

CORRELATION OF HIGH SERUM LEVELS OF TUMOR NECROSIS FACTOR- α WITH DISEASE SEVERITY IN HUMAN AFRICAN TRYPANOSOMIASIS

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Abstract. The levels of tumor necrosis factor- α (TNF- α) in sera from *Trypanosoma brucei gambiense*-infected patients from the endemic region of Boko Songho (Bouenza focus in Congo) were measured. An increase was observed in sera from patients (geometric mean = 53.75 pg/ml, n = 69) compared with control subjects from the same endemic area (6.72 pg/ml, n = 31). The patients were classified as being in the early (blood lymphatic) stage and late (meningo-encephalitic) stage of disease according to the presence of parasites and cells in cerebrospinal fluid (CSF). An increase in TNF- α was noted in late stage patients (68.42 pg/ml, n = 28) compared with early stage patients (43.68 pg/ml, n = 41). Those patients with fever, asthenia, and edema and those with neurologic signs had higher levels of TNF- α (89.36 pg/ml, n = 26) than others (38.07 pg/ml, n = 43). No differences in TNF- α levels were seen when trypanosomes were detected in one location (blood, lymph nodes, or CSF) or two or three locations. These data show that the levels of TNF- α in serum of *T. b. gambiense*-infected patients were correlated with disease severity (presence of signs of inflammation or presence of major neurologic signs) and indicate that TNF- α could be involved in some aspects of human African trypanosomiasis physiopathology.

Human African trypanosomiasis, or sleeping sickness, is caused by *Trypanosoma brucei gambiense* and *T. b. rhodesiense*.^{1,2} At least 45 million people in Africa are estimated to be exposed to trypanosomiasis. In the early stage of the disease (blood-lymphatic phase), the parasites invade the blood and the lymphatic system. The most common signs are intermittent fever, headache, joint pain, edema, hepatosplenomegaly, and lymphadenopathies. The late (meningo-encephalitic phase) stage of the disease starts when trypanosomes cross the blood brain barrier, at which time, besides nonspecific symptomatology, various neurologic signs are noted. Hypergammaglobulinemia, autoantibodies, immune complexes, and immunodepression have also been observed.^{3,4} Cytokines may contribute to the nonspecific symptomatology (fever) as well as these immunologic abnormalities.^{5,6}

Tumor necrosis factor- α (TNF- α) is involved in numerous physiologic functions and has also been implicated in the pathogenesis of septic shock and systemic inflammatory reactions.⁵⁻⁷ Increased TNF- α levels have been demonstrated in the sera of patients with bacterial, viral, and parasitic infections, such as malaria or leishmaniasis.⁸⁻¹² In human malaria, increased serum levels of TNF- α have been correlated with severity of disease.¹¹ Alterations in the cytokine network during African trypanosomiasis have not yet been established although certain clinical manifestations suggest such modifications. In this study, we evaluated the level of TNF- α in sera of patients with African trypanosomiasis and the correlation of these levels with the stage and activity of the disease.

SUBJECTS, MATERIALS, AND METHODS

Subjects. The study was carried out in 69 Congolese suffering from sleeping sickness (female:male ratio = 1.3:1, 4-72 years of age, mean \pm SD age = 31.4 \pm 21.1) and 31 uninfected subjects (female:male ratio = 1:1, 3-68 years of age, mean \pm SD age = 29.3 \pm 19.5) living in the endemic

region of Boko Songho (Bouenza focus in Congo). During a mass screening for sleeping sickness, patients with trypanosomiasis were diagnosed on the basis of serologic and parasitologic results.¹³ All patients analyzed in this study were serologically and parasitologically positive. Patients with trypanosomiasis infections and control subjects were all parasitologically negative for malaria and filariasis. No subject presented any additional clinical pathology. Pregnant women were excluded. Controls were all parasitologically and serologically negative for trypanosomes.

Serum samples. To minimize alterations of blood components and to standardize samples, blood was collected between 10:00 AM and noon and allowed to clot at room temperature for 2 hr. The serum was separated by centrifugation (600 \times g for 10 min), frozen at -20°C, and stored at -80°C until use.

Clinical and laboratory analysis. Serologic responses were determined with the Testryp® Card Agglutination Test (CATT) (Smith Kline RIT, Rixensard, Belgium) on whole blood and samples reacted positively in at least one of the following serologic tests: quantitative CATT on serum, indirect immunofluorescence, and indirect hemagglutination (Cellognost®; Behring Institut, Marburg, Germany). Parasites were found in one or more of the following biological fluids: blood, lymph node aspirate, and, when required, in cerebrospinal fluid (CSF) either by direct microscopic examination or by using mini anion-exchange columns.¹⁴ A questionnaire on previous illness, and general signs and symptoms of infection was completed and a physical examination for adenopathies and neurologic signs was performed on each patient by a physician. The involvement of the central nervous system (CNS) (late stage of African trypanosomiasis) was based on CSF analysis (presence of the parasite and cell count).

Determination of TNF- α levels. The TNF- α levels were determined for each sample in duplicate using a sandwich radio immunoassay (TNF- α IRMA; Medgenix, Brussels, Belgium). Several monoclonal antibodies directed to distinct

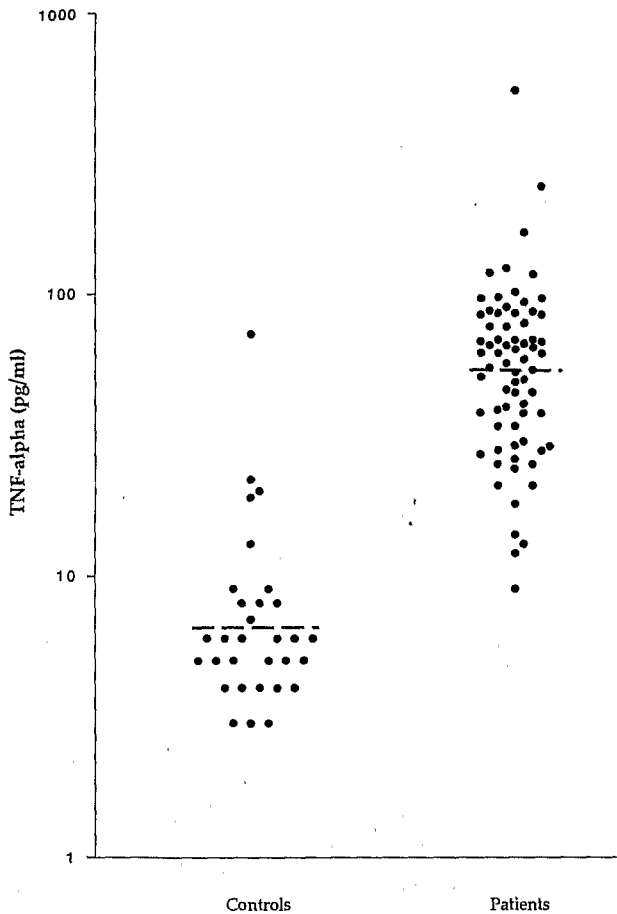


FIGURE 1. Levels of tumor necrosis factor-alpha (TNF-alpha) in sera of control subjects (Africans living in an endemic area, $n = 31$) and patients with human African trypanosomiasis ($n = 69$). The dashed lines represent the geometric means of each group.

epitopes of TNF- α and bound to the bottom of tubes were used as capture antibodies. Serum samples or standard amounts of TNF- α and ^{125}I -labeled anti-TNF- α (trace antibodies) were added to each tube. After the samples were washed with phosphate-buffered saline, pH 7.4, containing 0.5% Tween 20, counts were determined. Values of TNF- α in samples were obtained from a curve obtained from standards. This assay does not cross-react with TNF- β , interleukin-1 (IL-1), IL-6 or interferon- γ (IFN- γ), and its sensitivity is 1 pg/ml.

Statistical analysis. Geometric means, which were considered more representative of each data group of TNF- α levels than arithmetic means, and 95% confidence intervals (CIs) were calculated. The TNF- α levels were not normally distributed. Comparison between groups were made using a nonparametric test (Mann-Whitney U test). Tests of significance were two-tailed. All P values less than 0.05 were considered significant. The arithmetic mean \pm standard deviation was used for mean ages of control and patient groups.

RESULTS

Increase in TNF- α levels in trypanosomiasis. Figure 1 shows the levels of TNF- α in sera of control subjects and

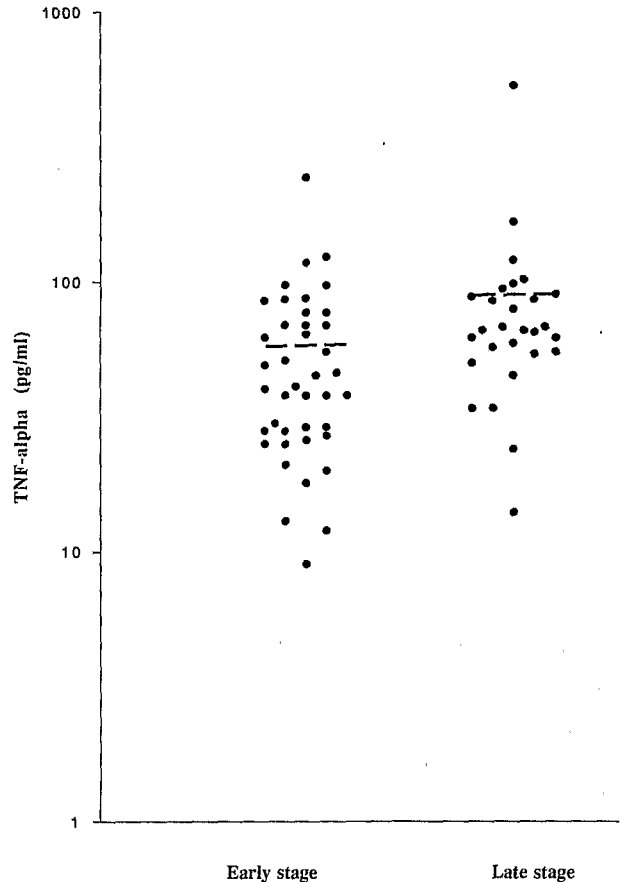


FIGURE 2. Levels of tumor necrosis factor-alpha (TNF-alpha) in early ($n = 41$) and late ($n = 28$) stage patients with human African trypanosomiasis. The dashed lines represent the geometric means of each group.

patients. The geometric mean of TNF- α in control subjects was 6.72 pg/ml (CI = 1.77–25.53). Significantly higher levels of TNF- α were observed in patients (53.75 pg/ml, CI = 13.52–201.94; $P < 0.0001$). High levels of TNF- α were also found in the CSF of eight patients but could not be measured in the others because of insufficient quantities.

Forty-one patients with four or less cells/mm 3 and no parasites in the CSF were considered to be early stage (blood lymphatic phase) patients and 28 patients with more than four cells/mm 3 or with parasites in the CSF were considered to be late stage (meningo-encephalitic phase) patients. Compared with the control group, significantly higher levels of serum TNF- α were measured in the two patient groups. An increase in serum TNF- α levels (Figure 2) was observed in late stage patients (68.42 pg/ml, CI = 36.19–238.34) compared with early stage patients (43.68 pg/ml, CI = 22.11–165.82, $P = 0.025$).

Levels of TNF- α and disease activity. Patients were classified into four groups according to clinical signs and stage of infection (Figure 3). Patients in the first phase were divided into two groups according to the intensity of general signs of inflammation (fever, asthenia, edema), indicating an active period of the disease. Patients with these clinical signs ($n = 14$) had higher levels of serum TNF- α than patients with no or mild symptoms ($n = 27$). The difference was

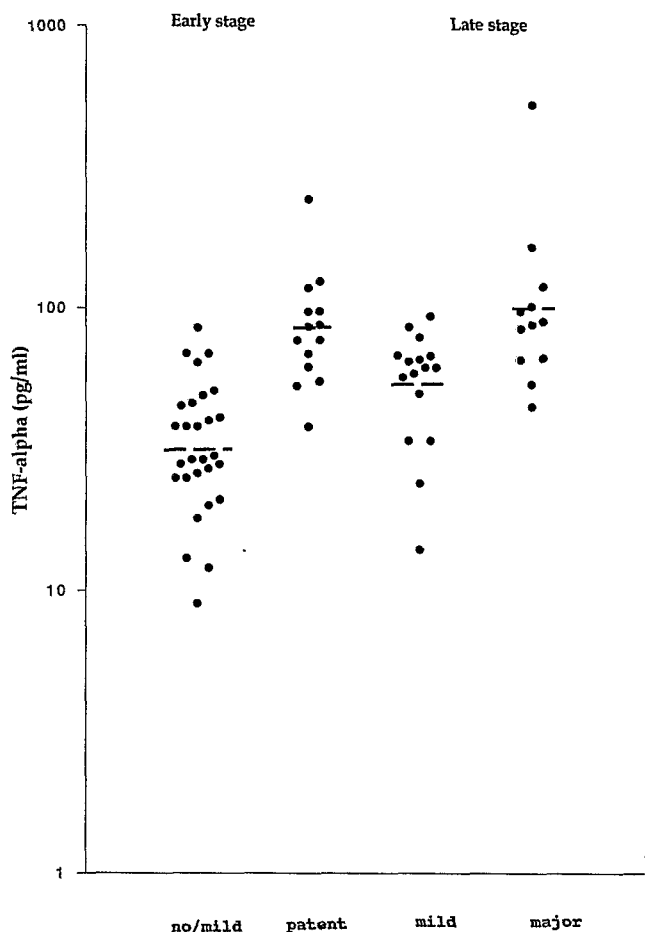


FIGURE 3. Levels of tumor necrosis factor-alpha (TNF-alpha) in individuals with human African trypanosomiasis in the early stage with no or mild symptoms ($n = 28$) (first column), in the early stage with clinical signs (patent) ($n = 15$) (second column), in the late stage with mild symptoms ($n = 16$) (third column), and in the late stage with major neurologic signs ($n = 12$) (fourth column). The dashed lines represent the geometric means of each group.

significant: 82.72 pg/ml (CI = 34.32–199.39) for the first group and 31.74 pg/ml (CI = 11.06–90.95) for the latter group ($P < 0.001$). The difference between the latter group and control subjects was also significant ($P < 0.001$).

Patients in the late stage were divided into two groups according to the presence or absence of major neurologic signs (sensory disturbances, ataxia, convulsions, chorea, tremors, disturbed circadian rhythms, endocrine abnormalities). Patients with major neurologic signs ($n = 12$) had higher levels of TNF- α than those in the milder late stage group ($n = 16$): 97.79 pg/ml (CI = 28.01–341.42) versus 52.34 pg/ml (CI = 19.52–140.32) ($P < 0.006$).

All patients of the early or late stage of the disease with clinical signs ($n = 26$) had higher levels of TNF- α than the other patients ($n = 43$): 89.36 pg/ml (CI = 31.08–256.92) versus 38.07 pg/ml (CI = 12.36–117.2) ($P < 0.001$) (Figure 4).

In the patients in whom parasites were observed in the blood, there was no correlation between TNF- α concentration and parasitemia. There were no differences in TNF- α serum levels between those patients with trypanosomes in

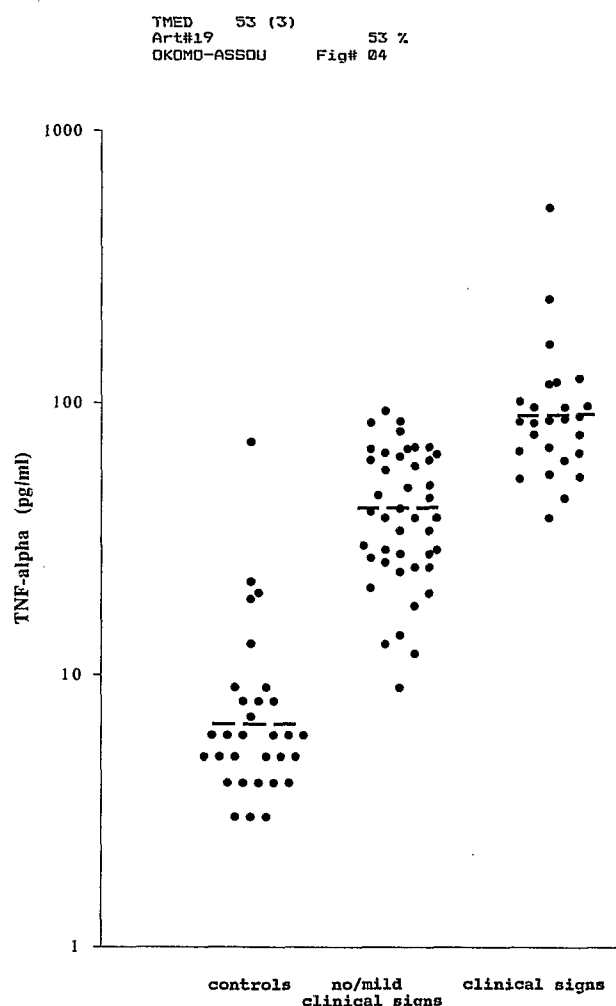


FIGURE 4. Levels of tumor necrosis factor-alpha (TNF-alpha) in sera of control subjects (Africans living in an endemic area, $n = 31$), in patients with human African trypanosomiasis with no or mild clinical signs ($n = 43$), and in patients with clinical signs ($n = 26$). The dashed lines represent the geometric means of each group.

both blood and lymph nodes and those with parasites in the blood alone.

DISCUSSION

The present study reports a marked elevation of TNF- α levels in patients with African trypanosomiasis and a correlation between high levels of TNF- α and disease severity. The difference between TNF- α levels in the early and late stages was less significant ($P = 0.025$). The results of this study were concerned with TNF- α levels observed in only one focus (Boko Songho focus in Congo). However, we were also able to obtain the sera of two patients from Cote d'Ivoire. In the sera of these two patients, an increase in the TNF- α level was also observed. This probably means that trypanosomiasis induces an increase in TNF- α levels and that the phenomenon is not limited to *T. b. gambiense* strains from Congo. Studies in other foci would be necessary to confirm this possibility. The search for endogenous mediators of cachexia in parasitic diseases led to the isolation of cachectin in sera of cattle and rabbits infected with trypano-

somes.¹⁵ Peptide sequence analysis revealed that TNF- α and cachectin are the same molecule.¹⁶

Tumor necrosis factor- α is known to induce fever, asthenia, anemia, and hypertriglyceridemia, which are observed in African trypanosomiasis. We observed that high levels of TNF- α are associated with the presence of patent inflammatory signs in the early phase of trypanosomiasis and of major neurologic signs in the late phase. Because of the mobility of the population and the limited duration of our stay, we were able to obtain serum samples after treatment from only three patients. In these, a decrease in the TNF- α level was found one week after treatment. This is in agreement with the association between increased serum TNF- α levels and disease severity in other parasitic diseases.^{11,12} In leishmaniasis, an increased TNF- α level is a marker of disease activity and a rapid decrease in the TNF- α level is observed after effective therapy. In malaria, high levels of TNF- α are associated with manifestations of severe malaria but are not specific to cerebral malaria. Moreover, a persistently increased serum TNF- α level probably contributes to the hypergammaglobulinemia observed in trypanosomiasis because the role of TNF- α on activation, proliferation, and differentiation of B lymphocytes has been shown.^{17,18}

Besides its role in the pathophysiology of African trypanosomiasis, TNF- α participates in the mechanisms leading to trypanosome elimination: TNF- α acts indirectly in a cascade of events leading to cell activation or directly on parasites through its cytotoxic properties.¹⁹ In murine models, macrophage activation induced by IFN- γ exerts a powerful antimicrobial effect on various microorganisms including trypanosomes. Tumor necrosis factor- α participates in IFN- γ -mediated antimicrobial activity and can act as an autocrine factor.²⁰⁻²⁴ In a recent study, initial control of parasitemia in *T. b. brucei*-infected mice was diminished by injection of anti-TNF- α antibodies.²⁵

The mechanism leading to enhanced production of TNF- α is still not understood. In vitro, *Leishmania amazonensis* are able to induce TNF- α production by human monocyte-derived macrophages.¹² Monocytes-macrophages are the most potent TNF- α -producing cells. Trypanosomes can be the direct stimulus for TNF- α synthesis by macrophages or can act indirectly through other cells. Recently, a factor released by *T. b. brucei* that binds and activates CD8⁺ lymphocytes has been found. This factor induces IFN- γ secretion that is an activator of TNF- α production.²⁶

The mechanisms leading to TNF- α production in trypanosomiasis deserve further work. Mice chronically infected with *T. b. brucei* after treatment with subcurative doses of a trypanocidal compound develop inflammatory lesions of the CNS. The presence of TNF- α RNA transcripts in the CNS of these mice suggest that TNF- α production plays a role in these lesions.²⁷ Also, TNF- α and other cytokines contribute to the generation of somnogenic molecules such as IL-1.²⁸ It is known that anti-TNF- α antibodies cause an improvement in septic shock patients.²⁹ This, along with the higher levels of TNF- α detected in the sera of patients with severe African trypanosomiasis, are in favor of the role of this cytokine in the generation of generalized inflammation and possibly of the associated neurologic signs.

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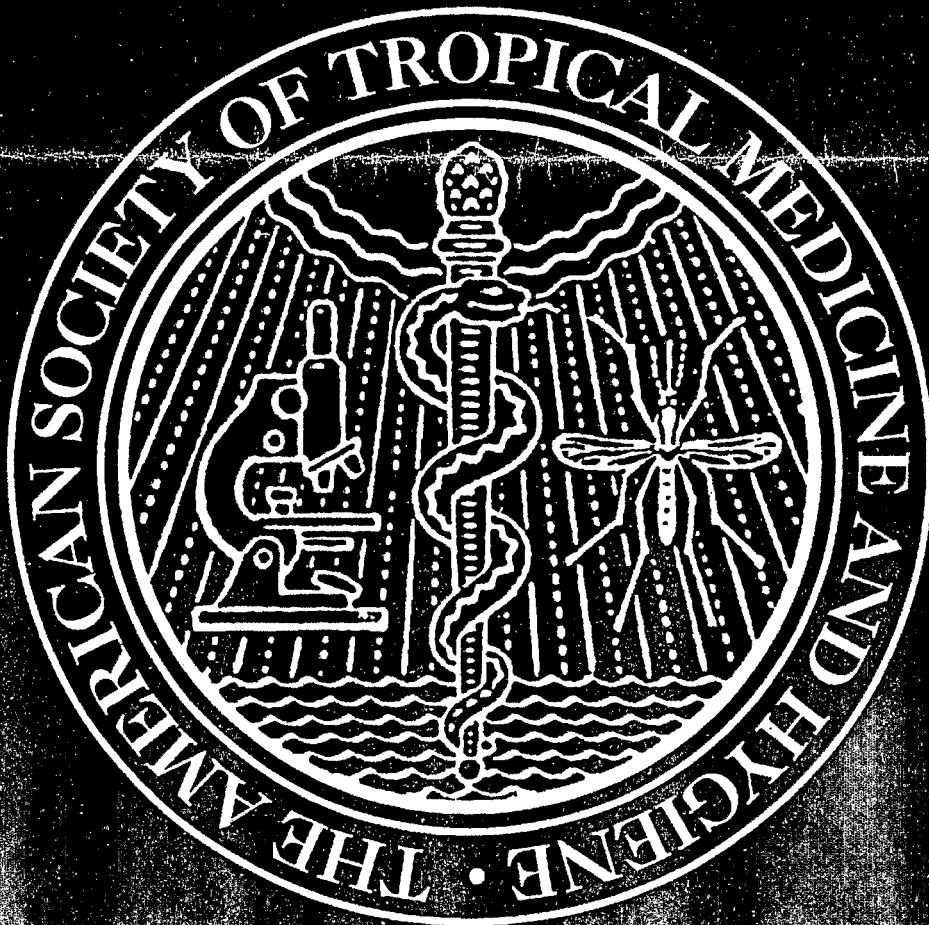
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