Original article

T-Helper 4 and Cytotoxic T-Lymphocytes, Interleukin- 4 and 10 in HIV-Seropositive Patients with Uncomplicated *Plasmodium Falciparum* Malaria.

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Abstract:

Background and Aim: The biological functions of anti-inflammatory cytokines appear to be crucial in the immune response. The aim of this study is to determine the levels of the anti-inflammatory (IL-4 and IL-10) cytokine response in malaria HIV and HIV-malaria coinfecting individuals with uncomplicated *Plasmodium falciparum* malaria. **Methods:** Levels of IL-4 and IL-10 of 111 malaria-infected, 97 HIV-malaria co-infected and 79 HIV seropositive subjects as well as 73 controls were determined by enzyme linked immunosorbent assay. CD4 and CD8 cells were counted using the Dynabeads T4-T8 quantification protocol while haematological parameters were estimated using standard haematological techniques. **Results:** IL-4 and IL-10 levels were significantly higher in malaria patients ($8.18 \pm 6.41\text{pg/ml}$ and $26.29 \pm 12.08\text{pg/ml}$) than in HIV ($5.208 \pm 1.69\text{pg/ml}$) or HIV-malaria ($4.415 \pm 3.23\text{pg/ml}$) coinfecting subjects ($P < 0.05$). On the other hand, the mean CD4 and CD8 counts (1434 ± 331.05 and 2003 ± 405.73 cells/ μl respectively) of malaria infected individuals were significantly elevated ($P < 0.05$) as compared to HIV, HIV-malaria and control groups. **Conclusion:** The results of this study suggest that malaria antigen may initiate a higher anti-inflammatory cytokine response than HIV or HIV associated with malaria.

Key words: anti-inflammatory cytokines, HIV, Malaria, Nigeria

INTRODUCTION

Malaria and human immunodeficiency virus (HIV) are among the most common infections in sub-Saharan Africa and, to a lesser extent, in other developing countries. It is estimated that 38 million Africans are infected with HIV-1, whereas 300 million to 500 million suffer from malaria each year [1]. Therefore, any interaction between these infections will have a significant impact on public health, even if the statistical effect is modest. On a population basis, an increased prevalence of malaria and increased parasite density in HIV-infected individuals could lead to increased malaria transmission and affect both HIV-positive and HIV-negative individuals [1]. The increased risk of clinical malaria in HIV-positive subjects could increase the burden on clinical services in areas where HIV-1 is prevalent.

Infection with HIV-1 causes progressive cellular immunosuppression, and any resulting impairment in the im-

mune response to malaria might be associated with failure to prevent infection or to suppress parasitemia and clinical disease [2]. Both the cellular and humoral arms of the adaptive immune system are pivotal elements in the eradication of *Plasmodium* from the body, and both are critically dependent on CD4⁺ lymphocytes [3]. It has been firmly established that CD4⁺ T cells are comprised of at least two functionally different subsets, distinguished on the basis of lymphokine secretion in Th1 (IFN- γ -producing) and Th2 (interleukin-4 producing) cells. Of particular interest is the fact that CD4⁺ T cells, of either Th1 or Th2 type, also have regulatory functions in human *P. falciparum* malaria. Both Th1 and Th2 responses seem to be required to control the infection, but they need to be adequately tuned in intensity and time [4, 5]. Thus, both Th1 and Th2 are activated during infection with *P. yoelii* 17XL in C57BL/6 mice, but early activation of Th2 cells is deleterious, conferring susceptibility to infection [6]. In addition, in *P. chabaudi* infection there is a shift from Th1 to Th2 during peak para-

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sitemia that is important for clearance of parasites [7]. In contrast, the effectiveness of CD8+ T cells against the asexual blood stage of *Plasmodium* appears to be limited, although CD8+ T lymphocytes might contribute to the fight against the parasite through secretion of cytokines as IFN- γ , IL-4 IL-10 etc. The effects of HIV on malaria in adults are now well documented. The true clinical impact of malaria on HIV progression remains to be determined [8]. However, the magnitude and clinical significance of these potential interactions need further research [9].

In this study we determine the strength of the anti-inflammatory cytokines (IL-4 and IL-10) response in malaria, HIV and HIV- malaria coinfecting individuals.

MATERIALS AND METHODS

Study Design

A total of 287 individuals enrolled for this study presented with symptoms of uncomplicated malaria (i.e. temperature ranging from 37-40 °C, joint pains, no nervous system involvement) between the months of January and June 2006 at medical centers in the Benin metropolis (Lahor Medical Centre, Suyi Hospital and Milestone Hospital). The distribution of study subjects was as follows: 111 malaria infected, 97 HIV-malaria co-infected and 79 HIV-seropositive subjects as well as 73 controls. Benin City is located in the southern region of Nigeria where malaria and HIV infections are endemic. All the participants were tested for HIV and malaria and were grouped accordingly. Written consent from the patients was sought before blood samples were collected for analysis. Patients who had started antimalarials or antiretroviral therapy and those with intestinal infections were excluded from this study.

Cytokine assay

IL-4 and IL-10 cytokines levels were assayed using enzyme linked immunosorbent assay (ELISA or EIA) [10] (MABTECH Inc. Company, Sweden).

CD4/CD8 Count

The CD4 and CD8 counts were estimated using Dynabeads T4-T8 Quantification Protocol (DynaL Biotech ASA, Oslo, Norway) following the manufacturer's instructions as follows.

Pack contents:

- 2ml of Dynabeads CD14 monoclonal antibodies
- 2ml of Dynabeads CD4 monoclonal antibodies
- 2ml of Dynabeads CD8 monoclonal antibodies
- Phosphate buffer saline
- Eppendor microfuge

Sample Preparation

EDTA or ACD whole blood was mixed for 2 minutes

at room temperature, and 225 μ l and 225 μ l of washing/diluent buffer 250 μ l and whole blood, respectively, were added to 1ml microfuge tubes.

Monocyte Depletion

After addition of 25 μ l of Dynabeads CD14, the tube was capped and mixed carefully by inversion, and the mixture was incubated with a tilting and rotation action for 10 minutes at room temperature. Each tube was placed in a Dynal MPC-S magnet for 2 minutes. 200 μ l of monocyte-depleted blood was carefully transferred to 2 sets of tubes, and the tubes containing the rosette monocytes were safely discarded.

T4-T8 lymphocyte isolation

200 μ l of washing buffer was added to each of the 2 tubes containing 200 μ l of monocyte depleted blood, followed by 25 μ l of Dynabeads CD4 to one of the tubes and 25 μ l of Dynabeads CD8 to the other. The tubes were capped and the content mixed carefully by inversion. Incubation with a tilting and rotating action was done for 10 minutes at room temperature. The tubes were placed in the Dynal MPC-S for 2 minutes, and the supernatant was discarded without removing the tubes from the MPC-S magnet. The isolated cells were washed by adding 500 μ l of buffer/diluent to each tube, and the tubes were removed from the MPC-S magnet. A pre-cut sheet of paraffin was placed over the tubes. The rosettes were resuspended by inverting the tubes a few times. The paraffin sheet was safely discarded and refitted in the MPC-S magnet for 2 minutes before the supernatant was discarded. The washing steps were repeated once.

T4 and T8 cell counting

The cells were resuspended in 100 μ l of 2% glacial acetic acid tainted with gentian violet and fixed properly. The Neubauer Counting Chamber was charged and the rosettes were counted immediately in one counting area of 1 mm by 1mm with a depth of 0.1 mm

Calculation

A dilution factor of 1/2 was applied because the isolated cells from 250 μ l of whole blood were resuspended in 225 μ l of buffer +25 μ l of Dynabeads CD14.

The volume of suspension in a counting area was equal to 1mmX1mmX0.1mm=0.1mm³. If X rosettes are counted in the counting area of 0.1mm³, T4 or T8=2X10Xcells/ μ l will be counted in 1mm³.

Haematological parameters

The haematological parameters of the subjects (differential white blood cells and total white blood cells) were assessed using the Swelab Automatic Counter (Boule Medical Stockholm, (Sweden).

Statistical analysis

Data obtained from this study were analyzed using

Table 1: Mean comparison of IL-4, IL-10 and haematological indices of HIV, malaria and HIV-malaria coinfectd subjects.

Cytokines	Malaria $\bar{x} \pm S.D$	HAR-M $\bar{x} \pm S.D$	HIV $\bar{x} \pm S.D$	CONTROL $\bar{x} \pm S.D$
IL-4 (pg/ml)	8.18 $\pm$ 6.41	4.415 $\pm$ 3.23	5.208 $\pm$ 1.69	5.846 $\pm$ 1.819
IL-10 (pg/ml)	26.29 $\pm$ 12.08	4.33 $\pm$ 10.39	6.86 $\pm$ 10.98	26.008 $\pm$ 13.23
CD4 (cells/ μ l)	1434 $\pm$ 331.05	207.0 $\pm$ 841.09	274 $\pm$ 465.08	1314 $\pm$ 207.03
CD8 (cells/ μ l)	2003 $\pm$ 405.73	275 $\pm$ 20.45	317 $\pm$ 11.00	1073 $\pm$ 119.13

Key: HIV-M: Malaria HIV co- infection

ANOVA

RESULTS

The mean IL-4 concentration was significantly higher in malaria patients (8.18 $\pm$ 6.41pg/ml) than the levels obtained in HIV (5.208 $\pm$ 1.69pg/ml) and HIV-malaria (4.415 $\pm$ 3.23pg/ml) coinfectd subjects ($P < 0.05$). Furthermore, mean IL-10 concentration in malaria patients (26.29 $\pm$ 12.08 pg/ml) was significantly higher than the levels obtained in HIV (6.86 $\pm$ 10.98pg/ml) and malaria-HIV (4.33 $\pm$ 10.39pg/ml) coinfectd subjects ($P < 0.05$). The mean haematological parameters revealed a significant difference in the CD4 count (1434 $\pm$ 331.05 cells/ μ l) of malaria-infected individuals when compared to values obtained in HIV (274 $\pm$ 465.0 cells/ μ l) and HIV-malaria (207.0 $\pm$ 841.09 cells/ μ l) coinfectd subjects respectively ($P < 0.05$). The CD8 count was significantly higher in malaria-infected subjects (2003 $\pm$ 405.73 cells/ μ l) than in controls, HIV (317 $\pm$ 11.00cells/ μ l) and HIV-malaria coinfectd (275 $\pm$ 20.5cells/ μ l) subjects ($P < 0.05$), while packed cell volume was significantly higher in malaria, HIV and HIV- malaria coinfectd individuals than in control subjects (Table1).

DISCUSSION AND CONCLUSION

The depletion in haematological cells may be associated with lowered serum levels of immunological mediators, a direct outcome of which could be the poor immune response observed in HIV-infected individuals [11]. This study revealed that the mean IL-4 and IL-10 concentrations were significantly higher in malaria patients than those obtained in HIV and HIV-malaria coinfectd subjects ($P < 0.05$). Although Torre *et al* [12] reported that IL- 4 and IL-10 did not seem to be involved during the acute phase of malaria, Wrinkler *et al* [13] observed a more pronounced Th2-driven immune response during the acute phase of untreated and uncomplicated malaria caused by *P. falciparum*, with a shift towards Th1 responsiveness induced by parasite clearance. The lowered levels of Th2 obtained in HIV and HIV-malaria coinfectd individuals could be associated with the

depletion of CD4 and other cells in these group of subjects. On the other hand, there was no significant difference when the levels in control subjects was compared to values obtained in HIV and malaria-HIV coinfectd subjects ($P > 0.05$). Several members of the cytokine network have been found to play an important role in controlling the replication of the human immunodeficiency virus (HIV) in several experimental systems. Their effects can be categorized in the following three functional groups: (1) HIV-inductive cytokines; (2) HIV-suppressive cytokines; (3) cytokines with both activating and inhibiting capacities. Studies on the mechanism of these molecules have highlighted the fact that several steps of the retrovirus life cycle, from binding to budding of progeny virions from the infected cell, are affected by cytokines. This general concept has been recently substantiated by the discovery that certain beta-chemokines can act as blockers of viral entry by interfering with HIV co-receptors [14].

In conclusion, this study revealed that *P. falciparum* infection seems to initiate a stronger IL-4 and IL-10 response than HIV or HIV associated with malaria. However, more cohort studies are required to assess the impact of malaria infection on other anti-inflammatory cytokines as well as pro-inflammatory cytokines.

REFERENCES

- 1 . Whitworth J, Morgan D, Quigley M, Smith A, Mayanja B, Eotu H, Omoding N, Okongo M, Malamba S, Ojwiya A. Effect of HIV-1 and increasing immunosuppression on malaria parasitaemia and clinical episodes in adults in rural Uganda: a cohort study. *Lancet* 2000; 356: 1051-6.
- 2 . Good MF, Doolan DL. Immune effector mechanisms in malaria. *Curr Opin Immunol* 1999; 11: 412-9
- 3 . Dong C, Flavell RA. Th1 and Th2 cells. *Curr. Opin. Hematol.* 2001; 8: 47-51.
- 4 . Torre D, Speranza F, Giola M, Matteelli A, Tambini R, Biondi G. Role of Th1 and Th2 cytokines in immune response to uncomplicated *Plasmodium falciparum* malaria. *Clin. Diagn. Lab. Immunol.* 2002; 9: 348-351.
- 5 . Troye-Blomberg M, Berzins K, Perlmann P. T-cell control of immunity to the asexual blood stages of the malaria

- parasite. *Crit. Rev. Immunol.* 1994; 4: 131-155.
- 6 . Kobayashi F, Morii T, Matsui T, Fujino T, Watanabe Y, Weidanz WP, Tsuji M. Production of interleukin 10 during malaria caused by lethal and nonlethal variants of *Plasmodium yoelii yoelii*. *Parasitol. Res.* 1996; 82: 385-391.
 - 7 . Helmbj H, Kullberg M, Troye-Blomberg M. Expansion of IL-3-responsive IL-4-producing non-B non-T cells correlates with anemia and IL-3 production in mice infected with blood-stage *Plasmodium chabaudi* malaria. *Eur. J. Immunol.* 1998; 28: 2559-2570.
 - 8 . Whitworth JA, Hewitt KA. Effect of malaria on HIV-1 progression and transmission. *Lancet* 2005; 365: 196-7.
 - 9 . Khoo S, Back D, Winstonley P. The potential for interactions between antimalarial and antiretroviral drugs. *Aids* 2005; 19: 995-1005.
 - 10 . Baron EJ, Peterson LR, and Finegold FS. Immunodiagnosis In: Bailey and Scott Diagnostic Microbiology, 9th Edition. Editors: James F.S., Lisa M.Potts, Chris M., and Julie T. Publisher, Mosby. 1994: 134-145.
 - 11 . Tatfeng YM, Agbonlahor DE. Proinflammatory cytokines and haematological indices in HIV seropositive adults with uncomplicated *Plasmodium falciparum* malaria. *JTMH.* 2008; 36(2): 73-75.
 - 12 . Torre D, Giola M, Speranza F, Matteelli A, Basilico C, Biondi G. Serum levels of interleukin-18 in patients with uncomplicated *Plasmodium falciparum* malaria. *Eur. Cytokine Netw.* 2001; 12: 361-364.
 - 13 . Winkler S, Willheim M, Baier K. *et al.* Reciprocal regulation of Th1- and Th2-cytokine-producing T-cells during clearance of parasitemia in *Plasmodium falciparum* malaria. *Infect. Immun.* 1998; 66: 6040-6044.
 - 14 . Vicenzi E, Biswas P, Mengozzi M, Poli G. Role of pro-inflammatory cytokines and beta-chemokines in controlling HIV replication. *J. Leukocyte Biol.* V 1997; 62(1): 34-40.