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COMPARATIVE BONE MARROW RESPONSES OF ALBINO RATS EXPERIMENTALLY INFECTED WITH SINGLE AND MIXED SPECIES OF *TRYPANOSOMA CONGOLENSE* AND *TRYPANOSOMA BRUCEI* AND ABILITY TO CONTROL ANAEMIA

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RUNNING TITLE: BONE MARROW CHANGES IN AFRICAN TRYPANOSOMOSIS

ABSTRACT

Effect of *Trypanosoma congolense* and *T. brucei* mixed infection on ability of the bone marrow to respond to anemia was investigated in albino rats. This was with the view of assessing the possible impact on recovery rate from anemia following chemotherapy of African trypanosomiasis. The investigation involved descriptive evaluation of packed cell volume and corresponding bone marrow cytological changes associated with single and mixed infection of *T. congolense* and *T. brucei*. It involved laboratory based experimental infection of albino rats as research models. A total of 32 adult albino rats of mixed sexes were used for this investigation. The rats were randomly grouped into three groups, A, B, C made up of 8 rats each, and infected with *T. congolense*, *T. brucei* and mixed infection of these species. Eight other rats served as the uninfected control group. Parameters measured included weekly packed cell volume (PCV) and differential bone marrow cytology of the different groups of infected and control rats at the end of 21 days post infection (PI). At the end of 21 days PI, the anemia characterized by drop in PCV was most severe in the mixed infection group, and least in *T. brucei* group with tendency for self-recovery from anemia. The bone marrow responses in the mixed infection group was however weak and inferior to that of *T. brucei* and *T. Congolense* groups. Poor erythropoietic response in the mixed infection group despite significant fall ($P < 0.05$) in PCV level was believed to arise from severe renal and hepatic pathology resulting to subnormal erythropoietin release and severe stem cell injury. This is believed would cause longer time to be taken by mixed infection animals to recover from anemia after chemotherapy. It is concluded that *T. congolense* and *T. brucei* mixed infection result to marked incapacitation of the bone marrow and ability for recovery from anemia. This suggests that supportive administration of synthetic erythropoietin may be required in trypanosome specie mixed infection situation due to severe pathological effects on the kidney and liver resulting to impaired erythropoietin biosynthesis and slow recovery from anemia following chemotherapy in African trypanosomiasis.

Keywords: Anemia, bone marrow, mixed infection, rats, trypanosomiasis, erythropoietin.

DES REPONSES COMPARATIVES DE LA MOELLE OSSEUSE DE RATS ALBINS INFECTES EXPERIMENTALEMENT AVEC DES ESPECES UNIQUES ET MIXTES DE *TRYPANOSOMA CONGOLENSE* ET DE *TRYPANOSOMA BRUCEI* ET LA CAPACITE DE CONTROLER L'ANEMIE

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TITRE COURANT: LES CHANGEMENTS DE MOELLE OSSEUSE DANS LA TRYPANOSOMIASE AFRICAINE

RESUME

L'effet de *Trypanosoma congolense* et *T. brucei* infection mixte sur l'aptitude de la moelle osseuse à répondre à

L'anémie a été étudiée chez les rats albinos. Il s'agissait d'évaluer l'incidence possible sur le taux de récupération de l'anémie consécutive à la chimiothérapie de la trypanosomiase africaine. L'étude portait sur l'évaluation descriptive de l'hématocrite et les modifications cytologiques correspondantes de la moelle osseuse associées à l'infection unique et mixte de *T. congolense* et *T. brucei*. Il a impliqué l'infection expérimentale de rats albinos commodes de recherche. Un total de 32 rats albinos adultes de sexmixte ont été utilisés pour cette enquête. Les rats ont été groupés aléatoirement en trois groupes, A, B, C, qui composait de 8 rats par groupe, et infecté par *T. congolense*, *T. brucei* et infection mixte de ces espèces. Huit autres rats ont servi de groupe témoin non infecté. Les paramètres mesurés incluaient l'hématocrite hebdomadaire (PCV) et la cytologie de la moelle osseuse différentielle des groupes différents de rats infectés et témoins à la fin de 21 jours après l'infection (PI). À la fin de 21 jours PI, l'anémie caractérisée par la chute du PCV a été la plus sévère dans le groupe infection mixte, et moindre dans le groupe *T. brucei* avec la tendance à l'auto-récupération de l'anémie. Les réponses de moelle osseuse dans le groupe infection mixte étaient cependant faibles et inférieures à celle de *T. brucei* et *T. congolense*. Une mauvaise réponse érythropoïétique dans le groupe d'infection mixte en dépit d'une chute significative ($P < 0.05$) du taux de PCV était considérée de provenir d'une pathologie rénale et hépatique sévère résultant au déchargement de l'érythropoïétine sous-normal et des lésions graves des cellules souches. On pense que ce la causerait plus de temps à être prises par les animaux d'infection mixte pour récupérer de l'anémie après la chimiothérapie. Il est conclu que l'infection mixte *T. congolense* et *T. brucei* résulte à l'incapacité marquée de la moelle osseuse et la capacité de récupération de l'anémie. Ceci suggère qu'une administration d'un grand soutien d'érythropoïétine synthétique peu être requise dans une situation d'infection mixte de ces espèces de trypanosomes due à des effets pathologiques graves sur le rein et le foie résultant de l'altération de l'érythropoïétine - biosynthèse et de la récupération lente de l'anémie après chimiothérapie dans la trypanosomiase africaine.

Mots clés: Anémie, moelle osseuse, infection mixte, rats, trypanosomiase, érythropoïétine.

INTRODUCTION

Bone marrow responses play key roles in the pathogenesis of anemia in African trypanosomiasis as they determine ability for haemopoietic cell regeneration and control of anemia in infected hosts (1-4). Anemia constitutes a major pathological feature of trypanosomiasis in man and animals (5,6). Bone marrow factors involved in dyserythropoiesis include ineffective erythropoietic activities and erythrophagocytosis by macrophages in the bone marrow, spleen and other tissues (5,7).

We had reported that even though *Trypanosoma congolense* and *T. brucei* are pathogenic to animals, they cause severe anemia under mixed infection which arises probably from combination of differences in the pathogenic mechanisms of the different species of trypanosome (8). Although trypanosome mixed infection occurs naturally in the field (9), the effect on ability of the animal to control the development of anemia has not been reported. Similarly, in parts of Central Africa, sleeping sickness in man arising from *T. rhodesiense* and *T. gambiense* mixed infection is not unlikely.

In this study, we investigated the effects of *T. congolense* and *T. brucei* mixed infection on ability to respond to and recover from anemia using rats as model. Such findings are likely to find relevance in the proper chemotherapy of trypanosomiasis due to mixed trypanosome species infections and prevention of the post treatment lingering effects of anemia, a major cause of death in African trypanosomiasis.

MATERIALS AND METHODS

A total of 32 adult albino rats were used for the investigation. The rats were bred at our Research Station in Vom, Plateau State, Nigeria and brought to Kaduna for the study. Commercially prepared rat cubes and water were fed to the rats *ad libitum* throughout the course of investigation. At the end of one week acclimatization period, the rats were randomly grouped into three groups; A, B, C and control groups made up of 8 rats each. Trypanosome species used were *T. congolense* (Bassa) and *T. brucei* (Lafia). Both parasites were obtained from cattle during field survey and cryopreserved in liquid nitrogen from where they were first sub-passaged into donor rats and then into the experimental rats.

The rats in group A were inoculated with *Trypanosoma congolense*, 1×10^3 parasites while group B was inoculated with *T. brucei* with the same number of parasites. Group C was inoculated with 0.5×10^3 each of *T. congolense* and *T. brucei*. All inoculations were intraperitoneal (IP). Parameters measured included packed cell volume (PCV) as described by Dacie and Lewis (10), and estimation of mean differential bone marrow counts of both control and trypanosome infected rats at the end of 21 days post infection. Bone marrow smears were obtained from the right femur of 5 rats randomly selected from each group, and three rats that remained in group C by day 21 post infection. The smears were air dried, fixed for 20 minutes in absolute alcohol, stained with Giemsa's stain and examined by light microscopy as described by Anosa et al (7). A minimum of 500 marrow cells were counted per rat and differentiated.

RESULT

At the end of 21 days PI, the anemia characterized by drop in the mean PCV was most severe in group C with *T. congolense* and *T. brucei* mixed infection ($P < 0.05$). The percentage overall drop in PCV at the end of 21 days PI was 8.5%, 6.3% and 17.0% for groups A, B, and C respectively (Table 1). However, the bone marrow in Group B, infected with *T. brucei* was most hyperplastic due to erythroid hyperplasia (Table II) followed by Group C and Group A. This led to the fall in myeloid: erythroid (M:E) ratio to the value of 1.02 ± 0.31 : 1, 0.50 ± 0.04 : 1 and 0.82 ± 0.1 in groups A, B and C respectively as against the value of 1.27 ± 0.05 : 1 for control rats. Similarly granulocyte maturation rate dropped from the value of 3.81 ± 1.10 in control rats to 3.09 ± 0.82 , 2.62 ± 0.77 and 1.37 ± 0.01 for rats in groups A, B, and C respectively.

Lymphocytes in the bone marrow of infected groups were less in number compared to those of control rats. Similar changes were observed in the marrow plasma cell counts. There was however increased cellularity of the monocyte cell lineages which was most ($1.6 \pm 0.45\%$, $P < 0.05$) in group C with mixed infection followed by group B and least in group A. Macrophage hyperplasia followed the same pattern with that of monocytes in the infected rats with macrophages being most numerous ($3.40 \pm 0.97\%$, $P < 0.05$) in the marrow of rats in group C with mixed infection. However more significant numbers of mitotic cells were encountered in the bone marrow of rats in group B infected with *T. brucei* following by group C and least in group A.

DISCUSSION

The overall changes in the mean PCV of infected rats resulting to severer anemia in the mixed infection group support our earlier findings in *T. congolense* and *T. brucei* mixed infection of rats (8). This is believed to arise from the combination of different mechanisms of pathology associated with the trypanosome species which have to do with differences in the preferential sites of localization in the tissues of infected hosts (11,12). The bone marrow of *T. brucei* infected rats was relatively most hyperplastic and responsive. This was characterized by marked erythroid hyperplasia and increase in numbers of mitotic figures which were dominantly of erythroid origin and severe fall in the M:E ratio. This supports earlier observations in *T. brucei* infected deer mice (13) and horses (14) in which marked erythroid hyperplasia also resulted in very high reticulocyte responses in the infected animals. Similar responses were observed in vervet

monkeys infected with the human infective *T. brucei gambiense* (15). Anosa et al (1,7) observed erythroid hyperplasia in cattle infected with *T. vivax* and *T. congolense* respectively. However, *T. congolense* resulted to myeloid hyperplasia in infected cattle (16) while mild reticulocyte responses occurred in *T. congolense* and *T. vivax* infected sheep (17). This study confirms that the superior reticulocyte responses in *T. brucei* infections arise from high erythropoietin activities resulting to selective stimulation of erythropoiesis above granulopoiesis. This seemed to have been responsible for the recovery from low PCV by day 21 PI in the *T. brucei* - infected group B while such improvement in PCV did not occur in the other groups, especially in the mixed infection group.

Even though anemia characterized by drop in PCV level was most severe in the mixed infection group, erythroid responses were relatively weak compared to the more superior responses in the *T. brucei* group. This is believed to have been responsible for the persistent and most severe anemia in the mixed infection group.

The inability of the bone marrow of the mixed infected group to respond well in the face of severe drop in the PCV value of infected rats suggests that, there were subnormal erythropoietin activities which probably arose from marked pathology of the liver and kidneys, organs involved in the biosynthesis of erythropoietin which controls erythropoiesis; and severe stem cell injury. Hepatic and renal pathology occur commonly in trypanosomiasis of man and animals (18, 19) and is believed to play roles in the pathogenesis of anemia.

Relatively marked increases in macrophage numbers in the mixed infection group suggest that there was also marked erythrophagocytosis by macrophages in the marrow of the rats which contributed to the severe anemia observed in this group. Increase in the monocyte numbers may have been also due to their increased demand as macrophages in the mixed infected group. This is supported by the identification of numerous macrophages with engulfed red and white blood cell lineages in the bone marrow of mixed infected group. The roles of macrophages in the pathogenesis of anemia have already been described (3, 4).

Further analysis of cytological changes in the bone marrow of infected rats also suggested that there was depression of the granulocytic precursors at all levels but particularly the more mature stages such as metamyelocytes, bands

and segmenters which constitute marrow storage pool or reserves. This resulted in the lower granulocyte maturation rate, which is the ratio of

non-mitotic to mitotic granulocytes. Similar observations were reported by Anosa et al (7) in *T. vivax* - infected calves.

TABLE I: SUMMARY OF THE PCV VALUES OF CONTROL AND INFECTED RATS

Experimental Group	Pre-infection	Day 21 PI (% Drop)
Control	51.7 ± 1.3	49.7 ± 3.8 (3.9)
Group A	47.0 ± 3.7	43.0 ± 2.8 (8.5)
Group B	48.0 ± 2.3	45.0 ± 2.9 (6.3)
Group C	47.0 ± 1.7	39.1 ± 2.3 (17.0)*

*= P<0.05

TABLE II: DIFFERENTIAL MARROW CELL COUNTS (%) OF CONTROL AND TRYPANOSOME INFECTED RATS AT 21 DAYS PI

Cell Types	Control Rats	<i>T.c.</i> Infected Rats	<i>T.c.</i> Infected Rats	Mixed Infected Rats
Erythroid Cells	35.2 ± 3.57	43.66 ± 6.59*	54.23 ± 2.71*	45.80 ± 1.12*
Myeloid Cells	44.5 ± 0.21	46.34 ± 0.27	27.17 ± 3.44*	37.40 ± 1.34
Myeloid:Erythroid Ratio	1.27 ± 0.05:1	1.02 ± 0.31:1	0.50 ± 0.04:1*	0.82 ± 0.1:1*
Granulocyte Maturation Rate	3.81 ± 1.10	3.09 ± 0.82	2.62 ± 0.77	1.37 ± 0.01*
Other Cells:				
Lymphocytes	15.6 ± 2.11	6.70 ± 3.82*	10.61 ± 2.89*	9.01 ± 0.09*
Plasma Cells	0.70 ± 0.35	0.16 ± 0.23	0.10 ± 0.14	0.4 ± 0.10*
Monoblasts/Promonocytes/ Monocytes	0.9 ± 0.87	0.53 ± 0.19	1.20 ± 1.42*	1.6 ± 0.45*
Macrophages	1.40 1.74	1.20 ± 0.09	2.00 ± 1.24	3.40 ± 0.97*
Unclassified	0.30 ± 0.29	0.35 ± 0.21	0.50 ± 0.71	0.40 ± 0.31
Damaged Cells	1.01 0.14	0.55 ± 0.49*	1.31 ± 0.16	0.80 ± 0.10
Mitotic Cell	0.21 ± 1.31	0.51 ± 0.45	2.88 ± 0.44*	1.20 ± 0.81*

T.c.= *Trypanosoma congolense*; *T.b.* = *Trypanosoma brucei*; *= P< 0.05

This drop was most severe in the mixed-infected group which may have resulted from marked granulophagocytosis by macrophages in the bone marrow of the mixed infected rats. Although similar marrow cytological changes occurred in the *T. congolense* - infected rats, they were inferior to those of *T. brucei* and mixed infection groups. This supports earlier reports of mild reticulocytosis associated with *T. congolense* infection in sheep (17). This confirms the beneficial effect of synthetic erythropoietin administration in the management of anemia in trypanosomosis due to mixed infections (20). It was concluded that *T. brucei* precipitated most superior marrow erythropoietic response in

infected rats resulting in apparent recovery from anemia in the *T. brucei* - infected group. Even though anemia was most marked in the mixed infection group, bone marrow responses were weak and inferior to those of *T. brucei* group as the marrow was relatively less hyperplastic. This arose probably from subnormal erythropoietin release due to severe pathology of the kidney and liver (21, 22) as a result of combined effects of the peculiar pathogenic mechanisms of the parasites which has to do in part, with the differences in sites of localization in infected hosts. Such peculiar differences may have also caused severe stem cell injury and marked antigenic activation of macrophages in the mixed infection group

leading to macrophage hyperplasia and massive erythrophagocytosis. These acting together may incapacitate the bone marrow's ability to respond to anemia and recovery following chemotherapy. Although further studies were needed to confirm such findings in more natural hosts such as cattle, sheep and goats, and humans with mixed *T. rhodesiense* and *T. gambiense* infections, these observations suggest that supportive administration of synthetic erythropoietin may be required to enhance

recovery from anemia arising from infections due to mixed species of African trypanosomes.

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