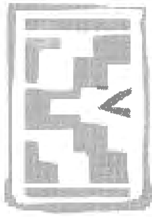

In vitro erythrophagocytosis by cultured macrophages stimulated with extraneous substances and those isolated from the blood, spleen and bone marrow of Boran and N'Dama cattle infected with *Trypanosoma congolense* and *Trypanosoma vivax*

V.O. TAIWO and V.O. ANOSA

Department of Veterinary Pathology, University of Ibadan, Ibadan, Nigeria

ABSTRACT

TAIWO, V.O. & ANOSA, V.O. 2000. *In vitro* erythrophagocytosis by cultured macrophages stimulated with extraneous substances and those isolated from the blood, spleen and bone marrow of Boran and N'Dama cattle infected with *Trypanosoma congolense* and *Trypanosoma vivax*. *Onderstepoort Journal of Veterinary Research*, 67:273–287

A standard radioactive chromium (^{51}Cr) release assay was used to assess the *in vitro* phagocytosis and lysis of bovine erythrocytes by cultured splenic, bone marrow and peripheral blood monocyte-derived (PBM) macrophages isolated from healthy and *Trypanosoma congolense* and *T. vivax*-infected cattle of the Boran and N'Dama breeds.

Recombinant cytokines (rHuTNF-α and rBoIFN-γ) and non-acid-dialysed peripheral blood mononuclear cell (PBMNC) culture supernatants stimulated these PBM for enhanced activities. The stimulants caused increases in the rate of erythrocyte phagocytosis and lysis by cultured PBM in a concentration-dependent manner. But very high stimulant concentrations caused deceased *in vitro* erythrophagocytosis. However, bacterial lipopolysaccharide (LPS) and acid-dialysed PBMNC culture supernatants did not cause any increase in cultured PBM erythrophagocytosis.

In vitro erythrocyte phagocytosis and lysis by splenic, bone marrow and peripheral blood monocyte (PBM)-derived macrophages of Boran breed of cattle infected with *Trypanosoma congolense* increased from 14 days post-infection (DPI) onwards and thereafter maintained at various levels above pre-infection. Cultured splenic macrophages showed the greatest erythrocyte destruction capability while PBM-derived macrophages was the least. The rates of *in vitro* erythrocyte phagocytosis and lysis were higher with the cultured PBM of the Boran than those of the N'Dama cattle during *T. congolense* infection. The rate of *in vitro* erythrocyte destruction was however, similar in both groups of cattle during *T. vivax* infection. These results correlated positively with the dynamics and degree of anaemia developed by these groups of animals during both *T. congolense* and *T. vivax* infections.

Cattle infected with *T. congolense* and *T. vivax* developed varying degrees of normocytic normochromic anaemia during infection. Boran cattle developed a more severe anaemia, and had to be treated with diminazine aceturate, than N'Dama cattle during *T. congolense* infection. Both breeds of cattle developed a milder but similar degree of anaemia during *T. vivax* infection. None of the animals were treated.

The results of this study indicated a role of *in vivo* macrophage stimulatory factors, notably cytokines such as TNF-α and IFN-γ in host's serum, as well as parasite antigens, which may act singly or in concert, in the process of enhanced erythrocyte destruction, hence anaemia by the mononuclear phagocytic system (MPS) during bovine trypanosomosis.

Keywords: Anaemia, Boran, cattle, cultured macrophages, cytokines, *In vitro* erythrophagocytosis, N'Dama, trypanosomosis

Accepted for publication 5 October 2000—Editor

INTRODUCTION

Macrophage, the central cell in the mononuclear phagocytic system (MPS), is the immediate precursor of the peripheral blood monocyte (PBM) (Lasser 1983). After entering the tissues, the macrophage often becomes a relatively quiescent resident and non-circulating cell unless challenged with one or more stimulatory signals in its local environment (Lasser 1983; Adams & Hamilton 1984). When exposed to a variety of antigens, notably lipopolysaccharide (LPS), and other host regulatory factors such as cytokines like gamma-interferon (IFN-γ) and tumour necrosis factor (TNF-α), resting macrophages proliferate *in situ* and undergo a range of changes resulting in altered morphology, biochemical and functional activities (Adams & Hamilton 1987; Unanue & Allen 1987). This phenomenon is termed "activation" (MacKaness 1970).

The avid phagocytosis of mature and immature erythrocytes by an expanded and activated MPS has been described as one of the major causes of anaemia in African animal trypanosomosis (Murray & Dexter 1988; Anosa, Logan-Henfrey & Shaw 1992). During trypanosome and other haemoprotozoan infections, the MPS have been shown to be activated (Kelly 1986) and phagocytose erythrocytes both in peripheral blood (Connal 1912; Wellde, Lotzsch, Diendl, Sadun, Williams & Warui 1974) and in tissues, notably the spleen, liver and bone marrow of infected animals (Anosa & Kaneko 1983).

Erythrocyte-macrophage interactions leading to erythrophagocytosis and the dynamics of erythrocyte destruction *in vivo* during trypanosome infection and *in vitro* assays have not been studied in cattle. Furthermore, comparative *in vitro* erythrophagocytosis by macrophages isolated from different tissues of cattle infected with trypanosomes has not been carried out. Hence, the main objectives of this study were to use *in vitro* techniques to monitor the rate of phagocytosis and destruction of erythrocytes by cultured macrophages from various organs of cattle during trypanosomosis and using extraneous stimulants such as bacterial LPS, and crude and recombinant cytokines (IFN-γ and TNF-α) in order to elucidate their roles in the pathogenesis of anaemia during bovine trypanosomosis.

MATERIALS AND METHODS

Animals

A total of 45 cattle were used. In the assays using extraneous stimulants, 16 healthy cattle, eight Boran breed and eight of the N'Dama breed (four cows and four steers of each breed) all aged between 2–4 years were used. Twenty-nine cattle were infected with trypanosomes and used for *in vitro* erythrophagocytosis assays in two separate studies. In the first study, cultured macrophages were isolated from the blood, spleen and bone marrow of nine 6 months old calves of the Boran breed (five males and four females). In the second study, cultured PBM were isolated from ten Boran cattle and ten of the N'Dama breed (five males and five females of each breed), aged between 2 and 4 years.

The Boran cattle were born at the Kapiti Plains ranch in the Machakos District of Kenya and transferred to the Large Animals' Unit of the International Livestock Research Institute (ILRI), Nairobi, Kenya immediately after weaning. The N'Dama cattle are progenies of the original N'Dama cattle born at ILRI after embryonic transfer from The Gambia (Jordt, Mahon, Toure, Ngulo, Morrison, Rawle & Murray 1986). All the animals were housed in open barns on concrete floors with adequate straw and wood-shavings and fed *ad libitum* with grass hay supplemented with pelleted concentrates and mineral salt blocks.

Radio-labelling of erythrocytes (^{51}Chromium)

Ten mℓ of blood were collected by jugular venapuncture from the donor cattle into a tube containing 1 mℓ of acid-citrate-dextrose (ACD) preparation in sterile Dulbecco's modified phosphate-buffered saline (DPBS), pH 7,4, as anticoagulant. The blood was centrifuged (Beckman J-6B, Beckman, USA), at 1 200 *g* for 25 min at 10 °C and the plasma, buffy coat and upper third of the erythrocyte pellet were removed. Two hundred μℓ of mixed middle and lower thirds of the erythrocyte pellet were washed once in 10 mℓ of DPBS by centrifugation at 750 *g* for 10 min at 10 °C. The erythrocyte pellet was re-suspended in 5 mℓ DPBS and the erythrocyte concentration determined by counting on an electronic cell counter (Coulter Electronics, USA), and thereafter adjusted to 4 x 10^6 erythrocytes/mℓ in 10 mℓ of DPBS.

Five mℓ of the erythrocyte suspension, containing 2 x 10^7 erythrocytes, was put into each of two 10 mℓ tubes and the erythrocytes were pelleted by centrifugation at 750 *g* for 10 min at 10 °C. The erythrocyte pellets were resuspended in 1 mℓ of DPBS. The erythrocyte suspension in one tube was labelled with radioactive $Na_2{}^{51}CrO_4$ (^{51}Cr; Amersham International PLC, UK) as described by I.C.S.H. (1971). The erythrocytes were resuspended at 10^6/mℓ in culture medium composed of 10 % foetal bovine serum (FBS) (Flow Laboratories, UK) in RPMI-1640 with 50 μg/mℓ gentamycin (Flow Laboratories, UK) and 200 I.U./mℓ penicillin and 15 μg/mℓ streptomycin (Gibco Laboratories, UK).

Stimulants used for *in vitro* PBM activation

Six stimulants were used for these studies. They include two batches of LPS (lipid A fraction) from

Salmonella enteritidis (SE) and *Serratia marcescens* (SM) (Sigma Chemical Company, USA), recombinant human TNF-α (rHuTNF-α; AMGEN Biologicals, California, USA), recombinant bovine IFN-γ (rBoIFN-γ) (courtesy Dr J. Peel, Centre de Recherches Agricoles, Ciba Geigy, Switzerland), and cytokine-rich supernatants from 48-h cultured PHA-stimulated or unstimulated peripheral blood mononuclear cells (PBMNC). The PBMNC culture supernatants were used either directly (undialysed) or acid-dialysed (pH 2,0) before use, as described by Townsend, Duffus & Williams (1988).

Assays using bacterial LPS (SE and SM)

In this experiment, as in the others described below, PBM were isolated from each of two Boran and two N'Dama cows. Two commercial preparations of bacterial LPS (SE and SM) at concentrations of 0,1, 1 and 10 μg endotoxin/mℓ final dilution in culture medium were added to the PBM cultures. This assay was carried out twice.

Assays using rHuTNF-α and rBoIFN-γ

PBM were isolated from four Boran steers and four N'Dama steers. The recombinant cytokines were added to each well of PBM cultures at final dilutions of 10, 100 and 1000 U/mℓ in medium. Three separate experiments were carried out using rHuTNF-α, while two experiments were carried out using rBoIFN-γ.

Assays using PBMNC culture supernatants

PBM were isolated from each of two Boran and two N'Dama cows. Acid-dialysed or undialysed 48-h PBMNC culture supernatants were used at 1:100, 1:10 dilution in culture medium, and in undiluted form. The PBM to which the undiluted PBMNC culture supernatant was added, had previously been centrifuged and all culture medium removed before adding 200 μℓ of the undiluted PBMNC culture supernatants. This assay was carried out twice.

Assay using cultured macrophages isolated from the blood, spleen and bone marrow

Four male and four female Boran calves were each exposed to bites from five male *Glossina morsitans centralis* infected with *Trypanosoma congolense* ILNat 3,1 (Geigy & Kauffmann 1973; Nantulya, Musoke, Rurangirwa & Moloo 1984) as described by Dwinger, Murray & Moloo (1987). One animal from the infected group was killed with a capture bolt gun and thereafter exsanguinated on each of the following days post-infection (DPI): 7, 14, 21, 28, 35, 42, 49 and 56. Immediately before being shot, a small sample of bone marrow (vide infra) was taken, and 50 mℓ of blood collected by jugular venapuncture from each animal into sterile beakers containing an equal volume of cold Alsever's solution (pH 6,2). The control calf was sampled, bled, killed and exsanguinated on the 65th day of the experiment. After euthanasia the spleen of each animal was sterilely removed and treated as is described below.

Haematology

Packed cell volume (PCV), haemoglobin (Hb) concentration and erythrocyte (RBC) counts of all the animals prior to infection with trypanosomes (day 0) and on 7, 14, 21, 28, 35, 42, 49 and 56 DPI were determined and mean corpuscular volume (MCV) and mean corpuscular haemoglobin concentration (MCHC) were calculated as described by Jain (1986).

Assays using cultured PBM of trypanosome-infected adult cattle

Two separate experiments, one using *T. congolense*-, and another using *T. vivax*-infected adult Boran and N'Dama breeds of cattle of both sexes were carried out. Five Boran cattle breed and five of the N'Dama breed were each tsetse-challenged with either *T. congolense* ILNat 3,1 or *T. vivax* IL2337 (Gardiner, Assoku, Whitelaw & Murray 1989) as described in the first study above. In the *T. congolense* experiment, PBM were isolated from the animals on 0, 14, 21, 28, 35, 42, 49 and 56 DPI, while in the *T. vivax* experiment, PBM were isolated from the blood of the animals on 0, 8, 15, 22, 29, 36, 43 and 50 DPI. In the *T. congolense* experiment, all the five Boran cattle were treated with Berenil® at the rate of 3,5 mg/kg body weight on 48 DPI when their PCV had dropped to 15 % in order to prevent them from dying.

Isolation of PBM from blood and macrophages from the spleen and bone marrow

PBM

Fifty mℓ of blood were collected by jugular venapuncture from each animal into an equal volume of sterile Alsever's solution at pH 6,2. PBM were isolated aseptically from the blood by the plasma/gelatin-coated flasks' method of Goddeeris, Baldwin, ole Moiyoi & Morrison (1986). The isolated PBM were resuspended in 2 mℓ warm culture medium and counted on a haemocytometer and viability was determined by trypan blue exclusion. More culture medium was added to the PBM to make a final suspension of 10^6 PBM/mℓ. One hundred μℓ of the PBM suspension (containing 10^5 cells) were dispensed per well in triplicate; into 96-well flat-bottomed tissue culture plates (Costar, Cambridge, USA) and cultured for 18 h at 37 °C in 5 % CO_2-in-air prior to erythrophagocytosis assays.

Splenic Mϕ

After euthanasia, the spleen of each animal was removed and placed in a beaker containing sterile

Alsever's solution (pH 6,2). Pieces of the spleen, about 10 mm^3 in size, were aseptically taken from the parenchyma and placed in sterile 50 mℓ tubes containing Alsever's solution. These pieces were rinsed twice in Alsever's solution and transferred into sterile 60 x 15 mm tissue culture petri dishes (Falcon 3002; Becton Dickinson, New Jersey, USA) containing 10 mℓ culture medium in a sterile horizontal airflow biological hood. The tissues were minced with sterile scissors to separate the cells. The cell suspension was pipetted into a 10 mℓ tube and allowed to stand for 1 min to allow large tissue particles to settle at the bottom. Adherent cells in the supernatant cell suspension were separated and cultured for 18 h as previously described for the PBM.

Bone marrow Mφ

Prior to euthanasia, each animal was restrained in a position of right lateral recumbency and its sternum prepared for surgery by shaving the hairs and disinfecting the area with a 10% solution of Savlon (May & Baker, UK) and 70% ethanol (Pronalysis, May & Baker, UK). Using a 3-inch, 14G stainless steel Salah bone marrow biopsy needle (Downs Surgical Ltd, England), 500 µℓ of marrow from the third sternabrum were aspirated into a 5 mℓ syringe which had been previously flushed with 5000 IU/mℓ final concentration of heparin in Dulbecco's phosphate buffered saline (DPBS; Novo Industri A/S, Copenhagen, Denmark). The aspirate was put into a tube containing 10 mℓ of Alsever's solution and placed on ice for about 1 min to allow large clumps and bony spicules settle at the bottom of the tube. Adherent cells in the marrow cell suspension were separated as described above for the PBM. Bone marrow adherent cells were cultured for 96 h prior to *in vitro* erythrophagocytosis assays as recommended by Pontzer & Russell (1989). The culture medium of the bone marrow adherent cell was replaced with fresh culture medium every 24 h.

In vitro erythrophagocytosis assays

Four uninfected adult cattle, two Boran cows and two N'Dama bulls were sources of the erythrocytes used throughout in these assays. Following the culture of isolated PBM and macrophages for 18 h, ^{51}Cr-labelled erythrocytes were co-incubated, in triplicate wells, with 10^6 PBM or macrophages in 300 µℓ culture medium to give an effector: target (E:T) ratio of 1:20 at 37 °C in 5% CO_2-in-air for 4 and 18 h. Controls, also in triplicate, of cultured PBM/macrophage alone and erythrocytes alone in 300 µℓ culture medium were set up for each assay. Before the beginning of the assay, 100 µℓ of the labelled erythrocyte suspension, in triplicate, was put into 3,5 mℓ gamma-counting (Rohren) tubes (Sarstedt, West Germany) and the radioactivity counted on the gamma counter (Beckman Instruments Inc., USA) fitted with the ^{51}Cr cartridge. The mean count was determined, and this represented the total erythrocyte radioactivity made available to 10^5 cultured PBM in each well.

At the end of each culture period, the plates were centrifuged at 200 *g* for 5 min at 10 °C and 150 µℓ of the cell-free supernatant was collected from each well into gamma-counting tubes and radioactivity in the supernatant determined. The mean count of the triplicates of each sample minus the mean count of triplicate control tubes, that is, PBM alone and erythrocytes alone in culture medium, was recorded as the percentage cumulative ^{51}Cr release, an indication of PBM-mediated erythrocyte lysis over time was calculated using the formula:

$$\text{Erythrocyte lysis (\%)} = \frac{\text{Supernatant radioactivity} \times 2}{\text{Total erythrocyte radioactivity}} \times 100$$

The percentage PBM phagocytosis per well, an indication of intracellular or phagocytosed labelled erythrocytes in the cultured PBM, was calculated as follows: The remaining half of the supernatant culture medium in each well was carefully aspirated, without agitating the cell pellet, and discarded. Unphagocytosed erythrocytes left in the wells were lysed with warm tris-ammonium chloride buffer and carefully discarded. The wells were thereafter rinsed gently once with culture medium to remove extraneous radioactivity while avoiding the removal of adherent PBM. Adherent PBM were harvested by lysis with warm 1N NaOH solution and the lysates put into labelled Rohren tubes. The radioactivity in these lysates was read on a gamma counter. Percentage phagocytosis, in triplicate samples, was calculated using the formula below:

$$\text{Phagocytosis (\%)} = \frac{\text{PBM lysate radioactivity}}{\text{Total erythrocyte radioactivity}} \times 100$$

Parallel assays using non-radio-labelled erythrocytes were carried out using adherent macrophages harvested onto cover slips put into the wells of tissue culture plates. Sequential staining with Diff-Quik (Anosa, Logan-Henfrey and Shaw 1992) at 15 min intervals was followed by light microscopic examination and photomicropgraphy (HM-LUX/Orthomat; Ernst Leitz Wetzler GmBh, Germany).

Statistical analysis

The mean values of both pre- and post-infection as well as inter-breed haematological parameters and indices, percentage phagocytosis and erythrocyte lysis were calculated and subjected to analysis of variance (ANOVA) (SAS 1987) and Duncan's multiple range test (Duncan 1959) for statistical differences, if any, at 95% confidence interval ($P < 0,05$).

RESULTS

In vitro PBM stimulation assays

The stimulation of cultured PBM with the two batches of bacterial LPS (SE and SM) had no significant effect on *in vitro* phagocytosis and lysis of erythrocytes by cultured PBM when compared with the unstimulated PBM (Table 1). The use of rHuTNF-α and rBoIFN-γ to stimulate cultured PBM caused an enhancement of *in vitro* erythrophagocytosis in a concentration-dependent manner. Stimulation of cultured PBM with 100 U/mℓ of both recombinant cytokines was optimal, as this concentration caused the highest erythrocyte destruction (both phagocytosis and lysis) by the PBM of all the animals (Table 1). At very high concentration of both cytokines (1 000 U/mℓ), *in vitro* erythrophagocytosis was not correspondingly increased in these assays as this concentration of the cytokines did not cause any enhancement of *in vitro* erythrocyte destruction significantly above those of unstimulated PBM in both breeds of cattle.

Undialysed 48-h PBMNC culture supernatants, with or without PHA stimulation, caused PBM activation for enhanced *in vitro* phagocytosis and lysis of erythrocytes in a concentration-dependent manner in both breeds of cattle used in this study (Table 2). At very high concentration of the crude cytokines (the undiluted form), however, lower cumulative release values were obtained, but PHA stimulation of PBMNC greatly enhanced PBM activation for *in vitro* erythrocyte phagocytosis and lysis by cultured PBM. Acid dialysis (at pH 2) of the PHA-stimulated or unstimulated PBMNC culture supernatants abolished their PBM stimulatory effect for enhanced *in vitro* erythrophagocytosis in both breeds of cattle (Table 2).

Assays involving *T. congolense*-infected Boran calves

The haematology results of the animals are shown in Table 3. The animals developed normocytic normochromic anaemia from 21 DPI onwards, with PCV and RBC count dropping from 30 % and 8,7 x 10^6/μℓ at pre-infection to 16 % and 4,1 x 10^6/μℓ, and 17 %

TABLE 1 *In vitro* erythrocyte phagocytosis and lysis by cultured PBM stimulated with bacterial LPS and recombinant cytokines

Stimulant	Phagocytosis (%)		Erythrocyte lysis (%)	
	4 h	18 h	4 h	18 h
LPS (μg/mℓ)				
Salmonella enteritidis (SE)				
0,0	3,9 ± 0,4*	7,3 ± 1,2	3,2 ± 0,6	11,0 ± 1,6
0,1	4,8 ± 1,1	7,4 ± 0,9	3,1 ± 0,3	10,1 ± 1,1
1,0	2,7 ± 1,2	7,2 ± 1,0	2,9 ± 0,5	10,2 ± 1,2
10,0	3,5 ± 0,9	6,8 ± 0,8	3,0 ± 0,3	9,5 ± 1,3
Serratia marcescence (SM)				
0,0	3,9 ± 0,4	7,3 ± 1,2	3,2 ± 0,6	11,0 ± 1,6
0,1	3,9 ± 0,3	7,3 ± 0,6	2,9 ± 0,4	9,5 ± 1,1
1,0	4,1 ± 1,1	6,8 ± 0,5	2,9 ± 0,6	10,5 ± 0,9
10,0	4,5 ± 0,9	7,0 ± 0,8	2,9 ± 0,9	11,0 ± 1,5
Recombinant cytokines (U/mℓ)				
rHuTNF-α				
0,0	7,0 ± 3,0	8,0 ± 2,7	6,5 ± 1,7	14,1 ± 1,4[c]
10,0	8,8 ± 0,5	9,1 ± 2,3	8,4 ± 1,9	21,4 ± 3,6[b]
100,0	14,9 ± 2,0[a]	12,6 ± 2,0[b]	14,1 ± 3,2[a]	35,0 ± 2,5[a]
1 000,0	11,4 ± 4,8[a]	13,0 ± 5,1[b]	8,3 ± 1,8	11,9 ± 2,1[b]
rBoIFN-γ				
0,0	8,5 ± 3,0	8,8 ± 0,8	2,1 ± 1,3	10,9 ± 1,6[c]
10,0	14,4 ± 1,5[a]	16,4 ± 2,1[a]	9,7 ± 2,1	30,2 ± 2,5[a]
100,0	12,8 ± 0,2[a]	15,4 ± 0,6[a]	9,9 ± 1,0	38,0 ± 3,3[a]
1 000,0	8,6 ± 4,5	9,7 ± 1,4	8,9 ± 1,9	12,9 ± 0,2[c]

* Data presented as mean ± S.E.M.
Values with no or same superscripts on the same column for each stimulant do not differ significantly ($P < 0,05$)

TABLE 2 *In vitro* erythrocyte phagocytosis and lysis by cultured PBM stimulated with undialyzed and acid-dialysed PBMNC culture supernatants

Supernatant dilution	Phagocytosis (%)		Erythrocyte lysis (%)	
	4 h	18 h	4 h	18 h
Unstimulated, undialysed PBMNC culture supernatant				
0	6,0 ± 0,5*	5,7 ± 0,4	4,0 ± 0,9	10,9 ± 0,8[c]
1:100	8,3 ± 0,8	8,3 ± 1,2	6,2 ± 1,5	11,3 ± 0,9[c]
1:10	16,5 ± 1,3[a]	18,4 ± 0,9[a]	4,6 ± 1,2	14,4 ± 1,1[b]
Undiluted	10,9 ± 1,5	12,3 ± 1,8[b]	7,8 ± 1,6	7,4 ± 1,3
PHA-stimulated, undialysed PBMNC culture supernatant				
0	8,4 ± 0,9	13,3 ± 1,3[b]	2,9 ± 0,5	10,5 ± 1,0[c]
1:100	17,3 ± 1,5[a]	18,3 ± 1,4[a]	6,2 ± 0,4	32,8 ± 3,7[a]
1:10	20,5 ± 1,6[a]	17,3 ± 2,0[a]	6,7 ± 1,1	23,0 ± 2,4[b]
Undiluted	18,7 ± 2,2[a]	12,4 ± 1,1[b]	9,9 ± 0,3	17,6 ± 1,9[b]
Unstimulated, acid-dialysed PBMNC culture supernatant				
0	5,9 ± 0,2	7,6 ± 0,3	3,0 ± 0,5	10,7 ± 1,1[c]
1:100	2,0 ± 0,4	5,3 ± 0,6	0,7 ± 0,1	9,8 ± 0,9[c]
1:10	6,2 ± 0,3	6,9 ± 0,4	3,1 ± 0,1	8,4 ± 0,3[c]
Undiluted	6,4 ± 0,4	3,5 ± 0,3	3,4 ± 0,3	3,1 ± 0,2
PHA-stimulated, acid-dialysed PBMNC culture supernatant				
0	4,1 ± 0,3	5,8 ± 0,4	2,8 ± 0,2	10,5 ± 0,6[c]
1:100	9,2 ± 0,5	4,7 ± 0,3	1,5 ± 0,1	7,8 ± 0,3
1:10	7,8 ± 0,2	8,2 ± 0,5	4,3 ± 0,2	4,5 ± 0,3
Undiluted	4,5 ± 0,3	4,7 ± 0,4	4,6 ± 0,1	4,3 ± 0,4

* Data presented as mean ± S.E.M.
Values with no or same superscripts on the same column do not differ significantly ($P < 0,05$)

TABLE 3 Haematology of Boran calves infected with *Trypanosoma congolense*

Animal No.	F362	F360	F363	F370	F367	F371	F369	F365	F364
DPI	0	7	14	21	28	35	42	49	56
PCV (%)	30,0	29,5	30,5	18,0	16,5	16,0	16,5	16,5	15,5
RBC count (x10⁶/μℓ)	8,7	8,4	8,6	5,5	5,1	5,1	4,5	5,0	4,6
Hb conc. (mg/dℓ)	10,9	10,5	11,5	6,5	5,7	6,2	6,4	6,4	6,3
MCV (fℓ)	34,5	35,1	35,5	32,7	32,4	31,4	36,7	33,0	33,7
MCHC (mg/dℓ)	29,0	28,5	28,2	30,5	30,9	31,9	27,3	30,3	29,7

and 5,8 x 10^6/μℓ, respectively, in calves killed on 28 and 42 DPI.

In vitro erythrocyte phagocytosis and lysis by cultured PBM and Mϕ isolated from the spleen and bone marrow increased during the course of *T. congolense* infection in Boran calves (Table 4). Percentage phagocytosis by cultured splenic macrophages increased from 8,3 % at pre-infection to 24,8 % on 14 DPI, with the highest value of 27,3 % on 35 DPI in the 4-h assays. Similar increases in percentage phagocytosis were observed with both cultured PBM and bone marrow macrophages, but these were generally lower than those of splenic macrophages. In the 18-h assays, percentage phagocytosis by both cultured PBM and bone marrow macrophages were higher than those of the 4-h assays. Percentage erythrocyte lysis by the cultured macrophages also increased with infection, especially in the 18-h assays (Table 4). This occurred from 21 DPI onwards and remained high until the termination of the experiment. As in the percentage phagocytosis, cultured splenic macrophages showed the highest percentage erythrocyte lysis, with a peak value of 42,6 % on 49 DPI.

TABLE 4 Erythrophagocytosis (%) by cultured Mϕ isolated from various tissues of Boran calves infected with *T. congolense*

DPI	0	7	14	21	28	35	42	49	56
4-h erythrocyte phagocytosis									
Splenic Mϕ	8,3 ± 1,2*	8,8 ± 0,3	24,8 ± 2,3[b]	8,8 ± 0,2	22,8 ± 1,9[b]	27,3 ± 2,0[b]	18,3 ± 1,3[a]	24,8 ± 2,2[b]	8,4 ± 0,3
Bone marrow Mϕ	1,4 ± 0,3	8,1 ± 0,1	9,2 ± 0,6	8,3 ± 1,0	8,7 ± 0,1	20,2 ± 1,6[a]	8,8 ± 1,2	8,5 ± 0,5	7,6 ± 0,4
PBM-derived Mϕ	6,1 ± 1,1	6,1 ± 0,6	7,6 ± 0,5	6,9 ± 0,2	7,2 ± 1,2	18,4 ± 1,1[a]	5,8 ± 0,2	13,6 ± 1,2[a]	17,4 ± 1,9[a]
18-h erythrocyte phagocytosis									
Splenic Mϕ	10,7 ± 1,0	11,5 ± 2,1	8,7 ± 1,2	7,3 ± 1,0	25,8 ± 2,3[b]	24,7 ± 1,1[b]	16,1 ± 0,9[a]	27,9 ± 2,1[b]	18,0 ± 2,0[a]
Bone marrow Mϕ	12,6 ± 1,2	6,2 ± 2,0	7,2 ± 0,3	15,4 ± 1,1[a]	24,5 ± 1,5[b]	11,0 ± 1,0	11,7 ± 0,5	9,5 ± 2,2	12,3 ± 1,3
PBM-derived Mϕ	9,4 ± 1,0	10,2 ± 2,0	6,4 ± 1,5	8,8 ± 0,8	18,3 ± 1,2[a]	25,2 ± 1,1[b]	18,6 ± 0,6*	14,6 ± 1,3[a]	15,7 ± 1,5[a]
4-h erythrocyte lysis									
Splenic Mϕ	1,0 ± 0,2	0,6 ± 0,2	3,1 ± 0,3[a]	1,7 ± 0,3	7,8 ± 0,3[b]	1,5 ± 0,1	10,7 ± 0,5[b]	15,1 ± 1,0[b]	6,1 ± 0,5[a]
Bone marrow Mϕ	1,0 ± 0,1	0,7 ± 0,0	0,5 ± 0,1	2,5 ± 0,2[a]	6,6 ± 1,0[b]	0,0 ± 0,0	0,0 ± 0,0	5,3 ± 1,3[a]	2,7 ± 0,0[a]
PBM-derived Mϕ	0,6 ± 0,1	1,4 ± 0,1	2,6 ± 0,3[a]	1,6 ± 0,3	1,5 ± 0,3	0,9 ± 0,2	4,5 ± 1,1[a]	8,7 ± 1,3[a]	7,3 ± 1,3[a]
18-h erythrocyte lysis									
Splenic Mϕ	4,1 ± 0,2	4,7 ± 1,3	6,6 ± 1,3	25,1 ± 2,1[b]	17,4 ± 2,3[b]	22,5 ± 1,6	26,3 ± 2,1[b]	42,6 ± 5,3[b]	29,7 ± 3,2[b]
Bone marrow Mϕ	9,8 ± 2,1	7,8 ± 1,5	5,1 ± 0,3	29,0 ± 1,0[b]	22,2 ± 0,0[b]	4,8 ± 1,1	30,1 ± 1,5[b]	26,3 ± 2,1[b]	25,0 ± 1,0[b]
PBM-derived Mϕ	5,3 ± 1,3	8,1 ± 0,0	8,0 ± 1,1	6,3 ± 1,0	5,4 ± 0,5	6,8 ± 0,3	15,7 ± 2,1[a]	33,5 ± 3,5[b]	28,6 ± 2,1[b]

Mϕ = Macrophage
* Data presented as mean ± S.E.M (triplicate values)
[a] Values significantly different from pre-infection levels at $P < 0,05$
[b] Values significantly different from pre-infection levels at $P < 0,01$

TABLE 5 Haematology of Boran and N'Dama cattle experimentally infected with *T. congolense*

DPI	0	7	14	21	28	35	42	49	56
PCV (%)									
N'Dama	33,3 ± 0,6*	33,5 ± 1,0	28,7 ± 1,5[a]	27,3 ± 0,6[a]	24,0 ± 0,7[a]	21,3 ± 1,2[a]	20,7 ± 0,6[b]	20,7 ± 1,5[b]	18,7 ± 1,2[b]
Boran	31,7 ± 2,9	32,3 ± 1,5	28,3 ± 2,5[a]	23,7 ± 0,3[b]	21,7 ± 0,3[b]	19,0 ± 1,0[b]	15,5 ± 0,7[b]	16,0 ± 1,4[b]	17,0 ± 0,0[b]
Hb conc. (mg/dℓ)									
N'Dama	11,0 ± 0,5	11,8 ± 0,2	10,0 ± 1,0	10,8 ± 0,5	10,1 ± 1,0	9,8 ± 1,1	9,6 ± 1,1	9,5 ± 0,6	8,9 ± 1,0
Boran	10,7 ± 1,4	11,5 ± 1,1	9,5 ± 1,5	9,6 ± 1,9	9,3 ± 2,1	7,7 ± 2,1[a]	7,9 ± 1,1[a]	6,6 ± 0,9[b]	6,3 ± 0,0[b]
RBC counts (x 10^6/μℓ)									
N'Dama	10,2 ± 0,9	10,6 ± 0,6	9,2 ± 2,8	8,7 ± 1,0	6,6 ± 1,1[a]	6,4 ± 1,3[a]	5,7 ± 0,7[b]	5,8 ± 0,9[b]	5,9 ± 1,0[b]
Boran	10,1 ± 1,5	10,5 ± 1,9	9,6 ± 2,3	7,5 ± 3,9	7,0 ± 2,2[a]	5,5 ± 2,2[b]	4,8 ± 1,6[b]	5,0 ± 1,8[b]	4,6 ± 0,0[b]
MCV (fℓ)									
N'Dama	32,6 ± 1,5	31,6 ± 2,3	31,2 ± 1,6	31,4 ± 0,8	36,4 ± 4,2	33,3 ± 2,2	33,3 ± 3,7	32,7 ± 3,1	35,7 ± 2,1
Boran	31,4 ± 1,3	30,8 ± 2,5	29,5 ± 4,5	31,6 ± 2,3	31,0 ± 2,0	34,5 ± 2,0	36,5 ± 5,3	32,0 ± 1,6	32,6 ± 0,0
MCHC (gm/dℓ)									
N'Dama	35,0 ± 2,2	35,2 ± 2,6	34,8 ± 2,5	39,6 ± 4,9[a]	40,1 ± 3,6[a]	41,1 ± 3,6[a]	40,0 ± 5,1	41,7 ± 4,5[a]	42,0 ± 2,1[a]
Boran	33,8 ± 3,5	35,6 ± 2,3	33,6 ± 2,4	40,3 ± 2,5[a]	41,2 ± 3,7[a]	40,2 ± 3,0[a]	49,1 ± 4,2[a]	41,2 ± 3,2[a]	37,9 ± 0,0

* Data presented as Mean ± S.E.M. ($n = 5$)

[a] Values significantly different from pre-infection levels at $P < 0,05$

[b] Values significantly different from pre-infection levels at $P < 0,01$

Assays using *T. congolense*-infected Boran and N'Dama cattle

The haematology results of the animals are shown in Table 5. The animals also developed normocytic normochromic anaemia. However, Boran cattle developed a more severe anaemia ($P < 0,01$) than the N'Dama. All the Boran cattle were treated with Berenil® on 42 DPI when their PCV had dropped to ~15 % to prevent imminent death. None of the N'Dama cattle required such treatment.

Assays using non-radio-labelled erythrocytes revealed that Mϕ-erythrocyte interaction and contact leading to phagocytosis started within 15 min of co-incubation and by 4 h, some macrophages had developed an extensive pseudopodia network inside which one or two erythrocytes had been trapped (Fig. 1A). At the end of the 18-h assays, three to four intact erythrocytes could be found in enlarged and foamy macrophages (Fig. 1B).

Percentage phagocytosis by cultured PBM isolated from Boran and N'Dama cattle in the 4-h and 18-h assays increased significantly ($P < 0,05$) above pre-infection levels from 21 DPI onwards and remained so throughout the course of the infection (Fig. 2A). Two peaks of enhanced phagocytosis were observed in the two groups of infected cattle. However, peak phagocytosis was attained earlier and the values were significantly higher ($P < 0,05$) in infected Boran (32,6 ± 1,9 % on 28 DPI) than in the N'Dama (22,8 ± 0,8 % on 35 DPI) cattle. Percentage erythrocyte lysis (Fig. 2B) also increased significantly from 21 DPI in both groups of cattle. The increase was more pronounced and persisted throughout the course of infection in Boran cattle, while it declined to near pre-infection levels on 49 and 56 DPI in N'Dama cattle.

Assays using *T. vivax*-infected Boran and N'Dama cattle

The haematology results of the animals are shown in Table 6. All the animals developed mild normocytic normochromic anaemia from 22 DPI onwards. However, in contrast to the *T. congolense* experiment, N'Dama cattle developed a more severe anaemia ($P < 0,05$) than the Boran cattle. None of the animals required treatment with Berenil®, as their PCV did not go below 18 %.

In the 18-h assays, percentage cultured PBM phagocytosis did not increase significantly above pre-infection levels until 22 DPI in both breeds of cattle (Fig. 3A). Thereafter, there was a decline to near pre-infection levels from 29 to 50 DPI when the experiment was terminated. Percentage erythrocyte lysis after 18 h of assay increased significantly ($P < 0,02$) above pre-infection levels from 22 DPI onwards (Fig. 3B) in both breeds of cattle and remained high until the end of the experiment. There were no significant differences ($P > 0,05$) in percentage erythrocyte phagocytosis and lysis between both breeds of cattle throughout the course of infection.

DISCUSSION

Undialysed 48-h PBMNC culture supernatants, which have been reported to contain crude cytokines

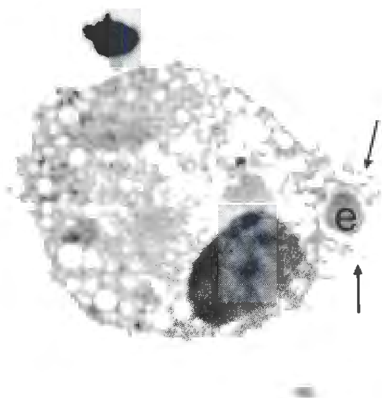

FIG. 1A Photomicrograph of an enlarged and foamy cultured PBM with extending pseudopodia network (arrows) trapping an erythrocyte (e) after a 4-h co-incubation period
H and E (x 1 250)

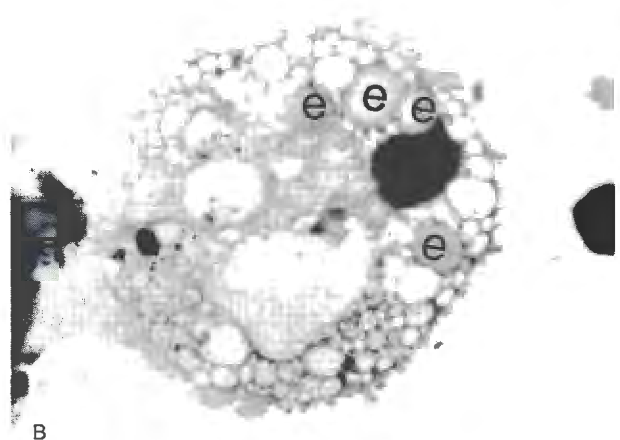

FIG. 1B Photomicrograph of an enlarged and intensity foamy cultured PBM containing four intact engulfed erythrocyte (e) after an 18-h co-incubation period
H and E (x 1 250)

TABLE 6 Haematology of Boran and N'Dama cattle experimentally infected with *Trypanosoma vivax*

DPI	0	8	15	22	29	36	43	50
PCV (%)								
N'Dama Boran	34,7 ± 2,3* 33,3 ± 3,2	33,0 ± 2,7 32,0 ± 2,1	32,3 ± 0,6 31,0 ± 1,7	28,3 ± 1,2[a] 29,7 ± 1,6[a]	20,3 ± 3,5[a] 25,3 ± 5,9[a]	20,3 ± 4,5[a] 26,3 ± 1,5[a]	23,3 ± 3,2[a] 29,3 ± 1,2	26,0 ± 1,0[a] 30,7 ± 0,6
Hb conc. (mg/dℓ)								
N'Dama Boran	11,0 ± 0,6 11,5 ± 0,6	10,3 ± 0,5 10,0 ± 1,2	10,7 ± 0,1 10,6 ± 0,7	9,3 ± 0,5 10,4 ± 1,1	6,5 ± 1,3[a] 8,8 ± 1,9[a]	7,2 ± 2,2[a] 9,3 ± 0,7[a]	7,9 ± 1,5[a] 9,9 ± 0,1	9,0 ± 1,0 10,6 ± 0,1
RBC counts (x 10^6/μℓ)								
N'Dama Boran	10,2 ± 0,7 10,8 ± 1,2	8,8 ± 0,1[a] 10,3 ± 0,9	8,9 ± 1,1[a] 10,6 ± 0,3	7,5 ± 0,6[a] 7,3 ± 0,8[a]	6,0 ± 2,2[a] 6,4 ± 1,7[a]	4,6 ± 1,6[b] 6,3 ± 1,1[a]	5,1 ± 1,5[b] 6,7 ± 1,1[a]	5,3 ± 1,2[b] 7,1 ± 1,0[a]
MCV (fℓ)								
N'Dama Boran	34,2 ± 2,4 31,4 ± 3,2	35,6 ± 4,2 31,3 ± 2,3	31,2 ± 3,6 30,6 ± 1,6	35,7 ± 4,8 35,6 ± 5,5[a]	33,8 ± 3,4 36,1 ± 5,0[a]	36,2 ± 5,1 37,1 ± 6,0[a]	39,6 ± 5,8[a] 38,5 ± 4,8[a]	43,3 ± 5,1[a] 41,3 ± 6,6[a]
MCHC (gm/dℓ)								
N'Dama Boran	32,7 ± 1,2 34,5 ± 1,1	31,5 ± 1,5 31,2 ± 0,9	33,2 ± 0,8 33,9 ± 1,4	32,9 ± 2,2 33,8 ± 1,3	31,8 ± 2,5 33,9 ± 1,7	34,9 ± 1,8 34,9 ± 1,0	33,5 ± 1,1 30,2 ± 1,2	34,4 ± 2,2 33,6 ± 1,8

* Data presented as Mean ± S.E.M. ($n = 5$)
[a] Values significantly different from pre-infection levels at $P < 0,05$
[b] Values significantly different from pre-infection levels at $P < 0,01$

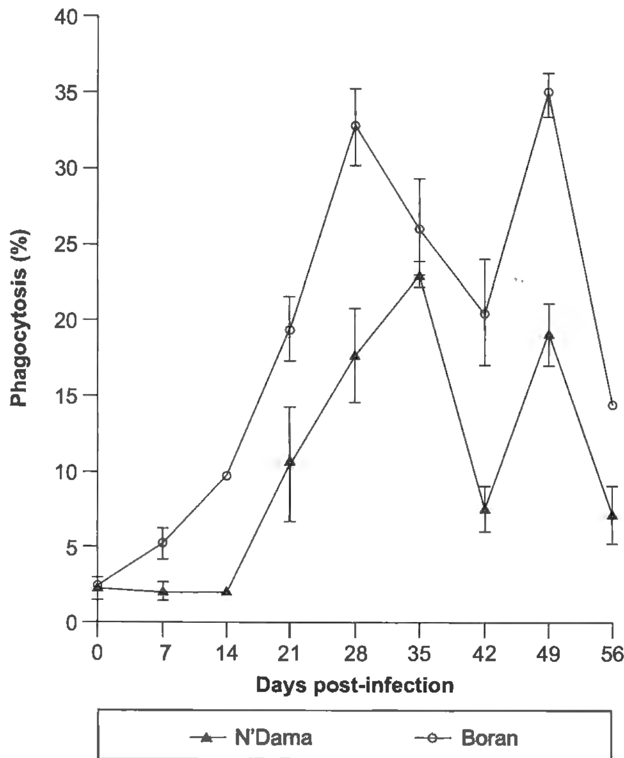

FIG. 2A *In vitro* phagocytosis of erythrocytes by cultured PBM in Boran and N'Dama cattle infected with *Trypanosoma congolense*

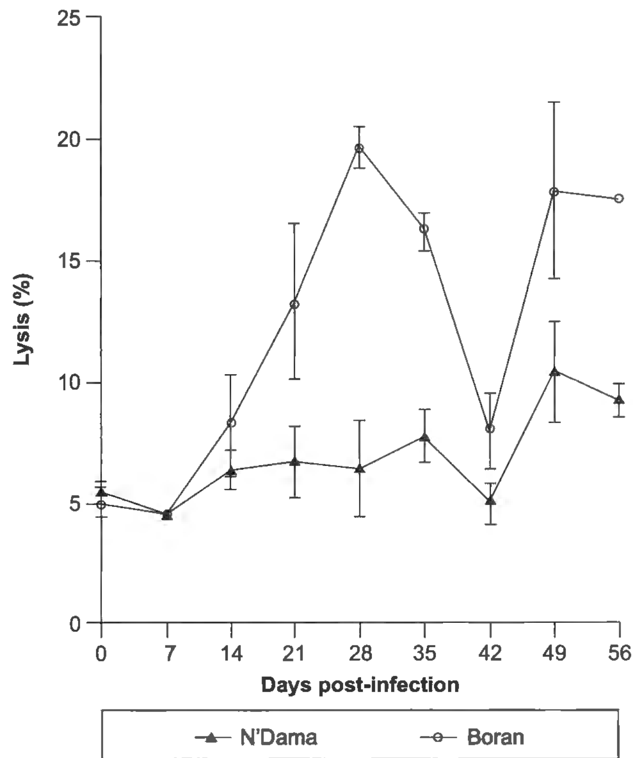

FIG. 2B *In vitro* lysis of erythrocytes by cultured PBM in Boran and N'Dama cattle infected with *Trypanosoma congolensecongolense*

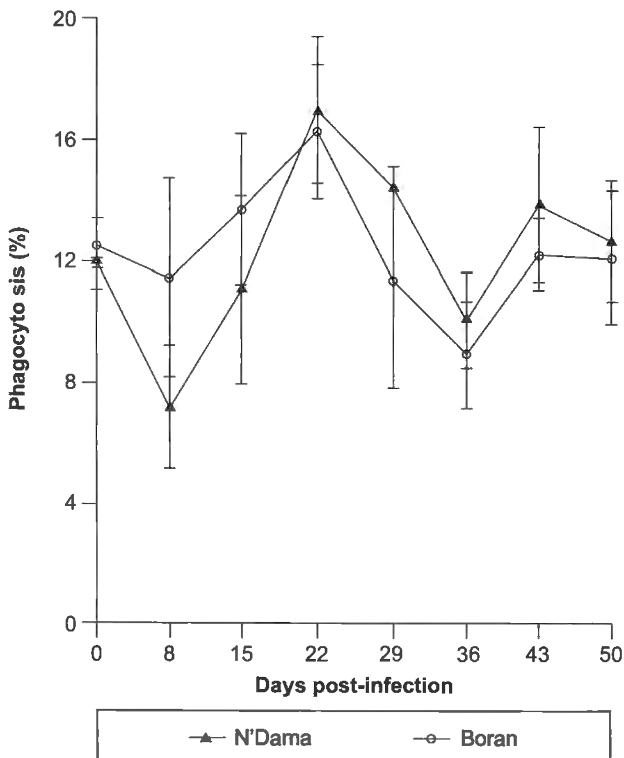

FIG. 3A *In vitro* phagocytosis of erythrocytes by cultured PBM in Boran and N'Dama cattle infected with *Trypanosoma vivax*

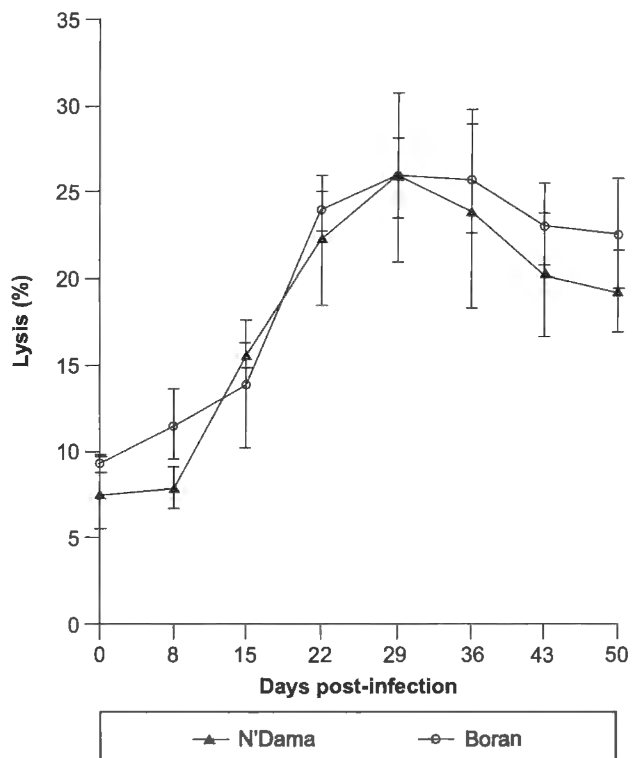

FIG. 3B *In vitro* lysis of erythrocytes by cultured PBM in Boran and N'Dama cattle infected with *Trypanosoma vivax*

(Townsend *et al.* 1988), rHuTNF-α and rBoIFN-γ caused PBM stimulation for enhanced *in vitro* erythrocyte destruction in all the animals used in this study. There was no significant difference in either the rate of phagocytosis or lysis of erythrocytes from the Boran or N'Dama breed of cattle. Each of the stimulants caused different levels of enhanced erythrophagocytosis by the cultured PBM of all the animals. The levels of erythrophagocytosis, which were stimulant concentration-dependent, peaked with increasing concentrations of the stimulants and then decreased at higher stimulant concentrations. It was however, observed that this decrease in erythrophagocytosis was not due to the death or degeneration of macrophages, since more than 75 % of the cultured PBM were found, during the assays, to be viable after 36 h in culture medium containing high stimulant concentrations. In contrast to the other stimulants, bacterial LPS and acid-dialysed 48-h PBMNC culture supernatant did not induce an increase in *in vitro* cultured PBM erythrophagocytosis above those of the unstimulated controls.

Macrophages are highly sensitive to endotoxin at concentrations in the range of nanograms/mℓ or less and are able to elicit detectable macrophages activation *in vitro* (Morrison & Ryan 1979). Endotoxin is reported to perturb the macrophage membrane, thus causing the cross-linking of surface membrane epitopes and the initiation of the cascade of events leading to activation (Adams & Hamilton, 1987). However, excessive perturbation has been known to cause the disruption of macrophage membrane integrity and consequent cytolysis (Peavy, Baughn & Mucher 1978). It is unclear whether the LPS concentrations used in the present study were too low or too high for the cultured PBM used in the present assays. It is also unclear whether a stimulation period of 18 h was too long to maintain cultured PBM functions and membrane integrity. However, several workers have used between 0,1–30 μg/mℓ endotoxin concentrations to stimulate bovine, human and murine monocytes and macrophages for the secretion of interleukin-1 (IL-1), TNF-α, leukotrine B_4, thromboxane and lysozyme (Warfel & Zucker-Franklin 1986; Charley, Larverne & Lavenant 1990; Lewis, McCarthy, Lorenzen & McGhee 1990; O'Sullivan, Fleisher, Olson, MacLachlan & Brown 1990). It is not surprising that both rHuTNF-α and rBoIFN-γ activated cultured bovine PBM for enhanced *in vitro* erythrophagocytosis and lysis in the animals used in this study.

Recombinant HuTNF-α has about 80 % protein homology with both recombinant murine and bovine TNF-α (Marmenout. Franson, Tavernier, Van Den Heyden, Tizard, Kawashima, Shaw, Johnson, Semon, Muller, Ruysschaert, Van Vliet & Fiers 1985; Pennica, Hayklick, Bringman, Palladion & Goeddel 1985) and it is known as a potent *in vivo* and *in vitro* enhancer of macrophage functions (Philip & Epstein 1986; Sherry & Cerami 1988; Singh & Sodhi 1990). The present study is the first of its kind to show that *in vitro* erythrophagocytosis can be enhanced using rHuTNF-α to stimulate PBM-derived bovine macrophages. The enhancement of macrophage erythrophagocytosis after rBoIFN-γ stimulation is in agreement with the reports of Pontzer & Russell (1989) on enhanced phagocytosis of opsonized sheep erythrocytes by bovine bone marrow macrophages stimulated with IFN-γ, a potent activator of macrophages which has been shown to be very important in macrophage activation for tumour regression and in the control of protozoan infections (Adams & Hamilton 1984; Wirth, Kierszenbaum, Sonnefeld & Zlotnik 1985; Kelly 1986).

Forty-eight-hour culture supernatants of PBMNC, in presence or absence of PHA stimulation, enhanced *in vitro* erythrophagocytosis by the macrophages of all the animals. However, PHA stimulation of PBMNC caused a greater secretion of cytokines. Dialysis of the PHA-stimulated and unstimulated PBMNC culture supernatants against acid buffer at pH 2 abolished their stimulatory activity for enhanced *in vitro* PBM erythrophagocytosis. Townsend, Duffus and Williams (1988) reported that both PHA-stimulated and unstimulated PBMNC of calves secreted predominantly IFNs α, β and γ. The biological effects of both IFN-α and β are destroyed at pH 2 (Trinchierri & Perussia, 1985). Hence, the dialysis of the culture supernatants against acid buffer at pH 2 would leave IFN-γ as the only IFN in the culture supernatant. Other cytokines, however, such as IL-1, IL-2, IL-3, IL-4, IL-6, TNF-α, $PGE_{2\alpha}$, among others, may be pres- ent in the culture supernatants. Since the presence or absence of these cytokines, and the relative concentrations of these cytokines in the supernatants were not determined in the present study, it was impossible to identify the role each of them might have played in *in vitro* PBM activation for enhanced erythrophagocytosis.

All the animals infected with either *T. congolense* or *T. vivax* in these studies developed varying degrees of normocytic normochromic anaemia. This is in agreement with earlier reports and confirms that anaemia is one of the major clinical features of trypanosomosis in both human and animal trypanosomosis (Dargie, Murray, Murray, Grimshaw & McIntyre 1979a, b; Paling, Moloo, Scott, Gettinby & McOdimba 1991). Boran breed of cattle developed a much more severe anaemia than N'Dama breed during the *T. congolense* infection. This also is in consonance with the reports of previous workers that taurine cattle, typified by the N'Dama breed, are trypanotolerant and hence develop less severe anaemia than trypanosusceptible Boran breed (a Zebu) during trypanosomosis (Dargie *et al.*, 1979a, b; Murray & Dexter 1988; Paling *et al.* 1991). All the five Boran cattle had to be treated with Berenil® on 42 DPI when their PCV had dropped to about 15 %, to prevent their imminent

death. None of the N'Dama cattle required such treatment. However, during the *T. vivax* infection, none of the animals were treated as their PCV did not drop below 18 %. In contrast to our observations in the *T. congolense* experiment, N'Dama cattle appeared to have succumbed more to infection with *T. vivax* than the Boran, even though a 6 % PCV differential that occurred pre-infection between the two breeds was maintained throughout the course of infection. N'Dama cattle were visibly more devastated and lost more weight (data not presented) than the Boran cattle.

In vitro erythrocyte phagocytosis and lysis by splenic and bone marrow macrophages and cultured PBM increased during *T. congolense* and *T. vivax* infections in Boran and N'Dama breeds of cattle. These results indicate that PBM and tissue macrophages were progressively activated *in situ* for erythrocyte phagocytosis and destruction during bovine trypanosomosis. This agrees with the findings of previous workers who reported *in vivo* macrophage activation and increased phagocytic activity during *T. gambiense* infection in man (Connal 1912), *T. congolense* infection in cattle (Dargie *et al.* 1979a), *T. brucei* infection in mice (Anosa & Kaneko 1984) and *T. vivax* infection in goats (Anosa & Kaneko 1989).

Cultured splenic macrophages showed the highest rate of *in vitro* erythrocyte phagocytosis and destruction followed by those from the bone marrow and cultured PBM. Monocytes are the recognized precursors of tissue macrophages (Gordon 1986), it can be expected that the splenic macrophages would have been fully differentiated, and more mature, and therefore would have shown more phagocytic activity than PBM. This was corroborated by their greater erythrophagocytic activity than those of the bone marrow and PBM in these *in vitro* assays. The spleen is one of the major sites where senescent or damaged erythrocytes are normally removed by the cells of the MPS in health (Jain 1986; Bartosz 1988). The spleen is also one of the major sites where erythrophagocytosis by the MPS has been reported to occur in African animal trypanosomosis (Losos & Ikede 1972; Anosa & Kaneko 1983, 1984). The findings in this study suggest that splenic macrophages were more activated than those from the bone marrow and cultured PBM during trypanosomosis. It may therefore be speculated that splenic macrophages may have come into contact more with trypanosomal antigens and soluble host-produced substances, for example cytokines, secreted into their tissue microenvironment during infection than the others. Splenic macrophages may also possess more receptors involved in the removal of senescent and damaged erythrocytes than PBM, which would have led to their higher *in vitro* erythrophagocytic activity.

The rates of erythrocyte phagocytosis and lysis in N'Dama and Boran breeds of cattle were similar prior to infection with *T. congolense* and *T. vivax*. This observation suggests parity in the state of activation of cultured PBM from these two breeds of cattle in health. During the *T. congolense* infection, *in vitro* erythrocyte phagocytosis and lysis occurred earlier and was significantly higher with PBM isolated from the Boran cattle than those from the N'Dama. This finding suggests that PBM activation occurred earlier and was more pronounced in Boran than N'Dama cattle, and is most probably associated with the development of a more severe anaemia in the Boran than in the N'Dama during *T. congolense* infection. This finding is also in agreement with the reports of Dargie *et al.* (1979a, b) and Paling *et al.* (1991).

During the *T. vivax* infection, cultured PBM from Boran and N'Dama cattle phagocytosed and lysed erythrocytes at the same rate, which was in contrast to what was observed during the *T. congolense* infection. This suggests that the PBM from the two breeds of cattle were more or less equally activated during the course of *T. vivax* infection. In this respect, it is noteworthy that both groups of cattle developed a similar degree of anaemia during the *T. vivax* infection suggesting that the rates of *in vivo* erythrocyte destruction may have been similar in the two breeds of cattle during the infection, even though N'Dama cattle appeared to have suffered more adversely from this infection than Boran cattle. It is also noted that the levels of erythrocyte phagocytosis and lysis were generally lower during *T. vivax* than during *T. congolense* infection. This observation suggests that erythrocyte destruction during trypanosomosis may be determined by the level of macrophage activation, parasite species or perhaps the level and sustainment of parasitaemia during infection.

In conclusion, the findings in these series of experiments have demonstrated that serum factors such as host-secreted or parasite-induced cytokines, can render erythrocytes more prone to destruction by cultured PBM. Also PBM from Boran and N'Dama cattle can be stimulated *in vitro* with cytokines in PBMNC culture supernatants and recombinant TNF-α and IFN-γ for enhanced erythrophagocytosis. The results have also demonstrated that cultured PBM and tissue macrophages showed increases in *in vitro* erythrophagocytosis with progression of trypanosomosis and these correlated positively with the development of anaemia which occurred earlier and more severe in the Boran breed of cattle than the N'Dama.

ACKNOWLEDGEMENTS

These experiments were undertaken at ILRI, Nairobi, Kenya by the first author as part of the fulfillment for the degree of Doctor of Philosophy (Veterinary Pathology), University of Ibadan, Nigeria. The Research Fellowship award for this purpose by ILRI is gratefully acknowledged.

REFERENCES

ADAMS, D.O. & HAMILTON, T.A. 1984. The cell biology of macrophage activation. *Annual Review of Immunology,* 2:283–318.

ADAMS, D.O. & HAMILTON, T.A. 1987. Molecular transductional mechanisms by which IFN-gamma and other signals regulate macrophage development. *Immunological Review,* 97:5–27.

ANOSA, V.O. & KANEKO, J.J. 1983. Pathogenesis of *Trypanosoma brucei* infection in deer mice (*P. maniculatus*). V. Macrophage ultrastructure and function. *Veterinary Pathology,* 20: 617–631.

ANOSA, V.O. & KANEKO, J.J. 1984. Pathogenesis of *Trypanosoma brucei* infection in deer mice (*P. maniculatus*). Ultrastructural pathology of the spleen, liver, heart and kidney. *Veterinary Pathology,* 21:229–237.

ANOSA, V.O. & KANEKO, J.J. 1989. Ultrasructural pathology of hemopoietic organs in *Trypanosoma vivax* infection in goats. *Veterinary Pathology,* 26:78–83.

ANOSA, V.O., LOGAN-HENFREY, L.L. & SHAW, M.K. 1992. A light and electron microscopic study of changes in blood and bone marrow in acute haemorrhagic *Trypanosoma vivax* infections of calves. *Veterinary Pathology,* 29:33–45.

BARTOSZ, G. 1988. Erythrocyte membrane changes during aging *in vivo,* in *Blood cell biochemistry, I. Erythroid Cells,* edited by J.R. Harris. New York & London: Plenum Press.

CHARLEY, B., LARVERNE, S. & LAVENANT, L. 1990. Recombinant porcine interferon-g activates *in vitro* porcine adherent mononuclear cells to produce interleukin 1. *Veterinary Immunology and Immunopathology,* 25:117–125.

CONNAL, A. 1912. Autoerythrophagocytosis in protozoal diseases. *Journal of Pathology and Bacteriology,* 16:503–517.

DARGIE, J.D., MURRAY, P.K., MURRAY, M. & McINTYRE, W.I.M. 1979b. Blood values and erythrokinetics of N'Dama and Zebu cattle experimentally infected with *Trypanosoma brucei. Research in Veterinary Science,* 26:245–247.

DARGIE, J.D., MURRAY, P.K., MURRAY, M., GRIMSHAW, W.M.T. & McINTYRE, W.I.M. 1979a. Bovine trypanosomiasis: red cell kinetics of N'Dama and Zebu cattle infected with *Trypanosoma congolense. Parasitology,* 78:271–286.

DUNCAN, R.D. 1959. Multiple tests and multiple F tests. *Biometrics,* 11:1–41.

DWINGER, R.H., MURRAY, M. & MOLOO, S.K. 1987. Potential value of localized skin reactions (chancres) induced by *Trypanosoma congolense* transmitted by *Glossina morsitans centralis* for the analysis of metacyclic trypanosome populations. *Parasite Immunology,* 9:353–362.

GARDINER, P.R., ASSOKU, R.K.G, WHITELAW, D.D. & MURRAY, M. 1989. Haemorrhagic lesions resulting from *Trypanosoma vivax* infection in Ayrshire cattle. *Veterinary Parasitology,* 31:187–197.

GEIGY, R. & KAUFMANN, M. 1973. Sleeping sickness survey in the Serengeti area (Tanzania) 1971. 1. Examination of large animals for trypanosomes. *Acta Tropica,* 30:12–23.

GODDEERIS, B.M., BALDWIN, C.L., OLE MOIYOI, O. & MORRISON, W.I. 1986. Improved methods for purification and depletion of monocytes from peripheral blood mononuclear cells. Functional revaluation of monocytes in response to lectins. *Journal of Immunological Methods,* 89:165–173.

GORDON, S. 1986. Biology of the macrophage. *Journal of Cell Science* (Suppl.), 4:267–286.

I.C.S.H. 1971. International Committee for Standardization in Haematology. Recommended methods for radioisotope red cell survival studies. *Blood,* 38:378–386.

JAIN, N.C. (Ed.) 1986. Schalm's veterinary haematology. 4th ed. Philadelphia: Lea & Ferbiger.

JORDT, T., MAHON, G.D., TOURE, B.N., NGULO, W.K., MORRISON, W.I., RAWLE, J. & MURRAY, M. 1986. Successful transfer of frozen N'Dama embryos from the Gambia to Kenya. *Tropical Animal Health and Production,* 18:65–75.

KELLY, J.M. 1986. The role of interferons in parasitic protozoan infection. *Parasitololgy Today,* 2:173–174.

LASSER, A. 1983. The mononuclear phagocytic system. A Review. *Progress in Pathology,* 14:108–126.

LEWIS, C.E., McCARTHY, S.P., LORENZEN, J. & McGHEE, J. O'D. 1990. Differential effects of LPS, IFN-gamma and TNF-alpha on the secretion of lysozyme by individual human mononuclear phagocytes: relationship to cell maturity. *Immunology,* 69:402–408.

LOSOS, G.J. & IKEDE, B.O. 1972. Review of the pathology of domestic and laboratory animals caused by *Trypanosoma congolense, T. vivax, T, brucei, T. rhodesiense* and *T. gambiense. Veterinary Pathology* (Suppl.), 9:1–71.

MACKANESS, G.B. 1970. The monocyte and cellular immunity. *Seminars in Hematology,* 7:172–184.

MARMENOUT, A., FRANSON, L., TAVERNIER, J., VAN DEN HEYDEN, J., TIZARD, R., KAWASHIMA, E., SHAW, A., JOHNSON, M.-J. SEMON, D., MULLER, R., RUYSSCHAERT, M.-R., VAN VLIET, A. & FIERS, W. 1985. Molecular cloning and expression of human tumour necrosis factor and comparison with mouse tumour necrosis factor. *European Journal of Biochemistry,* 152:515–522.

MORRISON, D.C. & RYAN, J.L. 1979. Bacterial endotoxins and host immune responses. *Advances in Immunology,* 28:293–449.

MURRAY, M. & DEXTER, T.M. 1988. Anaemia in bovine trypanosomiasis. A Review. *Acta Tropica,* 45:389–432.

NANTULYA, V.M., MUSOKE, A.J., RURANGIRWA, F.R. & MOLOO, S.K. 1984. Resistance of cattle to tsetse-transmitted challenge with *Trypanosoma brucei* or *T. congolense* after spontaneous recovery from syringe-passaged infections. *Infection and Immunity,* 43:735–738.

O'SULLIVAN, M.G., FLEISHER, L.N., OLSON, N.C., MACLACHLAN, N.J. & BROWN, T.T. 1990. Modulation of arachidonic acid metabolism by bovine alveolar macrophages exposed to interferons and lipopolysaccharide. *American Journal of Veterinary Research,* 51:1820–1825.

PALING, R.W., MOLOO, S.K., SCOTT, J.R., GETTINBY, G., MCODIMBA, F.A. & MURRAY, M. 1991. Susceptibility of N'Dama and Boran cattle to sequentila challenges with tsetse-transmitted clones of *Trypanosoma congolense. Parasite Immunology,* 13:427–439.

PEAVY, D.L., BAUGHN, R.E. & MUSHER, D.M. 1978. Strain-dependent cytotoxic effects of endotoxin for mouse peritoneal macrophages. *Infection and Immunity,* 21:310–319.

PENNICA, D., HAYKLICK, J.S., BRINGMAN, T.S., PALLADINO, M.A. & GOEDDEL, D.U. 1985. Cloning and expression in Escherichia coli of the cDNA of murine tumour necrosis factor. *Proceedings of the National Academy of Sciences USA,* 82:6060–6064.

PHILIP, R. & EPSTEIN, L.B. 1986. Tumour necrosis factor as immunomodulator and mediator of monocyte cytotoxicity induced by itself, IFN-gamma and IL-1. *Nature (London),* 323: 86–89.

PONTZER, C.H. & RUSSELL, S.W. 1989. Culture of macrophages from bovine bone marrow. *Veterinary Immunology and Immunopathology,* 21:351–362.

PONTZER, C.H. & RUSSELL, S.W. 1989. Culture of macrophages from bovine bone marrow. *Veterinary Immunology and Immunopathology,* 21:351–362.

SAS 1987. Statistical Analysis Systems Users' Guide, Version 7.03, Cary, North Carolina, USA.

SHERRY, B. & CERAMI, A. 1988. Cachectin/tumour necrosis factor exerts endocrine, paracrine and autocrine control on inflammatory responses. *Journal of Cell Biology*, 107:1269–1277.

SINGH, R.K. & SODHI, A. 1991. Effect of TNF priming on murine peritoneal macrophages on their activation to a tumoricidal state. *Immunological Letters*, 28:127–134.

TOWNSEND, J., DUFFUS, W.P.H. & WILLIAMS, D.J.L. 1988. The effect of age of cattle on the in vitro production of interferon by peripheral blood mononuclear cells. *Journal of Comparative Pathology*, 99:169–185.

TRINCHIERI, G. & PERUSSIA, B. 1985. Immune interferon: a pleiotropic lymphokine with multiple effects. *Immunology Today*, 6:131–136.

UNANUE, E.R. & ALLEN, P.M. 1987. The basis for the immunoregulatory control of macrophages and other accessory cells. *Science*, 236:551–557.

WARFEL, A.H. & ZUCKER-FRANKLIN, D. 1986. Down-regulation of macrophage lysozyme production by lipopolysaccharide and interferon. *Journal of Immunology*, 137:651–665.

WELLDE, B.T., LOTZSCH, R., DIENDL, G., SADUN, E., WILLIAMS, J. & WARUI, G. 1974. *Trypanosoma congolense*. I. Clinical observations of experimentally infected cattle. *Experimental Parasitology*, 36:6–19.

WIRTH, J.J., KIERSZENBAUM, F. SONNENFELD, G. & ZLOTNIK, A. 1985. Enhancing effect of gamma interferon on phagocytic cell association with and killing of Trypanosoma cruzi. *Infection and Immunity*, 49:61–66.