Original Article

Cytokines Responses to Human African Trypanosomiasis Infection in Abraka, Nigeria

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ABSTRACT

Background: The staging of human African trypanosomiasis (HAT) has been greeted with different benchmarks ranging from white blood cell counts to the use of immune component profiles across regions.
Objective: The aim of this study was to analyze an array of cytokines to identify potential markers that could be used in the staging of HAT in Nigeria.
Methods: Sera and cerebrospinal fluid (CSF) of 35 HAT seropositives from Abraka, Delta State, Nigeria were subjected to cytokines (interleukin-10 [IL-10], tumor necrosis factor-α, IL-1α, IL-7, and IL-13) analysis using enzyme-linked immunosorbent assay. Welch *t*-test and Tukey analysis of variance were used to analyze the data.
Results: Comparing mean cytokine levels of weakly, moderately, and strongly positives and between as early and late stages results showed significantly depressed CSF levels of IL-1α and IL-7 while IL-10 was significantly elevated in the strongly positives as well as in the late stage.
Conclusion: We strongly suggest that IL-10 could be playing a key role in the immuno-pathology of HAT, thus should be considered a biomarker for the late stage.

Key words: Cytokines, human African trypanosomiasis, late stage, Nigeria

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INTRODUCTION

Human African trypanosomiasis (HAT) is caused by the subspecies of *Trypanosoma brucei* vectored by *Glossina*. After infective bite, parasites initially proliferate in the hemolymphatic system (first stage); and as the disease progresses, the central nervous system (CNS) is invaded (second stage). However, because of the difficulty in the identification of parasites in the cerebrospinal fluid (CSF), it has been recommended that counts of white blood cells (WBCs) could be used in staging HAT[1] because incorrect staging of HAT could lead to complications from inappropriate application of therapy. However, the use of CSF WBCs counts have been challenged as different endemic regions have reported varied benchmarks.[2-4] Hence, the quest to using components of the immune system in the staging of HAT should be explored since the control of African trypanosomiasis (AT) partly requires the signaling of immune cells by cytokines.[5,6]

Cytokines orchestrate a Type I and/or a Type II immune response(s) that plays a role in AT disease outcomes.[6-10] Trypanosome-derived products activates the generation of pro-inflammatory mediators (interleukin-alpha [IL-1α] and tumor necrosis factor-alpha [TNF-α]);[11] and it has been proposed to be part of the immune process leading to the pathological conditions of HAT.[12,13] Similarly, IL-7, a Type I cytokine-triggered the production of IL-1α and TNF-α that reportedly influenced disease outcome.[14] TNF-α has been implicated in the dysfunction of the blood-brain-barrier, enabling entry of trypanosomes into the CNS and thus initiating late stage of infection.[15]

IL-10, a Type II cytokine has been suggested to be a critical immunomodulator of HAT such that it down-regulates a range of inflammatory and activation markers on macrophages including TNF-α.[16] However, up-regulation of TNF-α by IL-10 in *T. brucei gambiense*-infected patients has been demonstrated.[12] The role of Type II cytokines (IL-10 and IL-13) in conferring immunity to HAT positives is highly speculative because of the contrasting reports by authors who have advanced deleterious,[17] protective,[9] or null effects.[18]

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In addition, data from Uganda and Malawi have shown differences in TNF-α profile of HAT patients with early and late stages of infection.[19] In the middle of these varied suggestions regarding the roles of some cytokines in HAT disease progression, it was imperative we described for our locality. Thus, the roles of IL-10, TNF-α, IL-α, IL-7, and IL-13 among seropositives as well in the early and late stages of HAT are discussed.

MATERIALS AND METHODS

Study area

Volunteers were drawn from three communities (Umeghe, Urhouka and Ugono) of Abraka, a HAT endemic focus. Abraka which is in Delta State, Nigeria lies between latitude 5º47' to 6°15'N and longitude 5°42' to 6E. The predominant occupation of the over 5000 population is farming.

Ethical considerations

Ethical permission was obtained from the Delta State Ministry of Health and Eku Baptist Hospital. Informed consents were sought and granted by participants.

Staging human African trypanosomiasis

Of the 474 screened using card agglutination tests for trypanosomiasis, only 35 were recruited into the study being seropositive as well as measured up to the inclusion criteria. The demographics of the 35 positives are thus: 20 females and 15 males; age group ranged between 7 and 70 years with the following breakdown (7–10 years [$n = 5$], 11–18 years [$n = 14$], 19–50 years [$n = 13$] and 51–70 years [$n = 3$]). Double serial dilution were used to group seropositives into: Weak (1:2–1:4) ($n = 9$), moderate (1:8–1:16) ($n = 12$), and strong (≥1:32).[14] In addition, the 35 seropositives were further screened for parasites in the blood and CSF including determining the counts of white blood cells in the CSF. So, 16 persons were either at the early ($n = 12$) or late stage ($n = 4$) of HAT.[1] Following medical examinations, most of the seropositives had symptoms such as malaise, anemia, headache, pyrexia, weight loss, and weakness.

Exclusion criteria

We excluded volunteers with malaria particularly those with moderate/heavy parasite load including individuals showing symptoms of malaria. Similarly, individuals with overt diseases such as viral hepatitis B, HIV, sickle cell anemia, and diabetes were identified using standard procedures and excluded.

Cytokine assay

Sera and CSF were obtained from the positives and then analyzed for IL-10, TNF-α, IL-1α, IL-7, and IL-13 using standard enzyme-linked immunosorbent assay according to the manufacture's protocol (Abcam Plc, United Kingdom). Similarly, cytokine analysis was carried out on 20 control subjects who were HAT negative among the population.

Statistical analysis

Data obtained were subjected to the test instruments of Welch *t*-test and Tukey analysis of variance using InStat Graphpad Statistical Package, Inc., (CA 92037, USA).

RESULTS

Table I shows the distribution of cytokine levels in relation to seropositive status. The mean level of IL-10 in the strongly positive was highly elevated ($P < 0.0001$). Similarly, mean TNF-α for strongly positive was relatively high ($P < 0.001$). Other cytokines (IL-1α, IL-7, and IL-13) were not significant with the degree of positivity.

Table II compares the cytokine levels between early and late stages of HAT. Late stage of HAT for mean IL-10 was significantly elevated in the serum and the CSF. Raised mean levels were only observed in TNF-α in the serum of late stage, but in the CSF, no significant change was seen. Meanwhile, CSF IL-1α and IL-7 were significantly depressed in the late stage ($P < 0.05$). For IL-13, no change was recorded both in the serum and in the CSF ($P > 0.05$). However, our results should be viewed with caution because of the small sample sizes for individuals at the early ($n = 12$) and late ($n = 4$) stages.

DISCUSSION

High levels of IL-10 are associated with protection of CNS from inflammatory pathology particularly at the point when parasites first enter the brain.[20] Our result on serum IL-10 in the strongly positives and late stage of HAT corroborates the findings of Sternberg *et al*.[21] IL-10 is known to facilitate the proliferation, differentiation, and immunoglobulin secretion processes of B-cells.[3,22] Furthermore, raised IL-10 response has been ascribed to antigen inhibition properties.[23]

An *in vitro* study has implicated TNF-α as a growth control factor of *T. b. gambiense* in the midst of increased number and lifespan.[24] Thus suggesting that raised TNF-α could be conferring some form of protection to *T. b. gambiense*-infected

Table I: Cytokine levels of seropositive and negative volunteers

Level of positivity	IL-10 (pg/ml)	TNF-α (pg/ml)	IL-1 α (pg/ml)	IL-7 (pg/ml)	IL-13 (pg/ml)
Weak (n=9)	64.98±11.38	24.98±2.81	92.99±9.81	133.83±22.23	55.48±3.39
Moderate (n=12)	76±12.31	32.51±2.18	97.49±17.97	140.08±34.68	63.28±9.97
Strong (n=14)	235.52±22.83	73.51±22.83	117.34±27.19	125.82±7.45	66.68±7.87
Negative (n=20)	73.59±6.85	14.41±0.41	78.22±6.95	79.22±9.91	50.67±4.17
F	114.43	39.73	1.75	0.61	1.85

n: Number examined, IL: Interleukin, TNF-α: Tumor necrosis factor-alpha

Table II: Cytokine levels in early and late stages of human African trypanosomiasis

Cytokine (pg/ml)	Mean±SD		*t*	*P*
	Early (*n*=12)	Late (*n*=4)		
Serum (IL-10)	146.66±2.11	378.2±2.23	45.26	<0.01
CSF (IL-10)	65.67±1.07	128.61±1.27	10.69	<0.05
Serum (TNF-α)	43.09±2.08	81.61±1.52	5.85	>0.05
CSF (TNF-α)	25.53±1.53	25.85±0.13	0.41	>0.05
Serum (IL-1α)	95.41±10.51	110.75±9.66	1.04	>0.05
CSF (IL-1α)	103.85±14.85	48.87±17.65	7.06	<0.05
Serum (IL-7)	135.98±4.43	122.20±6.29	1.87	>0.05
CSF (IL-7)	90.83±8.89	57.56±0.34	3.25	>0.05
Serum (IL-13)	62.01±4	65.47±4.22	1.22	>0.05
CSF (IL-13)	23.49±2.65	22.41±1.74	0.76	>0.05

CSF: Cerebrospinal fluid, IL: Interleukin,
TNF-α: Tumor necrosis factor-alpha, SD: Standard deviation

individuals as this may be the case in the seropositives as well as in the serum of late stage. However, no change was seen in the CSF of the late and early HAT patients. Impaired secretion of TNF-α by macrophages has been associated with an increased expression of anti-inflammatory cytokines.[19] The interplay between pro- and anti-inflammatory cytokines could be the case here as it has been evidently argued that IL-10 could modulate.[16] We are therefore of the view that this likely interaction could be the narrative for CSF TNF-α being a possible attempt to ameliorating neuropathological conditions.[19,20] Similarly, the suppressed levels of CSF IL-1α and IL-7 could be the effect of the activity of other anti-inflammatory cytokines following infection.[16]

In this study, only IL-13 was unaltered among seropositives and between late and early stages of HAT in the serum and CSF. This is quite instructive in the sense that it shows noninvolvement in the immunopathology of HAT. In support of this, the data on mice suggested that IL-13 were not the main trigger of alternative macrophages because IL-13 signaling occurred independently of an anti-inflammatory cytokine (IL-4), thereby corroborating the natural propensity of animals to develop alternatively activated macrophages.[10,25]

CONCLUSION

It is evident that IL-10, TNF-α, IL-α, and IL-7 are interacting in a complex manner with itself and others in boosting immunity of HAT patients. However, prominent among these cytokines is IL-10 being raised in seropositives as well as in serum and CSF of late stage of HAT. We thus suggest that IL-10 should be considered in the staging of HAT as it has proved to be a potential biomarker.

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Conflicts of interest

There are no conflicts of interest.

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