Serum cytokine levels in patients with acute brucellosis and their relation to the traditional inflammatory markers

Kutbettin Demirdag a,⁎, Mehmet Ozden b, Ahmet Kalkan a, Ahmet Godekmerdan b, S. Sirri Kilic a

a Department of Clinical Microbiology and Infectious Diseases, Faculty of Medicine, University of Firat, Elazig, Turkey
b Department of Immunology, Faculty of Medicine, University of Firat, Elazig, Turkey
Received 14 January 2003; received in revised form 25 June 2003; accepted 26 June 2003
First published online 29 July 2003

Abstract

The number of clinical studies on gamma interferon (IFN-γ), tumor necrosis factor-alpha (TNF-α) and interleukin-4 (IL-4) in human brucellosis is limited. The present study was focused on IFN-γ, TNF-α and IL-4 levels in acute brucellosis cases, in the acute phase and at the end of the treatment. The relation of these cytokines to traditional inflammation markers was also investigated. The study included 27 cases of acute brucellosis and 20 healthy volunteers who had no complaints. It was found that mean IFN-γ and TNF-α levels, CRP (C-reactive protein) levels and ESR (erythrocyte sedimentation rate) values were significantly higher in acute brucellosis cases as compared to post-treatment values and values measured in the control group. In addition, IFN-γ and TNF-α levels measured in the acute phase correlated with the increase in CRP levels and ESR values. Our results confirmed that IFN-γ and TNF-α are involved in the pathophysiology of brucellosis and are closely related to the inflammatory activation of the disease. In view of the present findings, it is suggested that IFN-γ and TNF-α may be used for monitoring brucellosis.

© 2003 Federation of European Microbiological Societies. Published by Elsevier B.V. All rights reserved.

Keywords: Brucellosis; Cytokine; Inflammatory marker; Treatment

1. Introduction

Brucella is a facultative, intracellular pathogen that can reside within phagocytic cells (macrophages) of the host and is apparently resistant to the normal mechanisms of bacterial killing [1]. In addition to the macrophage, which plays a central role in Brucella infections, other cells of the innate immune response are recruited and influenced by the interactions between bacteria and host. The ability of Brucella abortus to persist in the body and cause chronic infections relates to its ability to survive in macrophages by interfering with phagolysosomal fusions. Macrophages are recruited by interleukin-1β (IL-1β) and granulocyte-macrophage-colony-stimulating factor (GM-CSF). Cytokines appear to have an important role in the pathogenesis of brucellosis, and the Th1/Th2 balance may be involved in the susceptibility or resistance to the disease [2]. In mice, Brucella infections result in Type1 (Th1) cellular immune response, which promotes a clearance of the bacterial organism. The development of this response is under the control of major cytokines like tumor necrosis factor-alpha (TNF-α), gamma interferon (IFN-γ) and IL-12, produced at the onset of the infection [3].

The Th1 cytokine, IFN-γ, plays an important role in activating macrophages and in limiting Brucella infections both in vitro and in vivo [4]. Conversely, the Th2 cytokine, IL-10, can suppress the macrophage function and increase the susceptibility to an infection [5,6]. TNF-α shows a direct anti-bacterial activity against Brucella and functions as a co-stimulator in IFN-γ production [7]. Increased levels of IFN-γ in acute human brucellosis have been reported [8,9]. Little is known regarding the exact role of IL-4 (a Th2 cytokine) and TNF-α in human brucellosis.

It has been reported that in some diseases a positive correlation exists between inflammation markers (erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP)) and cytokines such as IFN-γ and TNF-α. [10,11]. Circulating levels of cytokines are correlated with clinical activity in some diseases [10,11]. It is thought
that in some cases, determination of cytokine serum levels of may contribute to the diagnosis, treatment and follow-up of the disease. The number of clinical studies on IFN-γ, IL-4 and TNF-α levels in human brucellosis is limited. To date, there is no study reporting on the involvement of cytokines in human brucellosis and their relation to the traditional markers of inflammation (ESR and CRP). In the present study, IFN-γ, TNF-α and IL-4 levels in the acute phase of the disease and at the end of the treatment and their relation to traditional inflammation markers in acute brucellosis are investigated.

2. Materials and methods

2.1. Patients

The study included a total of 27 acute brucellosis cases (15 males and 12 females; between 18 and 62 years of age, 42.4 ± 18.7 years). The diagnostic criteria were (i) isolation of a Brucella species from blood (Becton Dickinson 9050, USA) or (ii) the finding of ≤1/160 antibody titer to Brucella by a standard tube agglutination method in association with compatible clinical findings. Brucella species were isolated from the blood cultures in 15 cases (55.5%). All Brucella species determined were identified as Brucella melitensis by specific PCR [12]. All cases were treated with doxycycline+rifampicin, standard treatment regimen, for 6 weeks. Two cases were excluded from the study, one of them being unable to adjust to the treatment and the other not showing a complete clinical improvement at the end of the treatment. The rest of the patients (n = 25) adjusted to the treatment and completely recovered clinically at the end of the treatment.

2.2. Control group

The control group included 20 volunteers, 10 of them male and 10 of them female, ages ranging between 18 and 60 (46.6 ± 14.8). The cases in the control group were STA negative, showed ESR within normal limits and did not have any complaints. Exclusion criteria for the healthy control subjects included smoking, medication, pregnancy and any abnormalities in renal and liver function tests. Approval of the ethics committee was taken for the study, and in the control group subjects were informed about the study.

2.3. Determination of cytokine levels

Venous blood samples (5 ml) were taken from all patients before and after the treatment. The blood samples were centrifuged at 5000 rpm for 10 min; sera collected were stored at −80°C until assayed. Similarly, blood samples were obtained from the control group; serum was separated and stored at −80°C. IFN-γ, TNF-α and IL-4 levels in all serum samples were measured at the same time by ELISA method. A commercial kit was used for this purpose, and the study was performed according the kit procedure (Medgenix, Biosource International, Camarillo, USA). The results were expressed as pg ml⁻¹. ESR values (Westergren method) and CRP levels (immunoturbidometric method, Schiapparelli Biosystems, The Netherlands) before and after the treatment were also measured.

2.4. Statistical evaluation

Mann–Whitney U-test, paired-T test and Spearman correlation tests were used to analyze the data (SPSS 10.01). Values P < 0.05 were considered significant.

3. Results

Mean serum IFN-γ, TNF-α and IL-4 levels, CRP levels and ESR values of the study group (before and after the treatment) and of the control group are presented in Table 1. Mean IFN-γ and TNF-α levels, CRP levels and ESR values appeared to be significantly higher in acute brucellosis as compared to control group (P < 0.005). At the end of the treatment, the serum levels of these cytokines and the inflammation markers significantly decreased (P < 0.001). Furthermore, high IFN-γ and TNF-α levels measured in the acute phase of the disease correlated with the increase in CRP levels and ESR values (r: 0.494, 0.624, 0.846, 0.583, respectively; P < 0.05) (Figs. 1–4). Similarly, there was a correlation between IFN-γ and TNF-α levels in the acute phase (r:0.44, P < 0.05)

Table 1
Mean serum IFN-γ, TNF-α and IL-4 levels, CRP levels and ESR values of the healthy control group and of patients in the acute phase and at the end of the treatment

<table>
<thead>
<tr>
<th>Cytokines and inflammation markers</th>
<th>Acute brucellosis cases (n=25)</th>
<th>Control group (n=20)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Acute phase</td>
<td>After the treatment</td>
</tr>
<tr>
<td>TNF-α (pg ml⁻¹)</td>
<td>32.6 ± 15.3*</td>
<td>9.3 ± 1.8</td>
</tr>
<tr>
<td>IFN-γ (pg ml⁻¹)</td>
<td>20.3 ± 14.1*</td>
<td>5.5 ± 0.8</td>
</tr>
<tr>
<td>IL-4 (pg ml⁻¹)</td>
<td>0.29 ± 0.13**</td>
<td>0.37 ± 0.39</td>
</tr>
<tr>
<td>ESR (mm h⁻¹)</td>
<td>47.3 ± 14.6*</td>
<td>19.3 ± 12</td>
</tr>
<tr>
<td>CRP (mg l⁻¹)</td>
<td>52.2 ± 6.3*</td>
<td>11 ± 3.7</td>
</tr>
</tbody>
</table>

*P < 0.005, **P > 0.05
IFN-γ and TNF-α levels, CRP levels and ESR values at the end of the treatment were not statistically different from those in the healthy subjects ($P > 0.05$). In terms of IL-4 levels, no significant difference was found between patient and control groups, and between the levels before and after the treatment.

### 4. Discussion

The results of the present study have shown that cytokines (IFN-γ, TNF-α and IL-4) and traditional inflammatory markers are involved in acute brucellosis cases. The importance of cytokine responses in the pathogenesis of brucellosis has previously been studied in animal models [2,13]. However, the number of studies on human brucellosis is rather limited, and our study is the first attempt to investigate changes in cytokine levels in acute brucellosis, the effects of treatment on cytokines and their relation to traditional inflammation markers.

Induction of Th1 response by *B. abortus* has been demonstrated both in vivo and in vitro [14–17]. In a limited number of studies, involving with acute brucellosis patients, IL-1 and IL-4 were reported to be at undetectable levels in the serum, while IL-2 and IFN-γ levels were significantly higher in brucellosis patients than they were in the control group [8,9]. In addition to the studies showing induction of Th1-type immune response by brucellosis, Galanakis et al. [2] have reported that in children with brucellosis, serum IL-4 levels increased in the acute phase of the disease. Deterioration of the course of the disease was attributed to the increased levels of this cytokine, leading to a reduction in *Brucella* immunity through extreme Th2 response. In our study, while serum IFN-γ lev-
els in acute brucellosis cases were significantly increased, IL-4 levels were relatively low, and they did not significantly differ from the control group. Based on these results and consistent with other studies, it is suggested that Brucella infections do induce the Th1 response, but not the Th2 response, despite some contradictory results in the literature. However, this topic should be addressed in further clinical studies.

In vitro experiments have demonstrated that B. abortus induces human and murine monocytes to secrete the proinflammatory cytokines such as TNF-α and IL-1β, where TNF-α is a co-stimulator in the production of IFN-γ [7,18]. Some studies have suggested that Brucella strains do not induce TNF-α in human macrophages [3]. Ahmed K et al. [8] reported TNF-α to be at undetectable levels in the serum of acute brucellosis patients. On the contrary, in our study TNF-α levels were found to be significantly higher in the acute phase of the disease in comparison to the post-treatment and the control group values. It was observed that in addition to high levels in the acute phase of the disease, TNF-α was also in correlation with the increase in IFN-γ levels.

We observed that mean serum IFN-γ and serum TNF-α levels in acute brucellosis cases significantly decreased at the end of the treatment and that there was no significant difference between the post-treatment and the control group values. In literature, we did not find any study investigating the change of cytokine levels by treatment in acute brucellosis patients. However, it has been reported that during the infection with another intracellular pathogen, M. tuberculosis, IFN-γ levels dropped as a result of treatment in both the sputum and serum, whereas TNF-α decreased in the sputum only. Based on these results, it was suggested that IFN-γ and TNF-α could be markers showing the disease activity and inflammation in tuberculosic and used in evaluating the response to the treatment [19].

It is known that CRP and ESR, which are recognized as traditional inflammation markers, increase in acute brucellosis cases and decrease to normal levels after treatment. It is reported in various studies that especially CRP is a useful marker in the diagnosis and activity of acute brucellosis and in monitoring the efficiency of the treatment [20–22]. Various studies investigating the relation between traditional inflammation markers and cytokines show that there is a positive correlation between CRP, ESR and IFN-γ, TNF-α in several diseases [10,11]. In our study, besides the finding that serum IFN-γ and TNF-α levels increased in the acute phase in acute brucellosis cases, one of the most important findings was that the increase in these cytokines correlated with the increase in CRP and ESR.

Our results confirmed that IFN-γ and TNF-α are involved in the pathophysiology of brucellosis and have a close relationship with the inflammatory activation of the disease. In view of the present results, it is suggested that IFN-γ and TNF-α may be used for monitoring brucellosis. Although this is the first report in this field, further studies with higher number of cases should be undertaken.

References