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Anemia in Experimental African Trypanosomiasis

EMMANUEL O. OGBADOYI[1)], AGWU I. UKOHA[2)] AND ELIZABETH K. KYEWALABE[3)]

[1] *Ahmadu Bello University, Zaria, Kaduna State, Nigeria,* [2] *Department of Biological Sciences, Federal University of Technology, Minna, Nigeria,* [3] *Tropical Diseases Research Centre, P. O. Box 71769, Ndola, Zambia*

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ABSTRACT

Anemia is a recognized feature of both animal and human trypanosomiasis. The mechanism of trypanosomal anemia is however unknown. Understanding the mechanism of anemia is important for the effective management of trypanosomiasis patients, especially in animals where anemia could be extremely severe. Anemia was monitored in five goats experimentally infected with *Trypanosoma vivax* by measuring the packed cell volume (PCV) and the hemoglobin level (Hb) up to 26 days post infection. Parasitemia and serum free fatty acid (FFA) levels were also estimated. Massive parasitemia was observed on days 5 and 12 post infection. There was 45-59% decrease in the PCV and 33-63% decrease in the Hb level. The serum FFA level progressively increased through out the course of infection. Anemia in the early stages of the infection is initiated and maintained by living trypanosomes, the severity of the anemia depending on the level of parasitemia. Persistent anemia as the disease process progresses is caused by factor(s) other than living trypanosomes. It is suggested that erythrocyte haemolysis and erythrophagocytosis are the underlying causes of trypanosomal anemia.

INTRODUCTION

African trypanosomiases are a spectrum of diseases in man and domestic animals caused by the tsetse transmitted protozoan parasite, trypanosome. Human African trypanosomiasis (sleeping sickness) is caused by *Trypanosoma brucei*

gambiense and *T. b. rhodesiense*. Animal trypanosomiasis (nagana in cattle) on the other hand is caused by three species of trypanosomes, namely *T. b. brucei, T. congolense* and *T. vivax*. Of these three trypanosome species, *T. vivax* is the most important trypanosome of veterinary importance in West Africa. Animal trypanosomiasis still constitutes a major constraint in the growth and development of the livestock industry in many countries of sub-Saharan Africa, thereby limiting total food production. Animal trypanosomiasis and East Coast fever are believed to account for the death of 3 million cattle a year and one or both of these diseases occur(s) across more than one third of the African continent (ILRAD 1987). The estimated annual loss in livestock due to African trypanosomiasis is put at US$ 5,000 million (WHO 1997).

Anemia has since been recognized as a major pathological feature of clinical trypanosomiasis in both man and animals (Fiennes 1954; 1970) and has been used as an indicator of trypanotolerance in animals infected with trypanosomes (Akinbamijo et al. 1998; d'Iteren et al. 1998). However, to date, the mechanism of the anemia is unknown. Rather, hypotheses which have failed to find common ground of acceptable specificity have been put forward. These include: hypoplasia of the bone marrow, hydraemia, immunological reactions, erythrophagocytosis, hemorrhage syndrome and severe haemolysis (reviewed by Murray and Dexter 1988). A more recent addition to this list of hypotheses is the report of Igbokwe et al. (1994) that peroxidative injury to erythrocytes may play a role in the pathogenesis of anemia in trypanosomiasis. A good understanding of the pathogenesis of the anemia is important for a better management of the patients, especially in animal trypanosomiasis where the anemia could be very severe. This study is part of our contribution to the efforts towards the elucidation of the mechanism of anemia in trypanosomiasis.

MATERIALS AND METHODS

Trypanosomes:

Trypanosoma vivax stabilate, stock Kabam/84/NITR/7.4 was obtained from the Nigerian Institute for Trypanosomiasis Research, Vom, Nigeria. This was inoculated in sheep's blood and kept in liquid nitrogen at -196°C before being used in goats.

Goats:

Goats were purchased from local markets in Zaria, Northern Nigeria and kept in fly-proof pens in the faculty of veterinary medicine, Ahmadu Bello University, Zaria. Appropriate steps were taken to ensure that all the goats were in good health and apparently free from trypanosomiasis and any other form of

parasitic infection before the experimental infection.

Chemicals:

Unless otherwise stated, all chemicals used were obtained from Sigma, England, and were all of analytical grade.

Experimental infection:

Ten goats were divided into 2 groups of 5 each. One group of 5 were each infected with 10^7 *T. vivax* cells while the second group of 5 were uninfected and served as control.

Parasitemia and packed cell volume (PCV):

Parasitemia and PCV were determined using the haematocrit centrifuge technique (Woo 1970).

Hemoglobin content:

The hemoglobin content was estimated by simply taking a little quantity of blood sample and then reading off the hemoglobin level in a Counter haemoglobinometer.

Estimation of serum free fatty acid (FFA) level:

FFA levels were estimated as described by Falholt et al. (1973). All the parameters were measured essentially twice a week, beginning from day 0 to day 26 post infection.

RESULTS

Parasitemia:

There were cyclical fluctuations in the number of parasites in circulation which resulted in 3 successive waves of parasitemia with peaks on days 5, 12 and 22 post infection (Fig. 1). Between days 5 and 8, the number of parasites in circulation decreased sharply, reaching a very low level (less than half the peak value) by day 8. The second phase of declining parasitemia which was more drastic than the first occurred between days 12 and 19 post infection. By day 19, the level of parasitemia was the same as that on day 2 (6 times less than the peak value on day 12). The peak values on days 5 and 12 were the same (about 4 trypanosomes per microscope field), while the value for day 22 was about 1 trypanosome per field. The values given are an average of the values for the five goats.

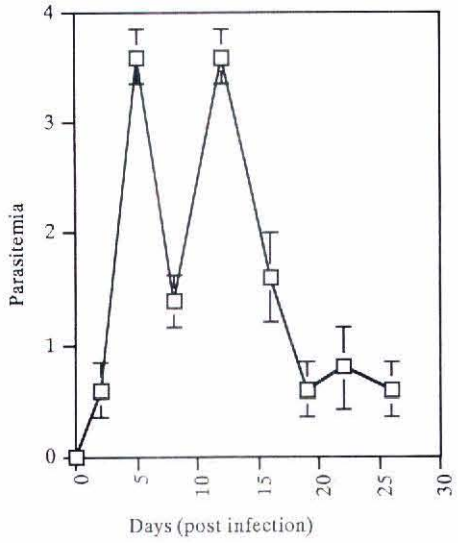

Figure 1. Development of parasitemia through the course of infection. An illustration of the cyclical fluctuations in parasitemia during the course of the experimental infection which lasted for 26 days. Each point on the vertical axis is the mean of the values of the five infected goats. The vertical bars represent standard errors of mean (SEM).

Packed cell volume (PCV):

With the exception of goat number 3808 which showed no apparent decrease in PCV by day 2 post infection, the PCV of all the infected goats showed significant decreases by day 2, with as much as 25% decrease in goat number 3845 (Fig. 2). The decrease at the end of the experiment ranged between 45-59%. In general, there was a progressive decrease in PCV of all the infected goats until the termination of the experiment.

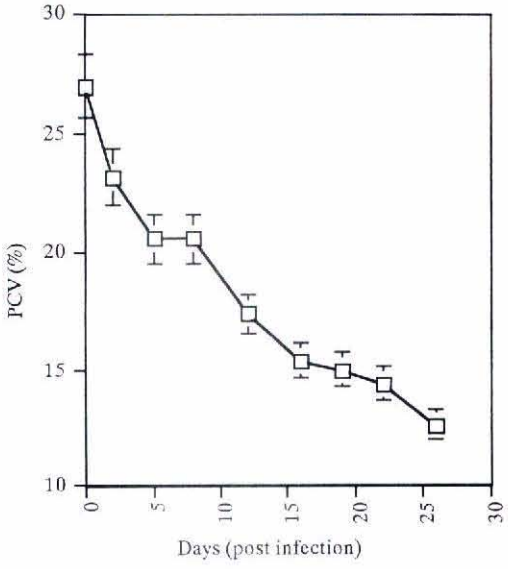

Figure 2. The packed cell volume of the infected goats during the period of infection. This shows the declining PCV during the 26-day period of experimental infection. Each point on the vertical axis is the mean of the values of the five infected goats while the SEM are represented by the vertical bars.

Hemoglobin (Hb) level:

Like the PCV, the Hb levels of all the infected goats decreased significantly

during the course of infection with an average of 48% reduction except for goat 3844 which had its hemoglobin level reduced by as much as 63% before it finally died (Fig. 3).

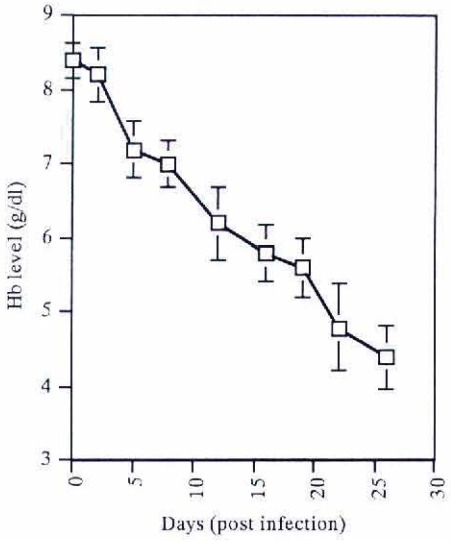

Figure 3. The hemoglobin levels of the infected goats during the period of infection. This illustrates the decrease in Hb levels with progression through the disease process. Each point on the vertical axis is the mean of the values of the five infected goats while the vertical bars represent SEM.

Serum free fatty acid (FFA) level:

There was a progressive increase in the serum FFA level of all the infected goats (Fig. 4). The values of all the parameters measured in all the control animals did not show any appreciable change but remained essentially constant with occasional drop or rise by 1 unit (data not included).

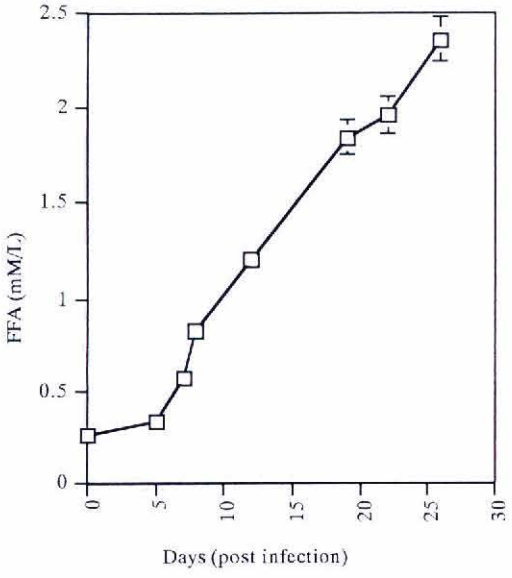

Figure 4. Serum free fatty acid (FFA) levels of the infected goats. Serum FFA levels of the infected goats measured over the 26-day period of infection. Each point on the vertical axis is the mean of the values of the five experimentally infected goats. Vertical bars represent SEM.

DISCUSSION

Anemia in trypanosomiasis proceeds in two distinct but overlapping phases, each phase being mediated by different mechanisms (Murray and Dexter 1988). The first phase is believed to be mediated by factors released by degenerating trypanosomes (Tizzard et al. 1978b; ILRAD 1987) and red cell damage by living trypanosomes (Banks 1979; Esievo 1983). Our observations in this study are in support of living trypanosomes playing a key role in the initiation and maintenance of the progressive development of anemia in trypanosomiasis. The onset of anemia and the severity in the first phase strongly correlate with the appearance, intensity, and duration of parasitemia. The observed considerable decline in the PCV and the Hb content between days 0 and 5 post infection and the relatively stable values between days 5 and 8 (Figs. 2 and 3) which coincided with increasing and decreasing parasitemia respectively (Fig. 1) could not have been a mere coincidence. The PCV and Hb content again decreased sharply between days 8 and 12 which was another period of increasing parasitemia. These findings suggest that living trypanosomes are essential for the progressive development of trypanosomal anemia in infected animals. Ikede et al. (1977) reported the absence of haemolysin in the plasma of *T. congolense* infected mice at least up to 15 days post infection but observed marked reduction in erythrocyte half-life and marked drop in PCV within the same period. In cattle infected with *T. congolense* increased red cell breakdown is believed to commence with the development of parasitemia (Holmes 1976; Preston et al. 1979). Therefore contrary to the widely held view that anemia in trypanosomiasis is induced by factors released by dying trypanosomes, we believe that the onset of anemia and its progression, at least in the early stages of infection, depends largely on the presence of living trypanosomes in circulation.

The persistent anemia even when parasites are barely detectable is the phase II of the anemia. We believe that this stage is mediated by factors other than those produced by living trypanosomes. FFAs and lysophospholipids are believed to be hemolytic factors released by dying trypanosomes and are responsible for trypanosomal anemia (Tizzard et al. 1978a; Nok et al. 1993). We observed steady and sharp increases in the FFA levels of all the infected goats through out the period of infection (Fig. 4) and this could have played a key role in the pathogenesis of the persistent anemia. Although serum albumin is known to bind to FFAs to neutralize their hemolytic effects (Starinsky and Shafrir 1970; Tizzard et al. 1978b), it is possible that the massive parasitemia that characterized the first two successive waves of parasitemia produced sufficient FFAs to saturate available serum albumin and some left unbound to exert their hemolytic effect on erythrocytes.

Elucidation of the mechanism of anemia in trypanosomiasis has remained an unsolved problem. Jennings et al. (1974) suggested that anemia in trypanosomiasis was mainly due to extravascular haemolysis and ruled out immune mechanisms since the onset of anemia was evident within a few days of infection. Murray and Dexter (1988) explained that immunological competence was not essential for the development of anemia. Dargie et al. (1979a; 1979b) and Dargie (1980) ruled out haemodilution as a contributory factor to the development of anemia. Hemorrhagic syndrome which is characteristic of acute infections caused by certain strains of *T. vivax* infections was not evident in the present study. The marked drop in PCV and the marked reduction in the Hb content observed in this work are good indicators of haemolysis. We therefore believe that the first phase of anemia is largely due to erythrocyte haemolysis. Erythrophagocytosis resulting from induced alterations in erythrocyte surface structure (Durocher et al. 1975; Esievo et al. 1982; Esievo 1983) is also a possibility as a cause of anemia in trypanosomiasis. We therefore conclude that trypanosomal anemia is multifactorially induced but the predominant factors depend on the stage of the disease process.

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