

# Interleukin (IL)-1 $\alpha$ , IL-7 and IL-13 Profiles among Nigerians with *Trypanosoma brucei gambiense* Infection

<sup>1</sup>Isaac, C., <sup>1</sup>\*Nmorsi, O.P.G., <sup>1</sup>Igbinosa, I.B., <sup>2</sup>Umukoro, D.O. <sup>3</sup>Imarenezor, E.P.K <sup>2,4</sup>Ukwandu, N.C.D.

1. Tropical Disease Research Unit, Department of Zoology, Ambrose Alli University, Ekpoma, Nigeria

2. Family Medicine Department, Delta State University, Abraka.

3. Department of Microbiology, Ambrose Alli University, Ekpoma, Nigeria

4. Department of Medical Microbiology, Ambrose Alli University, Ekpoma, Nigeria

Corresponding author: Prof. O.P.G. Nmorsi

E-mail: nmorsiopg@yahoo.com

**Background** Human African trypanosomiasis (HAT) is an endemic parasitic disease exclusively located in intertropical Africa where it is transmitted by the *Glossina* vector. The disease processes and in deed the control of this infection partly require an understanding of cytokine immune responses to the disease. The aim of this study therefore is to determine the profiles of IL-1 $\alpha$ , IL-7 and IL-13 among HAT positive volunteers.

**Methods and Findings** This investigation was carried out in Abraka, a HAT endemic area in Nigeria. Sera from 35 seropositive volunteers were categorized as weakly, moderately and strongly positive. Also, 16 of these 35 seropositive subjects demonstrated parasite in the blood and as a result were further grouped into early (n=4) and late (n=12) stages of HAT based on the demonstration of trypanosomes and the count of white blood cell in the cerebrospinal fluid (CSF). Sera of seropositive volunteers were subjected to interleukins (IL-) (IL-1 $\alpha$ , IL-7 and IL-13) assay. Similarly, serum and the CSF of the early and late stages of HAT were also subjected to interleukin test. In the serum, IL-1 $\alpha$  levels were significantly higher among the strongly positive volunteers than the control subjects (p<0.05). Conversely, depressed levels of IL-7 was seen for HAT late stage (p<0.001). IL-7 level was significantly lower in strongly positive individuals than the control volunteers. Also, late stage IL-7 level was significantly depressed (p<0.05). There was no significant difference in IL-13 levels among HAT infected individuals and their non infected counterparts.

**Conclusion** We suggest that the depressed levels of CSF IL-1 $\alpha$  and IL-7 should serve as markers of HAT late stage in Abraka, Nigeria.

## Introduction

Sleeping sickness or human African trypanosomiasis (HAT) is an endemic parasitic disease exclusively located in intertropical Africa where it is transmitted by the *Glossina* vector (1). HAT if not detected and treated at the early stage could progress to the late stage (2). The control of African trypanosomiasis requires among others the contribution of variant specific glycoprotein-specific B- and T-cell responses (3,4) as well as the macrophage/monocyte phagocyte system (5,6). Cytokines orchestrate a type I and/or a type II immune response, which influences the outcome of African trypanosomiasis (7,8,9,10).

Trypanosome-derived products have been shown to activate the generation of pro-inflammatory mediators including IL-1 $\alpha$  by macrophages (11). Chronic secretion of macrophage-derived mediators is in part responsible for the pathogenic aspects of HAT (12,13). In laboratory infected mice with *T.b. brucei*, an increase in the level of IL-1 $\alpha$  has been reported (7), including upregulation of granulocyte and macrophage due to IL-1 $\alpha$  administration in mice (14). Synergistic role of IL-1 $\alpha$  led to enhanced cytokine production and haematopoietic recovery (15,16).

IL-7, a type 1 cytokine is documented to be induced by IL-1 $\alpha$  and TNF- $\alpha$  (17), has been implicated in the pathology of trypanosomiasis where CD8+ predominance was reported (8). Also, Th2 cells produced anti-inflammatory cytokines due to B-cell

predominance following an increase in total cell number in spleen and lymph nodes of protozoan-infected mice treated with IL-7 (18). Altered genes encoding IL7R alpha and liquid IL-7 in the CSF compartment have been observed in individuals with disease of the central nervous system (19).

The role of type II cytokines (IL-4, IL-10 and IL-13) in resistance to African trypanosomiasis is speculative based on contrasting reports that it could exert deleterious (20), protective (9,21) or no effect on the outcome of trypanosomiasis disease (22). It was shown that IL-13, an anti-inflammatory cytokine, did not influence the susceptibility of *Trypanosoma* infection in mice and were not the main trigger to alternative macrophage activation (10). IL-13 has been suggested to be a good indicator for the course of CSF infections (23).

Despite the relative importance of cytokines in the control of HAT and their divergent roles in the immunopathology of this disease, there is paucity of information in relation to the status of IL-1 $\alpha$ , IL-7 and IL-13 in *T.b. gambiense*-infected individuals in our locality. This investigation therefore seeks to determine the profiles of these cytokines in HAT positive volunteers in Abraka, an endemic focus in Nigeria.

## Materials and methods

### Study area

This study was carried out in three communities (Umeghe, Urhouka and Ugono) of Abraka, a HAT endemic focus of Nigeria. The three Abraka communities surveyed are communities. Aforementioned communities lie between latitude 5°47'-6°15N and longitude 5°.42'-6°E with population of over 5,000, who are predominantly farmers.

### Ethical considerations

Ethical permission was obtained from the Delta State Ministry of Health and Eku Baptist Hospital. Before the commencement of the investigation, the nature, objectives and benefits of the investigation were thoroughly explained to inhabitants of these communities. Informed consents were obtained from 474 consented volunteers who were screened for Trypanosomiasis infection using card agglutination test for trypanosomiasis (CATT) kit (24).

### Population recruited

Of the 474 screened, sera of 35 seropositive subjects with T.b. gambiense infection were evaluated for interleukins (IL-1 $\alpha$ , IL-7 and IL-13) levels. Also, 16 volunteers from the 35 seropositive subjects, demonstrated trypanosomes in their serum.

### Staging of HAT

Sera obtained from venous blood were used to categorize the level of infection by double serial dilution as: weakly positive (1:2-1:4) (n=9), moderately positive (1:8-1:16) (n=12) and strongly positive ( $\geq$ 1:32) (n=14) according to the manufacturer's instruction (Intituit voor Tropische Geneeskunde, Antwerpen, Belgium). Of the 35 seropositives, 16 volunteers demonstrated parasite in the blood and were further categorized into early and late stage of HAT. Volunteers categorized as early stage, demonstrated the parasite in the blood only while late stage showed the parasite both in the blood and CSF. The staging of HAT was done on the recommendation of (25). Most of the seropositive volunteers were observed to suffer from malaise, anaemia, headache, pyrexia, weight loss and weakness following the medical history and physical examination carried out on the volunteers.

### Exclusion criteria

We excluded volunteers with overt diseases like viral hepatitis B, HIV or sickle cell anaemia using standard procedures.

### Interleukin assay

Sera and CSF were obtained from 35 HAT positive subjects and then assayed for IL-1 $\alpha$ , IL-7 and IL-13 using standard enzyme linked Immunosorbent assay (ELISA) according to the manufacturer's instruction (Abcam Plc, United Kingdom). Also, interleukin evaluation was carried out on 10 control group who were selected on the basis of being HAT negative and also not infected with the aforementioned diseases.

### Data analysis

Data obtained were subjected to statistical analysis, namely Welch t-test and Tukey-analysis of variance (ANOVA) using Instat statistical package.

## Results

Figure 1 shows IL-1 $\alpha$  profile among categories of seropositive human subjects. The differences in the levels of IL-1 $\alpha$  for weakly positive and moderately positive individuals compared with the control subjects were not significant ( $p>0.01$ ). Serum IL-1 $\alpha$  levels were significantly higher in strongly positive volunteers than control subject ( $p<0.05$ ). The serum IL-1 $\alpha$  concentrations for the categories of seropositive subjects were not significant ( $p>0.01$ ).

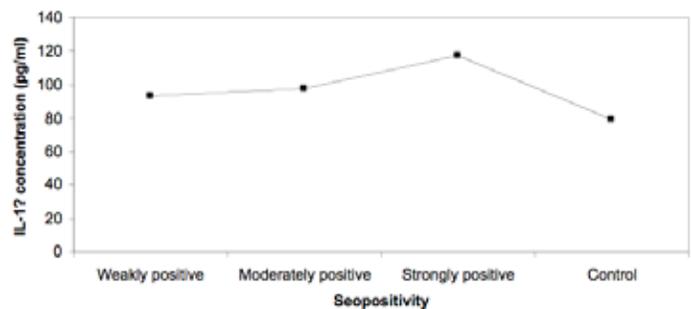


FIGURE 1: IL-1 $\alpha$  PROFILE AMONG CATEGORIES OF SEROPOSITIVE VOLUNTEERS

Table 1 presents the IL-1 $\alpha$  concentrations in serum and CSF of HAT volunteers with early and late stages of infection. The difference in serum IL-1 $\alpha$  concentrations between early and late stages of infection was statistically significant ( $p<0.001$ ). Significant depression of CSF IL-1 $\alpha$  concentrations was observed for HAT late stage ( $p<0.001$ ).

Stage of HAT	Early stage n=12	Late stage n=4	Mean difference
Mean			t-value = 5
Serum IL-1 $\alpha$ (pg/ml)	95.41 $\pm$ 10.51	125.75 $\pm$ 9.66	95% CI: 17.33 to 42.6
Mean			t-value= 7.06
CSF IL-1 $\alpha$ (pg/ml)	103.85 $\pm$ 14.85	48.87 $\pm$ 17.65	95% CI: -80.81 to -64.32

TABLE 1: IL-1 $\alpha$  PROFILE IN SERUM AND CSF OF HAT EARLY AND LATE STAGES

The levels of IL-7 for weakly positive and moderately positive volunteers were not significantly depressed ( $p>0.05$ ). However, IL-7 level in strongly positive volunteers was significantly lower than the control subjects ( $p<0.001$ ). There was no significant difference in the serum IL-7 concentration among seropositive subjects ( $p>0.01$ ) (Figure 2).

Table 2 shows IL-7 concentrations in serum and CSF of individuals with HAT early and late stages of infection. The mean difference in serum IL-7 concentrations between early stage and late stage of infection was statistically significant ( $p<0.05$ ). The CSF IL-7 concentration was significantly higher in volunteers with early stage of HAT than late stage ( $p<0.05$ ).

The mean differences of IL-13 levels between weakly positive, moderately positive and strongly positive volunteers com-

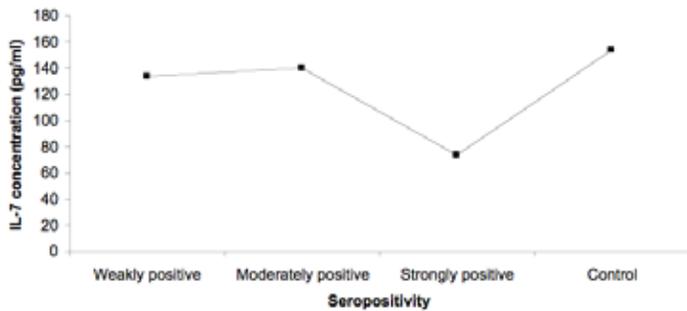


FIGURE 2: IL-7 PROFILES AMONG CATEGORIES OF SEROPOSITIVE VOLUNTEERS

Stage of HAT	Early stage n=12	Late stage n=4	Mean difference
Mean	135.98±4.43	122.20±6.29	t-value=4.87
Serum IL-7 (pg/ml)			95% CI: -19.8 to -7.69
Mean	90.83±8.89	57.56±0.34	t-value= 6.25
CSF IL-7 (pg/ml)			95% CI: -44.68 to 21.85,

TABLE 2: IL-7 PROFILE IN SERUM AND CSF OF HAT EARLY AND LATE STAGES

red with HAT negative volunteers were not significant ( $p>0.05$ ) (Figure 3). The differences in the levels of IL-13 among the categories of seropositive volunteers were also not significant ( $p>0.01$ ).

Table 3 shows IL-13 concentrations in serum and CSF of volunteers with early and late stages of HAT. The mean difference in serum IL-13 concentrations between HAT early stage and late stage of infection was not statistically significant ( $p>0.05$ ). There was also no significant difference in the mean CSF IL-13 concentration between HAT early stage and late stage ( $p>0.05$ ).

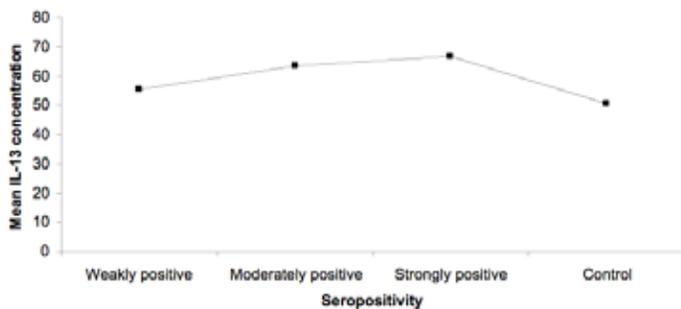


FIGURE 3: IL-13 PROFILE AMONG CATEGORIES OF SEROPOSITIVE VOLUNTEERS

## Discussion

Our data demonstrated elevated level of serum IL-1 $\alpha$  among the strongly seropositive volunteers. Similarly, serum IL-1 $\alpha$  levels of HAT late stage patients were increased compared to early stage. In contrast, we also showed depressed concentration of CSF IL-1 $\alpha$  in HAT late stage. The elevated levels of serum IL-1 $\alpha$  supports the report of (11) in which derived variant surface glycoprotein

Stage of HAT	Early stage n=12	Late stage n=4	Mean difference
Mean	62.01±4.0	65.47±4.22	t-value=1.22
Serum IL-13 (pg/ml)			95% CI: -1.94 to 7.06
Mean	23.49±2.65	22.4±1.74	t-value= 0.76
CSF IL-13 (pg/ml)			95% CI: -4.16 to 1.98

TABLE 3: IL-13 PROFILE IN SERUM AND CSF OF HAT EARLY AND LATE STAGES

(VSG) from *T. brucei*, elicit IL-1 $\alpha$ . This suggests that IL-1 $\alpha$  may be implicated in the immunopathogenesis of HAT infection. Also, we report depressed levels of CSF IL-1 $\alpha$  in HAT late stage. We observed that the profile of IL-13, an anti-inflammatory cytokine was unaltered in the CSF of HAT late stage. This data therefore suggests that the imbalance between IL-13 and IL-1 $\alpha$  could be due to the inability of IL-13 to immunomodulate IL-1 $\alpha$  in HAT late stage. This partly contradicts the hypothesis of a switch from dominant type I to a predominant type II cytokine (26, 27). However, we hypothesize that other anti-inflammatory cytokines such as IL-10 may be implicated in the down regulation of this cytokine in the CSF (28).

We observed depressed levels of IL-7 with severity of infection. These findings contradicts the report that human chronic trypanosomiasis was associated with elevated IL-7 production (8). It has been documented that natural killer cells are recognized as major effectors of innate resistance to protozoan infections through the control of the growth of this pathogens by indirectly producing cytokines (29). In a report, IL-7 deficient patients were observed to be deficient of T and natural killer (NK) cells (30). This study therefore suggests that depressed IL-7 concentration with disease progression may be associated with the pathogenic conditions in HAT positive patients which could be due to a regulatory role of anti-inflammatory cytokine in the CSF as documented by (28).

We reported unaltered IL-13 levels in seropositive volunteers and serum/CSF IL-13 levels in the early and late stages of HAT-infected human subjects. We therefore suggest that IL-13 is not a mediator to host immune response of HAT infection. This observation is in consonance with the report that IL-13 in trypanosome susceptible mice was not the main trigger of alternative macrophages because IL-13 signaling occurred independently of an anti-inflammatory cytokine (IL-4), corroborating the natural propensity of animals to develop alternatively activated macrophages (10, 31). The clearance of trypanosome parasites through innate immunity involves macrophage/monocyte phagocyte system (5,6).

This investigation showed elevated IL-1 $\alpha$  among the strongly positive volunteers. Conversely, depressed levels of IL-7 were observed in HAT late stage. These data suggests that IL-1 $\alpha$  and IL-7 could be major mediators in the immunopathology of *T. b. gambiense* infection. Additionally, we propose that depressed levels of CSF IL-1 $\alpha$  and IL-7 should serve as markers of HAT late stage in our locality.

## References

- Vicherman K (1985) Developmental cycles and biology of pathogenic trypanosomes. *Brit Med Bull* 41: 105–114.
- Greenwood BM, Whittle HCC (1980) The pathogenesis of sleeping sickness. *Trans R Soc Trop Med Hyg* 74: 716-725.
- Hertz CJ, Filutowicz H, Mansfield JM (1998) Resistance to the African trypanosome is IFN- dependent. *J Immunol* 161:6775-6783
- Reinitz DM, Mansfield JM (1990) T-cell-independent and T-cell-dependent B-cell responses to exposed variant surface glycoprotein epitopes in trypanosome-infected mice. *Infect Immun* 58:2337-2342.
- Dempsey WL, Mansfield JM (1983) Lymphocyte functions in experimental African trypanosomiasis. V. Role of antibody and the mononuclear phagocyte system in variant-specific immunity. *J Immunol* 130:405-411.
- Kaushik RS, Uzonna JE, Gordon JR, Tabel H (1999) Innate resistance to Trypanosoma congolense infections: differential production of nitric oxide by macrophages from susceptible BALB/c and resistant C57BL/6 mice. *Exp Parasitol* 92:131-143.
- Sileghem MR, Darji A, Hamers R, De Baetselier P (1998) Modulation of IL-1 $\alpha$  production and IL-1 $\alpha$  release during experimental trypanosome infections. *Immunol* 68: 137-139.
- Fonesco SG, Reis MM, Coelho V, Nogueira LG, Monteiro SM, Mairena EC, Bacal F, Bocchi E, Guiherma L, Zheng XX, Liew FY, Higuchi ML, Kalil J, Cunha-Neto E (2007) Locally produced survival cytokines IL-15 and IL-7 may be associated to the predominance of CD8+ T cells at heart lesions of human chronic chagas disease cardiomyopathy. *Scand. J Immunol* 66(2-3): 362-71.
- Bakhiet M, Jansson L, Busche P, Holmdahl R, Kristensson K, Olsson T (1996) Control of parasitemia and survival during Trypanosoma brucei infection is related to strain-dependent ability to produce IL-4. *J Immunol* 157:3518-3526.
- Noël W, Hassanzadeh G, Raes G, Namangala B, Deams I, Brys L, Brombacher F, De Baetselier P, Beschin A (2002) Infection-stage dependent modulation of macrophage activation in Trypanosoma congolense-resistant and susceptible mice. *Infect Immun* 70: 6180-6187.
- Tachado SD, Schofield L (1994) Glycosylphatidylinositol toxin of Trypanosoma brucei regulates IL-1 $\alpha$  and TNF- $\alpha$  expressions in macrophages by protein tyrosine kinase-mediated signal transduction. *Biochem Biophys Res Commun* 205: 984-991.
- Rhind SG, Sabiston BH, Shek PN, Buguet A, Muanga G, Stanghelli A, Dumas M, Radomski, WM (1997) Effect of Melarsoprol treatment on circulating IL-10 and TNF- $\alpha$  levels in human African trypanosomiasis. *Clin Immunol Immunopathol* 83: 185-189.
- Okomo-Assoumon, MC, Daulouede S, Lemesre J, Mouanda-N'Zila A, Vincendeau P (1995) Correlation of high serum levels of tumor necrosis factor- $\alpha$  with disease severity in human African trypanosomiasis. *Am J Trop Med Hyg* 53(5): 539-543.
- Hestdal, K., Jacobsen, S.E., Ruscetti, F.W., Dubois, C.M., Longo, D.H., Chizzonite, R., Oppenheim, J.J. and Keller, J.R. (1992) In vivo effect Interleukin-1 alpha on hematopoiesis role of colony-stimulating factor receptor modulation. *Blood*, 80(10): 2486-2494.
- Pang G, Couch L, Batey R, Clancy R, Cripps A (1994) GM-CSF, IL- $\alpha$ , IL-1 $\alpha$ , IL-6, IL-8, IL-10, ICAM-1 and VCAM-1 gene expression and cytokine production in human duodenal fibroblasts stimulated with lipopolysaccharide, IL-1 $\alpha$  and TNF- $\alpha$ . *Clin Exp Immunol* 96: 437-443.
- Kovacs CJ, Kerr JA, Daly BM, Evans MJ, Johnke RM (1998) Interleukin 1 alpha (IL-1 $\alpha$ ) and macrophage colony-stimulating factor (M-CSF) accelerate recovery from multiple drug-induced myelosuppression. *Anticancer Res* 18(3A): 1805-1812.
- Weitzmann NM, Cenci S, Ritas L, Brown C, Pacifici RC (2000) Interleukin-7 stimulates osteoclastogenic formation by up-regulating T-cell production of soluble osteoclastogenic cytokines. *Immunobiol* 96(5): 1873-1878.
- Gessner A, Will A, Vieth M, Schroppe K, Rollinghoff M (1995) Stimulation of B-cell lymphopoiesis by interleukin-7 leads to aggravation of murine leishmaniasis (1995). *Immunol* 84(3): 416-422.
- Landmark F, Duvefelt K, Lacobaeus E, Kockum I, Wallstrom E et al (2007) Variation in Interleukin-7 receptor alpha chain (IL-7R) influences risk of multiple sclerosis. *Nat Genet* 39(9): 1108-1113.
- Uzonna, JE, Kaushik RS, Gordon JR, Tabel H (1998) Immunoregulation in experimental murine Trypanosoma congolense infection: anti-IL-10 antibodies reverse trypanosome-mediated suppression of lymphocyte proliferation in vitro and moderately prolong the lifespan of genetically susceptible BALB/c mice. *Parasite Immunol* 20:293-302.
- Inoue N, Inoue M, Kuriki K, Yamaguchi H, Nagasawa H et al (1999) Interleukin 4 is a crucial cytokine in controlling Trypanosoma brucei gambiense infection in mice. *Vet Parasitol* 86:173-184.
- Schopf, LR, Filutowicz H, Bi XJ, Mansfield JM (1998) Interleukin-4-dependent immunoglobulin G1 isotype switch in the presence of a polarized antigen-specific Th1-cell response to the trypanosome variant surface glycoprotein. *Infect Immun* 66:451-461.
- Mukodzi S, Muller C, Ahrens N, Kuno S, Stenger R-D (2002). CSF cytokines (IL-6, IL-12, and IL-13): the right marker for shunt reimplantation after infections? *Critical Care*, 6(Suppl.1): P64.
- Magnus E, Vervoot T, Van Meirvenne NA (1978) A card agglutination test with stained diagnosis of T.b.gambiense. *Annales de la société belge de Médecine Troicale* 58: 169-76.
- World Health Organization (1998). Control and Surveillance of African trypanosomiasis. Report of a WHO expert committee. World Health Organization. Geneva, Switzerland. Technical Report. Series. No., 8881, 114.
- Goerdts S, Orfanos CE (1999) Other functions, other genes: alternative activation of antigen-presenting cells. *Immunity*, 10: 137-142.
- Namangala B, Noël W, De Baetselier P, Brys L, Beschin A (2001) Relative contribution of interferon-gamma and interleukin-10 to resistance to murine African trypanosomiasis. *J Infect Dis* 183: 1794-1800.
- de Waal, Malafy, R. and Moore, K.W. (1998). Interleukin-10. In: Thompson AW., Ed. The cytokine handbook. 3rd ed. San Diego: Academic Press. pp. 333-364.
- Scharton-Kersten, MT, Sher A (2002) Roles of natural killer cells in innate resistance to protozoan infections. *Curr Opin Immunol* 9(1): 44-51.

30. Ahmed A, Saha B, Patwardhan A, Shivprasad S, Nandi D (2009) The major players in adaptive immunity.1. Humoral immunity. Resonance 14(5): 455-471.

31. Mills CD, Kincaid K, Alt JM, Heilman MJ, Hill AM (2000) M-1/M-2 macrophages and the Th1/Th2 paradigm. J Immunol 164:6166-6173.

**Publish with iMedPub Journals**

<http://www.imedpub.com>

Archives of Clinical Microbiology (ACMicrob) is a new peer reviewed, international journal, with world famous scientists on the editorial board. ACMicrob is an open access journal with rapid publication of articles in all fields and areas of microbiology and infectious diseases. ACMicrob covers all aspects of basic and clinical microbiology relevant to infectious diseases including current research on diagnosis, management, treatment, preventive measures, vaccination, and methodology. Clinical microbiology relevant immunology, pathophysiology, genetics, epidemiological, and genomics studies are also welcome.

Submit your manuscript here:

<http://www.acmicrob.com>