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IL-15 serum levels in acute and chronic brucellosis

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ABSTRACT:

— ***Background:*** T helper (Th)1 cytokines have a critical role in facilitating the elimination of intracellular pathogens. Interleukin (IL)-15 is a Th1 cytokine, having biological activities similar to IL-2.

— ***Patients and Methods:*** In the present study, we evaluated IL-15 serum levels in patients with different clinical forms of brucellosis and healthy controls. We also aimed at assessing the potential role of serum IL-15 as a prognostic marker for human brucellosis.

— ***Results****:* Patients with acute brucellosis (n=50) had significantly higher levels of IL-15 in comparison with both patients with chronic brucellosis (n=32) (44.1±12.1 *vs* 5.2±3 ρg/ml, $p<0.01$) and healthy controls (n=65) (8.7±4.3 ρg/ml, $p<0.01$). IL-15 levels were significantly higher among patients with fever >38° C (n=65), in comparison with subjects who had no fever (n=17) (43.3±12.3 *vs.* 5.9±3.2 ρg/ml, $p<0.01$). On the contrary, IL-15 serum concentration was similar in patients with uncomplicated brucellosis (41.1±16.6 ρg/ml) and patients with visceral complications (44.9±11.1 ρg/ml). Following a complete antibiotic course, IL-15 levels showed a slight, not significant decrease among patients with acute brucellosis (from 44.1±12.1 ρg/ml to 37.3±16.1), whereas patients with chronic brucellosis had a significant increase in IL-15 serum concentration (from a pre-treatment value of 5.2±3 ρg/ml to a post-treatment level of 24±10.6 g/ml, $p<0.05$).

— ***Conclusions****:* IL-15 production seems to be defective in chronic brucellosis. Further studies should clarify the mechanisms regulating IL-15 release during the course of brucellosis.

— ***Key words:*** Acute, Brucellosis, Chronic, Interleukin-15, Zoonosis.

INTRODUCTION

Human brucellosis is widely spread in the Mediterranean basin. In Sicily alone the annual incidence ranges from 600 to 700 cases, mainly due to Brucella (B.) *melitensis* (B. Cacopardo, unpublished data).

Human brucellosis is often difficult to diagnose because of its proteiform manifestations; in fact, brucellosis may have an acute (either mild self-limiting or hyperacute), relapsing or chronic protracted course[1]. Routine laboratory tests are usually not helpful for diagnosis and white blood cell count is often normal or low[2]. Physical signs are generally non-specific, although lymphadenopathy, hepatomegaly or splenomegaly are often found[1]. Common presentations include osteoarticular[3-5] and hepatosplenic[6] involvement.

As for pathogenesis, the differentiation of CD4+ T lymphocytes into T helper (Th)1 or Th2 cells represents a crucial regulatory event which may orientate towards a mild, spontaneously healing or a chronic disease. Several studies demonstrated that polarization towards Th1 immune responses is the key to control infections caused by intracellular pathogens as it determines less dramatic and self-resolving clinical forms[7,8].

Interleukin (IL)-15 is a Th1 cytokine, sharing a number of functions and receptors with IL-2[9]. Its principal

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role is related with chemotaxis and activation of T lymphocytes and induction of natural killer (NK) cell activity[10,11]. IL-15 is secreted by macrophages early after infection by intracellular pathogens[12]. IL-15 may also favour the differentiation of Th0 cells into Th1 cells[13,14].

In the present study, we evaluated the potential *ex vivo* role of IL-15 in human brucellosis, by measuring IL-15 serum concentration in different clinical forms of brucellosis. We also aimed at assessing the potential role of serum IL-15 as a prognostic marker for human brucellosis.

PATIENTS AND METHODS

Patients

In our study, we enrolled 82 patients consecutively admitted to the Unit of Infectious Diseases of the Garibaldi Nesima Hospital of Catania, Sicily. All patients had been diagnosed with human brucellosis. Sera were taken from patients either at diagnosis or after a complete antibiotic course and stored at -80° C until use for IL-15 determination.

The diagnosis of brucellosis was based on clinical findings (fever, sweating, muscle weakness, arthralgias, appetite and weight loss) plus one among either positivity of blood cultures for Brucella *spp*. or complement fixation test ≥1:32 or standard serum agglutination test titer ≥1:320.

The diagnosis of chronic brucellosis was based on the persistence of symptoms and signs for more than 12 months. Exclusion criteria were: 1. coexistence of other infectious, neoplastic or autoimmune diseases; 2. administration of antibiotics for at least 15 days before entering the study; 3. recent (≤6 months) vaccination; 4. pregnancy; 5. active drug use.

Sera from 65 healthy individuals were used as controls. All subjects signed a written informed consent prior to study inclusion, in accordance with the Declaration of Helsinki.

Methods

Quantikine Human IL-15 Immunoassay (R&D Systems, Minneapolis, USA) was used to measure IL-15 serum concentration of patients with brucellosis and controls. The lower detection limit of the assay was 2 ρg/ml.

Statistics

Data are expressed as mean±standard deviation or number (percentage). When appropriate, one-way analysis of variance (ANOVA) and Student's *t* test were used to compare IL-15 serum concentration among groups.

RESULTS

We tested the sera of 82 patients with human brucellosis and 65 healthy controls. 49 patients (59.8%) were male and mean age was 47±13 years. In the control group, there were 42 males (64.6%) and mean age was 41±10 years.

Among patients with brucellosis, 52 (63.4%) had ingested raw milk or dairy products, whereas 30 patients (36.6%) were occupationally exposed to infection: 14 were shepherds, 9 veterinarians, 6 farmers and 1 butcher. Fifty cases (61%) were acute (mean duration of disease 27±4 days), whereas 32 cases (39%) were chronic, with a mean duration of disease of 234±55 days.

All patients with acute brucellosis had fever >38° C; the majority of patients complained of arthralgias and sweats. Only 15 of 32 chronic cases (47%) had fever >38° C; patients with chronic brucellosis reported arthralgias as the most frequent symptom. In 16 of 52 acute cases (30.8%), visceral complications were reported: 7 patients had arthritis, 4 meningoencephalitis, 2 endocarditis and 1 acute hepatitis; in 2 cases there was a combined articular and hepatic involvement. Of the 32 patients with chronic disease, 16 (50%) had visceral complications: 13 spinal brucellosis and 3 meningoencephalitis.

All patients were treated with 100 mg doxycycline and 300 mg rifampicin orally twice daily for six weeks, with the exception of patients having spinal or neurological involvement, who received a 12-week course of antibiotics. All patients completely recovered, with the exception of two cases of spondylodiscitis, who required a surgical approach.

Patients with acute brucellosis had significantly higher IL-15 serum levels in comparison with both patients with chronic brucellosis (44.1±12.1 *vs* 5.2±3 ρg/ml, $p<0.01$) and healthy controls (8.7±4.3 ρg/ml, $p<0.01$) (Figure 1). Comprehensively, mean IL-15 levels were significantly higher among patients who had fever >38° C (n=65), in comparison with subjects who had not (n=17) (43.3±12.3 *vs*. 5.9±3.2 ρg/ml, $p<0.01$) (Figure 2). On the contrary, IL-15 serum concentration was similar in patients with uncomplicated brucellosis (41.1±16.6 ρg/ml) and patients with visceral complications (44.9±11.1 ρg/ml).

Following a complete antibiotic course, IL-15 levels showed a slight, not significant decrease among patients with acute brucellosis (from 44.1±12.1 to 37.3±16.1 ρg/ml), whereas patients with chronic brucellosis exhibited a significant increase in IL-15 serum concentration (from a pre-treatment value of 5.2±3 pg/ml to a post-treatment level of 24±10.6 ρg/ml, $p<0.05$) (Figure 3).

DISCUSSION

The development of an effective immune response against intracellular pathogens relies on the activation of adequate cell-mediated responses. Brucella *spp*. usually replicate inside reticuloendothelial cells, resisting enzymatic degradation, inhibiting apoptosis and, sometimes, interfering with the production of Th1 cytokines[15-17]. It seems that the fate of brucellar infection critically depends on the predominant activation of Th1 or Th2 lymphocyte clones[8]. Either the recovery or the chronicization of brucellosis mirrors the balance between microbes and immune system, with Th1 responses facilitating microbial elimination and Th2 responses favouring bacterial intracellular survival[6-8].

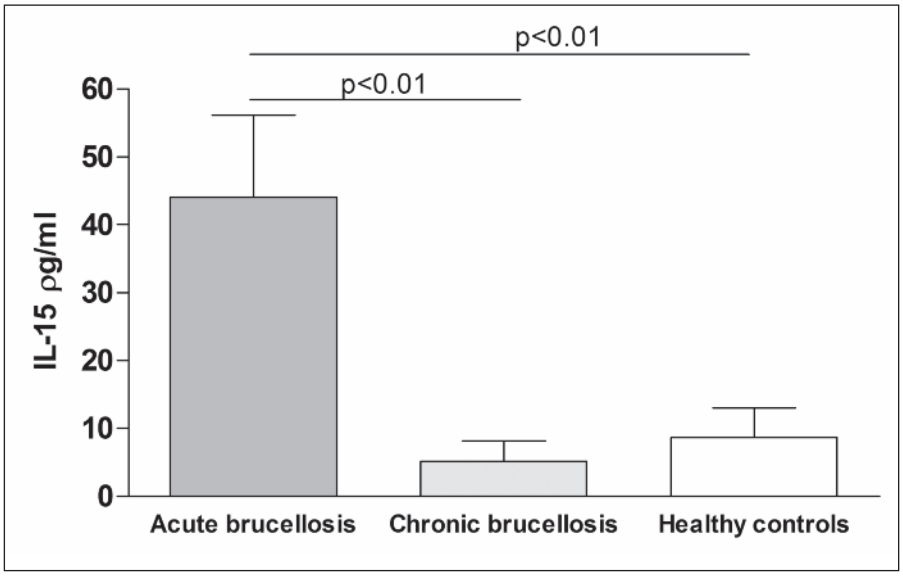

Figure 1. Interleukin (IL)-15 levels among patients with acute brucellosis, chronic brucellosis and healthy controls. Patients with acute brucellosis had significantly higher IL-15 levels (44.1±12.1 ρg/ml) than subjects with chronic brucellosis (5.2±3 ρg/ml, $p<0.01$) and controls (8.7±4.3 ρg/ml, $p<0.01$).

Giambartolomei et al[7] clarified the dynamic of Th1 response in patients with brucellosis: the authors observed a relevant production of protective cytokines, such as IL-2 and Interferon-γ, after T-cell stimulation with brucellar cytoplasmic proteins. On the contrary, levels of these cytokines, but also of Th2 cytokines, were significantly lower in cell cultures from patients with chronic brucellosis. In chronic brucellosis, Forestier et al[18] showed reduced expression of the major histocompatibility complex, which may result in a weak activation of Th1 cells.

IL-15 mRNA has been detected in a number of human tissues and cell types, including heart, lung, liver, placenta, skeletal muscle, adherent peripheral blood mononuclear cells, epithelial and fibroblast cell lines[10]. IL-15 has biological activities similar to IL-2 and has been shown to stimulate the growth of NK cells and to activate peripheral blood T and B cells[10,11,19-21].

In the present study we found significantly higher levels of IL-15 in acute brucellosis in comparison with chronic brucellosis and healthy controls. This observation is in keeping with the potentially protective role of Th1 cytokines during infections caused by intracellular pathogens. In the aforementioned study of Giambartolomei et al[7], the authors showed increased levels of Th1 cytokines in patients with acute brucellosis. Recently, Kalani et al[22] have reported IL-15 polymorphisms to affect resistance to brucellosis. IL-15 production seems to decline as long as brucellosis chronicizes. It might be explained either by a progressive exhaustion of Th1 protective responses or by the inhibitory action of specific brucellar antigens during protracted bacterial multiplication[17,18,23]. Actually, Brucella can parasitize within human antigen-presenting cells modifying phagocytosis, phagolysosome fusion, antigen presentation, cytokine secretion and apoptosis[18]. In addition, subversion of innate immune mechanisms by brucellar antigens may lead to defective Th1 immune responses and T-cell anergy in patients with chronic brucellosis[8].

IL-15 levels were also significantly higher in patients with fever, in keeping with the observation that IL-15 is able to trigger inflammatory cell recruitment during infectious diseases[12,24].

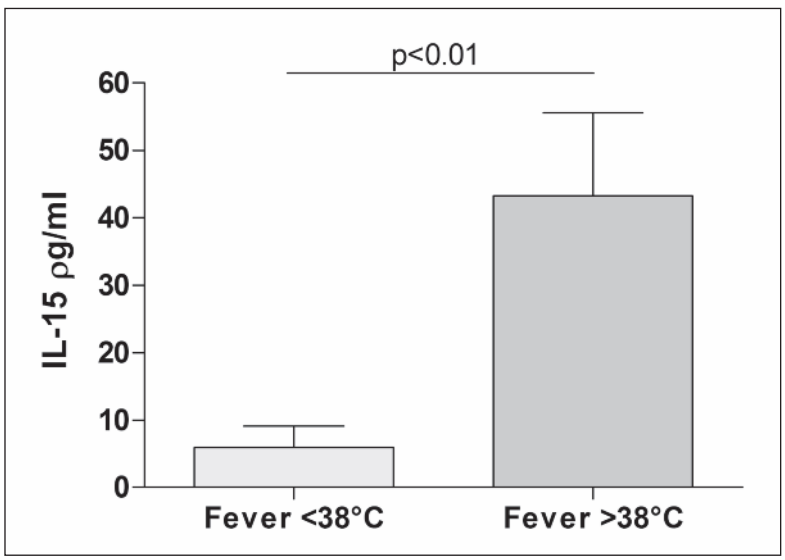

Figure 2. Interleukin (IL)-15 levels among patients with brucellosis presenting with fever >38° C and <38° C. Subjects with fever >38° C had significantly higher IL-15 serum levels than subjects with fever <38° C (43.3±12.3 *vs*. 5.9±3.2 ρg/ml, $p<0.01$).

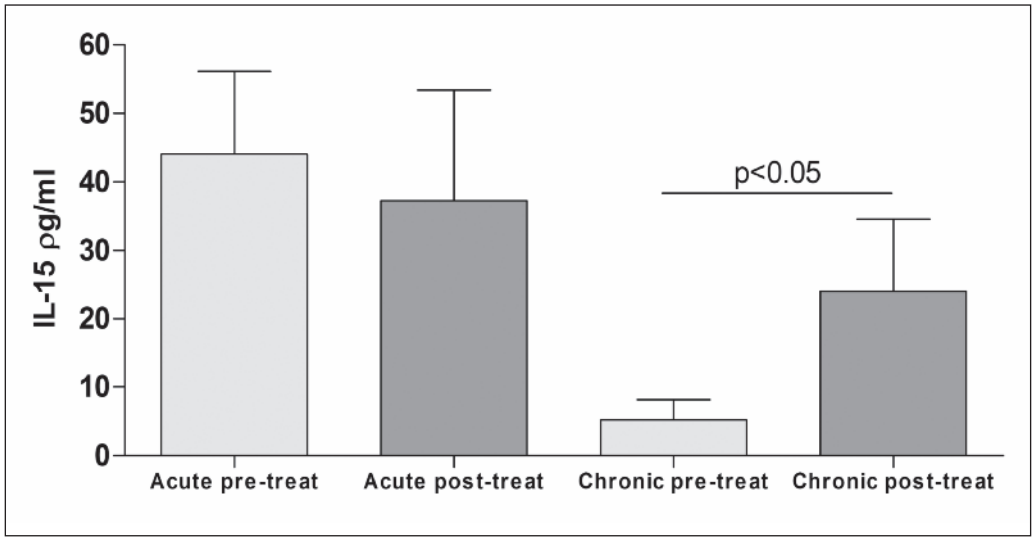

Figure 3. Evaluation of IL-15 levels among patients with acute and chronic brucellosis at baseline (pre-treat) and after antibiotic treatment (post-treat). Post-treatment IL-15 levels did not significantly differ among patients with acute brucellosis, whereas they were significantly higher among patients with chronic brucellosis (5.2±3 *vs*. 24±10.6 ρg/ml, $p<0.05$).

Further extensive studies are needed to assess the role of IL-15 as a prognostic marker of disease progression during the course of brucellosis.

Conflict of interest

No conflict of interest to declare. No funding sources

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