Ultrastructural and Immunological Aspects of the Phagocytosis of *Trypanosoma brucei* by Mouse Peritoneal Macrophages

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Trypanosome-activated mouse peritoneal macrophages phagocytized and digested *Trypanosoma brucei* in vitro and in vivo, but in the absence of specific antiserum and complement the degree of phagocytosis was minimal. Ultrastructurally, the parasites attached to the macrophage by their flagella, and ingestion proceeded flagellum first. Once ingested, *T. brucei* was degraded, presumably due to fusion of the parasite-containing phagosome with lysosomes. Contrariwise, normal mouse peritoneal macrophages displayed negligible ability to ingest *T. brucei*, even in the presence of specific antiserum and complement. During trypanosomiasis in deer mice (*Peromyscus maniculatus*), the development of hypergammaglobulinemia correlated with enhanced phagocytosis of *T. brucei* by macrophages, but only at early post-inoculation days (PID 5 to 15). Complement lysis of trypanosomes was not identified in these experiments. Between PID 20 to 30, antiserum and complement either had no phagocytosis-promoting ability or depressed the phagocytosis of *T. brucei* by macrophages. These results indicate that both specific antibody and complement contribute to the ingestion of *T. brucei* by activated macrophages, but that parasite antigenic variation effectively abrogates the phagocytic defense mechanism.

Although the interaction of mononuclear phagocytes and various microbial disease agents has been extensively examined, macrophage-African trypanosome interaction has received little critical investigation.

The early literature is concerned predominantly with *Trypanosoma lewisi*-macrophage interaction. One author (26) stated that the first probable example of immune adherence involved the in vivo attachment of *T. lewisi* to immune rat peritoneal exudate cells ("leukocytes") in the presence of specific antiserum (17). Unfortunately, others were unable to reproduce these results (18), although experimental details were brief or absent (22).

More recent accounts utilizing *T. lewisi* (16), *T. gambiense* (34, 35), and *T. brucei* (20) agree that immune serum enhances membrane attachment, erroneously equated with phagocytosis, but several important aspects of trypanosome-macrophage interaction remain either controversial or simply unknown due to lack of experimentation.

Among the neglected aspects of macrophage-trypanosome interaction are the ultrastructure of phagocytosis and the effect of antibody and

complement upon phagocytosis during sequential disease. It is with these aspects that this report is concerned.

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MATERIALS AND METHODS

Animals. Adult male and female deer mice (*Peromyscus maniculatus*) (24) were used for the induction of trypanosomiasis and for the isolation of peritoneal macrophages. Adult female Swiss-Webster (SW) mice were used for increasing stabilate titer before deer mice were inoculated and for [³H]adenine labeling of *T. brucei*. (Stabilate is frozen [-70°C] stock trypanosomes [7]. By starting each experiment with stabilate trypanosomes, each trial begins with the same variant trypanosome antigen.)

Infective agent/inoculation procedure. *T. brucei* (East African Trypanosomiasis Research Organization strain 110) organisms were stored in sealed capillary tubes at -70°C (7). Upon thawing, the protozoan agent was suspended in Eagle minimum essential medium (MEM)-Alsever solution mixture (50:50, vol/vol) (Grand Island Biological Co., Grand Island, N.Y.), and the suspension was injected subcutaneously into adult female SW mice. Approximately 96 h later (when the infected SW mice were near death due to overwhelming parasitemia), the infected SW mice were anesthetized with ether (Mallinckrodt, St. Louis, Mo.) and bled via axillary incision. The blood was

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pipetted into a 15-ml screw-cap polystyrene centrifuge tube (Corning Glass Works, Corning, N.Y.) containing 1 ml of heat-inactivated horse serum (GIBCO) and 9 ml of Alsever solution. The centrifuge tubes were placed at 4°C, and the erythrocytes were allowed to sediment for 30 min. The supernatant was pipetted into another 15-ml centrifuge tube and centrifuged at $950 \times g$ for 10 min at 4°C in a PR-2 International refrigerated centrifuge. The supernatant was decanted, and the trypanosome pellet was resuspended in approximately 6 ml of MEM-Alsever mixture. From this suspension trypanosomes were counted in a hemacytometer, and an appropriate volume containing 10^4 *T. brucei* organisms was injected intraperitoneally into anesthetized adult deer mice.

Serum immunoglobulin quantitation. For immunoglobulin determination, serum samples were collected from three *T. brucei*-injected deer mice at each of the following post-inoculation days (PID): 5, 10, 15, 20, 25, and 30. Control serum was collected from three normal deer mice. Quantitative radial immunodiffusion was performed by standard methods (8), using commercially prepared immunodiffusion plates for mouse immunoglobulin M (IgM), IgG₁, IgG_{2a}, and IgG_{2b} (Microbiological Associates, Bethesda, Md.).

Macrophage culture technique. Four milliliters of MEM containing a trace of sodium heparin was injected through 20-gauge syringe needles into the peritoneal cavity of exsanguinated experimental and control mice. After approximately 30 s of abdominal massage, the peritoneal cavity was exposed and the lavage fluid was recovered, using sterile pipettes. The cell suspension was pipetted into a 35-mm sterile plastic tissue culture petri dish (Falcon Plastics, Oxnard, Calif.) containing 0.5 to 0.8 ml of heat-inactivated fetal calf serum (GIBCO), approximately 100 IU of potassium penicillin G (E. R. Squibb & Sons, New York), and less than 1 mg of streptomycin sulfate (Pfizer Inc., New York). Cultures were incubated at 37°C in 5% CO₂ in a Wedco incubator (Wedco, Silver Spring, Md.). Peritoneal macrophages from normal mice were allowed to incubate for 5 to 6 h, and those from trypanosome-injected mice were allowed to incubate for 1 to 2 h before decantation, followed by three washes with phosphate-buffered saline (PBS) to remove nonadherent cells.

Hemacytometer counts of lavage fluid before and after incubation revealed that 10×10^6 to 12×10^6 normal peritoneal macrophages and 20×10^6 to 23×10^6 trypanosome-exposed (activated) peritoneal macrophages could be routinely cultivated by this procedure.

Viability of *T. brucei* in vitro. *T. brucei* organisms (1×10^8 to 2×10^8) were cultured in 35-mm plastic petri dishes containing 0.5 ml of MEM and 0.5 ml of Alsever solution. At 0, 0.5, 1, 2, 4, 8, 12, 24, 36, and 48 h, cultures were removed from the incubator and parasite viability (motility) was assessed with a Unitron inverted microscope and in microscopic slide wet-mount preparations.

Electron microscopy. *T. brucei* organisms (10^6) in 0.5 ml of MEM, 0.5 ml of Alsever solution, and 0.5 ml of heat-inactivated fetal calf serum were added to trypanosome-activated macrophage cultures, which were incubated at 37°C in 5% CO₂ for either 15, 30, 60,

or 90 min or 2.5, 4, 7, or 10 h. At these times, individual cultures were removed from the incubator, assessed for parasite viability (motility), washed with PBS to remove excess trypanosomes, and prepared for electron microscopy by methods described previously (31). For the in vivo study, deer mice were inoculated intraperitoneally with 10^5 *T. brucei* organisms. Peritoneal exudates were collected at 5, 15, and 30 min, 1, 2, 4, 8, and 12 h, and 1, 2, 3, 4, and 5 days and prepared for electron microscopy (31).

Labeling of *T. brucei* with [³H]adenine. Adult female SW mice were inoculated subcutaneously with 0.1 ml of a blood-PBS suspension containing three to four parasites per high-power field. The parasites were derived from infected deer mice according to the protocol given below. On PID 4 (the parasite titer is increased in SW mice in 4 days, without antigenic variation occurring [29]), parasitemic SW mice were inoculated subcutaneously with 1 ml of PBS containing 50 μ Ci of [³H]adenine (23 Ci/mmol; New England Nuclear Corp., Boston, Mass.). Eight hours later, these mice were killed by axillary incision and the radiolabeled trypanosomes were isolated by methods described above, except that resuspension was into 10 ml of a 70:30 (vol/vol) mixture of MEM and Alsever solution. After two washes, 0.1 ml of the final wash supernatant was mixed with 0.5 ml of NCS tissue solubilizer (Amersham Corp., Arlington Heights, Ill.); the digest was then pipetted into 10 ml of a standard liquid scintillation cocktail (Omnifluor [New England Nuclear Corp.] in toluene [Mallinckrodt]) in screw-cap glass scintillation vials (45 by 25 mm). These final wash supernatant samples are a control on washing efficiency, and were performed on every experimental and control sample. Radioactivity was counted in a Packard model 3320 liquid scintillation spectrometer. To verify that the radiolabel was within the parasites, the final trypanosome pellet was digested with NCS tissue solubilizer and prepared for scintillation counting. In addition, smears were prepared for autoradiography as follows. A final trypanosome pellet was resuspended in MEM-Alsever (1 ml), and one drop was streaked across microscope slides. After drying and fixation (methanol), the slides were overlaid with NTB-2 nuclear track emulsion (Kodak, Rochester, N.Y.) and exposed at -70°C for 24 to 48 h. Slides were developed for 3 min in D-19 (Kodak), fixed for 3 min in acid fixer (Kodak), and stained with Giemsa. Autoradiographic preparations were examined and photographed with a Zeiss Photomicroscope II, using Panatomic-X film (Kodak).

Phagocytosis assay. Radiolabeled parasites (2.5×10^6), originally isolated from *T. brucei*-infected deer mice on PID 5, 10, 15, 20, 25, and 30, were incubated for 1 h (37°C; 5% CO₂) with freshly prepared (approximately 2 h) mouse peritoneal macrophage cultures derived from *T. brucei*-injected deer mice. After 1 h of incubation, culture supernatants were pipetted into test tubes (10 by 75 mm), and the macrophage cultures were washed with PBS until free parasites and parasites attached to macrophages by flagella could no longer be seen in an inverted microscope. The washed macrophage cultures were flooded with PBS and resuspended by rubber-spatula agitation. Macrophages were pipetted into test tubes (10 by 75 mm), and after

centrifugation both macrophage culture supernatant pellets (containing excess parasites) and macrophage pellets were prepared for scintillation counting.

Experimental sequences, run in duplicate, varied only in incubation supernatants. The total volume of the incubation media varied from 0.8 to 1.2 ml, depending upon the various combinations of MEM, Alsever solution, and serum used. One sequence included homologous antiserum (isolated from the same mouse from which the trypanosomes were isolated), and another sequence included heat-inactivated (56°C, 30 min) homologous antiserum. Controls included samples with normal deer mouse serum and samples without serum (MEM and Alsever solution). Method controls included negative samples (2.5×10^8 radiolabeled trypanosomes incubated in MEM and Alsever solution without macrophages) and positive samples (stabilate parasites plus pooled antistabilate antiserum). Positive controls included heat-inactivated pooled antistabilate antiserum with and without the addition of guinea pig complement. Zero-time controls were also examined. To further explore the activation of macrophages derived from *T. brucei*-injected mice, a sequence using normal deer mouse peritoneal macrophages was included in the study.

RESULTS

Viability of *T. brucei* in vitro. In MEM-Alsever solution mixture, *T. brucei* re-

tained motility through 2 h of incubation. By 4 h, approximately half the parasites had ceased flagellation, indicating degeneration and parasite death. In smears from both 8- and 12-h cultures, individual motile parasites could be identified by light microscopy, but all parasites cultivated longer than 12 h were inactive. From these data we established a constant incubation period of 1 h in the phagocytic experiments.

Electron microscopy. Since the interaction of *T. brucei* and deer mouse peritoneal macrophages was similar in vivo and in vitro, no advantage accrues in discussing the two situations separately. In neither case was immune serum included; therefore the events described are not dependent on antibody or complement. The findings were similar for all incubation samples except the earliest samples and those in vitro samples that were incubated for longer than 2 h. By 15 min of incubation, the most frequent finding was various tangential and cross-sectional profiles of trypanosome flagellae within macrophage cytoplasmic vacuoles (Fig. 1). In samples incubated for longer than 2 h in vitro, excessive death of extracellular trypanosomes hindered morphological analysis. That is, lysed and fragmented parasites were rapidly ingested,

FIG. 1. Two cross-sections of a trypanosome flagellum are present within cytoplasmic vacuoles of a macrophage (in vitro). Uranyl acetate/lead citrate, $\times 10,000$.

filling most macrophages with numerous debris-laden vacuoles. At incubation periods of less than 2 h, morphologically intact trypanosomes were easily identified adjacent to macrophages.

T. brucei attaches to macrophages by its anterior end, that is, by its flagellum. The size and motility of the parasite inhibit immediate phagocytosis. The initial result of this struggle is a compromise of opposing forces, producing multiple membranous whorls around the parasite's flagellum (Fig. 2). Pseudopodia extend along the flagellum, and eventually the parasite is ingested (Fig. 3 and 4). Initially the vacuolar membrane is tightly adherent to the parasite's external membrane (Fig. 5). Degradation of the ingested trypanosome, presumably due to fusion of lysosomes with parasite-containing phagosomes, ensues (Fig. 6). The integrity of the parasite's external membrane is lost due to erosion or dissolution at multiple sites. Similarly, the parasite's submembranous microtubular array becomes disorganized, although some portions partially resist degradation. The parasite's cytosol begins to appear electron lucent, and the normal trypanosome silhouette is distorted. As degra-

dation continues, the once tight vacuolar membrane becomes loosely applied around the parasitic debris. Nuclear and kinetoplasmic deoxyribonucleic acid-related structures become dense and shrunken. Cytoplasmic organelles become unidentifiable and are replaced with irregular membranous, vesicular, and granular debris of varying electron-resistant densities. In the final stages, the last remnant identifiable is the flagellar microtubular array, still apparent in the universal "9-2" arrangement, but lacking intact external membranes.

Labeling of *T. brucei* with [^3H]adenine. Individual parasites did not contain equal amounts of radioactivity (as assessed by autoradiography) (Fig. 7), but radiolabeled trypanosome pellets contained hundreds of thousands of dpm compared with 100 to 300 dpm in final supernatants (as assessed by liquid scintillation counting) (Table 1).

Phagocytosis assay. Normal mouse peritoneal macrophages displayed meager ability to phagocytize [^3H]adenine-labeled *T. brucei*. Phagocytosis by normal macrophages was similar regardless of the content of the incubation

FIG. 2. Interaction of macrophages with trypanosome flagella results in multiple membranous whorls, which appear to fuse. Uranyl acetate/lead citrate, $\times 60,000$.

FIG. 3. *Phagocytosis of T. brucei by a mouse peritoneal macrophage. Pseudopodia are extending along a flagellum. Uranyl acetate/lead citrate, $\times 20,000$.*

FIG. 4. *Intact trypanosome within a mouse peritoneal macrophage. Uranyl acetate/lead citrate, $\times 20,000$.*

FIG. 5. Intact trypanosome within a "tight" cytoplasmic vacuole of a mouse peritoneal macrophage. Uranyl acetate/lead citrate, $\times 30,000$.

medium (Table 2). Activation of macrophages by exposure to *T. brucei* resulted in enhanced phagocytosis of the parasites (Fig. 8). There were no significant differences ($P < 0.05$) in the phagocytosis of radiolabeled trypanosomes by activated macrophages in the presence or absence of normal serum.

Both heat-inactivated and non-heat-inactivated antisera enhanced phagocytosis of *T. brucei* by activated macrophages at early PIDs. Heat inactivation reduced the stimulatory effect (Fig. 9). In the presence of antisera, phagocytosis increased from PID 0 to 5, peaked at PID 10, declined between PID 15, 20, and 25, and reached depressed levels (compared with no serum and normal serum controls) at PID 25 and 30 (Fig. 9).

Controls. *T. brucei* could adhere to plastic, as has been reported for other trypanosomes (11) (Table 3). The number of zero-time control dpm was low and does not invalidate the method (Table 3). Positive controls, consisting of stabi-

late parasites and specific agglutinating antisera, gave results similar to the experimental data, e.g., heat inactivation depressed the stimulatory effect, whereas addition of guinea pig complement enhanced the antiserum's phagocytosis-promoting ability (Table 3).

Immunoglobulin quantitation. The IgM concentration in serum from *T. brucei*-injected mice was normal at PID 5 (normal IgM concentration for deer mice ranges from 0.5 to 0.6 mg/ml, $n = 3$), 1.5 times normal at PID 10 (0.85 mg/ml), and approximately twice normal at PID 15, 20, 25, and 30 (Fig. 10).

IgG₁ concentration in serum from *T. brucei*-injected mice was below normal at PID 5 (3.7 mg/ml; normal IgG₁ concentration for deer mice ranges from 6.5 to 7.0 mg/ml; $n = 3$), steadily increased between PID 5 and 15, and remained approximately 30% elevated between PID 15 and 30 (Fig. 10).

Neither IgG₂ nor IgG_{2b} radial immunodiffusion plates yielded visible precipitin rings with

FIG. 6. Partially degraded trypanosome within a cytoplasmic vacuole of a mouse peritoneal macrophage. Abbreviations: (K) Kinetoplast, (F) flagellum, (N) parasite nucleus. Uranyl acetate/lead citrate, $\times 10,000$.

FIG. 7. Autoradiograph of $[^3\text{H}]$ adenine-labeled *T. brucei*. Giemsa, $\times 1,200$.

TABLE 1. Incorporation of $[^3\text{H}]$ adenine by *T. brucei* in vivo

<i>T. brucei</i>	Supernatant
320,861 ^a	198 ^b
271,464	121
270,985	291

^a Mean, duplicate samples (dpm/ 2.5×10^6 parasites).

^b dpm/0.1 ml.

either experimental or control deer mouse serum.

DISCUSSION

Macrophage activation is often correlated with enhanced resistance to infectious disease agents. For example, *Listeria monocytogenes* will proliferate within normal macrophages, resulting in macrophage lysis, but this bacterial growth pattern is abrogated within activated macrophages (21, 28). Not surprisingly, mechanisms have evolved that allow certain microbial

TABLE 2. Phagocytosis of [³H]adenine-labeled *T. brucei* by normal mouse peritoneal macrophages in vitro

PID	Incubation medium		
	Alsever and MEM	+ Normal serum	+ Homologous antiserum
5	681 ^a	ND ^b	420
10	219	120	354
15	109	185	123
20	362	542	418
25	214	282	365
30	178	63	130
Mean	294	238	302
SE ^c	±60	±59	±40
P < 0.05 ^d	Neg. ^e	Neg.	Neg.
Mean ± SE (total)	257 ± 30		

^a Values represent mean of duplicate samples, given in dpm.
^b ND, Not done.
^c SE, Standard error.
^d Difference of means, each group compared to the other two groups.
^e Neg., Negative.

FIG. 8. In vitro phagocytosis of [³H]adenine-labeled *T. brucei* by trypanosome-activated (A, B) and normal (C-E) mouse peritoneal macrophages. Incubation media: (A, C) MEM-Alsever solution, (B, D) plus normal mouse serum; (E) plus mouse antitrypanosome antiserum. Values represent mean ± standard error.

FIG. 9. Phagocytosis of [³H]adenine-labeled *T. brucei* by trypanosome-activated mouse peritoneal macrophages in vitro, showing the effect of antiserum and complement during sequential disease. Each point represents the mean of duplicate samples. Serum controls = normal deer mouse serum and no serum.

TABLE 3. Method controls for phagocytosis of [³H]adenine-labeled *T. brucei* by trypanosome-activated mouse peritoneal macrophages in vitro

Control	dpm ^a
Radiolabeled trypanosomes incubated without macrophages	75
Radiolabeled trypanosomes incubated with activated macrophages; zero-time incubation	52
Radiolabeled trypanosomes incubated with activated macrophages and: ^b	
Heat-inactivated antiserum	30,582
Heat-inactivated antiserum plus guinea pig complement	50,419
Non-heat-inactivated antiserum	55,078

^a Mean, duplicate samples.
^b Stabilize parasites plus specific pooled mouse antistabilate antiserum.

agents to evade the enhanced powers of activated macrophages. In this regard, the prototype organism is *Mycobacterium tuberculosis*, which survives within macrophagic phagosomes, but only when the bacteria are not damaged (intact cell wall) (3). Although *M. tuberculosis* inhibits phagolysosomal formation, evidence has been reported that *M. tuberculosis* is resistant to lysosomal degradation when phagolysosomal formation is induced by preincubation of the bacterium with either normal or immune rabbit

FIG. 10. Immunoglobulin concentration in sera from deer mice injected with *T. brucei*, PID 5 to 30. Each point represents the mean of three determinations.

serum (4). *Mycobacterium lepraemurium* does not inhibit phagolysosomal formation, but survives and multiplies within phagolysosomes despite the presence of hydrolytic enzymes (10). Other organisms, including *Mycoplasma microti*, *Toxoplasma gondii*, and *Encephalitozoon cuniculi*, survive within macrophages by inhibiting phagolysosomal formation (10, 15, 36). A third mechanism utilized by organisms to escape destruction by macrophages is illustrated by the ability of *Trypanosoma cruzi* to "escape" from phagosomes and to reside directly within the macrophage's cytoplasm (27). None of these mechanisms were apparent in this study of *T. brucei*-macrophage interaction. Similarly, lysis of macrophages in culture, as seen with *Toxoplasma gondii*, *Leishmania* spp., and *Trypanosoma cruzi*, was not apparent in macrophage cultures exposed to *T. brucei*.

The electron microscopic results reported here indicate that although *T. brucei* attaches by its flagellum to mouse macrophage membranes, rapid ingestion is unlikely. Multiple membranous whorls surrounding trypanosome flagella provide morphological evidence that the parasite's motility partially inhibits phagocytosis. Contrarily, degenerate trypanosomes are effectively ingested.

Flagellar attachment appears to be common in the interaction between macrophages and flagellated organisms. *T. lewisi*, *Leishmania donovani*, and *L. mexicana* have been reported

to attach to macrophages by their flagella (2, 9, 23). The biochemical and biophysical nature of flagella-macrophage attachment is not known, although previous reports conclude that flagellar attachment of *T. lewisi* to macrophages is enhanced when immune macrophages are utilized (9).

Morphologically intact trypanosomes were present within tight vacuoles, while degenerate trypanosomes were found within loose vacuoles. This is identical to a previous ultrastructural examination of *L. donovani*-macrophage interaction (1). Since preliminary experiments carefully defined the incubation medium and incubation duration necessary to insure extracellular parasite viability, we conclude that the sequence from tight vacuole to loose vacuole is physiological, not artifactual due to the ingestion of viable and nonviable parasites.

Morphological data indicate that trypanosome infection induces macrophage activation, and functional studies indicate that macrophage activation correlates with enhanced phagocytic capability. An immediate question arises since no effort was made to equilibrate the number of macrophages in activated macrophage cultures with the number of macrophages in normal macrophage cultures. Does twice the number of macrophages explain fourfold enhancement of phagocytosis? Probably not, for three reasons: (i) the uptake of [³H]adenine-labeled trypanosomes by normal macrophages was close to background counts, indicating negligible uptake; (ii) phagocytosis of radiolabeled trypanosomes by normal macrophages was similar regardless of the incubation medium content, including specific antiserum; and (iii) incubation controls ([³H]adenine-labeled *T. brucei* cultured without macrophages) gave background dpm of 75, and individual parasites were apparent through an inverted microscope attached to the plastic culture dish. Similar phenomena have been reported previously (11). In trypanosome-activated macrophage cultures, the plastic culture dishes were approximately 95% covered by macrophages, leaving a small surface area to which trypanosomes might attach. Contrarily, in normal macrophage cultures considerably more exposed plastic was available, and even though washing was extensive, any attachment of trypanosomes to the culture dishes was important since dpm were low. These factors indicate that the important difference between activated and nonactivated macrophage cultures was the relative functional maturity of the macrophages present, not the number of macrophages in culture.

In African trypanosomiasis, hypergamma-

globulinemia is widely recognized (12, 24, 30) and was not an unexpected finding. The secretion of IgM correlated with the peak of phagocytic enhancement afforded by serum at PID 10. The peak phagocytic activity at PID 10 correlated with reduction of parasitemia in vivo. Trypanosome-injected deer mice are heavily parasitemic at PID 10, but display greatly reduced peripheral blood parasite counts by PID 15. The reason for depression of IgG₁ serum concentration at PID 5 and 10 is not known, but may reflect increased vascular permeability due to direct and indirect effects of trypanosomes, e.g., kinin liberation (5). Alternatively, the depression of IgG₁ at PID 5 and 10 may reflect IgG₁ reaction with the parasites, indicating that IgG₁, and not IgM, is responsible for protection against trypanosomes at early PIDs. Despite hypergamma-globulinemia, phagocytosis of [³H]adenine-labeled *T. brucei* rapidly declined after PID 10 to 15, indicating that the trypanosomes were not recognized by the serum antibodies in the infected mice, presumably due to parasite antigenic variation.

The depressed in vitro phagocytic activity of deer mouse peritoneal macrophages at PID 25 and 30 may result from either of two possibilities. First, the macrophage's intrinsic phagocytic capabilities may be depressed. This seems unlikely (25), and could be examined by challenging trypanosome-infected deer mice with *Listeria monocytogenes*. Second, cytophilic antibody titers may be elevated, and nonvariant antigen-specific cytophilic antibody may interfere with macrophage-trypanosome interaction. During chronic trypanosomiasis, opsonins do not recognize the displayed trypanosome antigen(s), but cytophilic antibody attaches to macrophage Fc receptors, and the Fc receptor mediates antibody-trypanosome attachment to macrophages (33).

A previous report (34) that complement does not affect phagocytosis of trypanosomes is in conflict with results reported here. Although complement facilitated phagocytosis in our experiments, the mechanism was not clarified. A previous report (19) provides evidence that complement-mediated lysis of *T. rhodesiense*, *T. equiperdum*, and *T. brucei* occurs in vitro, but similar results, although of lesser degree, were reported for heat-inactivated as well as normal serum, suggesting that factors other than complement contributed to trypanolysis. Another report (13) that examined the clearance of *T. muscoli* in complement-deficient mice concluded that complement-mediated lysis was not a significant pathway in the elimination of the parasites, but did not exclude the possibility that C3 receptor-mediated phagocytosis was impor-

tant. In experiments reported here, lysis of trypanosomes in the presence of antiserum and complement was not apparent. Even when agglutination was evident, trypanolysis was not found.

The activation of macrophages by trypanosomes has been suggested by a previous study (25), and a more recent report (6) indicates that immunosuppression during trypanosomiasis may be partially mediated by macrophages, although conflicting results are available (14). Ironically, macrophage activation during trypanosomiasis may confer enhanced resistance to intracellular pathogens while simultaneously rendering the trypanosome-infected animal immunologically incompetent against other infectious disease agents.

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