

## Macrophage Function During *Trypanosoma musculi* Infection in Mice

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Quantitative and functional changes in peritoneal macrophages from mice infected with *Trypanosoma musculi* were investigated. Increases in the number and size and in the protein content of peritoneal macrophages and the presence of parasites in the peritoneal cavity were observed during the course of parasitemia. Induced releases of H<sub>2</sub>O<sub>2</sub> by macrophages were increased during parasitemia, but H<sub>2</sub>O<sub>2</sub> release returned to normal after infection. A correlation was also observed between parasitemia and <sup>99m</sup>Tc colloidal uptake by peritoneal macrophages. These changes in macrophage function may indicate an activation of these cells.

Macrophages play an important role in host resistance to intracellular pathogens, such as *Toxoplasma gondii* and *Trypanosoma cruzi* (10, 15). Immunity to these intracellular infections requires that sensitized lymphocytes stimulated by antigen interact with macrophages and that macrophages undergo a number of metabolic and functional changes generally referred to as "activation" (7, 11). The role of macrophages in parasitic infections by parasites which do not develop intracellularly is less well defined. But there is evidence that macrophages are modified during various parasitic infections (21). According to Viens et al., the immunological response of mice to *T. musculi* is thought to involve an interaction of both humoral and cellular factors (19). The role of the thymus has been revealed by experiments with thymectomized mice. The roles of serum and of the spleen cells from *T. musculi*-immunized mice were revealed by experiments in which they were transferred to another batch of mice just infected with *T. musculi* (18). Nevertheless, the immune effector mechanisms controlling the development and elimination of *T. musculi* remain unclear. As shown by Taliaferro and Pavlinova and by Brooks and Reed, the early control of *T. musculi* parasitemia may be due, at least in part, to macrophages (1, 17). As an initial step to clarify the role of macrophages in resistance to *T. musculi* infection in mice, we investigated changes in macrophage function at different periods of the infection.

### MATERIALS AND METHODS

**Mice.** Female Swiss mice weighing 18 to 20 g were purchased from Evic Ceba (Zone Industrielle, Blanquefort, France). The mice were matched for age before the start of each experiment.

**Infection with *T. musculi*.** The Partinico II strain of *T. musculi* used in this investigation was originally obtained from the London School of Hygiene and Tropical Medicine (5). Mice were infected intraperitoneally by injecting normal recipients with  $5 \times 10^4$  *T. musculi* that were freshly isolated from the blood of donor mice.

**Tissue culture of peritoneal macrophages.** The peritoneal cavity of each mouse was washed with 5 ml of NCTC 109 medium containing 10 U of heparin per ml, 100 U of penicillin per ml, 100  $\mu$ g of streptomycin per ml, and 25 mM HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid) buffer, and the cells were collected in siliconized centrifuge tubes. After centrifugation and cell count,  $1.5 \times 10^6$  macrophages were allowed to adhere at 37°C under 5% CO<sub>2</sub>-95% air for 45 min in a tissue culture petri dish (35 by 10 mm; model 3001F, Falcon Plastics, Oxnard, Calif.). The dishes were vigorously rinsed to remove nonadherent cells. The adherent cells were cultured in NCTC 109 supplemented with 10% heat-inactivated fetal calf serum, 100 U of penicillin per ml, 100  $\mu$ g of streptomycin per ml, and 25 mM HEPES buffer. Adherent cells were counted and examined morphologically by neutral red uptake and May-Grünwald-Giemsa stain; granulocytes did not exceed 1%.

**Uptake of <sup>99m</sup>Tc-sulfur colloid.** <sup>99m</sup>Tc-sulfur colloid was prepared as described elsewhere (20) and diluted to one-fifth with culture medium. A 1.5-ml amount of this radioactive solution was then added to each culture dish. Cells were incubated for 180 min at 37°C under 5% CO<sub>2</sub>-95% air. At the end of the incubation, the tissue culture dishes were thoroughly washed with phosphate-buffered saline, then dried, and assayed for their radioactivity. Each result is a mean of four dishes.

**Assay for H<sub>2</sub>O<sub>2</sub> release.** The assay for H<sub>2</sub>O<sub>2</sub> release was by the method of Root et al. (14). H<sub>2</sub>O<sub>2</sub> releases were recorded with and without a membrane-active agent, PMA (12-*O*-tetradecanoyl-phorbol 13-acetate) (Sigma Chemical Co., St. Louis, Mo.), triggering hydrogen peroxide release (9).

**Protein content of macrophages.** The amount of macrophage monolayer protein was determined by the method of Lowry et al. (6).

## RESULTS

**T. muscui infection.** A prepatent period of 3 to 4 days was followed by a phase of rapidly increasing parasitemia, reaching a peak around 11 days with  $4 \times 10^4$  to  $6 \times 10^4$  parasites per  $\text{mm}^3$ . The trypanosome numbers remained relatively constant during a plateau phase lasting about 9 days. The parasitemia declined sharply, and parasites disappeared from the blood around 24 days (Fig. 1). During the plateau phase of parasitemia many *T. muscui* were seen in the peritoneal cavity (around 6 to 10 million per mouse on day 15 of the infection). In the post-parasitemia period, no *T. muscui* were seen in the peritoneal cavity.

**Quantitative and qualitative changes in peritoneal cells during *T. muscui* infection.** Mice infested with *T. muscui* had increased numbers of peritoneal cells. A maximum number of cells were noted during the plateau phase of parasitemia. After infection, the number of cells returned to normal. The additional peritoneal cells consisted mainly of macrophages (Table 1).

During the plateau phase of parasitemia, macrophages appeared larger than normal and contained greater than normal numbers of granules. These granules, which are presumed to be lysosomes, were observed by phase-contrast microscopy. In the post-parasitemia period, the number of peritoneal macrophages returned to normal, and many macrophages appeared smaller than normal and had an irregular shape.

**H<sub>2</sub>O<sub>2</sub> release.** Without PMA, macrophages from infected mice released the same amount of H<sub>2</sub>O<sub>2</sub> as normal macrophages. An increase in

TABLE 1. Effect of *T. muscui* infection on total number of mouse peritoneal cells and number and protein content of peritoneal macrophages

Group (days after infection)	No. of peritoneal cells per mouse <sup>a</sup> ( $\times 10^6$ )	No. of macrophages per mouse <sup>a</sup> ( $\times 10^6$ )	Protein contents of macrophages plated $10^6$ <sup>a</sup> ( $\mu\text{g}$ )
Control	$4.6 \pm 0.6$	$2.5 \pm 0.5$	$85 \pm 21$
5	$5.2 \pm 0.7$	$2.9 \pm 0.5$	$89 \pm 25$
10	$6.8 \pm 1.0$	$4.1 \pm 0.7$	$171 \pm 36$
14	$7.7 \pm 1.2$	$5.3 \pm 1.1$	$163 \pm 31$
22	$5.6 \pm 0.8$	$3.2 \pm 0.6$	$132 \pm 31$
29	$5.1 \pm 0.7$	$2.7 \pm 0.6$	$95 \pm 22$

<sup>a</sup> Mean and standard deviation from five animals.

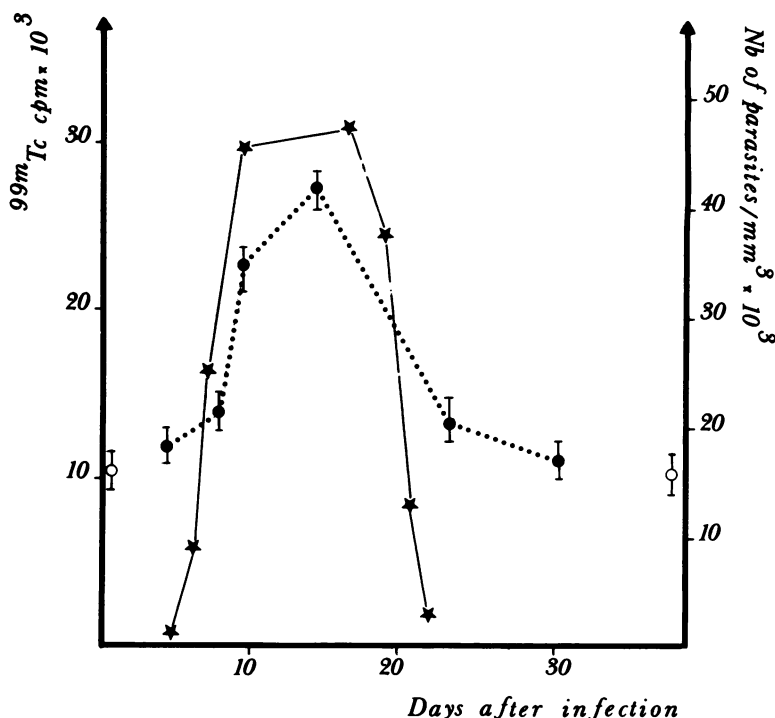


FIG. 1. Parasitemia expressed as the number of *T. muscui*  $\times 10^3$  per  $\text{mm}^3$  of blood ( $\star$ ) and time course effect of *T. muscui* infection on  $^{99\text{m}}\text{Tc}$ -sulfur colloid uptake by peritoneal macrophages ( $\bullet$ ). Each point represents the arithmetic mean (counts per minute  $\times 10^3 \pm$  standard deviation) of quadruplicate cultures. The points ( $\circ$ ) represent the colloid uptake by peritoneal macrophages from uninfected mice.

hydrogen peroxide release from macrophages of *T. musculi*-infected mice was induced by PMA. The increase in hydrogen peroxide release induced by PMA correlated well with the course of parasitemia and the presence of *T. musculi* in the peritoneal cavity (Table 2). There was no increase in induced releases of  $H_2O_2$  by macrophages from mice that had received intraperitoneally 4 days before  $10^6$ ,  $5 \times 10^6$ , or  $10^7$  *T. musculi*.

**Uptake of  $^{99m}Tc$ -sulfur colloid.** Peritoneal macrophages obtained at different days after infection were assayed for their ability to engulf  $^{99m}Tc$ -sulfur colloid. Compared to normal cells, macrophages from *T. musculi*-infected mice manifested an increase in colloid uptake. This increase was first observed at day 8 and peaked at day 14 after infection. An increase in the uptake of  $^{99m}Tc$ -sulfur colloid was well correlated with the course of parasitemia and the presence of *T. musculi* in the peritoneal cavity (Fig. 1).

**Protein content.** The protein content of macrophages from *T. musculi*-infected mice and of normal macrophages is given in Table 1.

## DISCUSSION

Morphological, biochemical, and functional changes of peritoneal macrophages of mice infected with *T. musculi* were observed. An increase in the number of these cells was noted between days 10 to 20 after infection, at the plateau phase of parasitemia. Peritoneal macrophages from *T. musculi*-infected mice ap-

peared larger than normal resident macrophages, and their protein content was about doubled. The rate of uptake of  $^{99m}Tc$ -sulfur colloid was higher for these macrophages than for the normal ones. The increase in uptake of colloidal gold is a reflection of certain kinds of macrophage activation (8). This feature has also been noted in peritoneal macrophage from *Mycobacterium bovis* BCG-treated Guinea pigs (12). An increase in the uptake of  $^{99m}Tc$ -sulfur colloid was noted in thioglycolate-elicited peritoneal mouse macrophages (20).  $H_2O_2$  releases induced by PMA were also higher for macrophages from parasitized mice than for normal ones. These modifications of macrophage functions were first measured at day 9, peaked during the plateau phase of parasitemia, and returned to normal value when no parasites were detected in the blood and in the peritoneal cavity.

Macrophages show a variety of metabolic and functional responses to various stimuli. Because of their late development it is not likely that the peritoneal macrophage alterations observed in *T. musculi*-infected mice are due to an inflammatory process. Also, there is no  $H_2O_2$  increase by macrophages from mice injected with  $10^6$ ,  $5 \times 10^6$ , or  $10^7$  *T. musculi* intraperitoneally 4 days before. Macrophages become activated in vitro after phagocytosis of certain particles (16). Phagocytosis is accompanied by an increase in  $O_2$  consumption, glycolysis, and hexose monophosphate shunt activity. The oxygen consumed is converted to a number of highly reactive products, among which are the superoxide anion ( $O_2^-$ ) and hydrogen peroxide ( $H_2O_2$ ). As found by Chang and Dusanic, in vitro, phagocytosis of *T. musculi* by mouse peritoneal macrophages occurs and is enhanced by mouse antiserum collected at 28 days after inoculation of *T. musculi* (2). A related parasite *T. lewisi* is killed after ingestion by rat macrophages (3). These data may suggest that in *T. musculi*-infected mice, peritoneal macrophages are activated by phagocytosis of *T. musculi* and that activation has a peak when the number of *T. musculi* is maximum.

*T. musculi* infection in mice gives rise to two immunological responses which are sequential and presumably distinct from each other (19). In the first response, there is a cooperative action of a thymus-dependent ablastin and a thymus-independent trypanocidal antibody. Viens et al. propose that the second response is apparently not dependent on a direct (trypanocidal) antibody, but rather appears to involve a thymus-dependent cellular mechanism (19). Complement-mediated lysis is probably not involved in the control of *T. musculi* infection, but it is not

TABLE 2. Spontaneous and PMA-induced  $H_2O_2$  releases by peritoneal macrophages

Animal group	nmol of $H_2O_2$ per $10^6$ cells per 5 min <sup>a</sup>	
	Without PMA <sup>b</sup>	With PMA <sup>b</sup>
Control	0.04 ± 0.02	0.14 ± 0.07
5 days after infection	0.03 ± 0.01	0.19 ± 0.08
10 days after infection	0.05 ± 0.02	0.64 ± 0.15
14 days after infection	0.05 ± 0.02	0.71 ± 0.22
22 days after infection	0.03 ± 0.03	0.21 ± 0.11
29 days after infection	0.05 ± 0.02	0.19 ± 0.09
Intraperitoneal injection of $10^6$ <i>T. musculi</i> 4 days before	0.04 ± 0.03	0.16 ± 0.11
Intraperitoneal injection of $5 \times 10^6$ <i>T. musculi</i> 4 days before	0.03 ± 0.02	0.15 ± 0.08
Intraperitoneal injection of $10^7$ <i>T. musculi</i> 4 days before	0.03 ± 0.02	0.18 ± 0.09

<sup>a</sup> Mean and standard deviation from five mice.

<sup>b</sup> Releases with or without PMA (0.1  $\mu$ g/ml) added to each tissue culture petri dish ( $1.5 \times 10^6$  macrophages per dish) for 15 min.

clear whether a C3-dependent function such as phagocytosis may facilitate elimination of the parasites (4). The role of lymphocytes in *T. musculi* infection is unclear and, if a direct effect of T cells on the control of *T. musculi* infection can be ruled out, the mechanisms involved may not be antibody mediated (13). Macrophages play a role in the early control of parasitemia (1, 17). They can modify the course of infection by nonspecific phagocytosis, and they also can be put into action by antibodies with cytophilic or opsonizing activity, or even by lymphocyte-derived factors. The involvement of macrophages would provoke some alterations of their functions. Whether macrophage activation contributes, per se, to the resistance of infected mice is unknown.

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