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Trypanosoma brucei Infection Stimulates Receptor-Mediated Phagocytosis by Murine Peritoneal Macrophages

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Trypanosoma brucei infection results in hypertrophy of the reticuloendothelial system. Peritoneal macrophages from infected mice were larger, spread out more on glass, and had increased receptor-mediated phagocytic activity both for C3b- and immunoglobulin G-coated erythrocytes. These observations suggest that macrophages were activated as a result of this infection.

African trypanosomiasis is characterized by multiple cycles of parasitemia and remission. In the course of infection, millions of parasites are destroyed by macrophages, which results in hyperplasia and hypertrophy of tissue macrophages, many of which contain remnants of trypanosomes (11). Macrophages often also show morphological evidence of erythrophagocytosis, suggesting that reticuloendothelial system activity is increased. The effect of trypanosomiasis on macrophage phagocytic activity has not been tested directly. Therefore, we measured receptor-mediated phagocytosis by isolated peritoneal macrophages from mice infected with *Trypanosoma brucei*.

CBA × C57BL/10 female mice, raised under specific pathogen-free conditions, were infected when they were 16 to 20 weeks old. *T. brucei* NIM6, derived from Serengeti/66/SVRP/42 (1), was cloned from stabilates as previously described (4). Four-hundred trypomastigotes in Kreb's glucose solution were injected intraperitoneally. The course of the resulting parasitemia was determined by counting the number of parasites in 1 drop of tail vein blood (10). Each group of six mice was infected on a different day so that on a single day we could test macrophages from mice at different stages of infection.

Macrophages were washed from mouse peritoneal cavities with iced phosphate-buffered saline (PBS). The peritoneal cells from four to six mice in each group were pooled and suspended in RPMI 1640 plus 1% heat-inactivated fetal calf serum (FCS) at a concentration of 2×10^6/ml for resident macrophages and 1.25×10^6/ml for cells from infected mice. We added 2.0 ml of the cell suspensions to 35-mm plastic petri dishes (Nunc, Copenhagen, Denmark), each of which contained three 13-mm glass cover slips. Peritoneal cells were allowed to adhere for 2 h at 37°C in a 6% CO_2 atmosphere, and then nonadherent cells were removed by rinsing with Hanks balanced salt solution (HBSS). The RPMI 1640–1% FCS was then replaced.

Phagocytosis was measured by using trinitrophenol-conjugated sheep erythrocytes (TNP_{10}-SRBC) (14). A 5% suspension of TNP_{10}-SRBC was incubated for 30 min at 37°C either with mouse anti-DNP-KLH diluted 1:500 in Veronal-buffered saline (VBS) or with K7, a monoclonal immunoglobulin M (IgM) anti-DNP (gift of Malcolm Kennedy, National Institute for Medical Research, London, England), diluted 1:3,000 in VBS. The IgM-treated cells were washed once in VBS and then suspended in an equal volume of a 1:4 dilution of fresh A/J strain mouse serum (C5 deficient) for 10 min at 37°C to make E-IgM-C. Complement fixation was ended by diluting the mixture 1:100 with cold PBS. After washing, all opsonized SRBC were diluted to 0.5% with RPMI 1640–1% FCS, and 200 μl was added to the macrophage monolayer. Petri dishes were then reincubated at 37°C in a 6% CO_2 atmosphere for 2 h, after which free SRBC were removed by washing with HBSS. The cover slips were dipped in either water or PBS and then fixed in 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer. Adherent macrophages were examined at 1,000× magnification, using a phase-contrast microscope (9). Slides were coded before they were examined. The number of SRBC per 100 macrophages (phagocytic index [PI]) was determined by examining at least 200 consecutive macrophages on each cover slip that had been dipped in water before fixation. Duplicate cover slips were prepared for each test group, and the mean PI from duplicate preparations was calculated. The cover slips that were dipped in PBS before fixation were

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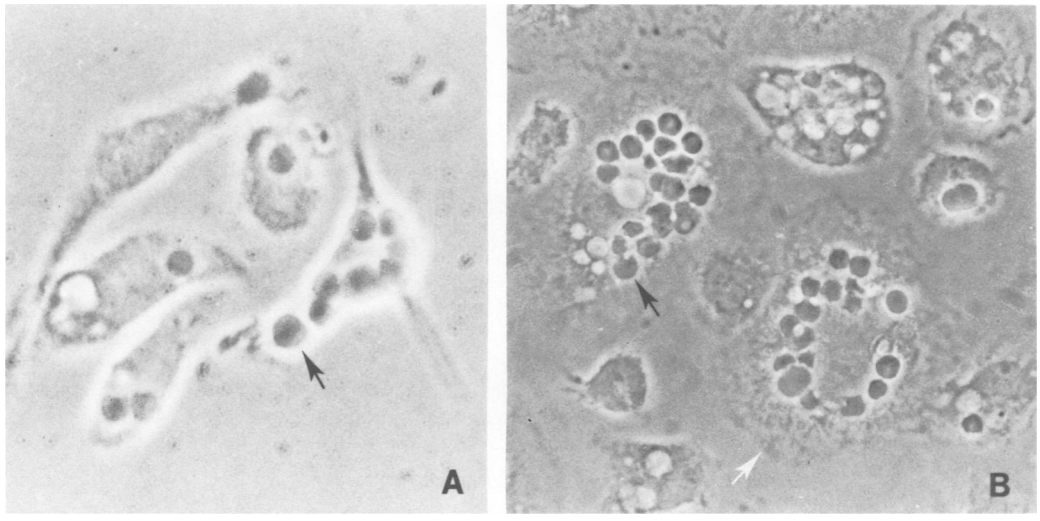

FIG. 1. Phase-contrast photomicrographs (×600) of adherent peritoneal macrophages from normal and *T. brucei*-infected mice after incubation with TNP_{10}-SRBC opsonized with anti-DNP-KLH. Coverslips were dipped in water before fixation to lyse uningested SRBC. Normal macrophages (A) appear spindly and have a compact cytoplasm. The macrophages from mice on day 14 of infection (B) are large, vacuolated, and have a spreading cytoplasm with indistinct borders (white arrow). Most cells contain darkly refractile SRBC (large arrows).

used to determine adherence. Each experiment was done twice.

Macrophages from infected mice were larger and spread out more on glass than did normal peritoneal macrophages (Fig. 1). There was no difference, however, in the percentage of adherent cells that bound C3b-coated TNP_{10}-SRBC; in all groups, SRBC were attached to 80 to 90% of glass adherent cells. We could not determine an adherence index because the large number of attached SRBC per macrophage precluded an accurate count.

All mice became infected with *T. brucei*. The course of the parasitemia is shown in Fig. 2. Parasites were present in the peritoneal wash on all days that macrophages were collected except day 4.

The PI for peritoneal macrophages obtained from mice at different intervals after infection is also illustrated in Fig. 2. C3b-mediated phagocytosis did not increase until day 6 of infection, but thereafter was increased. There was no phagocytosis (PI < 20) of TNP_{10}-SRBC that were incubated with only K7 or A/J serum. On day 4 of infection, the PI for IgG-coated TNP_{10}-SRBC was already three times the control value (normal macrophages). However, on day 13, there was a decrease in the PI to the control level. After that day, the PI increased again as the infection progressed. The transient decrease in IgG-mediated phagocytosis coincided with the stage of trypanosomiasis when there are circulating immune complexes (13), and these may have inhibited IgG-mediated phagocytosis (6).

This study confirms a recent report that trypanosomiasis increases the phagocytic activity of peritoneal macrophages (17). In that study, mice were infected with *T. musculi*, which causes a self-limited infection in mice. Phagocytosis was measured by the uptake of 99mTc sulfur colloid, a small particle that does not require opsoninization for phagocytosis. In this study, we measured receptor-mediated phagocytosis and found that both Fc and C3b receptor-mediated phagocytoses were increased during *T. brucei* infection. The increase in C3b-mediated phagocytosis is especially interesting because only activated peritoneal macrophages ingest SRBC via their C3b receptors (7). Although the increase in C3b-mediated phagocytosis was not as dramatic as has been reported in other systems (6, 7), we tested the macrophages after only 2 h of incubation in vitro; prolonged incubation in vitro increases the phagocytic activity of adherent macrophages (10). In our experiments, the PI of macrophages from infected mice was comparable to that of thioglycolate-elicited macrophages that were cultured in parallel (data not shown).

Both by morphological criteria and phagocytic activity, macrophages from infected mice were activated (3). It is not possible to determine the mechanism of macrophage activation from our experiments. The pathology of *T. brucei* in mice includes a mild peritonitis which results in increased numbers of macrophages in the peritoneal cavity during infection (2). Since peritoneal macrophages participate in the destruction of

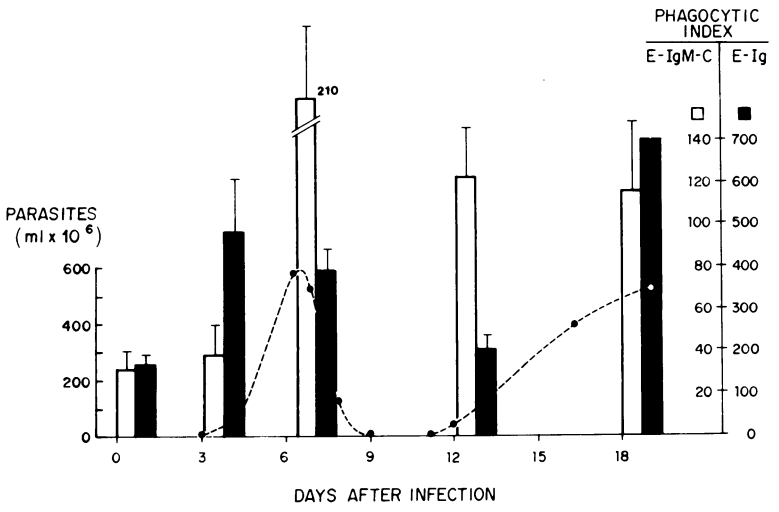

FIG. 2. The course of parasitemia is illustrated by the solid line. The PI for E-IgG (solid bars) and for E-IgM-C (open bars) is also illustrated. Each PI is the mean of two separate experiments and duplicate determination for each experiment. Mice on day zero were uninfected controls. The bars indicate one standard error of the mean.

trypomastigotes, it is possible that phagocytosis per se resulted in peritoneal macrophage activation (15). However, mice also develop delayed hypersensitivity against *T. brucei* (5), so that macrophage activation could have been due to production of a lymphokine by sensitized T cells (8).

It is well established that activated macrophages are necessary for host defense against intracellular trypanosomes and other intracellular protozoa (12). It is not clear whether macrophage activation is of any value to a host infected with an extracellular protozoan such as *T. brucei*. It may be that macrophage activation can increase the efficiency of phagocytosis and thereby speed the clearance of opsonized parasites (16). Since increased phagocytic activity is not parasite specific, it is likely that macrophage activation is also responsible for increased erythrophagocytosis by the reticuloendothelial system and thereby contributes to the anemia that invariably complicates this infection.

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