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Relationship between pro- and anti-inflammatory cytokines profiles and some haematological parameters in some Cameroonians infected with *Onchocerca volvulus*

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ABSTRACT

Objective: To investigate the relationship between white blood cells, lymphocytes, monocytes, and Interleukin(IL)-1 α , IL-6, IL-10 and IL-13 production in Cameroonians with *Onchocerca volvulus* (*O. volvulus*) infection. **Methods:** A total of 357 individuals from five sites at Upper Sanga, Lekkie, Nyong, Kelle and Sanaga Maritime divisions and located along Sanaga valley of Sanaga River in South Cameroon were screened for the presence of *O. volvulus* using the skin snip. The levels of the interleukins (IL-) namely IL-1 α , IL-6, IL-10 and IL-13 were evaluated using enzyme linked immunoabsorbent assay techniques. Haematological parameters were evaluated using standard laboratory automated analyser. **Results:** *O. volvulus* microfilariae were found in skin tissues of 85 (23.81%) volunteers. The mean interleukin (IL-) levels in the *O. volvulus* control and infected individuals were IL-1 α in (1.65 \pm 0.79 and 2.31 \pm 0.5) pg/mL; IL-6 in (278.36 \pm 55.34 and 201.74 \pm 34.56) pg/mL; IL-10 in (436.03 \pm 208.64 and 418.49 \pm 147.88) pg/mL and IL-13 in (8.98 \pm 7.28 and 38.06 \pm 11.92) pg/mL. There was a negative correlation between monocyte counts and IL-10 concentration in positive individuals. A negative correlation of IL-6 with white blood cell and lymphocyte counts was observed ($P < 0.05$). The level of IL-13 was positively associated with microfilarial load ($P < 0.05$). **Conclusions:** We observed depressed IL-6 and raised IL-13 concentrations in the sera of individuals with onchocerciasis which implicate these interleukins in the immunological responses of the disease. Therefore, these IL-6 and IL-13 are associated with *O. volvulus* infection among Cameroonians.

1. Introduction

Filarial nematodes infect more than 150 million people in tropical areas and cause widespread morbidity^[1]. Onchocerciasis remains a major public health problem in Cameroon where 50% of rural population is at risk of infection with an estimate of over 1.3 million people already infected with the disease^[2]. Filarial infections are associated with cellular responses to parasite antigens^[3] and adaptive immunity often characterized by the production of Th-1 and Th-2 associated cytokines and T regulatory cell production^[4].

Monocytes, lymphocytes, eosinophils and basophils are

recruited and activated by cytokines of which the interaction and balance between cytokines and these haematological parameters may determine the degree of adverse side effects and disease expression^[5–8]. Monocytes from patently infected individuals before treatment had a diminished proinflammatory capacity that was suggested to secondarily modulate some immune responses. Following chemotherapy which resulted in parasite clearance, monocyte capability was reversed with increased production of IL-1 α ^[9].

In humans, IL-10 is a key mediator of immunosuppression and immunosuppression is considered an important element of helminth infection^[10]. Generally, in chronic helminthic infection, production of high IL-10 are associated with a status of little pathology and high worm load^[11]. Similarly, in chronic onchocerciasis, Patients with relatively little sign of dermatitis despite the presence of millions of small worms

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in the skin, produced Tr1 cells associated with substantial amount of IL-10 without IL-2 or IL-4 isolation[12].

IL-13 is mainly produced by type 2 T helper cells and it plays a role as a potential trigger for immune intervention in *Onchocerca volvulus* (*O. volvulus*) keratitis[13]. In addition to inhibition of Th-1 type cytokines, monocytes producing cytokines have been shown to be regulated by IL-13[14,15]. Monocytes have also been observed to be target cells of immunomodulation[16,17].

In view of the roles cells of the immune system play in patients with onchocerciasis[6,7,18,19] and also the paucity of information on interleukin profiles of *O. volvulus* infected individuals in our study area, we focused our investigation on the relationship between white blood cells, lymphocytes, monocytes, and IL-1 α , IL-6, IL-10 and IL-13 production in positive patients. We also correlated parasite density and interleukin levels in *O. volvulus* infected Cameroonians.

2. Materials and methods

2.1. Study area

This study was carried out in five communities namely Upper Sanaga, Lekie, Nyong, Kelle and Sanaga Maritime divisions located along Sanaga valley of Sanaga River in South Cameroon. These communities lie between latitude 04° 43 N and 03° 51 longitude 12° 24 E and 10° 10 E. The main river in this area is Sanaga, which has a length of 912 km from the Adamawa zone to the Atlantic sea.

2.2. Study population and selection criteria

The total number of volunteers screened was 357. These consenting volunteers were earlier mobilized through proper education on the significant and objectives of the investigation. The participants were predominantly farmers while a few of them were civil servants and petty traders. We excluded those with overt diseases, intestinal parasites, malaria and HIV using standard diagnostic kits.

2.3. Microfilarial loads (Skin snips), interleukin analysis and blood cells counts

The skin was cleansed with a spirit swab and ether and allowed to air dry. Two skin snips from behind the shoulders were collected using sterile 2.0 sterile corneo scleral punch. The skins were incubated in saline solution in a micro-titration plate pre-labeled with patient identification code. These skin snips were transported to our Laboratory at Nkolbisson, Yaoundé for examinations. The skin snips were weighed and placed on a microscope slide and examined with the 10 \times objective for the presence of microfilariae. The number of microfilariae per weight of skin snip was noted.

Sera from 80 participants with positive skin snips and 16

control volunteers living with the same communities but are not infected with *O. volvulus* and other diseases highlighted above were analysed for cytokines (including IL-1 α , IL-6, IL-10 and IL-13) with ELISA according to the manufacturer's instruction (Abcam, UK) and correlated with microfilarial load in the blood.

Total white blood cells, lymphocytes and monocytes were measured in these volunteers using in automated Diana 5 Evolution.

2.4. Data analysis

Data were analysed using Microsoft Excel. Also we carried out *t*-test and correlation analysis using InStat statistical package.

3. Results

Of 357 participants, 85(23.81%) had *O. volvulus* microfilariae in their skin tissues. Table 1 shows the levels of cytokines of infected and non-infected individuals and association with parasite density. IL-1 α showed no significant difference between infected and control subjects ($P>0.05$). There was also no association between IL-1 α levels and microfilarial load ($P>0.001$). IL-6 was significantly depressed in infected subjects than non-infected control individuals ($P<0.05$). The difference in concentration of IL-10 between control and positive individuals was not statistically significant ($P>0.05$). Raised levels of IL-13 ($P<0.001$) and a positive correlation with microfilarial load ($P<0.05$) was seen among infected volunteers.

Table 2 shows the association of WBC, lymphocyte and monocyte counts with the levels of interleukins in infected individuals. There was no significant relationship between WBC, lymphocyte and monocyte counts and levels of IL-1 α and IL-13 ($P>0.05$) (Figure 1 and 2). However, there was a significant correlation of monocyte counts with IL-10 levels in infected subjects (Figure 3). Additionally, WBC and lymphocyte counts were negatively correlated with IL-6 concentration in volunteers infected with *O. volvulus* ($P<0.05$) (Figure 4).

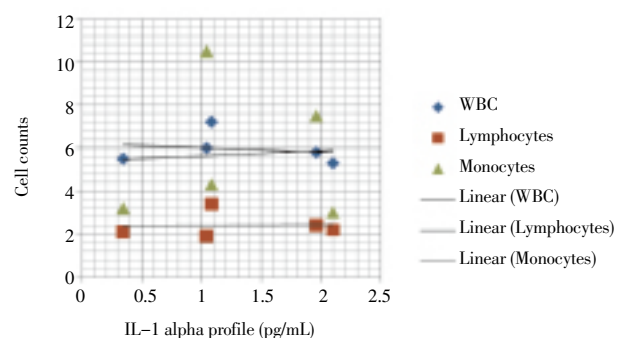


Figure 1. IL-1 α and WBC, lymphocyte and monocyte counts.

Table 1

Concentrations of Pro- and anti-inflammatory cytokines and association with microfilarial load in infected and control subjects.

Cytokines	Control (n=16)	Infected (n=80)	t-values	Correlation values with parasite load
IL-1 α (pg/mL)	1.65 \pm 0.79	2.31 \pm 0.50	1.76	0.48
IL-6 (pg/mL)	278.36 \pm 55.34	201.74 \pm 34.56	4.34*	-0.24
IL-10 (pg/mL)	436.03 \pm 208.64	418.49 \pm 147.88	0.65	0.22
IL-13 (pg/mL)	8.98 \pm 7.28	38.06 \pm 11.92	5.31*	0.73*

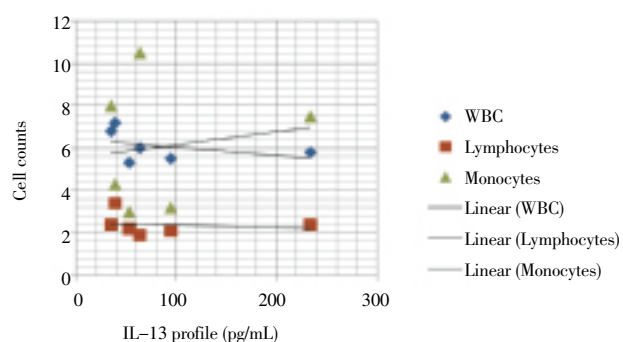
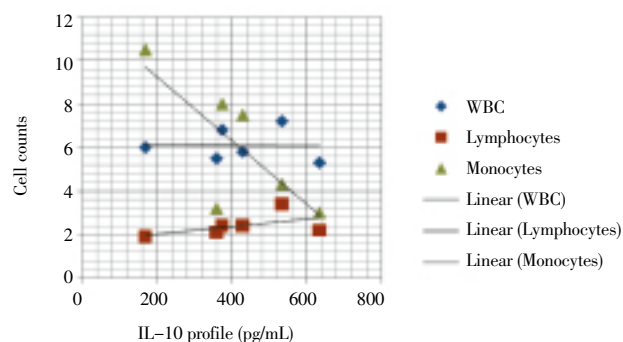
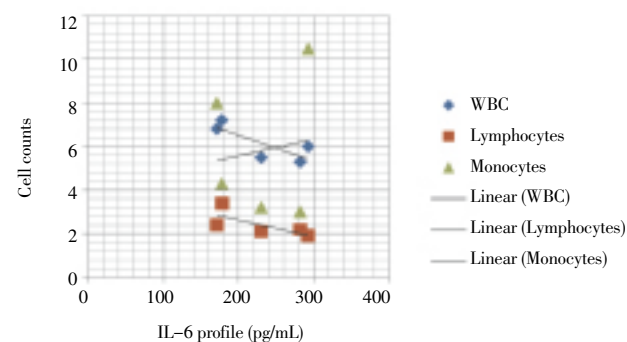
* $P < 0.05$ comparing with non-infected control subjects.

Table 2

Association of WBC, Lymphocyte and monocyte counts with cytokines profiles in infected individuals.

Cytokines	WBC (correlation coefficient values)	Lymphocytes (correlation coefficient values)	Monocytes (correlation coefficient values)
IL-1 α (pg/mL)	-0.21	0.02	0.05
IL-6 (pg/mL)	-0.76*	-0.70*	0.12
IL-10 (pg/mL)	-0.03	0.51	-0.77*
IL-13 (pg/mL)	0.38	-0.16	0.15

* $P < 0.05$ comparing with non-infected control subjects.

**Figure 2.** IL-13 and WBC, lymphocyte and monocyte counts.**Figure 3.** IL-10 and WBC, lymphocyte and monocyte counts.**Figure 4.** IL-6 and WBC, lymphocyte and monocyte counts.

4. Discussion

The presence of microfilariae in the skin tissues of 23.8% volunteers reported in this study denotes hypoendemicity of onchocerciasis. This level of microfilaridemia among the volunteers also denotes some aspects of dermatological morbidity in onchocerciasis[20–22].

In this investigation we observed that IL-1 α levels were unchanged while total white blood cells, lymphocytes and monocytes counts were not significantly correlated with IL-1 α production. Our finding is in contrast with the report of Semnai *et al*[9] where IL-1 α levels in infected patients due to parasite clearance were more in post-treatment than pre-treatment period. This pattern of result between infected and uninfected individuals was attributed to differences in gene expression particularly the heat shock genes[9] whose function includes immune activation and recognition[23]. We suggest therefore that infection with *O. volvulus* could not trigger differences in gene expression such that IL-1 α levels were unchanged. This implies that IL-1 α in patients with *O. volvulus* infection may not be involved in the recruitment of cells of the immune system at sites of morbidity.

We observed raised levels of IL-6 among the controlled volunteers when compared to the infected individuals. Also, white blood cell and lymphocyte counts were negatively correlated with the concentration of IL-6 in infected individuals. The depressed levels of IL-6 in *O. volvulus* found in the sera of individuals with *O. volvulus* parallels the findings of Njoo *et al*[24]. This result likely indicates a counter-regulatory mechanism between pro-inflammatory (IL-6) and anti-inflammatory (IL-13) cytokine

in an attempt to ameliorate adverse disease conditions in *O. volvulus* infected patients[25]. Furthermore, negative correlation between total white blood cell counts and IL–6 could have been as a result of the disease pathogenic effect leading to the probable release of some chemokines which may have inhibited granulocyte sequestration and suppress local adverse effects in *O. volvulus* positive patients[26]. Increased IL–6 has been associated with activated T–lymphocytes in onchocerciasis–negative human subjects[27] suggesting therefore that *O. volvulus* parasite may have prevented the recruitment of lymphocyte cells so that the level of immunity to the pathology of infection is negatively affected.

Our result showed unchanged levels in IL–10 though a little more elevated in control than infected subjects. This collaborates the report of Mai *et al*[18] in Togo within the same onchocerciasis endemic zone of Africa. The present investigation contradicts the report that *O. volvulus* infection induces IL–10 from human monocytes, leading to the down–regulation of major histocompatibility complex and costimulatory molecules which may result in impaired antigen presentation[28,29]. An important observation made earlier that the high IL–10 is associated with Th3 response which mediate hyporesponsiveness in onchocerciasis and thereby suppresses the on–going inflammation could account for the pattern of infection because the IL–10 were relatively not stimulated among the onchocerciasis infected volunteers[30].

A negative association was seen between the lymphocytes and IL–13. Also the increased level of IL–13 in positive individuals with a positive correlation between microfilarial load and the concentration of IL–13 contrast a report where IL–13 decreased with increasing microfilarial density[17,30]. IL–13 and proteins of filarial parasites have been documented to induce IgE and IgG4[31,32] which inhibits T– and B–cells activation and polyclonal antibody production[33,34]. This may likely have resulted in the negative association of these lymphocytes with IL–13.

In conclusion, we have demonstrated that depressed IL–6 and elevated IL–13 concentrations are associated with *O. volvulus* infection among Cameroonians. It is suggested that further research be encouraged in the manipulation of these interleukins in the design of possible therapeutic mechanism in the treatment of onchocerciasis.

Conflict of interest statement

We declare that we have no conflict of interest.

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