POSTER PRESENTATIONS

USE OF MACROPHAGES IN SPLEEN, LIVER AND BONE MARROW OF *TRYPANOSOMA CONGOLENSE (KAURA STRAIN)* INFECTED MICE FOR LABORATORY DIAGNOSIS

Ayoade, R.A.

Ibadan, MNIM

ABSTRACT

At the time of sacrifice of each of the three groups, mice infected for two weeks showed slight decreased mean body weight from 21.5±6.8 to 20.2±5.9($p<0.05$). This was probably due to the severity of infection in proportion to high parasiteamia level with the trypanosome parasite competing with the body cells for nutrients. However, increased body weight in 4 weeks infected mice, 22±6.9 to 24.5±5.3($p<0.02$) was probably due to lack of reduced nutrient demand as a result of absence or reduced *Trypanosoma congolense* parasite in the blood. The marked splenomegaly noticed could be as a result of increase cell population per unit area with concomitant decrease in interstitial space (Anosa and Kaneko, 1984), while there were no significant liver changes except for slight hepatomegaly, 9.2±1.99($p<0.001$) of about only twice the weight of that of control (4.4±0.5). After the acute phase of infection, it began to return to pre-infection size.

The acute phase of the infection was characterized by significant decrease in PCV, Hb concentration, thrombocyte count but increased lymphocyte, neutrophil, monocytes and eosinophil numbers in the heart blood, but this is untrue for the 4wk infected mice as the PCV was close to those of control mice,47.6±4.4 and 49±1.6 respectively showing no obvious sign of anaemia. This may imply compensated haemolytic anaemia similar to that reported in trypanotolerant deer mice infected with T. brucei (Anosa and Kaneko, 1983a). However, there was depression of leucocytes and thrombocytes at this stage, significantly below those of the two weeks infected mice. This was due to continued proliferation and activation of macrophages in the bone marrow, liver and spleen.

There was a more increased phagocytic activity in spleen and liver of group A mice infected for two weeks but significantly lower in 4 weeks infected mice except in the bone marrow where phagocytic activity persisted and increased at the end of 4 weeks infection. This corresponds to the variations in sizes of the macrophages examined for phagocytosis.

In conclusion, the results of this study demonstrate that the macrophage plays a very vital role in the events in the spleen, liver and bone marrow of *T. congolense* infected mice, particularly with respect to cytophagia and the control of haemopoiesis, thus could be used to establish a confirmatory diagnosis for the above named strain of *Trypanosoma congolense*, which is gradually becoming endemic in the Niger-Delta and South-Western parts of Nigeria.

INTRODUCTION

Trypanosomes are flagellated protozoan parasites that live in blood and body fluids of their hosts. The trypanosome infections of laboratory animals (rats and mice) are characterized by anaemia, which are often severe (Mackenzie and Cruickshank, 1973; Anosa *et al.,* 1977; Anosa and Kaneko, 1983).

Due to their presence in blood, they produce numerous changes in its cellular and biochemical constituents (Anosa, 1988). Similarity of the onset of appearances and composition of the cellular infiltration into the liver to the cellular responses observed in the spleen of *T.congolense* infected mice probably reflects the fact that both organs are directly accessible to trypanosomes in circulation (Morrison *et al*., 1982), with preferential adhesion of trypanosomes to red blood cells in blood vessels in various body parts including the spleen and the liver (Anosa and Kaneko, 1983).

Macrophages constitute a significant proportion of inflammatory cells in spleen, liver and bone marrow with reported consequences of macrophage activation being two-fold (Mackenzie *et al*.,1973; Anosa and Isoun,1983; Anosa and Kaneko,1983a,b; 1989; Anosa et al.,1992).This is followed by phagocytosis of inflammatory cells and damaged resident cells in tissue (Anosa *et al,*1992) as well as induction of immunosuppression (Corsini *et al.,*1977).

The aim of this work is to compare the haematological changes, body, splenic and liver weight changes, as well as change in macrophage numbers, size and phagocytic function produced by *Trypanosoma congolense* infection in spleen, liver and bone marrow with a view to highlighting the differences and similarities observed with 2 weeks and 4 weeks infected mice. An attempt will be made also to compare the findings in the infected group with a control group so as to be able to establish a confirmatory diagnosis of the trypanosome parasite infection.

MATERIALS AND METHODS

Experimental animals

22 albino mice aged 6 – 8 weeks were used for this study. These mice were reared at the Experimental Unit, Veterinary Physiology Laboratory, and University of Ibadan, Nigeria. They consisted of 15 males and 7 females and were stabilized with oral tetracycline for 5 days before the commencement of the infection. The mice were divided into 2 groups, the infected group and control group. The infected groups were randomly subdivided into two. Group A included 5 males and 2 females, which were the controls, Group B included 5 males and 3 females infected for 2 weeks and Group C were 5 males and 2 females infected for 4 weeks. Only 5 mice of each of these groups were sacrificed at 2 weeks and 4 weeks respectively. They were fed compounded pellet feed and kept in cages with wood shavings throughout the period of the experiment.

Trypanosome

The Kaura strain of *Trypanosoma congolense* used for this study was obtained from National Institute of Trypanosome Research (NITR), Jos, Plateau State, Nigeria. This strain was first isolated from cattle in Kaura, Kaduna State, Nigeria in 1995. It has since June 2001 been maintained as an isolate passage in rats and mice.

The mice were infected by inoculating about 2.5 million parasites intraperiotoneally. This produced an initial acute infection with death of two mice on day 9 in Group A and 3 mice on day 11 in Group B.

Haematological techniques

The mice were bled intracardially after rendering them unconscious with ether. The control group (Group A) mice were also sacrificed at the end of 4 weeks. The blood was collected into vacutainer tubes containing the disodium salt of ethylene diamine tetra-acetate (EDTA). The PCV was determined by the microhaematocrit method. The erythrocyte (RBC), total leucocyte (WBC) and platelets counts were determined using a Neubaer haemocytometer, multiplying the values counted by 10,000µl, 50µl and 1,000µl respectively (E-MIL GOLD LINE BS748, United Kingdom).

Haemoglobin concentration (Hb) was measured with a haemoglobinometer (Coulter Electronics).

The erythocyte indices, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration, were calculated using standard formulae (Jain, 1986).

This blood smears were stained with Giemsa's stain, these were used to evaluate changes in the peripheral blood cells and for differential leucocyte counts which were based on 100 cells per slide. Also reticulocyte count was based on 200 red blood cells per slide.

Collection of spleen, liver and bone marrow samples

After the mice were euthanized, the carcasses were opened up from the perineal region to the thoracic region. With the aid of a smooth tooth thumb forceps and a pair of scissors the spleen, liver, and kidney were dissected free and weighed with a meter balance (Mettler PM 600, Switzerland).

Impression smears of the spleen and liver of each mouse were made on two glass slides each and air dried. The femur of each mouse was dissected free; the head of each femur crushed and marrow contents milked out on a glass slide and immediately, a thin impression smear was made by sliding another glass slide in opposite direction to the one with marrow contents.

After air drying the impression smears from all the tissue samples, they were fixed for 3 minutes with methanol, air-dried again and then stained with Giemsa stain for 30 minutes after which the stains were then washed off.

Light microscopic examination of spleen, liver, and bone marrow smears

The phagocytic activities of macrophages in these smears were studied, Counting 50 macrophages from randomly selected fields of each smear. The differential and total number of cells engulfed by each macrophage was carried out using X 100 oil immersion Objective lens.

Statistical analysis was made by the Student's t test (Snedecor and Cochran, 1980). Data are given as mean ± standard deviation (SD).

RESULTS

Table 1. Mean body and organ weights of control and *T.congolense* infected mice

Mice Group	BODY WEIGHT		ORGAN WEIGHTS		
	Before Infection	At Sacrifice	Spleen (% body weight)	Liver (% body weight)	Kidneys (% body weight)
Controls(A)	19.4 ± 1.1	20.5 ± 2.5	0.4 ± 0.04	4.4 ± 0.5	1.3 ± 0.09
Infected(2 weeks)	21.5±6.8	20.2 ± 5.9***	3.4 ± 1.0*	9.2 ± 1.99*	1.9 ± 1.1***
Infected(4 weeks)	22 ± 6.9	24.5 ± 5.3**	1.7 ± 1.0*	5.1 ± 0.7**	1.3 ± 0.2#

* > Significant at P<0.001; ** > Significant at P<0.02; *** > Significant at P<0.05; # > Not significant at P>0.05

Table 2. Mean Erythrocyte Parameters and Thrombocyte counts of control and *T. congolense* infected mice

Mice Group	PCV (%)	Hb (%)	Reti-culocytes	MCH (pg)	MCHC (g/dl)	($X10^3\mu l$) Platelets
Controls(A)	49 ± 1.6	16.1 ± 0.4	0.6±0.9	28.1 ± 2.3	32.8 ± 0.4	173.6 ± 12.1
Infected(2 weeks)	33.4 ± 8.7**	10.8 ± 2.8*	8 ± 1.58**	17.5 ±2.2*	32.4 ± 0.3#	141 ±34.7**
Infected(4 weeks)	47.6 ± 4.4**	14.8 ± 1.2**	11 ± 3.4***	18.6 ± 0.7***	31.2 ± 0.7#	111.8 ± 9.3*

* > Significant at P<0.001; ** > Significant at P<0.02; *** > Significant at P<0.05; # > Not significant at P>0.05

Table 3. Mean absolute leucocyte differential values of control and *T.congolense* infected mice

Mice Group	WBC ($X10^3\mu$)	Lymphocytes	Monocytes	Segmented Neutrophils	Band Neutrophils	Eosinophils	Basophils
Control(A)	5.9 ± 0.9	4725 ± 606.8	13.5 ± 30.2	967.5 ± 182.4	99.3 ± 77.6	61.2 ± 60.55	13.5 ± 30.2
Infected (2 weeks)	8.4 ± 3.3*	7375.3 ± 3273.5*	189.2 ± 98.6*	1273± 1682.7*	119.6 ± 119.2**	157.2 ± 154.0*	–
Infected (4 weeks)	6.4 ± 1.5**	5640.5 ± 1250.1*	87.6 ± 27.4*	610.2 ± 370.4*	122.3 ± 119.5**	35.9 ± 33.5***	–

* > Significant at P<0.001; ** > Significant at P<0.02; *** > Significant at P<0.05; # > Not significant at P>0.05

Table 4. Mean number of cells phagocytosed by macrophages in bone marrow of control and T.congolense infected mice

Mice Group	No of MØ counted	RBC	Erythro-blasts	Reticu-locytes	Normo-blasts	Lympho-cytes	Neutro-phils	Baso-phils	Mitotic cells	Total No. of cells Engulfed
Control (A)	50	35.4 ± 5.7	0.2 ± 0.4	–	0.2 ± 0.4	–	0.4 ± 0.9	–	–	36.2 ± 4.7
Infected (2 weeks)	50	82.8 ± 8.5**	0.4 ± 0.9**	0.6 ± 0.9#	0.6 ± 0.5*	–	–	–	–	84.4 ± 7.9**
Infected (4 weeks)	50	103.2 ± 7.4*	2.2 ± 1.8*	0.4 ± 0.5*	2.4 ± 1.5*	0.2 ± 0.4*	1.8 ± 1.5**	0.2 ± 0.4**	–	110 ± 6.4*

Table 5. Mean number of cells phagocytosed by macrophages in spleen of control and T. congolense infected mice

Mice Group	No of Macrophage counted	RBC	Erythro-blasts	Reticu-locytes	Normob-lasts	Lympho-cytes	Neutro-phils	Baso-phils	Mitotic cells	Total No. of cells Engulfed
Control (A)	50	40.4 ± 5.5	–	–	0.6 ± 0.9	0.4 ± 0.9	0.4 ± 0.9	–	–	41.8 ± 5.6
Infected (2 weeks)	50	134.4 ± 20.9**	2.4± 1.1*	0.2 ± 0.4**	2.0 ± 1.6**	–	–	–	–	139 ± 22.6*
Infected (4 weeks)	50	84.6 ± 11.1*	1.0 ± 1.4**	–	2.2 ± 3.2*	1.4 ± 1.7**	–	–	-	89.3 ± 11.3*

* > Significant at P<0.001; ** > Significant at P<0.02; *** > Significant at P<0.05; # > Not significant at P>0.05

Table 6. Mean number of cells phagocytosed by Kupffer cells in liver of control and T. congolense infected mice

Mice Group	No of MØ counted	RBC	Erythro-blasts	Reticu-locytes	Normob-lasts	Lympho-cytes	Neutro-phils	Baso-phils	Mitotic cells	Total No. of cells Engulfed
Control (A)	50	34.8 ± 5.8	–	–	0.6 ± 0.9	–	0.2 ± 0.4	–	–	35.6 ± 5.2
Infected(2 weeks)	50	85.4 ± 26.5*	0.8 ± 1.1**	–	10.4 ± 23.2**	2.2 ± 3.0**	–	2.0 ± 3.5*	0.6 ± 0.9*	103.2 ± 13.5*
Infected(4 weeks)	50	68.4 ± 17.3*	4.4 ± 3.8**	–	5.0 ± 9.5**	0.8 ± 1.3*	3.0 ± 5.1*	3.8 ± 8.5**	–	85.4 ± 13.2*

* > Significant at P<0.001; ** > Significant at P<0.02; *** > Significant at P<0.05; # > Not significant at P>0.05

DISCUSSION AND CONCLUSIONS

The acute phase of the infection (2weeks infected mice) was characterized by significant decrease in PCV, Hb concentration, thrombocyte count but increased lymphocyte, neutrophil, monocytes and eosinophil numbers in the heart blood. In fact, this group of mice showed the highest and marked proliferation of granulocyte elements and proliferation and activation of macrophages with destruction of mature and maturing erythroids and granulocytic cells in the spleen and liver and to a lesser extent in the bone marrow at this phase of infection.

The chronic phase (4 weeks infected mice) did not show any obvious signs of anaemia but instead the PCV was close to the PCV of those of the control mice. This may imply compensated haemolytic anaemia similar to that reported in trypanotolerant deer mice infected with *T. brucei* (Anosa and Kaneko, 1983a). However, there was depression of leucocytes and thrombocytes at this stage, significantly below those of the two weeks infected mice. With continued proliferation and activation of macrophages (phagocytic activity) in the bone marrow and in the spleen and liver. There was phagocytosis of cells similar to those seen in the acute phase, erythroid hyperplasia, marked erythrocytes hyperplasia and return of the lymphocyte numbers (percentage) towards normal as shown by 5640 S± 1250.1 in 4 weeks infected mice and 4725 ± 606.8 in

control mice. This suggests that there is continued macrophage activation in the bone marrow with increased phagocytic activity as the infection progresses even when the phagocytic activity of the spleen and liver had decreased. This phenomenon in the bone marrow presumably contributes to progressive decrease in granulocytes and its precursors, thrombocytes and also erythroid series in the blood, leading to panleucopaenia. The changes explains why trypanosome infected animals and man succumb readily to secondary bacterial and other infections.

At the time of sacrifice of each of the three groups, mice infected for two weeks showed slight decreased mean body weight. This was probably due to the severity of infection in proportion to high parasiteamia level (acute phase of infection), with the trypanosome parasite competing with the body cells for nutrients. However, increased body weight in 4 weeks infected mice was probably due to lack of reduced nutrient demand as a result of absence or reduced *Trypanosoma congolense* parasite in the blood.

The splenic lesion in *T. congolense* infection in mice consisted of marked enlargement which could be as a result of a tremendous increase cell population per unit area with concomitant decrease in interstitial space and an obvious sludging of blood cells, particularly erythrocytes in many sinuses (Anosa and Kaneko, 1984) This increase in size and cell density could lead to splenic rupture which was the cause of death of two of the infected mice on day 9 post infection in this study. Another consequence of splenic enlargement and increase cell density is hypersplenism. There were no significant liver changes except for slight hepatomegaly, which after acute phase of infection begin to return to pre-infection size. The same trend was noticed for the spleen as well. There was marked bone marrow hyperplasia due to increased erythropoiesis.

A significant change in the spleen was paradoxical coexistence of splenic erythropoiesis and erythroclasis evidence as markedly increased numbers of normoblasts, reticulocytes and metarubricytes (late normoblast) on spleen impression smears. This agrees with the findings in mice infected with *T. brucei* (Anosa and Kaneko, 1983). The splenic macrophages were activated as indicated by their increased size and significant increase in numbers. There was marked increase in number of lymphocytes. Lymphocytes while decreasing in relative percentage increased overall due to the marked enlargement of the organ.

Splenic activities in *T. conglense* infected mice were major defensive with phagocytosis of parasites production of antibodies and cellular immunity involving transformed lymphocytes and macrophages, destruction of blood cells including erthrocytes, and to a lesser extent, neutrophils, thrombocytes, esinophils and erythropoiesis at tremendously increased rates (Anosa and Kaneko 1984).

The liver had no severe changes in *T. congolense* infection. There was proliferation and activation of Kupffer cells, (though scanty on the liver impression smears) with erythrophagocytosis as well as perivascular accumulation of lymphocytes. These two changes are related to the increased break down of red blood cells and intense antigenic stimulation respectively. Similar lesions have been found with light microscopy in deer mice infected with *T. equiperdum* (Moulton *et al.*, 1974), in *T. brucei* infected CFLP mice (Anosa *et al.*, 1977) and in

T. vivax infection of sheep and goats (Anosa, 1977). The cytophagia by macrophage in the bone marrow, first described in the bone marrow infected with *T. vivax* (Anosa *et al.,* 1992), definitely plays a major role in precipitating the pancytopeania and ineffective haemopoiesis in *T. congolense* infection. The process was selective for cell type and maturity with more matured cells of the erythroid and granulocytic series being preferentially phagocytosed, whereas lymphoid cell lies were seldom engulfed. This agrees with the fact that a macrophage simultaneously engulfed several apparently morphologically normal cell from different lineages suggest that the process is probably receptor mediated, presumably some receptors occurring in

some cell lines such as erythroid and granulocytic but not in lymphoid cells, and which are expressed as cells mature, are involved in producing the selectivity observed (Anosa *et al.,* 1997). Some non-cell-specific entity such as trypanosome antigen-antibody complex, which develops in trypanosomiasis and coats the target cells links the receptors on the target cells to other receptors on the macrophage leading to target cell macrophage adhesion and phagocytosis (Anosa *et al*., 1997)

There was a more increased phagocytic activity in spleen and liver of group A mice infected for two weeks but significantly lower in 4 weeks infected mice except in the bone marrow where phagocytic activity persist and increased at the end of 4 weeks infection. This corresponds to the variations in sizes of the macrophages examined for phagocytosis.

In conclusion, the results of this study demonstrate that the macrophage plays a very vital role in the events in the spleen, liver and bone marrow of *T. congolense* infected mice, particularly with respect to cytophagia and the control of haemopoiesis. Also, the results obtained could be used as a prototype for confirmatory laboratory diagnosis of the Kaura Strain of the *Trypanosoma congolense infection* which is rapidly spreading across various parts of Nigeria.

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REFERENCES

Anosa, V.O. (1988) Haematological and biochemical changes in human and animal trypanosomiasis. Review Elevere Medicine Veterinary Pay Tropical, 41(1):65–78,(2) 151–164.

Anosa, V.O., Isoun, (1983), T.T. Pathology of experimental *T. vivax* infections of sheep and goats. Zentralblat fur Veterinarmedizin, 30:685–700.

Anosa, V.O., Kaneko, J.J. (1983), Pathogenesis of *T. brucei* infection in deer mice (*Peromyscus maniculatus*). Haematologic, erythrocyte, biochemical and iron metabolic aspects. American Journal of Veterinary Research, 44:639–644.

Anosa,V.O., Kaneko, J.J. (1983b), Pathogenesis of *T. brucei* infection in deer mice (*P. maniculatus*) Light and electron microscope studies on erythrocyte pathologic changes and phagocytosis. American Journal of Veterinary Research, 44: 645–651.

Anosa, V.O., Kaneko, J.J. (1984), Pathogenesis of *T. brucei* infection in deer mice (*P. maniculatus*). Ultrastructural pathology of the spleen, liver, heart and kidney. Veterinary, Pathology, 21: 221–237.

Anosa, V.O, (1992)Logan-henfry, L.L. and Shaw, M.K. A light and electron microscopic study of changes in blood and bone marrow in acute haemorrhagic *T. vivax* infection in calves. Vetetrinary pathology 29:33–45.

Anosa, V.O., Logan-Henfrey, L.L., Wells, C.W. (1997). The haematology of *T. congolense* infection in cattle I. Sequential cytomorphological changes in the blood and bone marrow of Boran cattle. Comparative. haematology International. 7: 14–22.

Anosa, V.O., Logan-Henfrey, L.L., Wells, C.W. (1997) The Haematology of *T. congolense* infection in cattle II. Macrophages structure and function in the bone marrow of Boran cattle. Comparative Haematology International &: 23–29.

Corsini, A.C., Clayton, C., Askonas, B.A., Oglivie, B.M. (1977) Suppressor cells and loss of B-cell potential in mice infected with *T. brucei*. Clinical Experiment on Immunology, 29:122–137.

MacKenzie, P.C.I, Cruickshank, J.G. (1973), Phagocytosis of erythrocytes and leucocytes in sheep infected with *T. congolense* (Broden, 1904) Research on Veterinary Science, 15:256–262.

Morrison, W.I., Murray, M., Bovell, D.L. (1981), Response of the murine lymphoid system to a chronic infection with *T. congolense* I.The spleen. Laboratory Investigation, 45:547–557

Moulton, J.E. (1980), Experimental *T. brucei* infection in deer mice splenic changes. Veterinary Pathology, 17:218–225.