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Pathogenesis of Anemia in *Trypanosoma brucei*-Infected Mice

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The pathogenesis of anemia was studied in trypanosome-infected mice. A strain of *Trypanosoma brucei*, TREU 667, was used which first produces an acute phase marked by waves of parasitemia. Erythrocytes from infected animals were coated with immunoglobulin M during or just before the waves of anemia and parasitological crises. Erythrocytes from normal animals could be sensitized with "precrisis" sera presumably containing antigen and antibody. These data suggest that anemia during the acute phase is due to sensitization of erythrocytes with immunoglobulin M-antigen complexes. The anemia is partially compensated by a strong erythropoietic response. The acute phase is followed by a chronic phase marked by a constant high parasitemia and immunosuppression. The less marked anemia occurring during this latter phase is due to hemodilution and perhaps a low but significant immune response to the parasites, which causes continuing erythrocyte sensitization by immunoglobulin M-antigen complexes.

Anemia associated with the African trypanosomiases is a consistent and significant finding in humans (18, 56) and animals (44). Three mechanisms have been implicated: dyshemopoiesis (3, 11, 12, 14, 15), hemodilution (14, 17, 38, 50), and hemolysis (17, 24, 34, 41, 42).

Microcytosis (15), hypoferremia (47), and low plasma-iron turnover rates (9, 41) have been observed during chronic trypanosomiases, suggesting impaired erythropoiesis. In addition, the presence of massive hemosiderin deposits within the mononuclear phagocyte system may be indicative of defective iron utilization (36, 49). However, there have been observations of marked reticulocytosis (24, 50) and increased removal of iron from serum and incorporation into circulating erythrocytes (24), all of which are indicative of a functional erythropoietic response to lowered erythrocyte counts.

Holmes (17) found that infected calves developed a marked hypervolemia, with blood volume increases of about 30%. This is supported by the findings of Valli et al. (50) and Valli and Forsberg (49). These authors suggest that the dilution may be related to the histological findings of widespread microvascular damage. However, Preston and Wellde (41) and Dargie et al. (9) observed no increase in the blood volume of infected calves.

There is considerable evidence that the anemia in this infection is hemolytic and that it involves a significant increase in the rate of erythrocyte destruction (24, 25, 41, 42, 49); however, the mechanism responsible remains to be ascertained. In vitro, *Trypanosoma equiperdum* (28), *Trypanosoma brucei* (19), and *Trypanosoma congolense* (48) generate potent hemolytic activity when permitted to autolyze. Hemolytic material from *T. congolense* has been shown to consist of a mixture of free fatty acids and phospholipase A, whereas a hemolytic factor from *T. brucei* is a small-molecular-weight protein. Hemolytic factors have been found in the sera of trypanosome-infected cattle (35a). A number of immunological mechanisms have been proposed to be involved in the hemolysis. Trypanosome antigen has been demonstrated on the surface of erythrocytes (32; W. J. Herbert and M. D. Inglis, Trans. R. Soc. Trop. Med. Hyg. **67**:268, 1973). Woo and Kobayashi (54) reported that antigenic material from *T. brucei* was readily adsorbed in vitro onto normal rabbit erythrocytes. Antiglobulin tests showed that the erythrocytes of calves infected with *T. congolense* had absorbed immunoglobulins (26). However, some authors report inconsistent or fluctuating results (22). Preformed antigen-antibody complexes may be absorbed onto erythrocytes (55, 56), a phenomenon found in immune complex diseases (57); this would account for the detection of both antigen and immunoglobulin. Such immune complexes may fix complement on the erythrocyte surface, resulting in intravascular hemolysis or erythrophagocytosis or both. Complement has been detected on the surfaces of erythrocytes of patients infected with African trypanosomes (55, 56), and erythrophagocytosis has been observed in trypanosome-infected animals (22, 32, 33, 36, 50). These observations

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would be consistent with antibody or antibody-complement coating of erythrocytes.

We report here an examination of possible mechanisms of anemia and their relative importance, using a *T. brucei*-mouse model of trypanosomiasis.

MATERIALS AND METHODS

Reagents and solution. (i) Media. The tissue culture medium used was RPMI 1640 medium (GIBCO Laboratories, Grand Island, N.Y.) supplemented with 25 mM HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid; Aldrich Chemical Co., Milwaukee, Wis.), 11 mM glucose, and 1.0% bovine serum albumin (Sigma Chemical Co., St. Louis, Mo.), pH 7.4 (RPMI-HEPES). Dulbecco modified Eagle medium (GIBCO Laboratories) was also used.

(ii) Buffers. Electrophoresis buffer consisted of 0.097 M barbital, 0.003 M calcium lactate, and 0.01% (wt/vol) thimerosal (pH 8.6); phosphate-buffered saline (PBS) consisted of 0.1 M sodium phosphate and 0.073 NaCl (pH 8.0); phosphate glucose buffer (PG) consisted of 0.01 M sodium phosphate and 0.26 M glucose (pH 7.2); and sodium phosphate buffer consisted of 0.02 M sodium phosphate (pH 7.5).

(iii) Other materials. Other materials used were carrier-free potassium iodide (New England Nuclear Corp., Boston, Mass.); chloramine-T and poly-L-lysine (Sigma); salicylhydroxamic acid (SHAM; Aldrich Chemical Co.); goat anti-mouse immunoglobulin M (IgM), goat anti-mouse IgG, fluorescein isothiocyanate-conjugated IgG fraction of rabbit anti-goat IgG (Cappel Laboratories, West Chester, Pa.); rhodamine-conjugated bovine albumin (Microbiological Associates, Bethesda, Md.); printed microscope slides for fluorescence (Cell Line Associates, Inc., Minolta, N.J.); mouse IgM (Litton Bionetics, Kensington, Md.); glutaraldehyde (Polysciences, Inc., Warrington, Pa.); agarose (CoLab Laboratories, Inc., Chicago, Ill.); and ultrafiltration Centriflow cones (Amicon Corp., Lexington, Mass.).

Animals and parasite maintenance. Four-week-old female S/W mice weighing between 20 and 25 g (Taconic Farms, Germantown, N.Y.) were used in all experiments. All control animals were age-matched with experimental animals. Stabilates made from *T. brucei* TREU 667, obtained from F. Jennings of the University of Glasgow, Glasgow, U.K., were used for all experiments. Frozen stabilates were made as described by Brohn and Clarkson (5). All experimental infections were made after at least one mouse passage from stabilate, unless otherwise indicated. Mice were always infected by intraperitoneal injections of 1.0×10^4 trypanosomes.

Hematological determinations. Hematological parameters were determined daily for the first 21 days of infection and then once every 3 days throughout the rest of the infection. Tail blood (5 μl) was collected from each mouse and diluted in PBS. Parasites were counted with a Petroff-Hausser hemacytometer for phase-contrast microscopy, and erythrocytes were counted in an improved Neubauer chamber. Reticulocyte counts were done after staining with new methylene blue (4). Packed-cell volume was determined by the capillary microhematocrit method.

Blood collection. Blood for erythrocyte and plasma studies was collected from mice, using heparin (approximately 125 U/ml of blood) as an anticoagulant, from either the tail or the brachial artery for larger quantities. Blood was collected without anticoagulant for serum.

Indirect fluorescent-antibody technique. A 1:8 dilution of whole goat anti-mouse IgM serum (μ chain specific) or anti-mouse IgG (γ chain specific) and a 1:20 dilution of a fluorescein isothiocyanate-conjugated IgG fraction of a rabbit anti-goat IgG serum were employed in all immunofluorescence experiments. A 1:20 dilution of rhodamine-conjugated bovine albumin was used as a counter stain and diluent for the fluorescein isothiocyanate-rabbit anti-goat IgG.

Printed microscope slides were cleaned with 1 N NaOH, rinsed in distilled water, and air dried. A solution containing 1 mg of poly-L-lysine per ml in PG was applied to the slides for 30 min in a moist chamber at room temperature. The slides were then rinsed gently in distilled water, dried, and stored at −30°C until used.

For detection of IgM on mouse erythrocytes, approximately 200 μl of tail blood was obtained from infected and normal mice. The cells from these samples were washed two times by centrifugation at 1,000 × *g* and suspended in PG buffer (pH 7.2). Each sample of cells was divided equally into two groups; one group was suspended in 200 μl of goat anti-mouse IgM, and the other was suspended in normal goat serum. The cells were washed twice as described above and fixed by suspension in 0.5 ml of PBS with 1.5% glutaraldehyde. After three washes to remove the fixative, they were suspended in 1.0 ml of PG buffer, diluted 32-fold, and applied to poly-L-lysine-coated slides. The low-ionic-strength PG buffer was used to promote binding of erythrocytes to the slides. After allowing the cells to settle at 4°C for 30 min in a moist chamber, the slides were washed with RPMI-HEPES medium. The bovine serum albumin in the medium bound to reactive amino groups, preventing fluorescent reagents from binding nonspecifically to the slide. The slide wells were covered with 100 μl of fluorescein isothiocyanate-conjugated rabbit anti-goat IgG and incubated at 4°C in a moist chamber for 1 h. After incubation, the slides were rinsed in three changes of RPMI-HEPES medium, and cover slips were mounted with 40% glycerol–60% PBS. The stained slides were examined for fluorescence with a Zeiss microscope fitted with appropriate filters and incident light optics.

Erythrocyte sensitization in vitro. Mouse serum, the IgM peak of a Sephadex G-200 fractionation of mouse serum (as described in reference 39), and in vitro-formed immune complexes were tested for the ability to sensitize erythrocytes from uninfected mice. Blood was collected from uninfected mice, and the cells were washed seven times with PG as described above. Subsequently, 100 μl of a 2% suspension of cells was mixed with 200 μl of the mouse serum or serum fraction to be tested and incubated at 37°C for 30 min. The cells were washed twice with PG, and the indirect fluorescent-antibody procedure described above was used for detection of bound IgM.

Immune response to horse erythrocytes. Washed horse erythrocytes (2.4×10^9 cells per mouse) were injected intravenously into groups of three infected mice and three uninfected control mice. All mice were sacrified 7 days after injection of the horse erythro-

cytes, and serum was collected and stored at −30°C. These sera were inactivated at 56°C for 30 min, and the agglutination titers with horse erythrocytes were determined. To individual wells of a microtiter plate, 25 μl of a 0.5% washed horse erythrocyte suspension was added. The pattern of the settled cells was observed after overnight incubation at 4°C. The titer was recorded as the last dilution of serum which caused complete agglutination of all of the erythrocytes.

IgM quantitation. Quantitative rocket immunoelectrophoresis as described by Laurell (29) and modified by Weeke (52) was used. The sera were collected at appropriate times and stored at −30°C until assayed for IgM content. Immunoelectrophoresis was performed in 1% agarose gels prepared in electrophoresis buffer. Mouse IgM was used as a standard, and goat anti-mouse IgM was incorporated into the agarose gels at a final concentration of 3.0% (vol/vol). All serum samples were carbamylated with potassium cyanate before application to the gels. Electrophoresis was carried out overnight at 10°C at 5 V/cm.

Blood volume determination. The dilution of ^{125}I-labeled mouse serum proteins was used to estimate the blood volumes of mice infected for different lengths of time. Fresh normal mouse serum was collected, and small-molecular-weight components were removed by diluting 1.0 ml of serum to 10.0 ml with sodium phosphate buffer and reconcentrating with Centriflow membrane cones six times (these retain molecules with molecular weights greater than 5×10^4). The proteins were adjusted to 10 mg/ml as estimated by 280 nm absorbance and then labeled by the chloramine-T method (53). The labeled proteins were washed in sodium phosphate buffer as described above until the wash had no detectable radioactivity. The preparation was then diluted to 4.0 ml with sodium phosphate buffer.

Groups of mice were injected intravenously with 0.2 ml of the labeled serum proteins, and plasma was collected between 1 and 3 h after injection. The total plasma volume was calculated from the dilution of the injected radiolabel as determined from the counts per minute of the sample of the labeled proteins used for injection and the counts per minute of the plasma collected after injection. The calculated plasma volume combined with a hematocrit measurement allowed calculation of the total blood volume.

Immunosuppression by gamma radiation. Immunosuppression was accomplished by irradiating mice with 900 rads of whole-body irradiation given by an M-38 Gammator (Isomedix, Parsippany, N.J.) 24 h before inoculation with trypanosomes.

Preparation and administration of SHAM-glycerol. A stock SHAM-glycerol solution of 0.39 M SHAM and 4.3 M glycerol was prepared by suspending 0.30 g of SHAM in 2.0 g of glycerol and adding 1.96 ml of 1 N NaOH and 1.5 ml of distilled water. The suspension was heated to 60°C and stirred until the SHAM dissolved. The final volume was adjusted to 5.0 ml with distilled water. An intravenous dose of 0.5 ml/100 g of body weight was used.

RESULTS

Course of parasitemia, anemia, and erythrocyte sensitization in intact S/W mice. Figure 1 shows the parasitemias of mice after injection of 10^4 trypanosomes. The course of infection showed two phases: an early acute phase during which crises occurred and a later chronic phase marked by a high, steady parasitemia. Waves of anemia coincided with parasitological crises (Fig. 1). After day 28, the erythrocyte counts returned essentially to that of the control animals. The slight anemia seen in the controls during the first 4 weeks was undoubtedly caused by the frequent blood sampling. An increase in the percentage of IgM-positive erythrocytes occurred just before the first parasitological crisis and wave of anemia (Fig. 1). When parasitological crises ceased, do did the waves of anemia, and the proportion of IgM-sensitized erythrocytes decreased. When equivalent methods were used, no IgG was detected on host erythrocytes.

To characterize the nature of the sensitizing serum components, we performed in vitro experiments with sera from different stages of the infection and sera from irradiated, infected mice (Table 1). Serum containing variant specific exoantigen but little or no specific antibody was obtained from irradiated, infected mice (10). Intact mice were infected with the same strain used to infect the irradiated mice. Serum obtained from the intact mice 30 days after infection agglutinated parasites grown in the irradiated mice and therefore contained antibody to the variant-specific exoantigen present in the serum from irradiated mice. This day 30 serum would not, however, contain the same exoantigen present in the irradiated mice, since this variant would have been eliminated by the immune response. Serum obtained from intact mice on day 8 of infection ("precrisis") would be expected to contain both antibody and the variant-specific exoantigen since this parasite variant would not yet have been completely eliminated.

Erythrocytes incubated in serum obtained on day 8 of infection became IgM positive. Incubation of erythrocytes in the first peak of a Sephadex G-200 column fractionation of day 8 serum also caused IgM sensitization; this peak would contain IgM and IgM immune complexes but no free variant-specific antigen (molecular weight, 6.5×10^4 [8]). However, the day 30 serum did not cause fluorescence over background, although it contained sevenfold more IgM than that obtained on day 8 (Fig. 2). A volume of day 30 serum was slowly added to an equal volume of serum from irradiated, infected mice so as to form immune complexes in vitro. These mixed sera also caused IgM sensitization of erythrocytes in vitro.

We considered the possibility that parasite antigen may coat the erythrocytes first, thus leading to subsequent IgM binding. Exposure of erythrocytes to serum from irradiated, infected

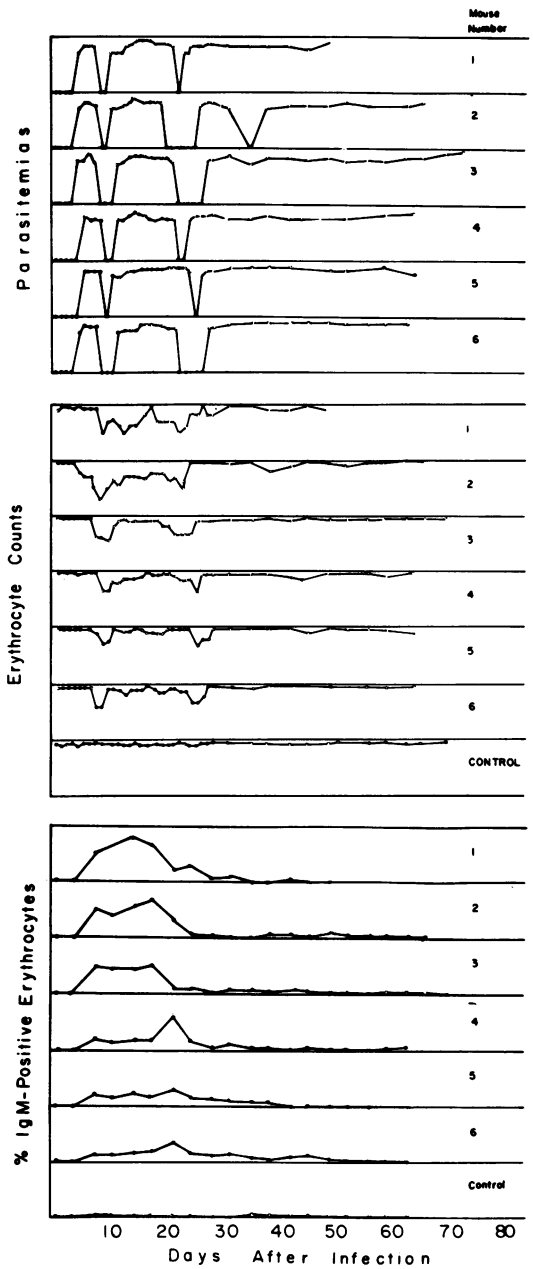

FIG. 1. Parasitemia, anemia, and IgM sensitization of erythrocytes. Parasitemias are plotted as the $\log_{10}$ of parasites per milliliter of blood; the scale is linear and ranges from 0 to 10. Erythrocyte counts are plotted on a linear scale which ranges from 0 to 12 × 10^9 erythrocytes per ml of blood. The percent IgM-positive erthrocytes scale is linear and ranges from 0 to 20%. In all cases, the ends of the individual curves represent the deaths of the individual infected animals. The same set of six infected animals was used for measurement of the three parameters. The control data represent the means of two uninfected animals sampled at the same time points as the infected animals.

TABLE 1. Detection of IgM on mouse erythrocytes after exposure to various sera in vitro

Serum	% IgM-positive cells
Normal mouse erythrocytes incubated in:	
Normal mouse serum	1.2 ± 0.2
Day 8 serum[a]	30.0 ± 3.2
Day 8 IgM serum peak[b]	22.8 ± 2.7
Day 30 serum[a]	0.9 ± 0.1
Premixed serum from irradiated, infected mice and 30-day serum	12.0 ± 2.1
Serum from irradiated, infected mice followed by 30-day serum	0.9 ± 0.1
Erythrocytes from irradiated, infected mice incubated in day 30 serum	0.8 ± 0.1

[a] Mice were infected on day 0 with 10^4 trypanosomes, always from the same frozen stabilate. Serum was collected 8 or 30 days after infection.
[b] First peak from a Sephadex G-200 column.

mice which contained exoantigen, followed by serum obtained on day 30, produced only background levels of IgM sensitization. To determine whether a longer exposure to soluble parasite antigen would allow binding of the antigen to erythrocytes, we collected erythrocytes from irradiated, infected mice and exposed them to the day 30 serum. Although these erythrocytes had extended exposure to parasite antigen in vivo, only background levels of IgM binding were observed.

Reticulocytosis. Packed reticulocyte volumes were estimated by multiplying the reticulocyte percentage by the hematocrit. The estimated volume of reticulocytes peaks after the first

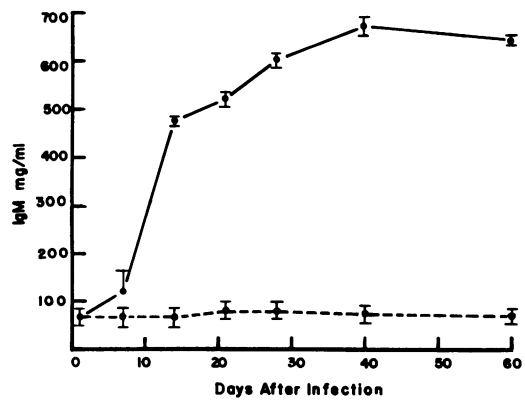

FIG. 2. Levels of serum IgM during *T. brucei* infection. IgM concentration was determined by rocket immunoelectrophoresis as described in the text. (—), Infected mice; (---), normal mice. Data represent the means of three mice per point ± standard error.

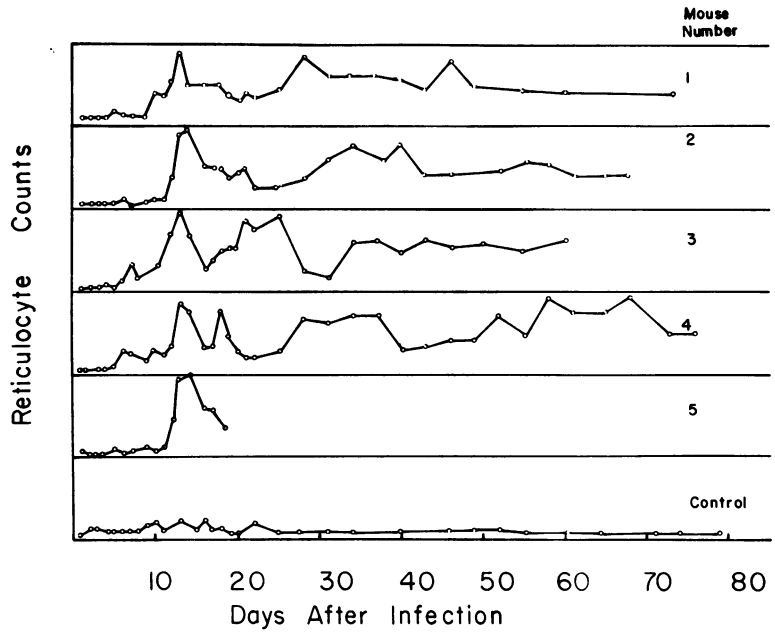

FIG. 3. Reticulocyte counts. The data represent the packed volume of reticulocytes expressed as a percentage of whole blood. These measurements were made as described in the text. The scale is linear and ranges from 0 to 15% of total blood as reticulocytes.

wave of anemia and then fluctuates during the rest of the course of infection (Fig. 3).

Suppression of immune response to horse erythrocytes. We hypothesized that the partial resolution of anemia in infected mice might be due to immunosuppression, as this is an often-reported finding (20, 21, 23, 37, 43, 46). Immunosuppression was determined by immunizing both uninfected mice and mice infected for different lengths of time with horse erythrocytes and comparing the hemagglutination titers of their sera 7 days after immunization (Fig. 4). Quantitatively, the mice became immunosuppressed to heterologous antigen earlier than they did to trypanosomes. The response to heterologous antigen given 7 days postinfection was much reduced, and the response to heterologous antigen injected at 14 and 21 days was severely depressed. In contrast, the first and second parasitological crises were equal (Fig. 1), indicating a sustained response to the parasites after the response to heterologous antigens was diminished.

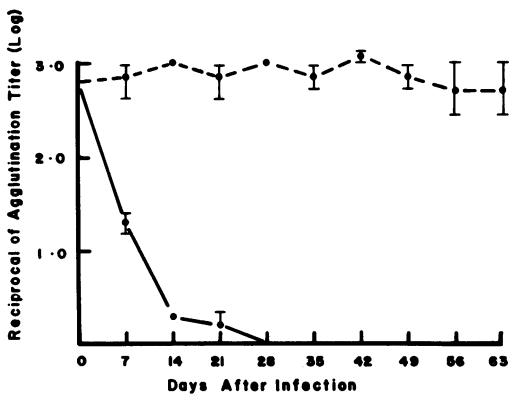

FIG. 4. Immunosuppression during *T. brucei* infection. (—), Infected mice; (---), control mice. The data points represent the means of the $\log_{10}$ of agglutination titers with horse erythrocytes of serum from three mice per time point ± standard errors. The time scale reflects the time of immunization with horse cells with respect to the time of inoculation with *T. brucei*. Serum for the agglutination assay was always collected 7 days after immunization with horse erythrocytes.

Splenomegaly and hepatomegaly. Both splenomegaly and hepatomegaly occurred (Table 2). Splenomegaly occurred sooner and was more prounced than hepatomegaly.

Blood, plasma, and total packed erythrocyte volumes. There was no change in plasma volume (as measured by dilution of ^{125}I-labeled serum proteins) 1 week after infection. The total blood volume was reduced by an amount equivalent to the loss in packed erythrocyte volume (Table 3). At weeks 3 and 5, total packed erythrocyte volume was the same as that in uninfected controls; however, plasma volume was increased. By week 8, the plasma volume was increased further but was fully compensated by an increase in erythrocyte volume, so that there

TABLE 2. Splenomegaly and hepatomegaly in mice infected with *T. brucei*

Mice	Mean spleen wt ± SE		Mean liver wt ± SE	
	Actual wt (g)	% Body wt	Actual wt (g)	% Body wt
Uninfected controls	0.23 ± 0.02	0.80 ± 0.06%	1.85 ± 0.15	6.14 ± 0.07%
Infected for 8 days	0.80 ± 0.03	2.59 ± 0.07%	1.92 ± 0.02	6.28 ± 0.05%
Infected for 30 days	0.85 ± 0.02	2.40 ± 0.03%	2.75 ± 0.05	7.74 ± 0.17%

was no anemia. By measuring the dilution of intravenously injected ^{51}Cr-labeled erythrocytes, we made an independent measurement of the total blood volume. By using this measurement and by measuring hematocrits, we observed essentially the same pattern of changes in plasma, blood, and packed erythrocyte volumes during the course of infection (data not shown).

Response of hematocrit after parasite lysis in vivo. To induce release of possible internal hemolysins from the parasites, we treated irradiated, infected animals with SHAM-glycerol, a combination known to produce rapid lysis in vivo (7). Irradiated mice were used to avoid the possibility of causing an increase in circulating immune complexes after disruption of the parasites. Four irradiated, infected mice had a mean hematocrit of 29.1% before treatment and 28.1% 5 h after intravenous injection of SHAM-glycerol. The same experiment done with intact, infected mice produced mean hematocrits of 38.5% before treatment and 36.2% after treatment. The slight reduction in hematocrit was most likely due to the intravenous injection of a volume of hypertonic solution equal to approximately 12% of the total blood volume. Uninfected control mice showed a shift from 47.8 to 46.1% after treatment. These experiments, therefore, do not support a role for a hemolysin released from lysed parasites.

DISCUSSION

The purpose of this study was to examine the causes of anemia associated with trypanosomiasis, using a rodent-*T. brucei* model. In mice, the TREU 667 strain produced a semi-acute infection lasting 60 to 70 days, which allowed a study of progressive pathology. In this respect, the model is more similar to natural infections of humans and domestic animals than are acute models, which are lethal within a few days and do not show an even temporarily effective immune response. Also, this strain reliably produces a central nervous infection, an important aspect of the course of the disease in humans (25). Finally, this model presents two distinct phases. The first stage is characterized by a relapsing parasitemia similar to that seen in natural infections, and the second stage is a prolonged chronic phase during which the animal is immunosuppressed and the parasitemia remains at a constant high level. Our studies indicate that some of the mechanisms causing anemia may be different in these two phases of the disease.

Causes of anemia during the acute phase. Marked reticulocytosis and loss of total packed erythrocyte volume early in the infection indicate that hemolysis plays an important role in the generation of anemia, as has been concluded in prior studies (17, 36, 37, 41).

The temporal association of waves of anemia with parasitological crises led us to conclude that the acute-phase anemia is caused by the immune response of the host. Our in vitro experiments suggested that this sensitization is due to the binding of antigen-antibody complexes since sera containing only antigen or only antibody did not bind to erythrocytes in vitro; however, a mixture of these two sera was effective, as was a high-molecular-weight fraction of acute-phase serum. This is in contrast with the findings of Brooks and Reed (6) with *Trypanosoma musculi*. They were able to clearly show that parasite antigen adheres to the host erythrocytes independent of antibody. Our conclusions concur with those of Kobayashi et al. (26), who proposed the same model based solely on find-

TABLE 3. Blood volume changes during *T. brucei* infection

Wk after infection	Mean vol (ml/kg of body wt) ± SE of:		
	Plasma	Blood	Total packed erythrocytes
Uninfected controls	40.6 ± 2.79	66.4 ± 2.08	29.7 ± 1.78
1	40.0 ± 3.83	60.7 ± 3.13	21.4 ± 2.16
3	48.6 ± 4.34	72.2 ± 2.27	29.6 ± 2.17
5	47.1 ± 6.77	73.4 ± 5.01	29.8 ± 2.71
8	55.9 ± 4.53	89.1 ± 5.04	36.3 ± 3.03

ing antibody on erythrocytes of calves infected with *T. congolense*. However, the evidence presented here provides much stronger support for this hypothesis. It would be desirable to pursue these studies with purified parasite surface coat antigen and monospecific antibody and to determine whether the waves of anemia could be mimicked by these more defined substances. Our conclusion that the acute-phase anemia is due to the immune response is further supported by the finding that, as the host becomes immunosuppressed, the parasitological crises and waves of anemia cease. These observations are in agreement with those of Balber (1), who reported that immunosuppression with cortisone attenuated the anemia seen in trypanosomiasis. In addition, our data show that hemodilution does not occur during the acute phase.

Our data suggesting that immune complexes bind to host erythrocytes correlate well with the observation of Herbert and Inglis (Trans. R. Soc. Trop. Med. Hyg. **67**:268, 1973) and MacKenzie et al. (32), who demonstrated trypanosome antigens on the erythrocytes of trypanosome-infected animals. Tabel et al. (46) found sporadic appearance of immunoglobulin on host erythrocytes in *T. congolense*- and *Trypanosoma vivax*-infected cattle; IgM was the most frequently found immunoglobulin. They also observed that only a subpopulation of cells agglutinated with antisera directed against host IgM. Dodd et al. (11) have also reported that only a subpopulation of erythrocytes from rabbits infected with *T. brucei* is agglutinated by antisera directed against host immunoglobulins. Our results extend these observations and provide a partial explanation. We clearly observed that only a portion of the host cells are sensitized at any one time; however, we have no concrete explanation for this observation.

It is likely that the sensitized erythrocytes fix complement and are then recognized by the mononuclear phagocytic cells of the spleen and liver. Splenomegaly occurred during the acute phase of infection (Table 2) and activation of macrophages has been observed (36, 37; J. A. Longstaffe, Parasitology **69**:xxiv, 1974). These phenomena would facilitate phagocytosis of IgM- and complement-sensitized erythrocytes via the C3 receptor (2) or possibly via a receptor for IgM (30, 51). Sensitization of erythrocytes with immunoglobulins has been observed in rodent (31) and human (13) malaria, and phagocytosis of both parasitized and nonparasitized erythrocytes by activated macrophages has been observed in the rodent malaria system (45). This may be analogous to the erythrophagocytosis seen in trypanosomiases (24, 32, 33, 36, 50).

Dargie et al. (9) studied two breeds of cattle infected with *T. congolense*. Although one breed was much more trypanotolerant, both had self-limiting trypanosomiases. There was a correlation between the parasitemia and degree of anemia. It would seem unlikely that a single immunological mechanism could underlie a pattern of an initial acute anemia followed by partial resolution, as seen in both the immunocompetent animals discussed above and the mice in this study which became immunosuppressed. However, if either antibody production is limited by immunosuppression or if antigen production is limited by an effective immune response, the effect on immune complex concentration and erythrocyte sensitization would be the same; i.e., they would be decreased. Such a decrease would reduce erythrocyte sensitization and resultant erythrocyte destruction.

Although clear evidence for a role of immune complexes in the generation of anemia is first described in this paper, immune complexes have been described previously in humans and animals with trypanosomiasis (16, 27, 40). This lends support to the hypothesis that the phenomenon we observed in mice is a general occurrence in the anemia produced by trypanosomiasis. However, this hypothesis cannot be accepted until relevant studies are done in cattle. In addition, this mechanism may not by itself account for the total anemia due to trypanosomiasis.

Causes of anemia during the chronic phase. We found an increase in total plasma volume after 3 weeks of infection; hemodilution did contribute to the anemia seen in the chronic phase of infection. However, the increase of spleen and liver size may account for some of the expanded blood volume late in the infection. At 30 days postinfection, there was an increase of 16 g/kg of body weight in spleen and 16 g/kg of body weight in the liver as compared with controls. This may account for some of the increase (7.0 ml/kg of body weight) in blood volume observed at 35 days postinfection.

McCrorie et al. (35) recently reported an increase in splenic sequestration of erythrocytes related to the degree of spleen enlargement in *T. brucei*-infected rabbits. Rheological changes may be less important than IgM sensitization in erythrocyte clearance during the acute phase but more important during the chronic phase when IgM sensitization is absent or below detectable limits. However, undetected, but physiologically significant, sensitization of erythrocytes may continue to occur. Using a derivative of the same parasite isolate as used in this study, Hudson and Terry (21) reported a low but significantly protective IgM response to new variants that continued even after the IgG response to sheep erythrocytes was totally suppressed. When the IgM response to sheep erythrocytes

was only 5% of the normal response, major parasitological crises ceased. Such a continuing but attenuated immune response could contribute to the anemia seen in the chronic phase despite our inability to detect IgM on host erythrocytes. A lesser degree of sensitization would predict a lesser degree of anemia. The recovery of a normal hematocrit shortly before death may, therefore, represent a final exhaustion of the immune response which alleviates the anemia but contributes to the death of the animal.

The lack of a hemolytic response to the rapid destruction of *T. brucei* in vivo by SHAM-glycerol does not support a role for a parasite-released hemolysin. This is an artificially induced parasitological crisis, however; perhaps a slower destruction of parasites by immunological factors would induce the formation and release of a hemolysin.

We conclude that, during the acute phase of *T. brucei* infection in mice, IgM sensitization by immune complexes together with splenomegaly causes waves of anemia which coincide with parasitological crises. As there was an increase in circulating reticulocytes during both the acute and chronic phases of this model, dyshemopoeisis cannot be a prime cause of anemia. During the chronic phase of infection, when the host is immunosuppressed, there are multiple factors which may contribute to the lesser degree of anemia. These are hemodilution, splenomegaly as well as hepatomegaly, and perhaps a continuing, low, but physiologically significant IgM sensitization of erythrocytes by immune complexes.

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