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The Haematology of *Trypanosoma congolense* Infection in Cattle I. Sequential Cytomorphological Changes in the Blood and Bone Marrow of Boran Cattle

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Abstract. Five adult Boran cattle (*Bos indicus*), infected with a clone of *Trypanosoma congolense* IL13-E3 three years earlier and treated, were re-challenged with the same clone. Changes in the peripheral blood were monitored twice weekly, and events in the bone marrow (BM) were assessed by weekly biopsies of the sternal BM, until day 98 postinfection (dpi) when the three surviving animals were treated with diminazene aceturate. One animal died on 57 dpi whereas another was treated on 63 dpi when the packed cell volume was 15%. The infected animals developed anaemia, leucopenia and thrombocytopenia during the first peak of parasitaemia which persisted until the experiment was terminated. Three phases of BM response were demonstrated on light microscopic examination of BM smears. The first, the preparasitaemic phase represented by samples taken on 15 dpi, was an immunological response with slight but significant increases in lymphoblasts, lymphocytes, plasma cells and macrophages (Mø) whereas erythroid and granulocytic cells were unchanged. The second, the early parasitaemic or acute phase (21–57 dpi) associated with the development of anaemia, leucopenia and thrombocytopenia, was characterised by intensification of the immunological response, and an early but transient granulocytic hyperplasia. The third, the late parasitaemic or chronic phase (63–98 dpi) associated with persisting pancytopenia, was characterised by erythroid, megakaryocytic and Mø hyperplasia, dyserythropoiesis, granulocyte hypoplasia and return of lymphoid cell counts to preinfection numbers. Transmission electron microscopy confirmed these findings and showed that intact trypanosomes were not observed in the sinusoids and haemopoietic compartment of the BM.

This study demonstrates that *T. congolense* infection affects haemopoiesis, downregulating or upregulating the various blood cell lineages depending on the stage of infection. This suggests a fine control mechanism, presumably cytokine-mediated. Erythropoiesis, thrombopoiesis and monocytopoiesis were generally upregulated, whereas granulopoiesis was downregulated. However, haemopoiesis was generally ineffective as numbers of circulating blood cells remained below preinfection levels throughout the period of the study.

Keywords: Anaemia; Bone marrow; Cattle; Haematological changes; Leucopenia; Thrombocytopenia; *Trypanosoma congolense*

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Introduction

Several studies have reported haematological changes in animals infected with *Trypanosoma congolense* (reviewed by Anosa 1988; Murray and Dexter 1988). These studies demonstrated that anaemia is a constant feature of the infection in cattle (Naylor 1971; Wellde et al. 1974; Valli et al. 1978; Paling et al. 1991; Williams et al. 1991), sheep (Mackenzie and Cruickshank 1973; Katunguka-Rwakishaya et al. 1992) and goats (Adah et al. 1993). The anaemia typically begins during the first wave of parasitaemia of the acute phase of the disease with the erythrocyte values decreasing rapidly over several weeks; the disease subsequently lapses into a

chronic phase with packed cell volume (PCV) ranging from 15–23%. Severely affected animals succumb to infection during the acute phase or early part of the chronic phase with PCV values decreasing to about 15%. Other animals may gradually recover from the anaemia over several months, whereas some may remain chronically infected with persistently low erythrocyte values.

T. congolense infection also induces leucopenia associated with neutropenia (Naylor 1971; Valli et al. 1979; Valli and Mills 1980; Maxie et al. 1979; Paling et al. 1991; Williams et al. 1991). A lymphocytopenia manifests in most cattle but lymphocytosis often develops in chronically infected or re-challenged trypanotolerant cattle. Thrombocytopenia commonly develops rapidly during the first wave of parasitaemia in *T. congolense* (Wellde et al. 1978; Forsberg et al. 1979; Davis 1982; Preston et al. 1982; Paling et al. 1991) and *T. vivax* (Davis 1982; Anosa et al. 1992) infections.

Although it has been established that *T. congolense* precipitates pancytopenia, the response of the bone marrow (BM) has received less attention. Erythroid hyperplasia associated with a drop in the myeloid:erythroid ratio has been reported (Naylor 1971; Mackenzie and Cruikshank 1973; Valli et al. 1978). The anaemia is usually macrocytic early in infection (Naylor 1971; Valli et al. 1978; Maxie et al. 1979; Valli and Mills 1980). Additional evidence of increased erythropoietic activity during *T. congolense* infection was the increased uptake of radioactive iron as reflected by accelerated disappearance of plasma iron (Mamo and Holmes 1975; Dargie et al. 1979; Valli and Mills 1980; Katunguka-Rwakishaya et al. 1992). However, the rate of incorporation of the iron into erythrocytes was normal in neonatal calves infected with *T. congolense* at 2 and 6 weeks postinfection (Valli and Mills 1980), whereas the percentage of the ^{59}Fe utilised by the BM was lower in the infected cattle than in the controls presumably due to iron-blockage in the recticuloendothelial system (Dargie et al. 1979); these findings suggest inadequacy of erythropoietic response. Moreover, reticulocytes did not increase in cattle infected with *T. congolense* (Valli et al. 1980; Williams et al. 1991), except following treatment of cattle with primary infection or during re-challenge infection of other cattle (Williams et al. 1991).

Since *T. congolense* infection induces unremitting anaemia, leucopenia and thrombocytopenia, thereby suggesting inadequate BM responses, serious attention should be directed to the events in the BM during infection to evaluate the extent to which the BM is responsible for, and responds to, the changes in the peripheral blood. Earlier studies of the BM of cattle infected with *T. vivax* demonstrated that the BM plays an important role in precipitating ineffective haemopoiesis by progressively downregulating granulopoiesis whereas erythropoiesis and monocytopoiesis became accelerated despite persisting anaemia (Anosa et al. 1992). This was accompanied by proliferation of BM macrophages (Mø) which phagocytosed large numbers of erythrocytes, neutrophils and eosinophils and their non-mitotic precursors, as well as thrombocytes. In the present study, we carried out a sequential study of the BM and peripheral blood using weekly biopsies of the sternal BM of five Boran cattle during a secondary infection with *T. congolense* to correlate the changes in the BM with those in the peripheral blood. The kinetics of haemopoietic progenitor cells and Mø function in these animals are reported elsewhere (Andrianarivo et al. 1994; Anosa et al. 1997).

Materials and Methods

Experimental Animals

Five Boran cattle (*Bos indicus*) aged 6–7 years were raised and maintained in a tsetse-free area of Kenya. Three years prior to the present study, the cattle were infected with *T. congolense* clone IL13–E3 (Williams et al. 1991) by tsetse fly (*Glossina morsitans centralis*) challenge. The cattle were successfully treated with diminazene aceturate (Berenil, Hoechst AG, Germany) 36–54 days postinfection (dpi). In the present study, the animals were re-challenged with *T. congolense* IL13–E3 by similar techniques. As is the practice in this institute, any animal which developed a PCV of 15% was treated with diminazene aceturate.

Haematological Techniques

Blood was collected biweekly from the jugular veins of the animals, before and after infection, into vacutainer tubes containing the disodium salt of ethylene diamine tetra-acetate (EDTA). The PCV was determined by the microhaematocrit method. The erythrocyte (RBC) and total leucocyte (WBC) counts were determined using a Coulter counter (model ZBI, Coulter Electronics Ltd, Harpenden, UK), and the haemoglobin concentration (Hb) was measured with a haemoglobinometer (Coulter Electronics). The erythrocyte indices, mean corpuscular volume (MCV) and mean corpuscular haemoglobin concentration (MCHC), were calculated using standard formulae (Jain 1986). Thin blood smears were stained with Diff-Quik (Baxter Healthcare Corporation, McGraw Park, Illinois, USA); these were used to evaluate changes in the peripheral blood cells and for differential leucocyte counts which were based on 100 cells per slide. The phase contrast method (Paris et al. 1982) was used to estimate the numbers of trypanosomes in the peripheral blood.

Collection of Bone Marrow Samples

The restraint of the cattle and the preparation of the sternal biopsy site for BM sampling have been described

(Andrianarivo et al. 1994). The marrow biopsy needle, a 4-inch 11-gauge Jamshidi type needle (Becton-Dickinson, Rutherford, NJ, USA), was introduced through a small incision in the skin and drilled through the sternal cortex into the marrow cavity. Approximately 0.5 ml of BM was aspirated using a 10 ml syringe and smears were made immediately on glass slides and later stained with Diff-Quik. The biopsy needle was then redirected into new areas to obtain core biopsies of 1–2 cm long.

The core biopsies were prepared for transmission electron microscopy as previously described (Anosa et al. 1992). Semi-thin sections, 0.5–1.0 μm thick, were cut and stained with 1% toluidine blue in 1% borax solution for light microscopy. Ultra-thin sections, 50–70 nm thick, were cut from selected blocks, counterstained with Reynolds's lead acetate and examined in a Zeiss EM 10A transmission electron microscope (Carl Zeiss GmbH, Oberkochem, Germany).

Light Microscopic Examination of Bone Marrow Smears

Differential BM cell counts were based on 500 cells from randomly selected fields of each smear. A differential count of megakaryocytes and their precursors was based on 50 cells per slide. Since reticulocyte counts of blood were not made at the time of the study, a reticulocyte count of the BM based on 500 erythrocytes in the BM smear stained with Diff-Quik; the reticulocytes were easily recognisable often as large cells with diffuse or punctuate basophilia. An ocular micrometer was used to measure the diameters of erythroblasts or rubriblasts (50 per slide), megakaryocytes (50), and as many promegakaryocytes and megakaryoblasts as could be found in each slide.

Statistical analyses were made by the student's *t* test (Snedecor and Cochran 1980). Data are given as mean ± standard deviation (SD).

Results

Parasitaemia

The five animals developed parasitaemia between 21 and 24 dpi. Two waves of parasitaemia occurred between this onset and 59 dpi, with a mean parasitaemia of $\log_{10}$ 2.7 parasites per day. One animal developed a PCV of 15% on 57 dpi and was euthanised, whereas another was treated on 63 dpi when its PCV dropped to 15%. The chronic phase of the infection, lasting from 63 to 98 dpi, was characterised by two or three waves of parasitaemia, with a mean parasitaemia per day of $\log_{10}$ 1.5.

Haematological Changes

The PCV, RBC count and Hb concentration remained unchanged during the incubation period. During the first two waves of parasitaemia, the erythrocyte values decreased progressively without remission, from mean PCV of 37.0 ± 5.1% on 24 dpi to 20.8 ± 5.3% ($p<0.001$) on 56 dpi (Fig. 1). During the chronic phase of the disease (63 to 98 dpi), the PCV values remained persistently low. The MCV was 43.4 ± 2.9 fl prior to infection and remained unchanged during the acute phase, and then rose progressively to 60.5 ± 7.0 fl ($p<0.001$) by 98 dpi (Fig. 1). Similarly, the MCHC which was 34.3 ± 1.8 g/dl before infection was unchanged during the acute phase between 21 and 56 dpi (33.8 ± 2.3) but dropped during the chronic phase, averaging 28.2 ± 1.8. Marked anisocytosis developed during the chronic phase of the infection consistent with the appearance of several macrocytes and microcytes. A few normoblasts were seen on two occasions in blood smears on 63 and 98 dpi (Table 1). The anaemia was therefore normocytic normochromic non-regenerative during the acute phase, and macrocytic hypochromic responsive during the chronic phase.

Total WBC count had dropped significantly

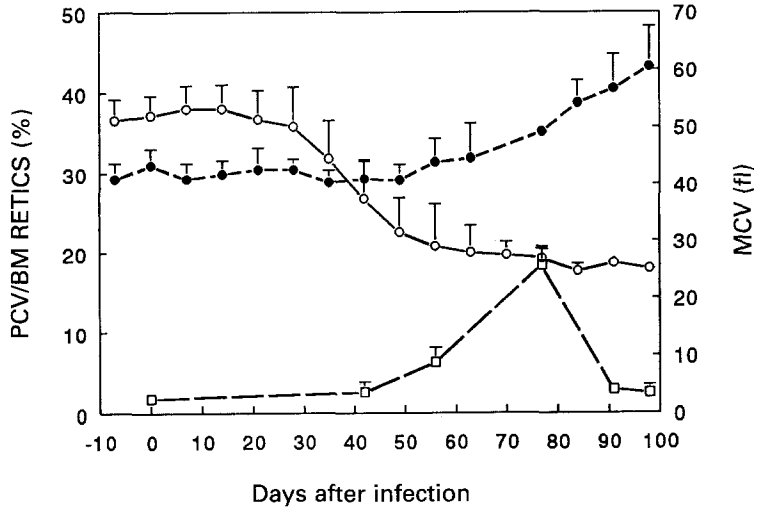

Fig. 1. The mean packed cell volume (PCV, ○) and mean corpuscular volume (MCV, ●) of peripheral blood, and the mean reticulocyte counts (BM RET, □) of sternal bone marrow of Boran cattle infected with *Trypanosoma congolense*.

Table 1. Mean leucocyte counts (per μl of blood) of five Boran cattle infected with *Trypanosoma congolense*

	Days preinfection		Days postinfection							Days posttreatment		
	−7 (*n*=5)[a]	−4 (*n*=5)	+14 (*n*=5)	+28 (*n*=5)	+42 (*n*=5)	+56 (*n*=4)	+63 (*n*=3)	+77 (*n*=3)	+98 (*n*=3)	14 (*n*=4)	17 (*n*=4)	132 (*n*=4)
Total leucocytes	8120±2268[b]	9040±2193	7540±2156	4800±1220	6880±2440	5540±1157	6025±466	5100±1137	4433±1144	8425±1684	7575±963	10525±1718
Neutrophil bands/ metamyelocytes	110±44	100±61	95±52	208±141	204±184	118±69	130±190	74±45	75±66	55±61	129±22	46±48
Segmented Neutrophil	1419±306	1938±545	1171±527	627±408	1029±446	633±157	1043±192	827±220	903±299	1710±406	997±239	1690±662
Eosinophils	1040±576	1071±454	986±302	337±120	316±156	179±181	74±79	123±49	75±68	352±140	515±271	606±174
Basophils	39±49	0	0	0	0	0	0	0	0	0	0	100±105
Lymphocytes	5020±1629	5138±1465	4622±1429	3405±801	4951±1943	4254±960	4195±530	3749±1140	3183±858	6044±1453	5659±251	7600±1438
Monocytes	394±102	578±260	628±265	183±89	402±241	356±96	566±117	327±180	186±84	260±109	348±279	474±177
Normoblasts	0	0	0	0	0	0	16±17	0	11±16	0	0	0

[a] Number of animals examined.
[b] Mean ± standard deviation.

($p<0.005$) during the first peak of parasitaemia (28 dpi) and remained below control levels throughout infection (Table 1). This was associated with significant decreases in numbers of neutrophils, eosinophils and lymphocytes. Monocyte numbers fluctuated during infection. Thrombocytopenia developed precipitously during the first peak of parasitaemia, abated slightly between the first and second waves of parasitaemia, again decreased during the second wave, and thereafter rose slightly during the chronic phase (Fig. 2). The lowest thrombocyte count ($1.4 \pm 0.6 \times 10^5/\mu l$) was recorded on 31 dpi.

After treatment, the total WBC counts and numbers of neutrophils and lymphocytes returned to control levels by days 14 and 17 post-treatment but eosinopenia persisted (Table 1) by day 132 post-treatment, WBC counts were above preinfection numbers ($10525 \pm 1718/\mu l$) due primarily to lymphocytosis ($7600 \pm 1438/\mu l$). Thrombocyte numbers rose markedly by day 7 post-treatment ($6.0 \pm 1.6 \times 10^5/\mu l$) compared to $3.6 \pm 0.5 \times 10^5/\mu l$ ($p<0.01$) recorded before infection (Fig. 2).

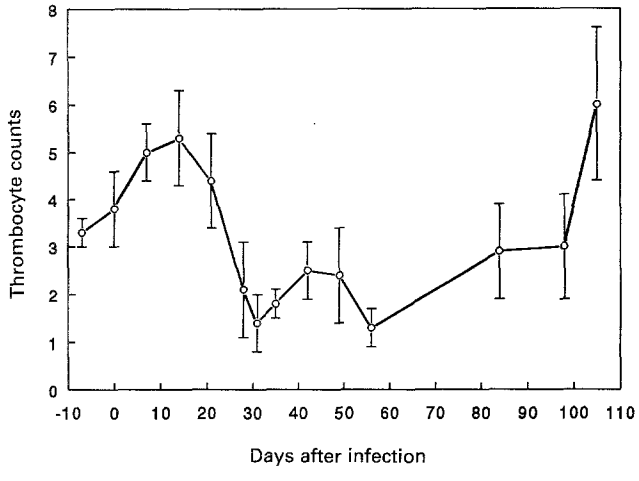

Fig. 2. The mean thrombocyte counts of Boran cattle infected with *Trypanosoma congolense*.

Light Microscopy of Bone Marrow Smears

T. congolense infection affected all cell lineages in the BM (Table 2). In the preparasitaemic phase (15 dpi), the percentages of lymphocyte/lymphoblast, plasma cell and Mø doubled ($p<0.025$). During the early phase of parasitaemia (29 and 43 dpi), the percentage of erythroid cells decreased significantly despite the rapidly developing anaemia, whereas granulocytic and lymphoid cells increased (Table 2). Thereafter, from 57 dpi until the termination of the study on 98 dpi, erythroid cells increased progressively while granulocytic and lymphoid cells decreased.

Erythroblast diameter decreased from 13.3 ± 1.4 μm (range 10.4–18.9) preinfection, to 12.6 ± 1.4 (range 9.3–16.4; $p<0.001$) during the acute phase (43 and 57 dpi) and 11.9 ± 1.6 (range 7.1–16.1; $p<0.001$) during the chronic phase (78 and 98 dpi). Marrow reticulocyte

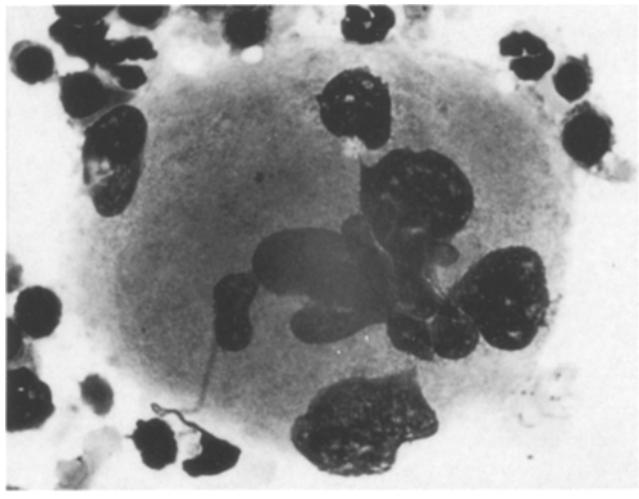

Fig. 3. Megakaryocyte with hyperlobulated nucleus from bone marrow of Boran cattle infected with *Trypanosoma congolense*, 57 days postinfection.

Table 2. Mean myelograms (%) of five Boran cattle pre- and postinfection with *Trypanosoma congolense*

Cell type	Day preinfection	Days postinfection						
	−7 (5)[a]	+15 (5)	+29 (5)	+43 (5)	+57 (5)	+64 (4)	+78 (3)	+98 (3)
Erythrocytic cells								
Erythroblast[c]	1.4±0.3[b]	0.7±0.2	1.7±0.9	1.5±0.7	1.9±0.6	2.2±0.7	1.2±0.3	2.3±1.6
Pronormoblast	2.4±0.6	2.0±0.4	2.5±1.0	2.5±0.7	3.2±1.1	3.3±0.5	3.1±0.5	2.9±0.9
Normoblast								
Basophilic	6.2±0.5	7.8±2.5	3.8±1.6	5.6±1.6	9.0±2.9	8.2±3.1	9.3±0.8	7.0±1.4
Polychromatophilic	28.3±1.3	27.9±0.9	18.4±0.9	23.1±2.7	27.5±2.5	30.3±1.5	27.5±3.5	34.2±3.0
Late	27.6±4.0	24.7±0.2	18.7±4.7	15.8±7.9	28.0±4.3	31.9±4.4	35.2±2.5	34.8±3.4
Total erythroid cells:	66.3±5.1	63.2±3.1	49.3±9.7	48.5±10.9	69.7±5.4	72.5±1.0	76.0±1.7	82.4±2.8
Granulocytic cells								
Myeloblast	0.3±0.1	0.7±0.7	0.8±0.4	0.4±0.1	0.3±0.3	0.4±0.2	0.3±0.1	0.1±0.1
Promyelocyte	0.6±0.3	0.3±0.3	1.8±0.9	0.4±0.2	0.3±0.2	0.4±0.2	0.4±0.2	0.1±0.1
Neutrophil								
Myelocyte	2.4±1.2	0.8±0.3	3.2±0.3	1.9±0.5	1.1±0.8	0.8±0.5	0.6±0.2	0.3±0.2
Metamyelocyte	4.2±1.4	3.7±0.8	5.4±1.1	4.4±0.6	2.8±0.9	1.3±0.2	1.3±0.6	0.8±0.4
Band	7.5±1.7	9.9±2.1	9.4±2.6	11.7±5.2	6.4±2.6	4.4±0.5	5.0±1.1	3.1±1.6
Segmenter	7.8±1.9	4.4±0.2	5.1±2.6	5.2±2.9	2.6±1.3	2.1±1.1	2.3±0.5	1.1±0.3
Eosinophil								
Myelocyte	1.5±0.3	1.7±1.0	0.8±0.7	0.8±0.2	0.7±0.2	0.2±0.2	0.3±0.2	0.3±0.2
Metamyelocyte	1.7±0.5	2.1±1.5	0.7±0.3	1.0±0.6	0.3±0.2	0.1±0.1	0.5±0.1	0.1±0.1
Band	1.6±0.9	2.9±0.5	2.9±0.9	1.6±1.3	0.8±0.3	0.6±0.4	0.4±0.3	0.5±0.2
Segmenter	2.1±0.9	1.6±0.5	2.0±0.6	0.6±0.3	0.3±0.1	0.5±0.2	0.3±0.1	0.1±0.1
Basophil	0	0.3±0.3	0.2±0.1	0.04±0.06	0.08±0.16	0	0	0
Total granulocytic cells	29.4±4.2	28.1±3.4	33.2±5.6	28.1±7.5	15.6±5.0	10.7±2.1	11.6±2.1	6.7±2.4
Myeloid:erythroid ratio	0.45±0.09	0.45±0.07	0.73±0.31	0.64±0.29	0.24±0.10	0.15±0.02	0.15±0.03	0.08±0.03
Granulocyte maturation rate	5.4±1.2	9.7±4.4	4.1±1.0	7.3±2.3	6.4±3.2	5.6±1.6	6.6±2.0	6.3±2.6
Lymphoblast	0	0.2±0.2	2.2±0.8	2.0±0.6	0.6±0.3	0.7±0.3	0.6±0.5	0.2±0.2
Lymphocyte	2.3±0.8	4.2±1.1	9.7±3.7	11.4±3.6	5.1±0.8	5.0±2.4	3.5±1.0	2.5±1.0
Plasma cell	0.8±0.4	1.7±0.5	2.7±0.8	2.8±1.0	2.1±1.1	1.1±0.8	0.7±0.4	0.5±0.3
Monoblast/promonocyte	0.1±0.2	0.4±0.2	0.5±0.3	1.1±0.5	1.0±0.6	1.3±0.5	1.9±0.6	0.7±0.3
Monocyte	0.3±0.2	0.1±0.1	0.5±0.5	1.0±0.4	0.5±0.2	0.8±0.6	0.7±0.3	0.5±0.1
Macrophage	0.7±0.3	1.9±0.4	1.4±0.6	4.9±1.4	5.2±1.3	5.4±1.8	4.8±0.4	6.4±1.1
Total MPS[d]	1.1±0.6	2.4±0.6	2.4±1.0	7.0±1.3	6.7±1.2	7.2±1.8	7.3±2.0	7.6±0.9

[a] Number of cattle sampled; [b] Mean ± standard deviation; [d]MPS, mononuclear phagocyte system. [c] An erythroblast is equivalent to a rubriblast, the immature erythroid cell with prominent nucleoli; pronormoblast is prorubricyte; normoblast is rubricyte.

Table 3. Mean reticulocyte counts, and reticulocyte and erythrocyte diameters in bone marrow smears of five Boran cattle infected with *Trypanosoma congolense*

	Days postinfection					
	−7	43	57	78	92	98
Reticulocyte count (%)	1.8±0.4[a]	2.6±1.3	6.3±1.8	18.4±2.3	2.9±0.7	2.5±1.2
Reticulocyte diameter (μm)	6.6±0.8	7.2±0.6	7.2±0.8	7.0±0.7	6.8±0.6	6.8±0.7
Erythrocyte diamenter (μm)[b]	5.4±0.6	ND	5.7±1.0	ND	ND	5.7±0.7

[a] Mean ± standard deviation.
[b] Includes randomly selected erythrocytes and reticulocytes.
ND = not done.

Table 4. Mean percentage of megakaryocytes and their precursors in bone marrow smears of Boran cattle infected with *Trypanosoma congolense*

	Days preinfection	Days postinfection			
	−7 (n=5)[a]	36 (n=5)	43 (n=5)	57 (n=5)	78 (n=3)
Megakaryoblasts	9.6±3.4[b]	4.8±1.6	6.0±2.2	5.2±2.0	7.3±0.9
Promegakaryocytes	10.4±2.0	10.8±3.3	8.4±2.3	12.8±3.9	12.0±2.8
Megakaryocytes	80.0±5.7	84.4±4.6	85.6±2.3	82.0±5.8	80.7±3.8

[a] Number of animals examined.
[b] Mean ± standard deviation.

Table 5. Mean diameters (μm) of megakaryocytes and their precursors in the bone marrow smears of Boran cattle infected with *Trypanosoma congolense*

	Days preinfection	Days postinfection			
	−7 (n=5)[a]	36 (n=5)	43 (n=5)	57 (n=5)	78 (n=3)
Megakaryoblasts	28.0±6.0[b] (43)[c]	33.6±7.9** (27)	28.2±6.5* (30)	31.1±5.1* (20)	40.6±7.1*** (17)
Promegakaryocytes	39.7±5.7 (43)	48.0±6.3** (52)	46.9±8.0*** (34)	51.2±7.5*** (45)	49.2±6.1*** (22)
Megakaryocytes	54.9±10.0 (250)	64.5±12.3*** (250)	62.2±11.4*** (250)	63.4±11.2*** (250)	60.9±11.2*** (150)

[a] Number of animals examined.
[b] Mean ± standard deviation.
[c] Total number of cells counted.
* Not significant compared to corresponding pre-infection values.
** $p<0.025$ compared to corresponding preinfection values.
*** $p<0.001$ compared to corresponding preinfection values.

counts increased by 57 dpi, peaked on 78 dpi (18.4 ± 2.3%) and dropped to almost preinfection numbers by 98 dpi (Fig. 1; Table 3). There was expansion of the mononuclear phagocyte system (Table 1).

A differential count of megakaryocytes and their precursors showed only minor changes in the relative percentages of these cells during infectioni (Table 4), but their sizes increased significantly (Table 5). Whereas the nuclei of the megakaryocytes of control cattle were usually ovoid or spherical, several megakaryocytes of infected cattle showed marked convolution and sometimes segmentation of the nuclei (Fig. 3). This occurred on 36 dpi in 10% of megakaryocytes and subsequently it became more common.

Variation in Severity of Disease Between Animals

Two infected cattle developed severe acute disease with PCV dropping to 15% in both animals on 56 dpi. One animal became moribund and was euthanised on 57 dpi whereas the second was treated on 63 dpi when the PCV was still 15% and recovered from the disease. The other three animals had less severe chronic disease and were not treated until the experiment was terminated on 98 dpi, at which time their mean PCV was 18.0 ± 0.1%. Prior to infection, group A that developed acute disease had significantly ($p<0.001$) lower PCV (35.3 ± 1.4%) than the group B with chronic disease (40.7 ± 1.9), as well as lower total WBC, eosinophil and lymphocyte counts. These differences became accentuated during infection. For instance, although the PCV of group A had dropped to 42.5% of preinfection values, the PCV of group B were 60.7% of preinfection values on 56 dpi. The myelograms showed slightly greater percentages of erythroid cells and fewer granulocytic cells in group A than in group B before infection and, except on 42 dpi, this persisted until 57 dpi when group A animals were treated or euthanised.

Electron Microscopic Study of Bone Marrow

Core biopsies of the sternal BM, taken preinfection and on days 57, 78 and 98 postinfection, were examined by TEM, augmented by light microscopic examination of toluidine blue-stained sections. TEM of the preinfection BM showed many fat-cells and small numbers of sinusoids lined by flattened endothelial cells. Occasional adventitial cells were present on the abluminal surface of the endothelial cells. The cells in the haemopoietic compartment (HC) were loosely packed with little

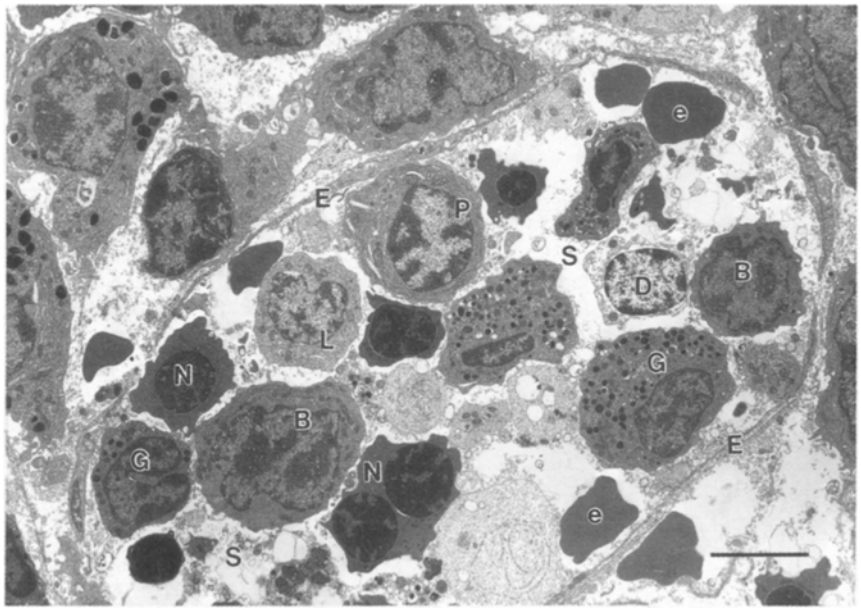

Fig. 4. Transmission electron micrograph of bone marrow sinusoid (S) of Boran cattle infected with *Trypanosoma congolense* showing normoblast (N), neutrophil precursors (G), lymphocyte (L), plasma cell (P), erythrocytes (e), blast cells (B) and degenerating cells (D), 98 days postinfection. E = sinusoidal endothelium. Bar = 5.0 μm.

contact between cells. The erythroid cells, granulocytic cells (neutrophils, granulocytes and occasionally basophils) and megakaryocytes were well represented. Small numbers of lymphocytes, plasma cells, monocytes and a few Mø were also present.

During infection, TEM and light microscopy of the toludine-stained sections showed a general increase in the cellularity of the HC in all animals on 57 dpi which persisted until 98 dpi. There was also an increase in the numbers of sinusoids and a marked decrease in the numbers and sizes of the fat cells. One animal (F149) had several large fat globules in the sinusoids on 78 dpi. There was proliferation of erythroid cells and Mø, whereas granulocytes, particularly eosinophils, were considerably depleted. The precursors of erythroid and granulocytic cells and the endothelial and adventitial cells had no obvious morphological abnormalities. Several neutrophils and their precursors phagocytosed thrombocytes and erythrocytes.

A study of the cell populations of the sinusoids was made because they link the BM to the general circulation. The dominance of erythrocytes in the sinusoids preinfection was diminished during infection by the considerable increases in normoblasts, reticulocytes, monocytes and Mø (Table 6; Fig. 4). There were many degenerate cells in the sinusoids of two of the three surviving animals on 78 dpi, whereas few of these cells were seen in the sinusoides of the third animal on 98 dpi (Fig. 4). Primitive blast cells were also seen in the sinusoids of one animal on 98 dpi; these cells were lymphoblastoid in nature but differed from lymphoblasts by the presence of many mitochondria and rough endoplasmic reticulum and by the greater electron density of their cytoplasm compared to lymphoblasts.

Free trypanosomes were not observed in the sinusoids and HC of the BM examined throughly by TEM. Two trypanosomes were encountered in arterioles within the HC.

Table 6. Cells in the sinusoids of the bone marrow of Boran cattle infected with *Trypanosoma congolense*[a]

	Days postinfection			
	−7	57	78	98
Total cells counted	219	387	315	354
Erythrocytes	91.3[b]	65.9	54.9	41.8
Reticulocytes	0	0	0.3	6.8
Normoblasts	0.5	4.4	19.4	24.6
Neutrophils	5.0	2.6	6.0	6.8
Eosinophils	2.7	0	0.6	0.3
Monocytes	0	2.3	1.9	2.0
Macrophages	0	14.5	10.5	10.5
Lymphocytes	0.5	4.4	6.3	3.1
Plasma cells	0	0	0	0.6
Blast cells	0	0	0	3.7[c]

[a] As seen with transmission electron microscopy.
[b] Per cent of total cells.
[c] Lymphoblastoid cells.

Discussion

The development of pancytopenia, i.e. anaemia, leucopenia and thrombocytopenia, which started during the first wave of parasitaemia, is consistent with findings in *T. congolense* infections of ruminants (Naylor 1971; Valli et al. 1978, 1979; Maxie et al. 1979; Paling et al. 1991; Williams et al. 1991), and in *T. vivax* infections of cattle (Anosa 1983; Anosa et al. 1992).

Sequential comparisons of the cytology of the BM revealed three phases of haematological response: the preparsitaemic, the acute and the chronic phases. The preparsitaemic phase (15 dpi) was characterised by significant increases in Mø numbers and size, and in lymphocyte and plasma cell counts, whereas percent-

ages of erythroid and granulocynic cells remained essentially unchanged. In the absence of detectable parasites in the circulation at this time, these changes could only have been induced by lesions in the tsetse bite site, the chancre, and presumably the lymph node draining the chancre. Since Mø, lymphocytes and plasma cells are the main inflammatory cells of the chancre (Gray and Luckins 1980; Akol and Murray 1982), it is probable that products of the interaction between these cells and trypanosomes in the chancre and the draining lymph node may have reached the BM and induced the cellular reactions observed. The response of the BM in this phase was typically immunological, and its development is favoured by the secondary challenge status of the infection (they had received an earlier challenge by the same clone 3 years earlier).

The acute phase was characterised by significant decreases in PCV, and in eosinophil, neutrophil, lymphocyte and thrombocyte numbers in the peripheral blood, and by intensification of the immunological reponse of the BM. In the early stages of the phase, there was also slight proliferation of granulocyte elements and proliferation and activation of Mø with destruction of mature and maturing erythroid, granulocytic cells and thrombocytes in the BM.

The chronic phase was characterised by persisting anaemia and depression of leucocyte and thrombocyte numbers, continued proliferation and activation of Mø in the BM with phagocytosis of cells similar to those seen in the acute phase, erythroid hyperplasia and dyserythropoiesis, marked granulocyte hypoplasia and return of the percentages of lymphocytes, plasma cells and lymphoblasts towards normal. This phase was therefore distinctly pro-erythropoietic, although severe anaemia persisted. The ability of the animal to respond to bacterial infections and other heterologous antigens seems to be seriously compromised because of the downregulation of granulopoiesis and lymphopoiesis with the resultant panleucopenia. These changes provide a clue to why trypanosome-infected animals succumb readily to secondary bacterial and other infections.

The TEM studies confirmed many of the above light microscopic findings such as increased cellularity of the BM, proliferation of Mø with cytophagia, hyperplasia or erythroid elements and hypoplasia of granulocyte elements. In addition, they raised other noteworthy issues. First, the increased contacts between Mø and haemopoietic cells (Anosa et al. 1997) suggest that Mø play a central role in the control of haemopoiesis, providing a means, presumably through cytokines, for the regulation of proliferation of cell lineages observed in this study. Secondly, the degenerate cells seen in the sinusoids probably represent cells in apoptosis. This process is precipitated by withdrawal of specific cytokines required for blood cell maturation (Arends and Wyllie 1991). It might be expected to occur in trypanosome infection due to the fluctuating populations of some cells such as lymphoid cells and granulocytes as well as the cytokines that control their populations, and because of loss of contact between the Mø and haemopoietic cells in the sinusoids.

Thirdly, primitive blast cells appeared in the sinusoids later in infection (78 and 98 dpi). These cells may include pluripotential and committed stem cells, and such cells (CFU-E, BFU-E) were detected in cultures blood at this time of infection (Andrianarivo et al. 1994). Their destinations may be the sites of extramedullary haemopoiesis.

It is noteworthy that these numerical and functional changes in the BM during the acute and chronic phases occurred despite the absence of *T. congolense* in the HC. The changes may have been induced by trypanosome products, as well as factors such as cytokines and primed lymphocytes derived elsewhere that reach the BM to induce these changes.

Some specific observations on the cell lines in the BM deserve mention. This study showed that trypanosome infection induced ineffective erythropoiesis which was manifested by persistence of severe anaemia despite an intense erythroid shift in the BM. Thus, in addition to the marked phagocytosis of normoblasts, reticulocytes and erythrocytes in the BM (Anosa etal. 1997), there was generation of progressively smaller-sized erythroblasts during the acute and chronic phases, delayed erythropoietic response associated with increasing MCV during the chronic phase but which fizzled out terminally as BM reticulocytes numbers fell. Although the presence of macrocystosis and several shift reticulocytes during chronic infection suggests an elevated erythropoietin level, the concurrent presence of many microcytes suggests a partially unhealthy BM manifesting in small-sized erythroblasts and perhaps iron blockade.

Similarly, ineffective granulopoiesis occurred in the BM during *T. congolense* infection as demonstrated by the hypoplasia of the granulocyte elements, granulocytic cells dropping from $29.4 \pm 3.8\%$ preinfection to only $6.7 \pm 2.4\%$ terminally, and by the phagocytosis of immature and segmented granulocytes by Mø (Anosa et al. 1977). The proliferation of lymphoblasts with the increases in numbers of lymphocytes and plasma cells in the preparasitaemic and acute phases of the infection suggest that lymphopoiesis was accelerated in the BM during this time presumably in response to the intense antigenic stimulation associated with the infection.

The marked increase in megakaryocyte mass associated with the increases in numbers and sizes of megakaryoblasts, promegakaryocytes and megakaryocytes increased promegakaryocyte endomitosis and ploidy with accelerated but inadequate thrombopoiesis. These increases are mediated by elevated thrombopoietin levels in animals (McDonald 1992). The thrombocytopenia may therefore be due mainly to thrombocyte phagocytosis observed in the BM (Anosa et al. 1997) and presumably other haemopoietic organs. Other studies have shown that trypanosome infections induce thrombocyte phagocytosis (Anosa and Kaneko 1983, 1989; Anosa et al. 1992) and clumping (Davis 1982; Anosa and Kaneko 1989; Anosa et al. 1992). Increases in megakaryocyte mass have also been described in *T.*

congolense (Forsberg et al. 1979) and *T. vivax* infections (Anosa and Kaneko 1989; Anota et al. 1992); however, it was suggested that there may be ineffective thrombopoiesis since the uptake of [^{35}S]methionine into the peripheral blood thrombocytes in the infected calves was reduced (Forsberg et al. 1979). The convolution or segmentation of megakaryocyte nuclei observed may reflect ageing or a 'shift to the right' of the megakaryocytes, suggesting some defect in thrombocyte release. The thrombocytosis observed 7 days after treatment reflects a rebound effect due to the increased megakaryocyte mass induced by the infection.

In conclusion, this study demonstrates that the cellular changes in the BM are dynamic and related to the cellular events in the peripheral circulation, with specific cell lineages in the BM being upregulated or downregulated at particular periods of the infection. Even in those cases where cell lineages were upregulated in the BM, there was failure to restore peripheral blood numbers of the definitive cells to preinfection levels, indicating that *T. congolense* induced generalised ineffective haemopoiesis. These observations further indicate that the concentrations of factors in the BM, such as cytokines, which modulate these changes would fluctuate in consonance with the changes and are subject to very fine controls.

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