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Hemolytic Complement and Serum C3 Levels in Zebu Cattle Infected with *Trypanosoma congolense* and *Trypanosoma vivax* and the Effect of Trypanocidal Treatment

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Total hemolytic complement and C3 levels were found to drop to 6.25% and 50% of preinfection levels, respectively, during trypanosome infections. Chemotherapeutic elimination of the trypanosomes with Berenil led to recovery of preinfection levels within 7 days and 11 days when cattle infected with *Trypanosome congolense* and *Trypanosoma vivax*, respectively, were treated 37 days after onset of infection. Recovery was slower in *T. vivax*-infected cattle treated on day 50. Berenil treatment had no effect on complement levels in control animals. The possible causes and implications of these low complement levels in bovine trypanosomiasis are discussed.

Hypocomplementemia has been reported to occur in trypanosome infections of humans (5, 10), monkeys (12), cattle (15, 19), mice (21), and rats (7). Depression of serum C3 (8, 12, 15), C4, and factor B (5) and changes in properdin with parasitemia (15) have also been reported.

The role of complement in antibody production has been reviewed by Pepys (16), and there exists a possibility that low complement levels in cattle with trypanosomiasis may contribute to the immunodepression that has been demonstrated in cattle infected with *Trypanosoma congolense* (6, 17, 18) or *Trypanosoma vivax* (17, 20). Since Berenil treatment leads to immediate recovery of immune responsiveness in zebu cattle (F. R. Rurangirwa, E. Mushi, H. Tabel, G. J. Losos, and I. R. Tizard, Tropenmed. Parasitol., in press), it was decided to measure the complement levels and to relate them to the immunodepression observed in trypanosome-infected cattle and to the rapid return of immune responsiveness after Berenil treatment. In this communication we report total hemolytic complement and C3 levels in cattle before and during infection with *T. congolense* or *T. vivax* and after Berenil treatment.

MATERIALS AND METHODS

Cattle. Boran steers (*Bos indicus*), aged 9 to 10 months, were used in all experiments. They were obtained from a trypanosome-free area and were shown to be free of trypanosomes before use.

Trypanosomes. *T. congolense* EATRO 1800 and *T. vivax* EATRO 1721 were used. They were passaged through 1-week-old calves, and each experimental animal was infected intravenously with 10^5 trypanosomes from the donor calf.

Parasitemia. Parasitemia in each experiment was determined as previously described (Rurangirwa et al., submitted for publication) by relating the number of trypanosomes in 100 or 400 fields of a thin blood smear stained with Giemsa to the total number of leukocytes determined by Coulter Counter.

Serum. All animals in each experiment were bled daily for 7 days before infection and thereafter until the termination of each experiment. The blood was left for 2 h at room temperature to clot. It was then centrifuged at 4°C, and serum was collected, filtered through a 1.2-μm membrane (Sartorius-membrane GMBH, Gottingen, W. Germany), and stored at −20°C in four aliquots. The average duration of storage was 30 days.

Estimation of total hemolytic complement. The number of 50% hemolytic complement units per milliliter of serum was estimated by adaptation of the method of Barta and Barta (2) to a microtiter method, using guinea pig erythrocytes sensitized with normal bovine serum as the indicator system.

Hemolysin. Sera were collected from normal cattle. Doubling dilutions of these sera in sucrose-Veronal buffer (pH 7.0) in 0.05-ml volumes were carried out in U-bottom microtiter plates, to which equal volumes of 2.5% washed guinea pig erythrocytes were added and incubated at 37°C for 1 h. The degree of hemolysis was read for each serum after the erythrocytes had settled. The animal with the highest titer was bled, and its serum was separated, inactivated at 56°C for 30 min, distributed in 5-ml samples, and stored at −20°C. This serum was used at one dilution below the 100% hemolysis endpoint. The inactivated serum was also titrated for agglutinins against guinea pig erythrocytes, and during sensitization it was used at a dilution above the agglutination titer.

Test procedure. Doubling dilutions of the sera to be tested were made in 0.05-ml volumes in U-bottom microtiter plates, commencing with undiluted serum (0.100 ml), using an automatic diluter (Cooke Engi-

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neering Co., Alexandria, Va.). After dilution, 0.05 ml of 2.5% sensitized guinea pig cells was added, and the plates were sealed, mixed well, incubated at 37°C for 1 h, and then removed to 4°C. The 50% hemolysis endpoint was read against a standard after the erythrocytes had settled. The number of 50% hemolytic complement units per milliliter was taken as the reciprocal of the serum dilution giving 50% hemolysis.

Determination of serum C3 levels. C3 was assayed by single radial immunodiffusion according to the method of Fahey and McKelvey (4). Antiserum to C3 (Cappel Laboratories, Downingtown, Pa.) was used at a concentration of 5% in 1% agarose in Veronal buffer-ethylenediaminetetraacetic acid. The volume of serum used in each well was 5 μl. After addition of the test sera, the agarose plates were incubated at 4°C for 72 h, washed in 2% sodium chloride for 48 h, then washed for another 24 h in distilled water; after this, the plates were dried and stained with Coomassie Blue dissolved in acidified alcohol. Ring diameters were measured with calipers and recorded.

Experiment 1. Twenty-four steers were used, divided into three groups of eight animals each. The first and second groups were infected with *T. congolense* and *T. vivax*, respectively. The remaining group of animals served as controls. All the animals were treated with Berenil (7 mg/kg of body weight) 37 days after the initiation of the trypanosome infection.

Experiment 2. The design of this experiment was as in experiment 1, except that the animals were treated with Berenil 50 days after initiation of the trypanosome infection.

RESULTS

Experiment 1. (i) Course of *T. congolense* and *T. vivax* infection. The daily parasitemia ($\log_{10}$) is recorded in Fig. 1A. The parasitemia remained fluctuating but patent until 1 day after Berenil treatment, when trypanosomes were no longer detectable in the blood.

(ii) C3 levels. Figure 1B shows the C3 levels in each group of animals. The C3 levels began to drop 10 days after infection, coinciding with the fall of the first peak of parasitemia. They remained low, though fluctuating, until the animals were treated with Berenil.

There was a rapid rise of C3 levels in the *T. congolense*-infected animals immediately after Berenil treatment. Control levels were reached 6 days posttreatment.

Unlike the *T. congolense*-infected group, the recovery of C3 levels in the *T. vivax*-infected animals was delayed for 2 days post-Berenil treatment before the levels began to rise; C3 reached the preinfection level 10 days after Berenil treatment. There was also a slight spontaneous rise in the C3 levels from day 29 up to the day of Berenil treatment (day 37).

(iii) Hemolytic complement levels. Hemolytic complement levels dropped rapidly 6 days after infection (Fig. 1C). The complement in *T. congolense*-infected animals reached a minimum level ($\log_2 = 2.26$) in 26 days, whereas in *T. vivax*-infected animals it reached the minimum level ($\log_2 = 2.55$) in 23 days. The hemolytic complement levels fluctuated more in *T. vivax*-infected animals than in *T. congolense*-infected animals.

After Berenil treatment, there was a rapid rise of the mean hemolytic complement levels of serum of *T. congolense*-infected animals. They reached control levels 8 days posttreatment.

There was a rapid rise of the mean hemolytic complement levels of serum of *T. vivax*-infected animals until 6 days posttreatment. This was followed by a slower rise until 13 days posttreatment, at which time the control levels were reached. An apparent recovery in hemolytic complement was observed 28 days postinfection in the *T. vivax*-infected group, similar to that observed in the C3 levels.

Experiment 2. All the *T. congolense*-infected animals in this group died of trypanosomiasis between 21 and 35 days after infection. Their complement levels were excluded from the experiment.

(i) Course of *T. vivax* infection. The daily parasitemia was similar to that recorded in Fig. 1. The parasitemia remained patent up to the time of Berenil treatment on day 50.

(ii) C3 levels. There was a drop in the C3 commencing 10 days after infection. The low level was maintained until treatment with Berenil. The C3 levels rose slowly for 7 days after Berenil treatment, then more rapidly for another 4 days. Except for day 12 after Berenil treatment, the serum C3 remained somewhat below control levels until the end of the experiment.

(iii) Hemolytic complement. The hemolytic complement began to drop 8 days after infection, reaching a minimum level ($\log_2 = 2.52 \pm 0.38$) by 28 days after infection. After Berenil treatment, the mean hemolytic complement levels of serum rose rapidly for 6 days posttreatment, then more slowly for 16 days, until the control level was reached.

In all cases, Berenil treatment did not alter the complement levels of control animals.

DISCUSSION

In this study, it has been clearly demonstrated that in trypanosome-infected animals, consumption of complement takes place. Thus C3 and total hemolytic complement levels were decreased to one-sixteenth and one-half preinfection levels, respectively, throughout most of the infection. Changes in the levels were detected at or soon after the first peak of parasitemia and remained low until Berenil treatment removed the trypanosomes.

Complement activation in bovine trypanoso-

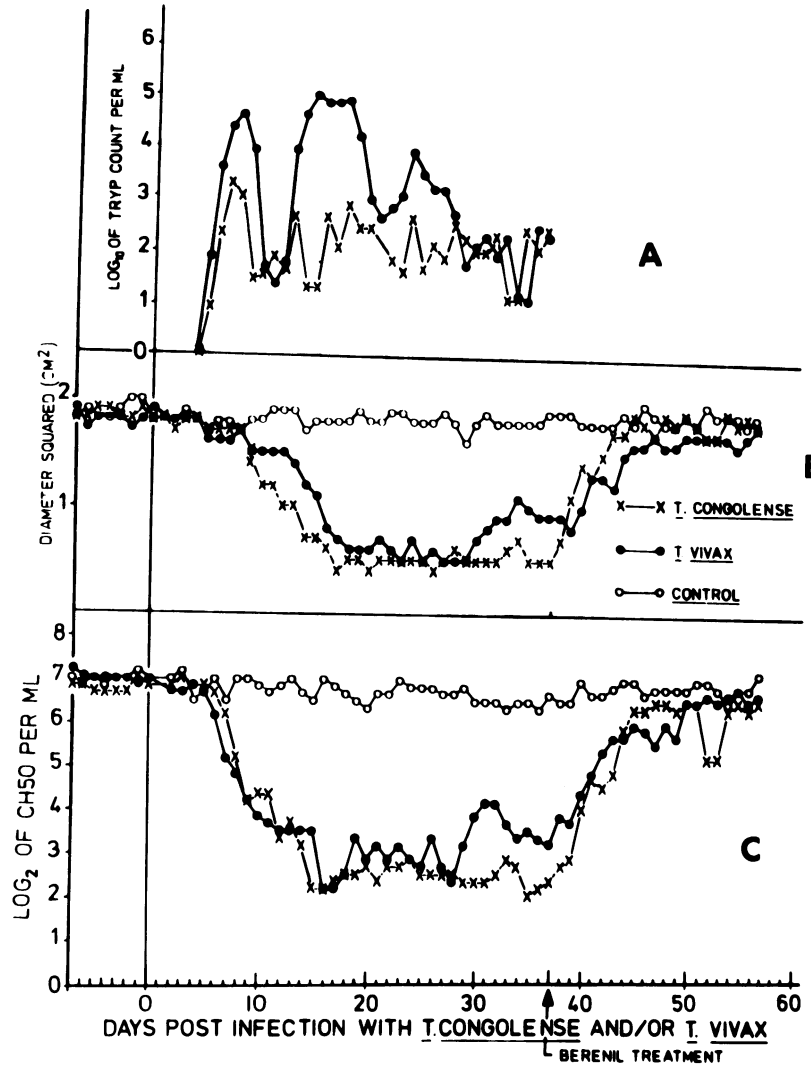

FIG. 1. *(A) The daily parasitemia, (B) the serum C3 level, and (C) the serum hemolytic complement levels in groups of T. congolense-infected, T. vivax-infected, and control cattle treated with Berenil 37 days after onset of infection. Each point represents the average of values from eight animals. The standard deviation of the C3 levels was ±0.1 to ±0.25 throughout the experiment; that of the hemolytic complement level was ±0.13 to ±0.41.*

miasis has been reported previously. Kobayashi and Tizard (8) found that anemia in cattle infected with *T. congolense* coincided with a drop in parasitemia, the appearance of complement-fixing antibody, and a progressive decrease in serum C3 levels. Tabel et al. (19) and Nielsen et al. (15) also found low complement levels in cattle infected with *T. vivax* and *T. congolense*, respectively. The low hemolytic complement and C3 levels reported in this communication are in agreement with their findings.

The cause of the drop in the complement levels in the trypanosome-infected cattle has not been determined, although in vitro activation of C1 and C4 by trypanosome components has been demonstrated (11, 13). Nielsen and Sheppard (11) showed that 2×10^8 *T. congolense* incubated at 20°C for at least 3.5 h generated a factor which inactivated 2 50% hemolytic complement units of bovine origin. It is therefore possible that the low complement levels we detected might have been an in vitro phenomenon due to complement inactivation after bleeding. However, the few trypanosomes that were taken when bleeding (ca. 10^5 *T. congolense*) cannot account for the activation of 124 50% hemolytic

complement units observed in our study. Moreover, the blood was kept at room temperature (20°C) for only 2 h, centrifuged at 4°C, and filtered through a 1.2-μm membrane to remove any contaminating trypanosomes. In vivo activation of both classical (7) and alternate (12) pathways by trypanosome has been reported before, and our findings therefore support the view that in vivo activation of complement occurs in bovine trypanosomiasis.

The more rapid drop in the hemolytic complement levels reported in this work, as compared to the fall in the levels of C3, suggests that C3 may not be the limiting component for the hemolytic complement levels. The faster rise of hemolytic complement as compared to the rise of C3 levels after Berenil treatment also supports this suggestion, and indicates that depressed C3 synthesis occurs in cattle infected with *T. vivax*. Normal macrophages are known to synthesize C3 (9). On the other hand, activated macrophages have been shown to release cleavage products C3a and C3b instead of intact C3, and C3b in turn stimulates the macrophages (1). Since proliferation of mononuclear phagocytes is a feature of lymphoid organs in cattle infected with *T. congolense* or *T. vivax* (G. J. Losos, personal communication), it is possible that these cells contribute to the hypocomplementemia.

Increased catabolism of some complement components (C3, C1, C1q) has also been reported in calves infected with *T. congolense* (15), and this may also contribute to the decline in complement levels.

Immune complexes occurring as a result of antigenic variation may also be partly responsible for the decreased complement levels. The apparent increase in complement levels corresponding to low parasitemic waves in the *T. vivax*-infected animals might support this view. However, Greenwood and Whittle (5) attributed the low levels of C3 found in Gambian trypanosomiasis to the formation of large-molecular-weight immunoglobulin M. There is no evidence to suggest that hypocomplementemic nephritis occurs in bovine trypanosomiasis.

The implications of in vivo activation of complement are manifold. Experimental decomplementation has been shown to have profound effects on the immune response (reviewed by Pepys [16]). Decreased complement levels in bovine trypanosomiasis may not only be involved in the changes in immunoconglutinin (8) but also in the immunosuppression phenomenon (6, 17, 18, 20).

If complement, especially C3, plays a role in bovine immune response, as has been shown in mice (16), then faulty T-B-cell cooperation may be envisaged as a cause of the immunosuppression observed in bovine trypanosomiasis. However, Rurangirwa et al. (submitted for publication) have shown that the bovine immune response to rinderpest vaccine was not affected by these trypanosome infections even when administered on day 25, when the complement levels were at their lowest. In addition, it has been shown (Rurangirwa et al., submitted for publication) that recovery of immune responsiveness returns very rapidly after Berenil treatment (2 to 5 days). This is much more rapid than the recovery in complement levels. Thus the role of low complement levels in the immunosuppression observed in bovine trypanosomiasis remains equivocal.

It has been suggested that hypocomplementemia may be responsible, at least in part, for the increased susceptibility of trypanosome-infected animals to secondary infections (14, 19), a phenomenon which has been known since the time of the early reports of the sleeping sickness commission (3). Indeed, Nielsen et al. (14) have demonstrated increased susceptibility of rats to *Salmonella typhimurium* after decomplementation with extracts from *Trypanosome lewisi*, a result which tends to strongly support this suggestion.

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