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Original Article

Significant Elevation of Serum Soluble CD14 Levels in Patients with Brucellosis

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SUMMARY: Activation of macrophages represents one of the initial events in innate immunity to intracellular infections. CD14 is expressed principally by cells of monocyte/macrophage lineage and plays a pivotal role in innate recognition of bacterial cell wall components, particularly lipopolysaccharides. We measured serum concentrations of soluble CD14 (sCD14) in serum samples obtained from 37 patients with brucellosis and 36 healthy controls. Serum levels of sCD14 were significantly increased in patients with brucellosis compared with those in healthy controls ($P < 0.001$). Re-analysis of serum samples after treatment in 25 patients demonstrated that treatment did not result in any significant decline in sCD14 levels. Despite a limited study population, these findings may implicate CD14 signaling as an important component of the initial anti-brucellar host response and suggest that activation of mononuclear phagocytic system is sustained even following effective treatment.

INTRODUCTION

Brucellosis is a systemic infectious disease that usually presents as a febrile illness with malaise, profound sweating, chills, arthralgia, hepatomegaly, and splenomegaly (1,2). Similar to host defense against other intracellular bacterial pathogens, that against *Brucella* is mediated primarily by the cellular immune system involving activated macrophages and T cells and their cytokines (3-5).

CD14 is expressed principally by monocytes/macrophages and plays a pivotal role in innate immunity. Bacterial cell wall components, particularly lipopolysaccharides (LPS), are recognized by macrophages using the CD14 receptor (6-8). Subsequently, a soluble form of CD14 (sCD14) is released into the serum. The sCD14 levels have been shown to be significantly elevated in some clinical conditions characterized by local or systemic activation of monocytes/macrophages (6,9). sCD14 also mediates activation of cells lacking the CD14 surface receptor, including endothelial and epithelial cells (10). Alternatively, sCD14 can reduce endotoxin-induced activities by competing with mCD14 for LPS, and is a regulatory factor capable of modulating the cellular and humoral immune response.

The aim of the present study was to elucidate sCD14 levels in the sera of patients with active *Brucella* infection. In addition, serum soluble interleukin-2 receptor (sIL-2R) status was evaluated as a marker of T-cell activation.

MATERIALS AND METHODS

Patient and control groups: This prospective study was approved by the Ethics Committee of Ankara University Faculty of Medicine, and included 37 patients with brucellosis, who had been diagnosed at the Infectious Diseases and Clinical Microbiology Department of Kirikkale University Faculty of Medicine between October 2002 and September 2003. The diagnosis was based on the presence of compatible clinical findings in conjunction with either serum agglutinin titers of ≥1:160 (by standard tube agglution test) or isolation of *Brucella* spp. (in blood or other body fluids) (1,2). Blood culture samples were obtained twice, and *Brucella* spp. were isolated in 25 patients (67.6%) by using the BACTEC 9050 blood culture system.

The patients had no clinical, serological, or laboratory evidence of other chronic infectious disorders. Their mean age was 35.2 ± 15.1 years (range: 13 to 62 years), and 19 (51.4%) were males. The mean duration of the symptoms prior to diagnosis was 10.91 ± 11.31 weeks (range: 1 to 52 weeks). Clinical findings of patients with brucellosis are shown in Table 1. All patients received the usual treatment schedule recommended for brucellosis (1): doxycycline 100 mg twice daily at least for 6 weeks, combined with streptomycin 1 g/day for 2 weeks or rifampin 600 mg/day for 6 weeks. However, treatment was extended to 3 months in 5 patients administered rifampin and doxycycline combination therapy. All patients were cured by anti-brucellosis treatment. Post-treatment serum samples were available for 25 patients, and all were obtained immediately after the termination of

Table 1. Clinical findings in patients with brucellosis

Symptom /Sign	*n* (%) (*n* = 37)
Fever	29 (78.4)
Arthralgia/arthritis	28 (75.7)
Splenomegaly	20 (54.1)
Hepatomegaly	18 (48.7)
Orchitis	2 (5.4)
Paravertebral abcess	1 (2.7)
Pneumonia	1 (2.7)

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brucellosis therapy.

Thirty-six healthy individuals without serologic evidence of brucellosis were assigned to the control group. Their mean age was 32.57 ± 10.94 years (range: 18 to 62 years), and 20 (55.6%) were males.

sCD14 and sIL-2R assays: Five mililiters of venous blood was drawn from patients and controls, immediately centrifuged, and stored at –70°C. Serum levels of sCD14 were measured by a commercially available solid phase enzyme-amplified sensitivity immunoassay (EASIA) (Biosource Europe S.A., Nivelles, Belgium). This technique involves an oligoclonal system in which several monoclonal antibodies directed against distinct epitopes of sCD14 are used. For sIL-2R determination, enzyme-linked immunosorbent assay (ELISA) (Biosource International, Camarillo, Calif., USA) was utilized. The detection limits for sCD14 and sIL-2R assays were 1 ng/ml and 16 pg/ml, respectively.

Erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) were also determined by using Western green and nephelometric methods.

Statistical analysis: Mann-Whitney U, Spearman correlation coefficient, and Wilcoxon's signed rank tests were used to analyze the data (SPSS 10.01). *P* values below 0.05 were considered significant. Results were expressed as median and interquartile range (IQR).

RESULTS

Statistical analysis revealed no significant difference between gender ratio and mean ages of patient and control groups ($P > 0.05$). The median serum levels of sCD14, sIL-2R, CRP, and ESR in patient and control groups are shown in Table 2. Serum levels of sCD14, sIL-2R, ESR, and CRP were all significantly elevated in patients with brucellosis as compared with those in controls ($P < 0.001$). There was a significant correlation between ESR and CRP values in the patient group ($r = 681$, $P < 0.001$). However, ESR or CRP values did not correlate with sCD14 or sIL-2R levels.

Twenty-five of 37 patients were re-evaluated in the post-treatment period. Pre- and post-treatment serum sCD14 levels of these patients are shown in Fig. 1. Serum levels of sCD14 declined slightly from 9.96 (5.21) to 8.14 (6.89), although the difference between pre- and post-treatment levels was not significant ($P > 0.05$). However, serum sCD14 levels in the post-treatment period in these patients remained significantly higher than those in healthy controls ($P < 0.001$). The serum levels of sIL-2R decreased significantly from 389.73 (337.10) to 311.27 (171.60) after treatment ($P = 0.001$), but remained significantly above that of healthy controls ($P < 0.001$). CRP and ESR values of patients significantly decreased following treatment ($P < 0.001$) to levels comparable to those of controls ($P > 0.05$).

Table 2. Serum levels of sCD14, sIL-2R and acute phase reactants in patients with brucellosis and in healthy controls

	Patients with brucellosis ($n = 37$) Median (IQR)	Healthy controls ($n = 36$) Median (IQR)	*P**
sCD14 (μg/ml)	10.04 (5.20)	4.85 (1.88)	<0.001
sIL-2R (pg/ml)	398.20 (327.85)	146.43 (145.35)	<0.001
CRP (mg/dl)	1.59 (3.90)	0.17 (0.27)	<0.001
ESR (mm/h)	25.50 (22.00)	8.00 (9.25)	<0.001

* Data was analyzed by using Mann-Whitney U test.
IQR, interguartil range; sCD14, soluble CD14; sIL-2R, soluble interleukin-2 receptor; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate.

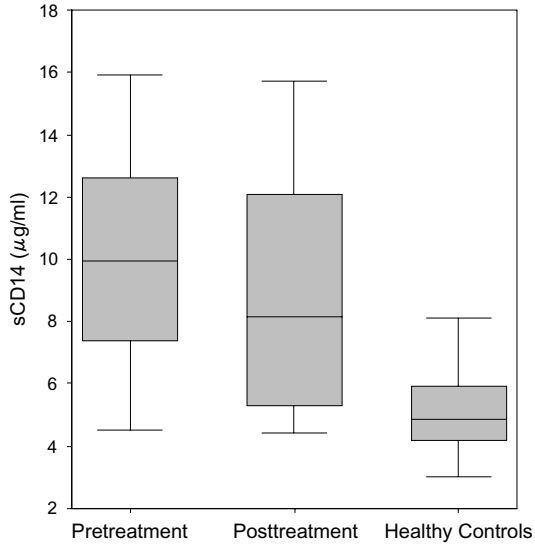

Fig. 1. Serum levels of sCD14 in 25 patients with brucellosis before and after treatment and in 36 healthy controls.

DISCUSSION

Although the function of sCD14 in human disease has not yet been clarified, a potential pathogenic role of sCD14 in several infectious diseases has been proposed. A wealth of evidence suggests that CD14 signaling is part of host response to intracellular bacterial pathogens such as *Mycobacterium tuberculosis*, and increased sCD14 levels were documented in sera and bronchoalveolar lavage fluid of patients with active tuberculosis (9,11,12). Moreover, serum sCD14 levels have been shown to correlate with disease progression and cognitive dysfunction in HIV-infected patients (13,14). Patients with Gram-negative septic shock have been shown to have elevated levels of sCD14, which correlated with high mortality (15). To our knowledge, the status of serum sCD14 in brucellosis has not previously been investigated.

Activation of macrophages represents one of the earliest events in the innate defense against intracellular bacterial infections. Numerous studies have reported on the close association between *Brucella* and professional phagocytes, particularly macrophages (16,17). However, the underlying exact molecular mechanisms and the receptors involved in this interaction are still unclear. The LPS is one of the most important virulence determinants for *Brucella* spp. It has been demonstrated that LPS of *Brucella abortus* induces human monocytes to secrete high levels of interferon-γ (IFN-γ), tumor necrosis factor-α (TNF-α), and interleukin-12 (IL-12) (18,19). The induction of cytokine secretion has been blocked by anti-CD14 monoclonal antibodies, suggesting that CD14 is a candidate molecule in recognition of *B. abortus* by monocytes (19). In the present study, a limited number of patients were recruited. However, serum levels of sCD14 were significantly increased in our patients with brucellosis, suggesting a role of CD14 signaling in anti-brucellar host response.

Analysis of pre- and post-treatment serum samples revealed that the values of acute phase reactants (ESR and CRP) and sIL-2R levels significantly declined following treatment. In

contrast, treatment had no significant effect on sCD14 levels. However, both sCD14 and sIL-2R levels remained elevated in the patient group compared to those in controls in post-treatment serum samples. These findings are in accordance with those of previous studies, and indicate that activation of mononuclear phagocytic system is sustained even after therapy (9). Furthermore, based on the results of the present study, it seems plausible to suggest that sCD14, unlike ESR and CRP, is not a useful marker of disease activity in brucellosis,.

CD14 induced activation of macrophages results in the release of proinflammatory cytokines such as TNF-α, IL-1, and IL-6 (20). These inflammatory cytokines lead to the production of acute phase reactants. Demirdag et al. demonstrated a positive correlation between cytokine levels (IFN-γ and TNF-α) and acute phase reactants (ESR and CRP) (21). Although sCD14 levels were significantly elevated in our patients, the levels did not correlate with CRP or ESR values. The lack of a positive correlation is not surprising, given the fact that several other accessory molecules such as human toll-like receptors (TLRs) are involved in CD14 signaling and in subsequent release of inflammatory cytokines (22,23).

In conclusion, serum sCD14 levels were found to be elevated in patients with brucellosis. Despite a small study population, this finding may indicate involvement of CD14 signaling in cell-mediated immunity against *Brucella* spp. Further studies are needed to define the precise function and cellular expression of CD14; its interaction with other recognition molecules such as TLR; and the bacterial constituents that elicit CD14 signaling.

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