

Cluster of differentiation expressions among Nigerians with *Ascaris lumbricoides* infection

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Received 3 March 2015; received in revised form 17 July 2015; accepted 7 August 2015

Abstract. Clusters of differentiation (CDs) are membrane proteins expressed on cells of the immune system and are associated with parasitic infection. Most of the work on CD expressions to helminth infection has mainly been on mouse model. Thus there is a dearth of information on the influence of *Ascaris lumbricoides* to CD expressions in human blood. White blood cell (WBC), eosinophil and platelet numbers were estimated alongside their CD14, CD40 and CD130 concentrations in *A. lumbricoides*-infected human subjects. Similarly, serum CD levels in relation to *Ascaris*-infected and helminth co-infected with healthy controls were estimated. The relationship between *Ascaris* load and CD profile was also shown. Our data indicated that >6 eosinophil-counts group expressed raised mean level of CD14 with a highly depressed mean CD40. Furthermore, mean CD40 for individuals with low platelet count was highly depressed. Additional analysis of infected vs control and the correlation of CD levels with parasite load showed null effect of *A. lumbricoides* on CD14 and CD40 expressions. Meanwhile, CD130 was raised as platelet number and parasite load increased. Thus the involvement of CD130 in the immuno-pathology of Ascariasis is likely. CD130 could therefore be a marker of infection and a potential target for improved anthelmintic therapy.

INTRODUCTION

Helminth parasitic infections contribute significantly to the disease burden of developing countries (Colley & LoVerde, 2001). In Nigeria, epidemiological surveys largely show the preponderance of *Ascaris lumbricoides* and hookworm infections across varied demographics (Obiukwu *et al.*, 2008; Nmorsi *et al.*, 2009; Omorodion *et al.*, 2012). The majority of helminth-infected individuals experience minor symptoms in comparison to protozoan, bacterial or viral infection. This might be a consequence of the human-protective nature being partly influenced by immune cells in association with cluster of differentiation (CD) antigens as observed in mice (Amiri *et al.*, 1992; Dunne & Doenhoff, 1983).

The CD antigens are membrane proteins expressed mainly on leukocytes while small amounts are expressed on endothelial cells, erythrocytes and stem cells. The total leucocyte (white blood cell) count is an important diagnostic tool to assess the host immune status and resistance to infection (Garside & Behnke, 1989). The differentials of leucocyte especially eosinophil have been heavily implicated in helminth infection by mediating host protection (Anthony *et al.*, 2007) and ensuring regulatory functions (Reiman *et al.*, 2006); and sometimes could assume antigen-presenting cells (Padigel *et al.*, 2006). It has also been shown that platelet cells help to fight infections by releasing considerable quantities of secretory products and expressing a multitude of immune

receptors on their membrane (Elzey *et al.*, 2003).

The role of CD system includes its use as cell markers in immunophenotyping (Chan *et al.*, 1999) as well as assuming the functions of receptors or ligands in order to initiate signal cascade involved in changing cell behaviour (Tundup *et al.*, 2014). Two commonly-implicated CD molecules are CD4 and CD8 being markers of two different subtypes of T-lymphocytes; helper and cytotoxic T cells respectively. Upon infection with HIV, CD4 destroys CD4⁺ T cells; and consequently, the relative abundance of CD4⁺ and CD8⁺ T cells is often used to monitor the progression of HIV infection.

Similarly, in helminth-infected mice, an up-regulation of CD14 expression impacted on macrophage plasticity and the biasing of CD4⁺ T cell (Tundup *et al.*, 2014); while CD40 ligand which is primarily expressed on activated CD4⁺ T-helper cells facilitated the priming of CD8⁺ T cells to becoming cytotoxic effector cells (Ma & Clark 2009). In addition, CD130 being a transmembrane protein forms one subunit of the type 1 cytokine receptor within the Interleukin (IL)-6 receptor family in which IL-6 drives the differentiation of CD4⁺ T cells into IL-4-secreting T_H cells (Rincón *et al.*, 1997). In spite of helminth-infection complexities due to a demonstration of a range of pathological conditions arising from their developmental stages within their host, host-immune responses are remarkably similar being T_H2-prone. T_H2 type responses are typically characterised by increases in the levels of IL-4, IL-5, IL-9, IL-10 and IL-13 including activation and expansion of CD4⁺ T_H2 cells resulting in the development of strong IgE, eosinophil and mast cells, a protective attempt of the host from helminth parasite (Wynn *et al.*, 1995). However, in a separate study of murine bone marrow-derived dendritic cells, the pseudocoelomic body fluid of *A. lumbricoides* which also promoted T_H2 ability *in vivo*, could not stimulate enhanced expressions of CD14 and CD40 (Dowling *et al.*, 2011).

Majority of work on CD expressions has been demonstrated mainly in mouse models with few studies conducted on *A.*

lumbricoides in human host. Data on the relationship between leucocytes, eosinophil and platelet counts and the amount of CD expressed in sole infection of *A. lumbricoides* and co-infection with other helminths is non-existent. Therefore, providing information on the profile of CD in human blood will enrich existing data and possibly identify marker(s) involved in the pathology of infection. We thus report for the first time the possible effect of *A. lumbricoides* infection on the expressions of CD14, CD40 and CD130 with the view of highlighting their likely roles.

MATERIALS AND METHODS

Study area

This study was conducted in April 2013. A cross section of people in communities, namely Ibule and Ilara (rural) and Akure (urban) in Ondo state voluntarily participated in the survey. The study sites are located between coordinates 5° 30'2" N–5° 35'2" N and 7° 15'2" E–7° 20'2" W. There are seven months of rainy season (April to October) and five months of dry season (November to March). Typically, the vegetation is the tropical continental rainforest. The majority of the people source their drinking, cooking, laundry and bathing water from the stream. The preoccupation of the rural dwellers is farming while those in Akure are mainly civil servants. A majority of rural dwellers still use crude means in disposing of their faeces, whereas Akure flooded with modern houses predominantly uses water-closet toilets.

Study population

A total of 1,272 volunteered themselves to be screened for helminth infections. Prior to the screening exercise, ethical clearance from the Ondo State Ministry of Health was obtained; and subsequently, community mobilisations were carried out. Informed consents were equally sought and obtained from the volunteers directly or from parents/caregivers on behalf of their children/wards. Here is the gender structure by communities: Akure (M: 411, F: 281); Ibule (M: 205, F: 200), Ilara (M: 66, F: 109). The age structure of the 1272 screened was as follows: ≤5 yrs (n=201);

6–16 yrs (n=671); 17–50 yrs (n=308); 50 yrs and above (n=92). A total of 151 were positive of one or more intestinal parasites. However, for the purpose of this study, the blood of only 60 positives having met the inclusion criteria of sole infection with *A. lumbricoides* were collected for both cell count and CD analysis. Before arriving at these 60 volunteers, various tests of other parasites including malaria were carried out and positives were ruled out. Individuals with viral infections and/or any other form of disease were equally excluded. The gender ratio of the 60 individuals is; M=23: F=37; while age structure was thus: ≤5 yrs (n=8); 6–16 yrs (n=25); 17–50 yrs (n=15); 50 yrs and above (n=12). Also, the number of individuals with only helminth co-infection was 12 of which *A. lumbricoides* + hookworm (n=7), *A. lumbricoides* + *Trichuris* (n=2), *A. lumbricoides* + *Strongyloides* (n=1), *Trichuris* + hookworm (n=2) were seen. Within the same population, 20 volunteers without any known infection or disease were selected as our control group.

Stool analysis

Fecal samples were collected in clean, sterilized containers and processed using formo-ether concentration and Kato-Katz techniques (Katz *et al.*, 1978). The intensity of infection of *A. lumbricoides* was categorized in egg per gram (epg) as light (1-4999), moderate (5000-49999) and heavy (≥50000) (WHO, 1998).

Haematological analysis

10mL of blood samples were obtained from positive volunteers and the control subjects. 5mL was separated and introduced into an anticoagulant container for white blood cell, platelet and eosinophil estimations for the 60 volunteers with only *A. lumbricoides* infection using Automated Haematology Analyser (BC-2300, Fortress diagnostic-2006 UK). The other 5mL was equally separated in different bottles (without anti-coagulant) and the blood was allowed to clot, and thereafter centrifuged (Haematopsin-800D). The serum was separated into another container for CD analysis.

Cluster of differentiation analysis

The sera were analysed using the Enzyme-Linked Immunosorbent Assay (ELISA) antigen kits for CD14, CD40 and CD130 after been reconstituted according to the manufacturer's instruction (Abcam, UK). The absorbance of the reaction in each microplate well which was coated with human antibodies specific for antigens of the different CDs was determined using Perkin-Elmer spectrophotometer (Vu 7804 print UK). The standard curve was plotted from the results of its serial dilutions; and CD concentrations of positives and controls were then extrapolated.

Statistical analysis

CD levels in relation to the three cell-count groups were analysed using Kruskal-Wallis One Way Analysis of Variance (Q=test value). CD expressions at the various levels of cell counts were further compared using Dunn's method and significance was reported at $P < 0.05$. For the comparison of single/co-infected against the healthy control, Man-Whitney test (T= test value) was applied and significant difference was noted at $P < 0.001$. Logistic regression was used to determine the relationship between parasite load and levels of CD expressed. All these analysis were done using a statistical package (SigmaStat 3.0, 2003).

RESULTS

A total number of 1,272 individuals were examined for helminth infections in three communities with overall incidence of 11.87%. Four helminths were recovered from volunteers: *A. lumbricoides*, hookworm, *Trichuris trichiura* and *Strongyloides stercoralis*. Infection with *Ascaris* was seen to be most dominant with highest incidence recorded in Ibule. In Akure, there was no volunteer with double infection whereas in Ilara three individuals had more than two helminth infections. The level of infection in the different communities is a demonstration of the level of personal hygiene/environmental sanitary conditions enhanced by provision of sanitation facilities.

Table 1. Prevalence of helminth infection in the different communities

Communities	Akure	Ibule	Ilara	Total
No. examined	644	400	128	1272
Total no. infected (%)	13 (2.01)	108 (27)	30 (23.43)	151 (11.87)
No. of single infection	13	91	13	117
<i>A. lumbricoides</i>	12	53	6	71
Hookworm	–	25	4	29
<i>Trichuris trichiura</i>	–	13	2	15
<i>Strongyloides stercoralis</i>	1	–	1	2
No. of double infection	–	17	14	31
No. with more than 2 infections	–	–	3	3

Table 2. Association between cell-count groups with *A. lumbricoides* infection and CD levels

WBC($10^9/L$)	No.	CD14 (ng/mL)	CD40 (ng/mL)	CD130 (ng/mL)
<4-10	14	100.23±7.37	62.45±15.38	1111.32±69.76
4-10	29	119.43±17.38	67.23±12.37	1345.32±43.06
>4-10	17	117.11±18.03	50.17±9.87	1234.56±86.22
Eosinophil ($10^9/L$)	No.	CD14	CD40	CD130
<2-6	8	115.54±17.13	81.52±14.18	1421.67±55.08
2-6	31	110.04±13.54	79.30±15.83	1324.61±67.18
>6	21	167.31±15.38	5.10±1.69	1516.06±80.03
Platelet count ($10^9/L$)	No.	CD14	CD40	CD130
<100	17	210.63±29.26	15.05±3.2	972.3±16.23
100-300	26	198.69±16.32	79.30±8.46	1455.1±32.24
>300	17	193.74±10.07	50.10±7.05	1465.9±60.64

CD concentration = mean±SD (ng/mL)

CD expressions for the three categories of cell counts (low, normal and high) in helminth-infected volunteers are shown in Table 2. Generally, differences were observed in CD levels among the three groups; however, some showed very high variations ($P<0.001$). These were eosinophil (CD14, CD40) and platelet (CD40 and CD130). There was high expression of CD14 in >6 eosinophil-counts group (>6vs2-6, $Q=6.25$, $P<0.05$; >6vs<6, $Q=3.89$, $P<0.05$) and a highly depressed CD40 (<6vs>6, $Q=4.49$, $P<0.05$; 2-6vs>6, $Q=5.98$, $P<0.05$).

For platelet-count analysis, mean CD40 was highly depressed in individuals with low (<100 counts) in relation to the normal (100-

300 counts) ($Q=6.98$, $P<0.05$). Meanwhile, for CD130, similar trend of decreased mean level in low-platelet group compared to normal ($Q=5.410$, $P<0.05$) and high (>300) ($Q=5.21$, $P<0.05$) were observed.

Table 3 presents the levels of CDs in relation to helminth-infected and non-infected volunteers. Data analysis involving CD14 of helminth positives of single and co-infection against healthy controls showed no significant variations ($T=97.00$, $P=0.006$; $T=39$, $P=0.731$) respectively. Similarly, there were no significant differences in the CD 40 levels between single vs control ($T=67$, $P=0.003$) and co-infected vs control ($T=39$, $P=0.042$). Conversely, higher levels of

Table 3. The concentration profile of CD with helminth-infected and non-infected groups

Infection/CD level	CD14 (ng/mL)	CD40 (ng/mL)	CD130 (ng/mL)
Single (n=60)	202.82 ± 42.41	68.42 ± 21.66	1529.56 ± 142.41
Co-infection (n=12)	211.27 ± 41.01	71.32 ± 20.35	1989.68 ± 140.71
Control (n=20)	207.08 ± 27.78	79.30 ± 30.89	1076.95 ± 150.73

CD concentration = mean±SD (ng/mL)

Table 4. Relationship between CD levels and *A. lumbricoides* load

Parasite load/CD level	CD14 (ng/mL)	CD40 (ng/mL)	CD130 (ng/mL)
Light (n=40)	206.21 ± 37.89	61.35 ± 25.11	1140.79 ± 85.33
Moderate (n=11)	193.95 ± 58.37	76.63 ± 30.11	1464.88 ± 73.31
Heavy (n=9)	205.08 ± 36.98	71.80 ± 28.11	1856.38 ± 112.38
R ² -value	0.0069	0.44	0.76

CD concentration = mean±SD (ng/mL)

CD130 were seen in individuals with single and co-infection than healthy controls (T=378, $P<0.001$).

The relationship of serum CD levels with parasite (*Ascaris*) load is shown in Table 4. The results demonstrated that there is a positive relativity between CD and the parasite loads. Although CD14 showed weak correlation, CD130 was relatively strong and statistically significant ($P<0.001$). This result should be interpreted carefully owing to the relatively small sample size of individuals under the categories of moderate and heavy infection.

DISCUSSION

Haematological studies in helminthiasis reveal alterations in the white blood cells and its various components (Parvathi & Karemungikar, 2011). Following helminth infection, eosinophil numbers increase dramatically in the blood and these eosinophils rapidly migrate to the site of infection where they are directly involved in killing (Galioto *et al.*, 2006) or modulate an array of mechanisms (Hogan *et al.*, 2008). We observed therefore that helminth-infected

individuals with >6 eosinophil count showed highly expressed CD14. Eosinophil trafficking into inflammatory sites has been shown to involve T_H2 cytokines and chemokines (Lampinen *et al.*, 2001; Banwell *et al.*, 2002). Chemokine CCL11 has been implicated in eosinophil recruitment in ulcerative colitic conditions, and that intestinal myeloid cells (CD14⁺) are a source of CCL11 (Lampinen *et al.*, 2013). Since CD14 is expressed mainly by macrophages and by neutrophils, we thus hypothesize a situation where in the face of increased eosinophils with *A. lumbricoides* infection, CD14 being raised assumed an intermediary role in eosinophils' sequestration, a likely protective response.

Eosinophil activation can be enhanced by increased expression of CD40 (26). Our result on CD40 profile in infected individuals showed a reduced expression at >6 eosinophil count. CD40 pathway may help to establish efficient adaptive B- and T-cell immunity to expand the precision of protection after the initial innate immune cell response (Ohkawara *et al.*, 1996). However, after a given period, it has been shown that anti-proliferative and anti-apoptotic genes could be upregulated

(Tummers *et al.*, 2014); hence, providing the condition for a probable inhibition of the expression of CD40 over time in spite of increase in the number of eosinophils. Deregulation of CD40 ligands could be a protective reaction in response to symptoms of infection (Peters *et al.*, 2009; Caproni *et al.*, 2007).

The eggshell of an intestinal parasite has been shown to adhere to platelet cells leading to its non-viability and halting development into the adult stage (Wu *et al.*, 2007). Also, platelets have been implicated in the killing of helminths larvae (Pancre & Auriault, (1995). Inflammatory cells including platelets produce a lipid mediator platelet-activating factor (PAF) which when released activates PAF-receptors which results in diverse biological activities Ishii & Shimizu (2000) and decrease in intestinal inflammation, enhanced worm survival with decrease in fecundity (Negrao-Correa *et al.*, 2004). Platelet aggregation in response to inflammation has been shown to be CD40-dependent (Yacoub *et al.*, 2010). We thus showed that the group with low platelet count had equally low expression of CD40; a probable indication of greater susceptibility to the pathological effect of infection.

Glycoprotein 130 (CD130) is a trans-membrane protein and it forms a subunit of the type I cytokine receptor within the IL-6 receptor family. IL-6 and IL-6 receptor associate with CD130 to form a hexameric complex producing intracellular signals (Murakami *et al.*, 1993). Our data indicate a rise in CD130 with increased platelet count. In addition, there was raised CD130 with increased *A. lumbricoides* load. These are probably strong indications of the influence of CD130 in the immuno-pathology of *A. lumbricoides* infection. In a study, IL-6 deficient mice, showed evidence of resistance to chronic infection (Bordon (2013); while human platelet number increased by the administration of IL-6 (Clarke *et al.*, 1996). Since there must be a priming involving CD130 and members of IL-6 family to accomplish immunological functions, we hypothesise that the high-platelet group may have been impacted by an increased expression in CD130 in

association with the IL-6 family. Thus, we suggest that CD130 could be implicated in the pathogenesis of *A. lumbricoides* infection in relation with other host immune component(s). Similarly, among helminth co-infected and single-infected groups, there were raised expressions of CD130 in comparison to the healthy controls. Again, emphasizing a marked influence of CD130 in the immunology of these infections.

In conclusion, we have shown that CD14 expression was higher in high-eosinophil group than other groups. This result may not be a consequence of *A. lumbricoides* infection since the result between infected and the control group does not suggest such. Also, the relationship of CD14 with the intensity of infection showed null influence to a large extent. Similarly, the highly depressed CD40 among low-eosinophil group could not be corroborated with the data of infected vs control and CD40 with parasite load. Conversely, increased platelet number showed upregulation of CD130. Likewise, increased *A. lumbricoides* load correlated with raised expression of CD130 including an upregulation in infected groups when compared with healthy controls. These results should be viewed with caution on the basis that some other unforeseen factors could have contributed in influencing CD responses given the complex nature of the immune system. However, the implication of our result is such that CD130 could be a marker of Ascariasis; and hence might be a target for the development of an improved anthelmintic therapy.

Acknowledgements. We thank all the volunteers that participated in this study and Dr, Ihimire of Department of Biochemistry, Ambrose Alli University, Ekpoma for his technical input.

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