (74.4%), lethargy (48.8%) and headache (46.5). The most frequently affected organ was the liver, as indicated by the elevation of liver enzymes in the plasma (21.1%). Abdominal ultrasonographies revealed splenomegaly and hepatomegaly in eight (18.6%) and four (9.3%) patients, respectively. The most commonly affected joints were the waist and knee (18.6% for both). The physical and laboratory findings are shown in Table 1.
Total lymphocyte and natural killer (NK) cell counts were higher in patients both before and after treatment than in healthy subjects; treatment had no significant effect on the counts (P > 0.05). In contrast, the CD3+ and CD8+ cell counts in the patients were higher before than after treatment, but even after treatment they remained higher than in the healthy subjects. However, the CD19+ levels and the CD4+/CD8+ ratio were higher in the healthy subjects than the patients and higher in the patients after treatment than before. Treatment did not affect the CD4+ cell count but healthy subjects had more of these cells. These results are summarized in Table 2.

Counts of the T cell population and its subsets in patients with positive blood cultures were not statistically different from those with negative blood cultures (Table 3).

**Discussion**

In most infections, including those by *Brucella* species, the different arms of the immune system, namely antigen presenting cells (APCs), NK, T and B cells, act together to provide a coordinated response (2). NK cells are part of the first line of defense against pathogens and following activation can kill infected targets. However, removal of NK cells in vivo does not alter the ability of mice to contend with *B. abortus* infections. This result shows that the immune response is sufficient to control *Brucella* infection even in the absence of functional NK cells (6). In
In this study, we found results consistent with many of the publications mentioned above; peripheral blood CD8+ T cell counts were higher in patients than in healthy subjects. In addition, the patients’ CD8+ T cell counts decreased with treatment. The CD4+/CD8+ ratios were inverted between the pre- and post-treatment periods. These findings suggest that the first step in the development of immunological protection against brucellosis is an increase in CD8+ T cell numbers. This contrasts with the findings of Moreno-Lafont et al. (8).

Many serum passive-transfer experiments suggest that humoral immunity is significant in murine brucellosis (10). Hoffmann et al. (11) indicated that high levels of antibody block the complement-mediated killing of B. abortus, which would make resistance to the disease dependent on cell-mediated immunity. Gazapo et al. (9) indicated that CD20+ cells did not change in brucellosis patients during the follow-up period. In our study, the CD19+ cell levels in healthy subjects were significantly higher than those in acute brucellosis patients; they were higher before than after treatment in the patients.

We found no literature in English addressing the correlation between blood culture results and T lymphocyte subset counts. We found no significant differences between patients with positive and negative blood cultures. Therefore, the correlation between blood culture results and T lymphocyte subset counts is not significant.
blood cultures from the point of view of total T cell and T-cell subset counts.

In conclusion, CD8⁺ T cell counts increased in acute brucellosis. However, these counts decreased after treatment, though not as far as the levels in healthy control subjects. The CD4⁺ T cells counts were higher than in healthy subjects. CD19⁺ and NK cell counts increased after treatment. Blood culture results did not correlate with the lymphocyte subpopulation counts.

References