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Serum cytokines profiles in Nigerian children with *Ascaris lumbricoides* infection

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ABSTRACT

Objective: To investigate the cytokines profile in the serum of some Nigerian children with *Ascaris lumbricoides (A. lumbricoides)* infection and relations between the intensity of infection, age and the concentration of these cytokines. **Methods:** Feacal samples from consenting children were examined using formol ether concentration techniques and Kato-Katz thick smear technique. Sera of 96 children comprising 76 *A. lumbricoides* infection and 20 controls were subjected to enzymes-linked immunosorbent assay. **Results:** The mean sera concentration of tumor necrosis factor alpha (TNF- α), interleukin-4 (IL-4), interleukin-5 (IL-5), interleukin-10 (IL-10) were (16.7±7.6) pg/mL, (4.6±0.8)pg/mL, (391.6±52.0) pg/mL and (181.2±30.4) pg/mL, respectively. The control subjects had the following mean serum cytokines: TNF- α (2.9±1.8) pg/m, IL-4 (4.0±0.1) pg/mL, IL-5 (125.1±50.1) pg/mL, IL-10 (74.5±54.2) pg/mL, respectively. The difference between the sera cytokines concentration of the *A. lumbricoides* infected children and their controls were statistically significant for TNF- α, IL- 5 and IL-10(χ^2= 9.99, P< 0.05; χ^2 = 137.24, P < 0.05; χ^2 = 44.30, P < 0.05, respectively). The intensity of infection correlated positively with TNF- α, and IL-5 (r = 0.93 and 0.98, respectively) while IL-4 and IL- 10 correlated negatively with the intensity of infection (r = -0.62 and 0.99, respectively). TNF- α, IL-4 and IL-5 correlated positively with age (r = 0.19, 0.33 and 0. 66, respectively). The mean cytokines between those with mild and moderate infections were statistically significant for TNF- α, IL-5 and IL-10 (χ^2 = 3.60, 4.52 and 5.30, respectively). The ratio of TNF- α : IL-10 was 0.092. **Conclusions:** Elevated IL-5, IL-10 and TNF- α found in the sera of the volunteers with Ascariasis implicates these cytokines as key mediators in the host responses to *A. lumbriocoides* infection in our studied area.

1. Introduction

Human gastrointestinal parasites have been generally recognized as a major public health problem in the tropic countries of the world. It is estimated that 1 billion people are infected world wide[1]. Geohelminths parasites are highly prevalent in conditions of poor sanitation and hygiene with children being more affected than adults[1,2]. Ascariasis caused by *Ascaris lumbricoides (A. lumbricoides)* is the most prevalent of the intestinal helminths that colonise the small intestine[3]. This infection is common in places where toilet facilities are inadequate and human feaces are disposed indiscriminately within our surrounding environment[4]. Here, food and water are easily contaminated which can subsequently constitute sources of infection. Ascariasis have been associated with eosinophilic pneumonia during the migratory phase through the lungs[5] as well as abdominal symptoms. Immune responses as expressed by production of cytokines in ascariasis have been documented globally[6-16]. Some of these investigations potentiate the relevance of interleukins in vaccine and chemotherapy[17,18]. There is dearth of information in this regard in this part of the globe as most data are epidemiological[2,19,20].

We therefore investigated and reported the cytokines profile in the serum of some Nigerian children with *A. lumbricoides* infection. The relationship of the intensity of infection, age and the concentration of these cytokines were highlighted.

2. Materials and methods

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This survey was carried out in Igwe-Oke, Owan East Local Government Area of Edo State, Majority of the inhabitants use pit latrines while the children defecate indiscriminately in their neighbourhood.

Ethical permission was obtained from Edo State Ministry of Health, Benin City, Edo State, Nigeria. The villagers were mobilized by a community mobilization campaign where the objectives, all procedures were explained to the children and their parents for their consent. Feacal samples were obtained from the consenting children. The samples were processed and examined using formol ether concentration techniques. The intensity of infection was ascertained by counting the egg per gram of feaces according to WHO standard using the Kato-Katz thick smear technique as described by Montresor *et al*[21]. Sera were obtained from blood samples of 76 individuals with *A. lumbricoides* infection and 20 control subjects. The sera were immediately subjected to enzyme - linked immunosorbent assay for the analysis of interleukin-4 (IL-4), interleukin-5 (IL-5), interleukin-10 (IL-10) and tumor necrosis factor-α (TNF-α) according to the manufacturer's instructions.

The data obtained from the investigation were subjected to statistical analysis namely chi-square and correlation using Microsoft Excel package.

3. Results

The cytokine profile of 76 *A. lumbricoides* infected volunteers with their age groups and intensity of infection and their control are presented in Table 1. The children between the age of 6 and 10 years had the highest level of parasite load (12 650 epg) while the least (2 010 epg) was observed among the children below 5 years of age. These children had the highest serum TNF-α [(25.1±4.1) pg/mL] and IL-5 [(430.4±150.7) pg/mL), respectively. The highest serum IL-4 [(4.9±3.1) pg/mL] and IL-10 [(212.8±81.1) pg/mL] were observed among the children below 5 years age. In all, the mean concentration of the TNF-α, IL-4, IL-5 and IL-10 were (16.7±7.6) pg/mL, (4.6±0.8) pg/mL, (391.6±52.0) pg/mL and (181.2±30.4) pg/mL, respectively. The relationship between intensity of infection and TNF-α, IL -4 and IL-5 were positively correlated at r = 0.19, 0.33, 0.66, respectively while the relationship between IL-10 and the intensity of infection was negatively correlated at r = - 0.46. The ratio of TNF-α /IL-10 was 0.092.

Moderate infection was reported among 44 children while 32 of them had mild infection. The difference between the mean TNF-α concentration of those with mild infection [(11.5±3.5)pg/mL] and moderate infection [(22.5±6.1)pg/mL] was statistically significant (χ^2 = 3.60; P< 0.05). The children with mild infection had a mean IL-4 concentration of (3.8±1.1) pg/mL while those with moderate infection had a mean concentration of (4.9±2.2) pg/mL and this difference was not significant (χ^2 = 0.02, P>0.05). The mean IL-5 concentration for those with mild infection was (361.0±117.2) pg/mL and this was statistically different from those with moderate infection [(420.5±73.3) pg/mL] (χ^2 = 4.52, P<0.05). The difference between the mean IL-10 concentration of the children with mild infection [(202.9±102.9)pg/mL] and those with moderate infection [(159.1±112.5)pg/mL] was statistically significant (χ^2 = 5.30, P< 0.05). Also the differences between the sera cytokines concentration of *A. lumbricoides* infected and their control children were statistically significant for TNF-α (χ^2 = 9.99, P<0.05), IL-5 (χ^2 = 137.24, P<0.05), and IL-10 (χ^2 = 44.30, P<0.05). The differences for the IL-4 was not significant (χ^2 = 0.02, P>0.05).

Table 1
The cytokines profile of 76 *A. lumbricordes* infected volunteers with their age groups.

Age groups in years Number infected	Mean parasite load (epg)	Mean TNF-α (pg/mL)	Mean IL-4 (pg/mL)	Mean IL-5 (pg/mL)	Mean IL-10 (pg/mL)
< 5	27 (2 010)	10.0±3.5	4.9±3.1	332.5±20.6	212.8±81.1
6-10	31 (12 650)	25.1±4.1	3.7±0.8	430.4±150.6	152.5±93.6
>10	18 (9 000)	15.4±4.5	5.2±2.5	411.9±135.0	177.5±51.9
Mean	(7 886.7)	16.7±7.6	4.6±0.8	391.6±52.0	181.2±30.4

Table 2
The cytokines profile of 76 *A. lumbricoides* infected volunteers and their control subjects according to the intensity of infection.

Intensity of infection mber of infected	Intensity of infection (epg)	Mean TNF-α (pg/mL)	Mean IL-4(pg/mL)	Mean IL-5 (pg/mL)	Mean IL-10 (pg/mL)
Mild(1-4 999)	32 (3 850)	11.5±3.5	3.8±1.1	361.0±117.2	202.9±102.9
Moderate (5 000-49 999)	44 (11 910)	22.5±6.1	4.9±6.1	420.5±73.3	159.1±112.5
Mean	(7 880)	17.0±7.8	4.4±0.8	390.8±43.1	181.0±31.0
Control	20 (0)	2.9±1.8	4.0±0.1	125.0±50.1	74.5±54.2

4. Discussion

Our study showed the production of low level of the anti inflammatory IL-4. This data deviates from the observation of Turner et al where they reported relatively higher IL-4 profile among the subjects investigated[22]. The low mean concentration of this interleukin reported in our present study could be responsible for maintenance of *A. lumbricoides* in Igwe - Oke, Nigeria. This assertion is proved valid considering the primary roles of IL-4 in helminthic infection where it had been demonstrated by Else *et al* that IL-4 had a blocking effect on pro-inflammatory cytokines[18]. Furthermore, IL-4 is implicated in promotion of B cells and in enhancing the switch to IgE and IgA which could assist in immune responses and parasite clearance. The pattern of the IL-4 which is found to be low could not counter the pro-inflammatory function of TNF-α and IL-5 and thereby assisted in establishing ascariasis among our volunteers studied.

We reported a significantly high TNF-α concentration. This implicates TNF-α in immunopathogenesis of *A. lumbricoides* infection among our volunteers especially during the tissue migratory phase. It has been documented that this phase may clinically cumulate in Ascaris pneumonitis accompanied by eosinophilia known as Loeffler´s syndrome[23].

IL-10 inhibits the release of pro inflammatory cytokines including TNF-α by human monocytes and neutrophils[24,25]. In addition, IL-10 is particularly important in promotion of resistance and prevention of the host from developing lethal immunopathology[9]. It is therefore obvious that the relatively higher concentration of IL-10 reported among our studied infected group than their controls were not sufficient to block and modulate the effects of TNF-α and IL-5 as manifested by the very low ratio and imbalance proportion between TNF-α and IL-10. This deduction supports the earlier information that IL-10 plays a major role in the regulation of the intensity of both Th 1 and Th 2 responses during helminths infections. It plays a principal role in minimizing immunopathology[26-29]. Also, TNF-α had been revealed to play a critical role in regulation of Th2 cytokine medicated host protection[10]. The net effect of the absence of this sufficient counter effect of the IL-10 and IL-4 on the pro inflammatory cytokines namely the IL-5 and TNF-α in our data may account for the maintainence of ascariasis in Igwe - Oke, Nigeria.

It had been emphasized that in most of the worm infections, the immune responses of the host tend toward the Th 2 like responses[30, 31] with production of significant quantities of IL-4, IL-5, IL-10 among other cytokines and with consequent development of strong immunoglobin (IgE), eosinophil and mast cells responses[30]. The net effect could be protective immunity. This phenomenon of induction of highly polarized Th 2 cytokine responses by the relatively greater production of IL-4 comparing other cytokines such as IFN-γ in response to adult and larval stage antigen in human infection with *A. lumbricoides* has been documented[32]. This shift from Th 1 to Th 2 can in part be proved valid by our report as we recorded adequate stimulation and production of high level of IL-5. This had been documented earlier[15,17,22,33,34]. This assertion deviates slightly from our investigation where we recorded low IL-4 and IL-10, hence it could be involved in the maintenance of chronicity of ascariasis in our studied area. Also we found that the concentration of IL-10 correlated inversely with age and intensity of infection. This observation is consistent with the finding of Jackson *et al*, Geigner *et al*, and Turner *et al*[14,16,22].

Conclusively, the elevated level of IL-5, IL-10 and TNF-α found in the sera of our volunteers from Igwe - Oke, Nigeria implicates these cytokines as the key mediators in the host response to *A. lumbricoides* infection in our studied locality.

Conflict of interest statement

We declare that we have no conflict of interest.

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