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Complement in Experimental *Trypanosoma lewisi* Infections of Rats

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The role of complement in host resistance to infection with *Trypanosoma lewisi* was studied in normal, C4-deficient, and C3-depleted rats. Complement levels were measured in normal rats throughout the course of infection. A drastic reduction of total complement and C4 hemolytic activities occurred, and C3 levels measured immunochemically were decreased. Although total complement and C4 levels were regularly reduced to less than 10% of preinfection levels regardless of parasite numbers, the degree of C3 consumption correlated with the parasitemia. C3 levels varied from 100% of preinfection in rats with light infections to 35% in animals with heavy parasitemias. Recovery to normal levels followed trypanosome elimination from the peripheral blood. The infection had no significant effect on C6 hemolytic activity. Parasitemias and C3 levels in C4-deficient rats did not differ from those of normocomplementemic controls. Depletion of C3 and late-acting components by cobra venom factor during the reproductive or adult stages of infection did not alter the parasitemias. In addition, *T. lewisi* and immune serum caused complement activation in vitro, which could be inhibited with ethylene glycol-bis-(β-aminoethyl ether)*N*,*N'*-tetraacetic acid or ethylenediaminetetraacetic acid. It is concluded that *T. lewisi* infections in rats result in activation of the classical complement pathway with extensive consumption of the early-acting components, as well as a low degree of activation of the alternative pathway. However, complement does not appear to play a major role in the control and termination of the infection.

Rats are able to control infections with the hemoflagellate *Trypanosoma lewisi* primarily through two specific immunological processes. A reproduction-inhibiting antibody called ablastin appears early in the course of infection (35, 36). As the result of ablastic activity, which is complement independent in vitro (15), the rapidly reproducing trypanosomes are converted to a population of monomorphic adults. A second immune response is characterized by the production of two classes of trypanocidal antibodies that eliminate the parasites and terminate the infection (14, 36). Several reports indicate that the complement system may play an important role in the latter process. Disintegrated trypanosomes, presumably destroyed by lytic antibody, have been observed in the blood during the course of infection (5) and after passive transfer of immune serum to infected rats (13). Complement-fixing lytic and opsonic antibody activities have been demonstrated in immune serum in vitro (21, 22, 36). Studies from this and other laboratories have demonstrated almost total loss of complement activity during infections (J. A. Jarvinen and A. P. Dalmasso, Abstr. 50th Annu. Meet. Am. Soc. Parasitol., p. 71, 1975; 19).

In the experiments reported here, we examined the role of complement in host control of *T. lewisi* infections. We found that during infections of normocomplementemic rats a massive activation of complement occurred via the classical pathway. There was extensive depletion of C4 with little inactivation of C3 and C6. The degree of C3 depletion was similar in infected C4-deficient and normocomplementemic rats. The course of infection was not altered in rats genetically deficient in C4 or in rats depleted of C3 and late-acting components by treatment with the anticomplementary factor of cobra venom. In vitro incubation of *T. lewisi* with immune serum resulted in activation of complement primarily by the classical rather than the alternative pathway.

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MATERIALS AND METHODS

Experimental animals. Two- to three-month-old Wistar rats raised from stock obtained from Simonsen Laboratories, Gilroy, Calif., were used. C4-deficient rats as described by Arroyave et al. (C. M. Arroyave, R. M. Levy, and J. S. Johnson, Fed. Proc. 33: 795, 1974) were raised from the Wistar stock by selective breeding. The rats were apparently free from *Bartonella* infection since neither *Bartonella* nor anemia was observed after the splenectomy of randomly selected individuals (26).

Maintenance of parasite and infection of rats. *T. lewisi* (Taliaferro strain obtained from Philip D'Alesandro, Columbia University) was maintained in Wistar rats by weekly syringe passage of infected tail vein blood in 0.9% NaCl. For infection of experimental animals, parasites were separated from the blood of donor rats by the method of Lincicome and Watkins (23) on day 6 or 7 of infection. A suspension of the trypanosomes in sterile 5% dextrose-0.9% NaCl was counted by hemocytometer and adjusted to the desired concentration. Rats were infected intraperitoneally with 3.5×10^5 to 8×10^5 *T. lewisi*; controls received an equivalent volume of sterile diluent.

Course of infection. Parasitemias were followed with smears and hemocytometer counts of tail vein blood samples obtained every 2 days. For hemocytometer counts, the blood samples were diluted in erythrocyte-diluting pipettes with 1% formalin-0.9% NaCl containing 10% Giemsa stain. The percentage of reproductive forms was determined by examination of 100 to 1,000 trypanosomes on Giemsa-stained smears. Epimastigotes and small trypomastigotes (approximately 20 μm or less in length) as well as actual dividing cells were considered as reproductive forms.

Complement determinations. Glucose-Veronal-buffered saline containing 0.05% gelatin, 0.5 mM $MgCl_2$, and 0.15 mM $CaCl_2$ ($GGVB^{++}$) was prepared as described (29) and used as diluent for total C, C1, C4, and C6 hemolytic activity titrations. Optimally sensitized sheep erythrocytes (EA) (32), $EAC4^{gp}$ (8; gp refers to complement component derived from guinea pig serum), and $EAC1^{gp}$ (33) were used at a concentration of 10^8/ml. Partially purified guinea pig C1 was prepared from guinea pig serum by precipitation as described by Nelson et al. (29). Titrations of C1 and C4 (10, 29) were performed with functionally pure guinea pig C2 (Cordis Laboratories, Miami, Fla.) and C3-C9 supplied by pooled normal rat serum (NRS) diluted 1:20 in Veronal-buffered saline containing 0.1% gelatin (GVB) and 0.04 M ethylenediaminetetraacetic acid (EDTA). For the titration of C6, 1 volume of EA, 1 volume of C6-deficient rabbit serum diluted 1:20, 1 volume of serially diluted rat serum, and 2 volumes of $GGVB^{++}$ were incubated for 1 h at 32°C. A total reaction volume of 1 ml was used in all hemolytic activity titrations. The percentage of lysis was determined spectrophotometrically, and the 50% end point was calculated by interpolation on semilog paper. C3 levels were measured by the radial immunodiffusion method of Mancini et al. (24), using rabbit anti-rat C3 at a 1:8 dilution. Titers were calculated as the percentage of NRS standard. For purposes of comparison, all complement titers are expressed as a percentage of preinfection values or the appropriate control.

Preparation of anti-rat C3. This was done by using a modification of the procedure of Mardiney and Müller-Eberhard (25). One hundred milligrams of zymosan (Mann Research Laboratories, Rutherford, N.J.) was processed and incubated with 6 ml of NRS as described. After six washes with isotonic saline, the zymosan-complement complex was resuspended in 3 ml of saline and homogenized with an equal volume of complete Freund adjuvant. Three milliliters of the mixture was injected into each of two rabbits in the footpads, intramuscularly and subcutaneously. The animals were bled 1 month after immunization. To remove contaminating antibodies to serum proteins other than C3, an immunonadsorbent was prepared as follows. A sample of NRS was subjected to Pevikon block electrophoresis using Veronal buffer, pH 8.6, $\mu = 0.05$, and a potential gradient of 3.5 V/cm for 18 h (27). The non-C3-containing β_2 and γ fractions were eluted, pooled, and concentrated. This material was made insoluble with gluteraldehyde and used to absorb the antiserum (3). A single line characteristic of C3 was obtained when the antiserum was tested against NRS by immunoelectrophoresis (25).

CoF treatment. Cobra venom factor (CoF) was injected intraperitoneally in two doses of 150 U per kg of body weight 12 h apart. In one experiment, CoF was given on day 5 in order to deplete C3 during the reproductive stage of infection. In a second experiment, depletion during the early adult stage was accomplished by administering the CoF on day 8. After treatment, C3 levels remained at less than 5% of the NRS standard for at least 4 days. C3 depletion was maintained for at least 8 days in the third experiment by giving an additional dose of 100 U/kg 4 days after treatment on day 8. Infected and uninfected controls received intraperitoneal injections of an equivalent volume of sterile 0.9% NaCl. CoF was isolated from the venom of *Naja naja* (Sigma Chemical Co., St. Louis, Mo.) as described by Ballow and Cochrane (4) and used in experiments 1 and 3. Purified CoF obtained from Cordis Laboratories was used in experiment 2.

In vitro studies. A complement fixation system was used to assess complement activation by *T. lewisi* in immune or nonimmune serum. Sera used as sources of antibody were obtained as follows. Immune sera were collected from infected rats between days 20 and 40 of infection. Two rats that had recovered from infections 14 months earlier were challenged intraperitoneally with doses of 5×10^5 and 3×10^6 trypanosomes administered 4 days apart. The animals were bled 9 days after the second challenge for hyperimmune sera. Nonimmune sera were obtained from normal uninfected rats.

T. lewisi used as antigen were harvested from the blood of donor rats on day 5 or 6 of infection as previously described and adjusted to a final concentration of 27×10^6/ml in (i) $GGVB^{++}$, (ii) GGVB (no metals) containing 10 mM EDTA, or (iii) GGVB containing 4 mM ethylene glycol-bis-(β-aminoethyl

ether)*N*,*N'*-tetraacetic acid (EGTA) and 6 mM $MgCl_2$.

Equal volumes of antibody source and *T. lewisi* were combined and incubated at 37°C for 30 min. When appropriate, the trypanosomes were removed by centrifugation at 15,000 rpm for 15 min at 4°C (Sorvall RC2-B, SS-34 rotor). The residual complement activity of the serum was then measured as described above. Samples containing EDTA or EGTA were recalcified with an equal volume of 4 mM $CaCl_2$ and incubated for 15 min at 0°C before determination of residual complement activity. Controls consisted of sera incubated with the appropriate buffer without trypanosomes.

Statistical analyses. Student's t test was used to determine statistical significance. Values of P less than 0.05 were considered as significant.

RESULTS

Effect of *T. lewisi* infection of normal rats on serum complement levels. Complement activity was measured in normal infected rats at 4-day intervals throughout the course of infection and in uninfected controls bled on the same schedule. The results of a typical experiment are presented in Table 1. The levels of total complement and C4 fell abruptly in infected rats as the number of parasites increased. Low total complement and C4 activities persisted from the peak of infection through the first precipitous decline in parasite numbers. The titers began to return to preinfection levels when most of the parasites had been eliminated. Frequently, the C4 titers after recovery greatly exceeded the preinfection values. A significant reduction of C3 levels also occurred during the infection, but was considerably less pronounced than the reduction of C4. After elimination of the parasites, C3 approached preinfection levels. There was no significant alteration of C6 activity during infection. The uninfected controls showed an increase in complement levels, apparently as a result of the repeated bleedings and the maturation of C4 activity with age, which will be discussed below.

The relationship between number of parasites, duration of infection, and loss of complement activity is best illustrated in Fig. 1, which shows data on individual representative animals. Rat number 1 demonstrated that even a light parasitemia (peak of 5,500 *T. lewisi*/mm^3) resulted in a precipitous drop in C4 activity, but a stimulation of C3 production. With parasitemias of greater numbers and/or duration, C4 activity became reduced to undetectable levels for increasing periods of time. Animals exhibiting total loss of C4 activity also showed a greater overproduction of C4 on recovery from infection. There was a time lag of C3 consumption relative to that of C4, which is not apparent when the values for the infected rats are averaged. C3 consumption correlated well with the maximum number of trypanosomes present in an infection ($r = 0.83$) as seen in the linear regression model of Fig. 2.

Infections of C4-deficient rats. *T. lewisi* infections of C4-deficient rats were compared with those of normal rats. Mean preinfection C4 titer of the normocomplementemic rats was 22,527 50% hemolytic complement (CH_{50}) units/ml of serum (range: 11,800 to 36,000) and that of the C4-deficient rats was 246 CH_{50} units/ml (range: 6 to 475). During the course of these studies, it was noted that weanling Wistar rats exhibited C4 activity ranging from undetectable to normal adult levels. The hypocomplementemic weanlings either developed normal C4 activity by approximately 4 months of age or maintained low C4 levels throughout life (unpublished observations). For this reason, C4 levels of the experimental animals were measured again 3 months after completion of the experiment. At this time the C4-deficient group

TABLE 1. *Serum complement levels[a] in rats infected with T. lewisi and in uninfected controls*

Day of infection	No. of *T. lewisi* in blood[b]	Total C		C4		C3		C6	
		Infected	Controls	Infected	Controls	Infected	Controls	Infected	Controls
4	5,000	78 ± 20	74 ± 26	94 ± 38	103 ± 75	100 ± 6	96 ± 3	97 ± 12	90 ± 17
8	159,000	3 ± 1[c]	111 ± 18	8 ± 5[d]	226 ± 78	72 ± 11[d]	118 ± 10	116 ± 16	97 ± 16
12	62,000	2 ± 1[c]	114 ± 26	4 ± 4[d]	468 ± 146	71 ± 9[e]	114 ± 14	108 ± 21	106 ± 16
16	35,000	21 ± 10[c]	172 ± 32	68 ± 42[e]	425 ± 158	73 ± 7[d]	119 ± 9	111 ± 17	114 ± 17
20	6,000	80 ± 32	144 ± 22	197 ± 192	189 ± 84	79 ± 10	100 ± 7	122 ± 18	116 ± 18
24	0	131 ± 23	115 ± 15	657 ± 260	215 ± 101	87 ± 5[d]	105 ± 5	113 ± 12	117 ± 18

[a] Mean ± standard error of seven infected and six uninfected rats expressed as percentage of preinfection value.

[b] Mean number of *T. lewisi* per cubic millimeter of blood in seven infected rats.

[c] $P < 0.001$. Determined by comparison with uninfected control rats.

[d] $P < 0.01$. Determined by comparison with uninfected control rats.

[e] $P < 0.05$. Determined by comparison with uninfected control rats.

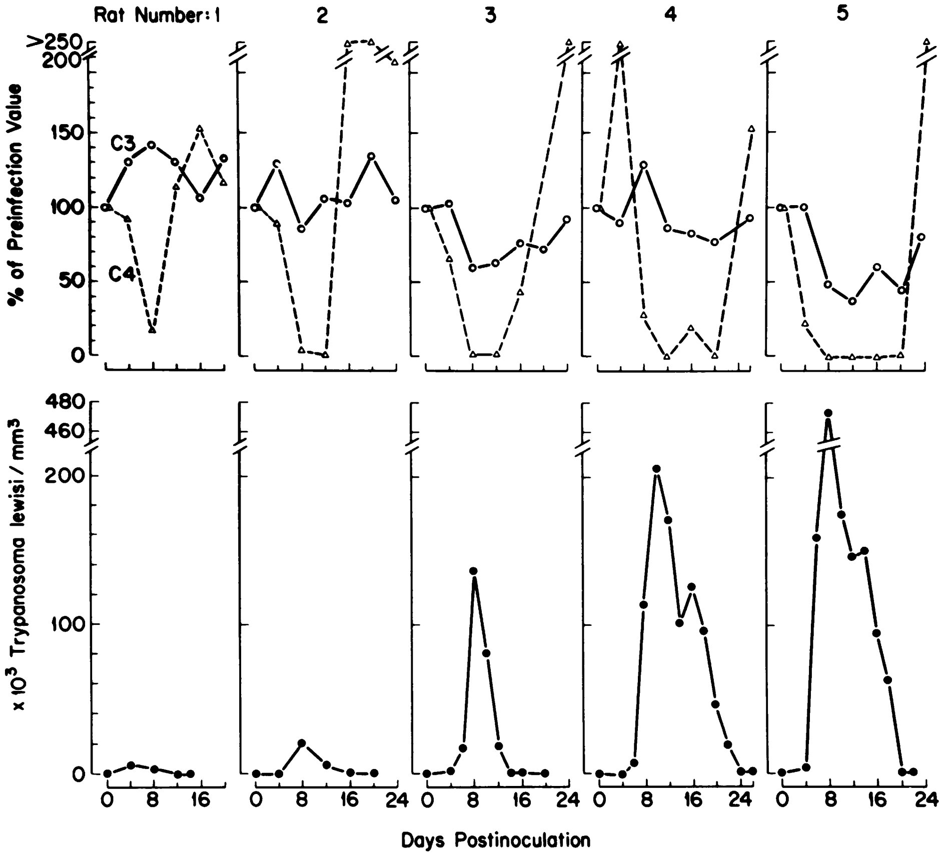

FIG. 1. *Relationship of number of parasites (●) and duration of infection to serum levels of C3 (○) and C4 (△) in representative individual rats infected with T. lewisi.*

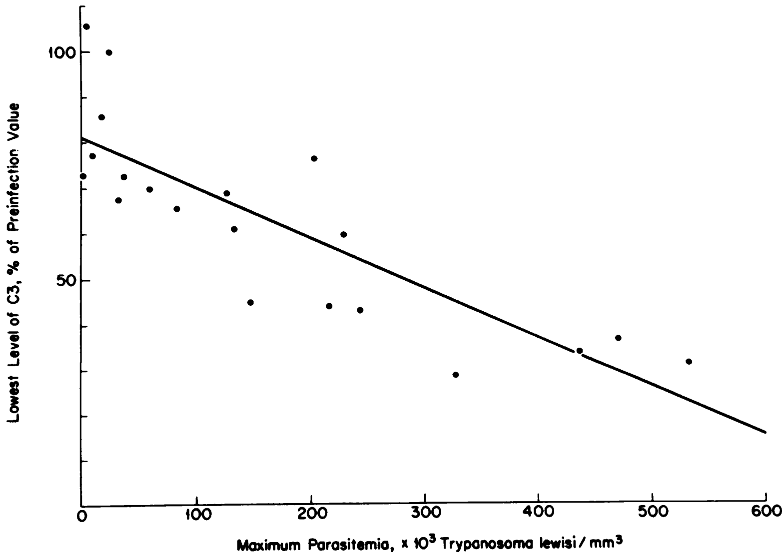

FIG. 2. *Correlation between the maximum decrease of C3 and the maximum number of T. lewisi per cubic millimeter of blood occurring during infection of normal rats.*

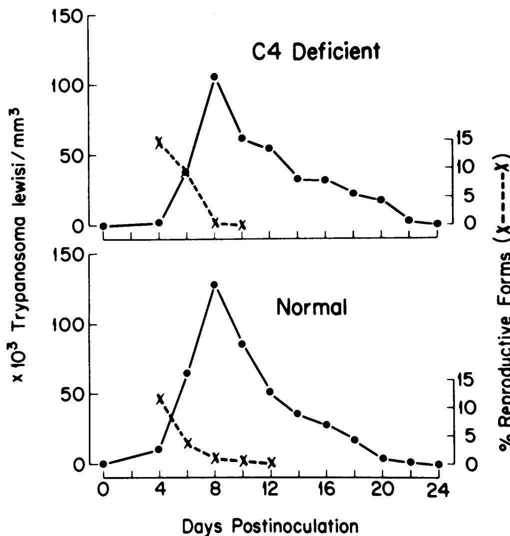

FIG. 3. *Comparison of parasitemias (●) and percentages of reproductive forms (×) in C4-deficient and normocomplementemic rats infected with T. lewisi. The points represent the mean values of 5 C4-deficient and 11 normocomplementemic rats.*

had a mean C4 titer of 4,475 CH_{50} units/ml (range: 1,750 to 7,100) compared with a mean of 82,620 CH_{50} units/ml (range: 33,300 to 158,000) for the normocomplementemic rats. The results of one experiment are seen in Fig. 3. The infections did not differ significantly in number of parasites, percentage of reproductive forms, duration of infection, or rate of parasite elimination. Similar results were obtained when the experiment was repeated with a second group of four C4-deficient and nine normocomplementemic rats. A significant reduction of C3 levels also occurred in infected C4-deficient rats (Table 2). However, there were no significant differences between the C3 levels in C4-deficient infected and normocomplementemic infected animals.

Infections of CoF-treated rats. Infected rats were treated with CoF to determine the effect of C3 depletion on the course of infection. Because the depletion of C3 and late-acting components produced by CoF persists for only 4 to 5 days (12), it was administered at various stages of infection (Fig. 4). In the first experiment, CoF treatment was effected during the reproductive phase. Normal infected rats with a mean of 11.8% reproductive forms on day 4 were randomly divided into two groups. One group received CoF on day 5, whereas the second group remained untreated. There was no significant alteration of the course of infection in terms of numbers of parasites, time of maximum parasitemia, or duration of infection as a result of the CoF treatment (Fig. 4). No differences were observed in the percentage of reproductive forms (Table 3, experiment 1).

CoF was administered on day 8 in the second experiment, which is normally the time of the peak parasitemia. At this time the majority of the parasites were adults, with less than 4.5% present as reproductive forms. The CoF-treated group appeared to have experienced greater parasitemias than the untreated controls (Fig. 4). This, however, was due to the response of a single rat that had a persistent low level of reproduction (2 to 2.5%) through day 14, a peak parasitemia of 1.8 × 10^6/mm³, and a 30-day duration of infection. The infections of the remaining CoF-treated rats lasted from 12 to 22 days compared with 14 to 20 days for the untreated controls. Statistically, none of the differences observed between the CoF-treated and untreated groups was significant (Table 3, experiment 2 for percentage of reproductive forms). To determine whether sustained C3 depletion would result in greater parasitemias, CoF was administered on day 8 and again on

TABLE 2. *Comparison of C3 levels in C4-deficient and normocomplementemic rats infected with T. lewisi and in uninfected controls*

Day of infection	C3 (% of preinfection value)			
	C4-deficient		Normocomplementemic	
	Infected[a]	Control[b]	Infected[c]	Control[d]
4	104 ± 4	117 129	106 ± 4	100 ± 3
8	88 ± 8[e]	156 80	73 ± 7[g]	115 ± 8
12	69 ± 7[f]	136 74	68 ± 5[g]	104 ± 11
16	69 ± 7[g]	145 98	72 ± 4[g]	110 ± 9
20	76 ± 10[e]	117 83	76 ± 5[f]	102 ± 7
24	89 ± 5[e]	125 89	86 ± 3[f]	103 ± 6

[a] Mean ± standard error (SE) of nine infected C4-deficient rats.
[b] Individual values of two uninfected C4-deficient rats.
[c] Mean ± SE of 12 infected normocomplementemic rats.
[d] Mean ± SE of 6 uninfected normocomplementemic rats.
[e] $P < 0.05$. Determined by comparison with uninfected normocomplementemic controls.
[f] $P < 0.01$. Obtained by comparison with uninfected normocomplementemic controls.
[g] $P < 0.0025$. Obtained by comparison with uninfected normocomplementemic controls.

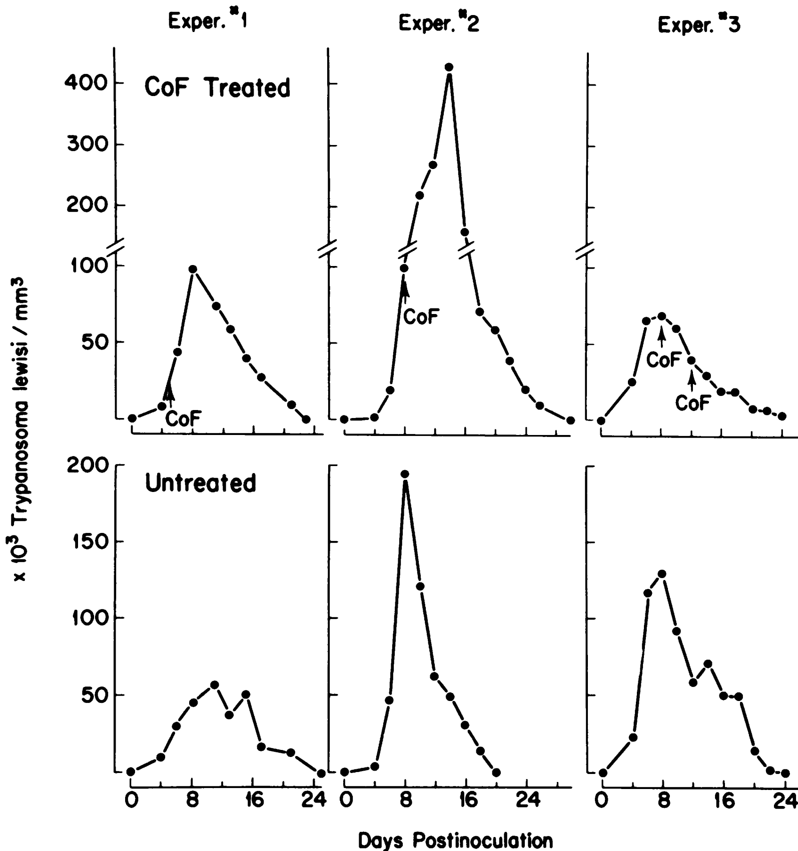

FIG. 4. *Influence of cobra venom factor treatment of rats on course of infection with T. lewisi. Experiment 1: CoF treatment on day 5 during reproductive phase. Experiment 2: CoF treatment on day 8 during early adult stage. Experiment 3: Prolonged CoF treatment (days 8 and 12) through adult stage. Points represent mean values of five to nine animals in each group.*

day 12 in experiment 3. C3 remained at less than 5% of the standard through day 16, yet no significant differences in parasitemias were observed (Fig. 4).

The effect of CoF treatment on the course of infection was also studied in C4-deficient rats. CoF was administered on day 8 of infection. These animals had no increase in the parasitemia or duration of infection when compared with either untreated C4-deficient or CoF-treated normocomplementemic rats.

In vitro studies. Incubation of *T. lewisi* with immune serum usually resulted in a considerable loss of total complement activity (Table 4). An occasional serum (e.g., no. 7) showed no loss of activity. When complement activation via the classical pathway was selectively inhibited by including EGTA in the incubation mixture, complement consumption was substantially reduced or eliminated in most sera. Immune serum no. 7, however, showed an unexpected loss of activity under these conditions. When both classical and alternative pathways were inhibited by incorporating EDTA into the incubation mixture, complement activity of all sera was completely preserved. In contrast to the results with immune serum, there was no loss of complement activity on incubation of trypanosomes with nonimmune serum.

DISCUSSION

The experiments with normocomplementemic rats infected with *T. lewisi* demonstrated a very pronounced activation of the complement system through the classical pathway. Although C4 activity was usually undetectable in the infected rats, C3 levels were only slightly decreased and C6 activity was unaffected. A similar intensive activation of the early components with minimal involvement of C3 and the late-acting components has been observed in vivo in experimental human and simian *Plasmodium* infections (2, 18, 30) and in patients with hereditary angioneurotic edema (34).

TABLE 3. *Comparison of reproductive activity of T. lewisi in untreated and CoF-treated rats*

Day of infection	Percentage of reproductive forms[a]			
	Expt 1		Expt 2	
	Treated	Untreated	Treated	Untreated
4	12.0 ± 3.2	11.5 ± 4.8	7.1 ± 0	7.7 ± 0.9
6	6.0 ± 2.4	6.9 ± 2.3	4.4 ± 1.1	4.3 ± 0.8
8	0.3 ± 0.2	1.4 ± 1.1	1.2 ± 0.9	0.1 ± 0.02
10	0.1 ± 0.02	0	0.4 ± 0.4	0
12	0		0.5 ± 0.5	
14			0.4 ± 0.4	
16			0	

[a] Mean ± standard error of 6 animals per group in experiment 1 and 5 animals per group in experiment 2.

Activation by immune complexes appears to be responsible for the reduced complement levels observed during infections with *T. lewisi*. Live trypanosomes activated complement in vitro in immune serum primarily by the classical pathway, whereas no complement consumption occurred on incubation of *T. lewisi* with nonimmune serum. Current work in this laboratory indicates that immune complexes formed with soluble *T. lewisi* antigens in plasma (7, 16) also activate complement and are at least partially responsible for early depletion of C1 and C4. Further studies are needed to clarify the interactions of *T. lewisi* and its soluble antigens with ablastic and trypanocidal antibodies and complement. Although the decreased complement levels of *T. lewisi*-infected rats can be attributed primarily to immune activation, the possibility of decreased synthetic rates must also be considered in view of recent studies of *Babesia rodhaini* infections. Decreased serum C3 levels in rats infected with this hemosporidian resulted from both an increase in C3 catabolism and a decrease in C3 synthesis (11).

Although complement activation by *T. lewisi* both in vivo and in vitro occurs primarily via the classical pathway, some evidence suggests that activation may also occur through the alternative pathway. C3 levels were similarly reduced during infections in both normocomplementemic and C4-deficient rats. In addition, in vitro complement activation with *T. lewisi* was not regularly and completely inhibited by EGTA in all immune sera tested. It is of interest that *T. cruzi* epimastigotes, but not trypomastigotes, can activate complement through the alternative pathway without the participation of natural or specific antibodies (31).

In spite of the observations of complement activation in *T. lewisi*-infected rats, our work does not support a major role for the complement system in control of these parasites. All parameters of infection were similar in C4-deficient, CoF-treated, and normocomplementemic rats. Although it is possible that the low levels of C4 present in the C4-deficient rats and the low levels of C3 remaining in the CoF-treated animals may have been sufficient to support a crucial complement-dependent function, this is highly unlikely since we did not observe any aberrations in the course of infection in these animals. There was no significant prolongation of either the reproductive or adult stages of infection, nor was there any change in the rate of parasite elimination. In addition, similar studies with the closely related *T. musculi* demonstrated that C3 depletion by CoF treatment of infected mice was sufficient to interfere with parasite elimination (J. A. Jarvinen and A. P. Dalmasso, Abstr. 49th Annu. Meet. Am. Soc. Parasitol., p. 19, 1974). In interpreting the results, it is also important to consider the course of events in normal infected rats. The increasing parasitemia effectively renders the

TABLE 4. *In vitro activation of complement by incubation of immune serum with T. lewisi*

Source of serum	Residual total C activity[a] after incubation for 30 min at 37°C with:		
	T. lewisi + buffer[b]	*T. lewisi* + EGTA[c]	*T. lewisi* + EDTA[d]
Immune rat no.			
1	1.5	127.4	89.2
2	0.6	ND[e]	ND
3	10.6	74.3	ND
4[f]	32.7	73.4	ND
5	51.4	ND	127.0
6[f]	67.1	81.0	ND
7	101.7	58.8	94.6
Nonimmune rat no.			
8	96.8	ND	91.0
9	99.4	ND	ND
10	93.6	ND	ND
11	107.3	87.4	79.4
12	106.6	85.1	ND
13	112.1	81.9	ND

[a] Expressed as the percentage of total C activity of serum after incubation with the appropriate buffer without trypanosomes.

[b] Gelatin-Veronal-buffered saline (GVB) containing 0.5 mM Mg^{2+} and 0.15 mM Ca^{2+}.

[c] GVB containing 2 mM EGTA and 3 mM Mg^{2+}, final concentration.

[d] GVB containing 5 mM EDTA, final concentration.

[e] ND, Not done.

[f] Hyperimmune serum as defined in Materials and Methods.

animals C4 deficient very early in the infection. This deficiency is maintained throughout most of the infection, possibly precluding the participation of complement-mediated opsonization and lysis in the critical stages of parasite elimination. Thus, if a complement-dependent process acts in the control of *T. lewisi*, either it functions extremely efficiently at very low levels of complement or it plays only a secondary role.

The participation of complement has been suggested by studies of infections with other species of trypanosomes. Simian infections with *T. rhodesiense* were characterized by regular reductions of C3 and occasional reductions of C4. The infected monkeys developed a proliferative glomerulonephritis with deposits of immunoglobulin M, C3, and properdin (28). Low total complement and C3 levels in association with disseminated intravascular coagulation were reported in a patient with East African trypanosomiasis (6). Two strains of mice genetically deficient in C5 had courses of infection with *T. musculi* comparable to those in normal controls (17); however, as stated above, C3 depletion by CoF treatment interfered with parasite elimination. Thus, complement-mediated opsonization probably functions in control of this trypanosome whereas immune lysis is unimportant. Mice infected with *T. cruzi* and then treated with CoF had increased parasitemias and early mortality (9, 20), whereas recent results from this laboratory showed no differences in parasitemia or mortality rates between C5-deficient and normocomplementemic mice. Although complement-mediated lysis of *T. cruzi* has been demonstrated with both epimastigotes (1, 31) and trypomastigotes (9, 20) in vitro, the in vivo studies suggest that complement-mediated opsonization rather than lysis may be an important control mechanism in acute infections with this species also. It is apparent that the exact role complement plays in trypanosome infections varies with the species of both host and parasite as well as other factors.

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