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Interleukin (IL)-10 and tumor necrosis factor-alpha (TNF-α) profiles of Nigerians with *Trypanosoma brucei gambiense* infection

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Abstract

We evaluated the profile of interleukin (IL)-10 and tumor necrosis factor-alpha (TNF-α) of 35 volunteers infected with Trypanosoma brucei gambiense in three human African trypanosomiasis (HAT) endemic communities in Abraka, Nigeria. Serum IL-10 levels were significantly higher in strongly positives than control subjects (P<0.001).The difference in serum IL-10 concentrations between early and late stages of infection was statistically significant (P<0.001). Significant elevation of CSF IL-10 concentrations was observed for HAT late stage (P<0.001). The level of TNF-α for weakly positive, moderately positive and strongly positive were significantly elevated when compared with the control subjects at χ^2 =6.37, P>0.01; χ^2 =22.79, P<0.001, χ^2=35.57, P<0.001, respectively. The mean difference in serum TNF-α concentrations between early and late stages of infection was statistically significant (P<0.0001) but not significant for CSF TNF-α levels (P>0.05). We therefore suggest that the elevated levels of IL-10 and TNF-α may implicate these cytokines as mediators of host response to HAT infection in our locality.

Key words: Interleukin -10; Tumor necrosis factor-alpha (TNF-α); *Trypanosoma brucei gambiense;* Human African Trypanosomiasis (HAT), Nigeria.

INTRODUCTION

Human African trypanosomiasis (HAT) is caused by subspecies of *Trypanosoma brucei*, transmitted by *Glossina* sp. This parasitic infection is confined to defined geographical foci in about 36 countries of sub-Saharan Africa. After infective bite, parasites initially proliferate in the hemolymphatic system (the first, or hemolymphatic, disease stage), and as the disease progresses, the central nervous system is invaded which constitute the second, or meningoencephalitic disease stage (1, 2). Also, first stage of HAT has been defined as the demonstration of the parasite in the blood or lymph fluid (3) while the late stage is the presence of trypanosomes in the CSF. However, because of the difficulty in identification of parasite in the CSF, it has been recommended that patients with white blood cell count of >5cells/μL should also be categorized into the late stage (4). Elevated levels of TNF-α and IL-10 has been implicated in HAT pathogenesis (5, 6, 7).

It has been advanced that IL-10 in both early and late-stage sleeping sickness patients were elevated when compared with the control subjects (6). High levels of IL-10 are associated with protection of the central nervous system (CNS) from inflammatory pathology when parasites first enter the brain (8). Interleukin (IL)-10 has been suggested to be a critical immunomodulator in human African trypanosomiasis. For example, IL-10 down regulates a range of inflammatory and activation markers on macrophages, including TNF-α, and it up-modulates the synthesis of soluble TNF receptors I and II (9). Also, up-regulation of TNF-α by IL-10 synthesis in *T.b. gambiense* patients has been proposed (10).

Disease severity of *T. b. gambiense*-infected patients has been correlated with increase in TNF-α (11). Also, studies using mouse models have indicated that TNF-α is associated with immune dysfunction (12) and neuropathogenesis (13). Inflammatory mediators such as TNF-α has been proposed to play a role in blood-brain-barrier dysfunction, enabling entry of trypanosomes into the CNS and thus initiating late stage of infection (14). The early stage of trypanosomiasis infection is characterized by up-regulated synthesis of the Th1 and proinflammatory cytokines such as TNF-α (15,16). Extended survival of host depends on a change of cytokine profile to a counterinflammatory and Th2 pattern (17). In Uganda and Malawi, differences in TNF-α profile of HAT patients with early and late stages of infection were reported (7).

In the face of contrasting interleukin profiles of HAT positive individuals based on geographical locations (7) and its associated impact in respect to the stage or level of infection, we investigated the TNF-α and IL-10 status of *T.b.gambiense*-infected volunteers in Abraka, Nigeria, an endemic focus where these data are lacking.

MATERIALS AND METHODS

This study was carried out in Umeghe, Urhouka and Ugono communities in Abraka, Delta State, Nigeria. These communities lie between latitude 5˚47'-6˚15N and longitude 5˚.42'-6˚E with population of over 5,000. The vegetation cover ranges from the mangrove thick forest to mixed rain forest and grass lands. The people in our studied communities are predominantly farmers.

The ethical permission was approved by the Delta State Ministry of Health, Asaba, Delta State and Eku Baptist Hospital, Eku, Delta State, Nigeria. Prior to the commencement of this investigation, community mobilization campaign was carried out where we explained the nature, objectives and benefits of the investigation so as to obtain informed consents. A total of 474 consented volunteers were screened using card agglutination test for trypanosomiasis (CATT) kit (18). Out of the 44 seropositive volunteers, 35 of them subsequently consented to participate in further investigations. Sera obtained from venous blood were used to categorize the level of infection by double serial dilution as: weakly positive (1:2-1:4), moderately positive (1:8-1:16) and strongly positive (≥1:32) according to the manufacturer's instruction (Intituut voor Tropische Geneeskunde, Antwerpen, Belgium). Staging of HAT into early and late stages was determined using (4) criteria. Volunteers with the overt diseases such as malaria, viral hepatitis B, HIV, measles, sickle cell anaemia were excluded from this study using standard procedures. CSF collected by lumbar puncture and venous blood were obtained from 35 seropositive volunteers and the white blood cell counts were determined using an automated heamatology analyzer (BC-2300). The sera and CSF were analysed to determine IL-10 and TNF-α concentrations using standard Enzyme linked Immunosorbent Assay (ELISA) according to the manufacturer's instruction (Abcam Plc, United kingdom).

Data obtained were subjected to statistical analysis, namely, Welch t-test, Chi-square test and Tukey-analysis of variance (ANOVA) using Instat and Microsoft Excel packages.

RESULTS

Table 1 shows IL-10 concentration among 3 categories of seropositive individuals. The level of IL-10 for weakly positive (64 .98 ±11.38 pg/ml) and moderately positive (76 ±20.3 pg/ml) when compared with the control subjects (73.59±6.85pg/ml) were not significant at χ^2 =1.01, P>0.01; χ^2 = 0.078, P>0.01, respectively. Serum IL-10 levels were significantly higher among strongly positive volunteers (235.5±22.83pg/ml) than control subjects (χ^2 =1.01, P<0.001). There was significant differences in the serum IL-10 concentrations of the 3 categories of the seropositive volunteers (F =294.43, P<0.0001).

Table 2 presents the IL-10 concentrations in serum and CSF of HAT early and late stages. The difference in serum IL-10 concentrations between early (146.66±2.11pg/ml) and late (378.2±2.23pg/ml) stages of infection was statistically significant (t = 188.26, 95% CI: 229.55-234.85, P<0.001). Significantly higher CSF IL-10 concentrations was observed for HAT late stage (128.61±1.27pg/ml) than early stage (65.67±1.07pg/ml) (t = 97.69, 95% CI: 61.55-64.32, P<0.001).

The level of TNF-α for weakly positive (24 ±2.81pg/ml), moderately positive (32.5±2.18 pg/ml) and strongly positive (73.5 ±22.83) were significantly elevated when compared with the control subjects χ^2 =6.37, P>0.01; χ^2 =22.79, P<0.001, χ^2=35.57, P<0.001, respectively. The differences in the serum TNF-α concentrations for the 3 categories of seropositive subjects, was statistically significant (F = 39.73, P<0.001) (Table 3).

Table 4 shows TNF-α concentration in serum and CSF of HAT early and late stages. The mean difference in serum TNF-α concentrations between early (43.09±2.08pg/ml) and late

(81.6±1.52pg/ml) stages of infection was statistically significant (t = 33.85, 95% CI: 36.07-40.95, *P*<0.0001).The CSF TNF-α concentration was not significantly elevated between HAT early (25.53±1.53pg/ml) and late ((25.85±0.1pg/ml) stages (t = 0.408, 95% CI: 61.55-64.32, *P*>0.05)

Table 1: IL-10 concentrations among categories of seropositive volunteers

Level of infection	Weakly positive n = 9	Moderately positive n = 12	Strongly positive n = 14	Control n = 15
Mean	64 .98 ±11.38	76 ±20.3	235.5±22.83	73.59±6.85
χ^2	1.01	0.078	355.78	
F-value	294.43			

Table 2: IL-10 concentrations in serum and CSF of HAT early and late stages

Stage of HAT	Early stage n = 12	Late stage n = 4	Mean difference
Mean SerumIL-10 (pg/ml)	146.66±2.11	378.2±2.23	t-value = 188.26, 95%CI: 229.55-234.85,
Mean CSF IL-10 (pg/ml)	65.67±1.07	128.61±1.27	t-value= 97.69, 95% CI: 61.55-64.32,

Table 3: TNF-α concentrations among categories of seropositive volunteers

Level of infection	Weakly positive n = 9	Moderately positive n = 12	Strongly positive n = 14	Control n = 15
Mean	24 ±2.81	32.5±2.18	73.5 ±22.83	14.41±0.41
χ^2	6.37	22.79	35.57	
F-value	39.73			

Table 4: TNF-α concentrations in serum and CSF of HAT early and late stages

Stage of HAT	Early stage n = 12	Late stage n = 4	Mean difference
Mean Serum TNF-α (pg/ml)	43.09±2.08	81.6±1.52	t-value=33.85, 95% CI: 36.07-40.95,
Mean CSF TNF-α (pg/ml)	25.53±1.53	25.85±0.1	t –value= 0.408 95% CI: 61.55-64.32

DISCUSSION

Increased levels of sera IL-10 was observed in the strongly positives and among volunteers with late stage HAT infection. Also, CSF IL-10 levels were elevated in late stage infection. These

observations suggest that IL-10 may be implicated in the immunopathology of *T.b.gambiense* infection. Our findings corroborates the report of (5) who observed higher levels of IL-10 in the serum and CSF of HAT individuals in late stage than early stage. The elevation of IL-10 may be ascribed to antigen inhibition properties which results in abrogated proliferative responses (19). Also, this result is expected considering the fact that IL-10 enhances proliferation, differentiation and immunoglobin secretion of B-cells (20, 21).

We observed that serum TNF-α concentration was higher among the HAT seropositive individuals than the control subjects. Also, late stage infection had higher serum TNF-α level than HAT early stage. Furthermore, the levels of TNF-α in the CSF of late and early stages of HAT was not different. Our finding of increase in serum TNF-α concentrations with disease progression supports the report of (11) where it was documented that the levels of serum TNF-α of *T. b. gambiense*-infected patients correlated with disease severity. *In vitro* study suggests that components of the glycosyl phosphatidyl inositol (GPI)-anchored trypanosome variant surface glycoprotein (VSG) triggers macrophages (22, 23) to produce TNF-α (24). TNF- α has been suggested to be involved in *T.b. gambiense* growth control in the face of increase in the trypanosome number and lifespan when anti-TNF-α was introduced into cultures of microphages and trypanosome parasite (24). We therefore hypothesize that the greater the severity of HAT infection the more GPI-anchored trypanosome VSG in blood circulation and hence greater production of TNF-α. This therefore suggests that the elevated serum TNF-α level in HAT patients may implicate this cytokine in the immunopathogenesis of the disease. Furthermore, the imbalance between pro and anti-inflammatory cytokines could contribute to the chronicity of this infection as the elevated TNF- α could not be sufficiently down-regulated by the anti-inflammatory property of IL-10 in the serum of HAT infected individuals. TNF-α was detected in the CSF as reported by (5). Our observation of no variation in TNF- α concentrations between first and late stages supports the report of (9) who implicated IL-10 in the reduced levels of TNF-α. Impaired secretion of TNF- α by macrophages has been associated with an increased expression of anti-inflammatory cytokines (25, 7) which could be an attempt to ameliorate neuropathological conditions (8, 7) associated with *T. b. gambiense* infection. Since IL-10 levels were elevated in the CSF of HAT late stage, we propose a regulatory interplay between IL-10 and TNF-α in the CSF of late stage *T.b. gambiense*- infected individuals in our locality.

We showed elevated levels of IL-10 in both serum and CSF of HAT infected subjects. Also, TNF-α levels were elevated in the serum of infected volunteers. We therefore conclude that these cytokines may be implicated in the immunopathogenesis of HAT infection. Furthermore, our data suggests that IL-10 could be a biomarker of HAT late stage and hence could be used in stage determination of *T. b. gambiense* infection.

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