

Differences in Microbicidal Activities of Human Macrophages against *Toxoplasma gondii* and *Trypanosoma cruzi*

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The microbicidal activities of normal human pelvic macrophages against infection by the intracellular protozoa *Toxoplasma gondii* and *Trypanosoma cruzi* were examined. Macrophages allowed infection by *T. gondii* and yet possessed remarkable microbicidal activity against this organism. In contrast, human macrophages were not microbicidal against *T. cruzi*.

Acquired (postnatal) infection with *Toxoplasma gondii* is an important cause of disease in individuals with impaired immunologic responses, whereas otherwise healthy individuals rarely have serious sequelae or require treatment for infection with this organism. In contrast, *Trypanosoma cruzi* causes considerable morbidity and mortality in areas in which this organism is endemic, regardless of the underlying immunologic condition of persons infected with this organism. Since macrophages serve an important effector function in host defense against the development of toxoplasmosis (1-4) and South American trypanosomiasis (5), we investigated the microbicidal activity of putatively normal human macrophages against *T. gondii* and *T. cruzi*. We observed a remarkable difference in how much the human macrophage allowed replication of these intracellular protozoan parasites.

Human peritoneal macrophages were collected from otherwise healthy women aged 21 to 41 years who were undergoing laparoscopy for purposes of tubal ligation or infertility. No evidence of inflammation, infection, or neoplasia was present in any of the women. At the start of laparoscopy, 30 to 50 ml of physiological saline was introduced into and then aspirated from the peritoneal cavity into test tubes. The tubes were placed in an ice bath and transported to the laboratory. The time elapsed from aspiration to processing of the fluid was from 0.5 to 2 h. Murine peritoneal macrophages were collected from peritoneal lavage fluid of Swiss Webster mice 6 to 8 weeks old (Simonsen Laboratories, Gilroy, Calif.) as previously described (8).

The human peritoneal lavage fluid was centrifuged at 225 × g for 10 min at 4°C. Pelleted cells were washed twice with phosphate-buffered saline (pH 7.2) and recentrifuged. The cells were suspended in RPMI medium (GIBCO Laboratories, Grand Island, N.Y.) containing 100 U of penicillin and 100 µg of streptomycin per ml and supplemented with 30% heat-inactivated fetal calf serum and 10% heat-inactivated autologous human serum (hereafter referred to as medium). In all experiments, more than 90% of the leukocytes were mononuclear cells in Diff-Quik (American Scientific Products, McGaw Park, Ill.)-stained cytocentrifuge preparations. The concentration of mononuclear cells was adjusted to 2 × 10⁶/ml with medium, and 200 µl of this suspension was

placed into individual wells of four- or eight-chamber tissue culture slides (Miles Scientific, Div. Miles Laboratories, Inc., Naperville, Ill.). The cells were incubated at 37°C for 2 h in air containing 5% CO₂ and were then washed twice with phosphate-buffered saline. The monolayers were used immediately or after 18 h of incubation.

Tachyzoites of the RH strain of *T. gondii* were harvested from the peritoneal cavities of mice, processed as previously described (8), suspended in medium at concentrations of 2 × 10⁶ to 8 × 10⁶ organisms per ml, and immediately added to the monolayers at the multiplicity of infection desired. The organisms were incubated with the cells for 2 h, and then the extracellular organisms were removed by washing the monolayers five times with phosphate-buffered saline. Immediately (time zero) and at various intervals thereafter, duplicate monolayers were fixed and stained with Diff-Quik stain and examined for morphologically distinct intracellular tachyzoites.

Blood-form and culture-form trypomastigotes of the Y (6) and Tulahuen (7) stocks of *T. cruzi* were suspended in medium at concentrations of 2 × 10⁶ to 8 × 10⁶ organisms per ml and immediately added to monolayers at the multiplicity of infection desired. The organisms were incubated with the cells for 6 h to allow for an infection rate comparable to that achieved with *T. gondii*. The monolayers were then processed as described above for *T. gondii*. The number of macrophages infected with either *T. gondii* or *T. cruzi* and the number of intracellular organisms per 100 macrophages were determined by counting at least 200 macrophages in each of the duplicate wells.

Monolayers of peritoneal macrophages were ≥95% mononuclear phagocytes according to nonspecific esterase staining. The remainder of the cells morphologically resembled fibroblasts or epithelial cells.

Human pelvic macrophages, while allowing infection by *T. gondii*, consistently demonstrated remarkable toxoplasma-macidal activity. In seven experiments, by 20 h after infection there was a more than 90% reduction in the number of infected cells (Fig. 1A). This result was reproducible even when the inoculum size was increased from a ratio of two to a ratio of eight parasites per macrophage. Microscopic evaluation revealed macrophages to be rapidly toxoplasma-macidal. At 2 to 6 h after infection of monolayers, >90% of the infected cells contained only remnant particles of *T.*

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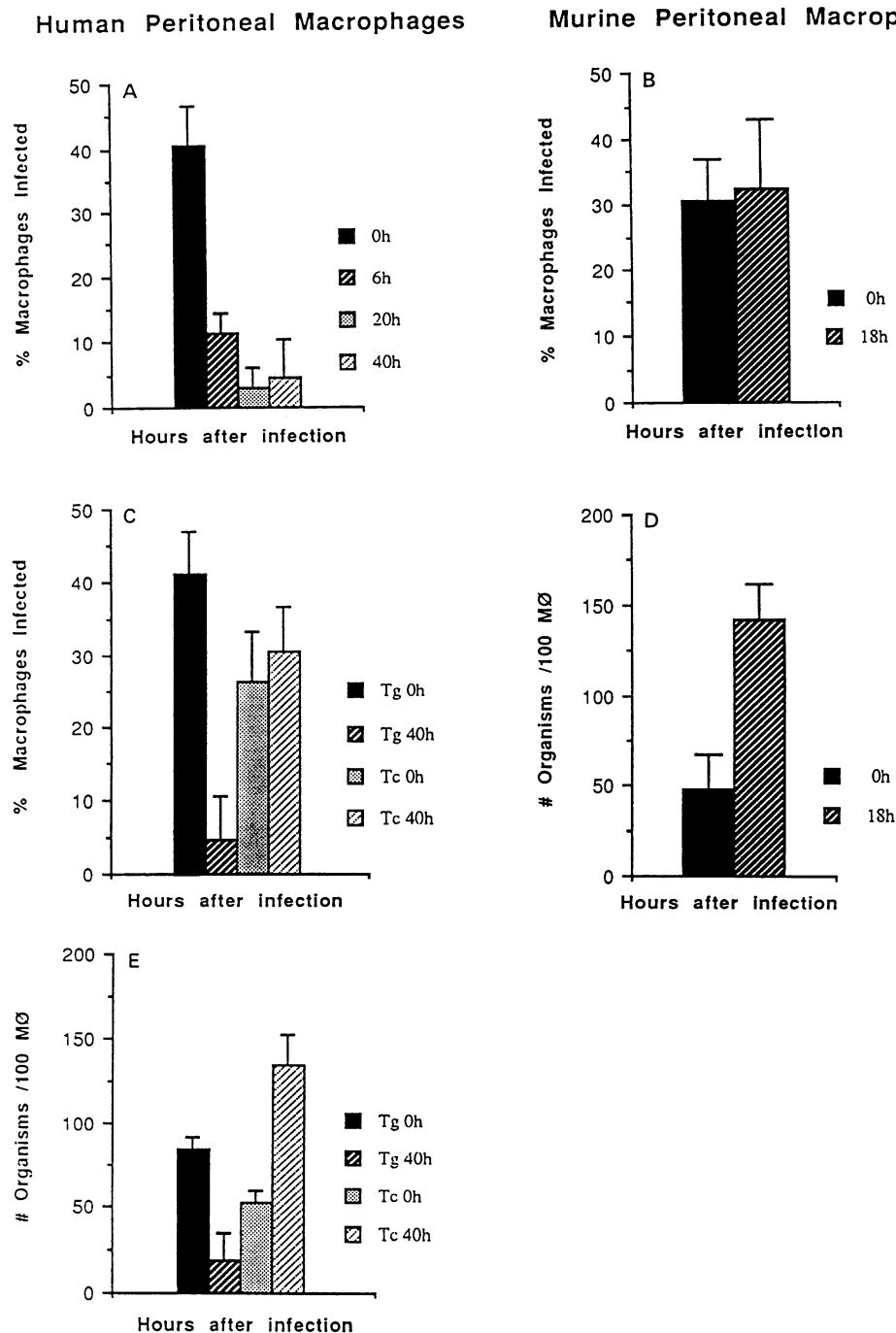


FIG. 1. Fate of *T. gondii* in human (A) and murine (B and D) macrophages ($n = 3$). Characteristics of the activity of human peritoneal macrophages against *T. gondii* (Tg) and *T. cruzi* (Tc) are shown in panels C and E. Seven experiments on the infection of human peritoneal macrophages with *T. cruzi* and *T. gondii* were performed in parallel. Results are means \pm standard errors (bars).

gondii. The remainder of the infected cells contained morphologically intact parasites. At 40 h after infection, all cells infected with *T. gondii* contained only viable-appearing organisms (Fig. 1A). To control for viability of the inoculum, mouse peritoneal macrophage monolayers were infected with *T. gondii* in parallel with the human macrophage monolayers; in contrast to the rapid toxoplasmicidal activity of the human macrophages, mouse peritoneal macrophages were highly permissive for replication of *T. gondii* and

revealed intracellular organisms with intact morphology each time they were assayed (Fig. 1B and D).

In contrast to the situation with *T. gondii*, an infection period of 6 h was necessary to infect between 25 and 35% of the human macrophages with *T. cruzi* (Fig. 1C). Human pelvic macrophages were permissive for both infection and replication of *T. cruzi* (Fig. 1C and E). By 40 to 60 h after infection, the number of *T. cruzi* cells per 100 infected cells had increased four- to fivefold. Regardless of the strain of *T.*

cruzi used in the experiment, both blood- and culture-derived trypanosomes were capable of infection and replication within the human macrophage.

Pooled data from four experiments (Fig. 1C and E) revealed that the percentage of human macrophages infected with *T. gondii* decreased from 41 (range, 35 to 47) at 0 h to 5 (range, 0 to 11) at 40 h after infection ($P < 0.005$). Similarly, the number of *T. gondii* per 100 human macrophages decreased from 84 (range, 78 to 92) at 0 h to 19 (range, 0 to 35) at 40 h after infection ($P < 0.01$). In contrast, the percentage of human macrophages infected with *T. cruzi* slightly increased from 26 (range, 22 to 33) at 0 h to 31 (range, 21 to 33) at 40 h after infection ($P > 0.4$), while the number of *T. cruzi* per 100 human macrophages increased from 52 (range, 41 to 60) at 0 h to 134 (range, 116 to 152) at 40 h after infection ($P < 0.05$).

The results of these studies demonstrate a remarkable difference in the microbicidal activity of normal macrophages against different protozoal parasites. These findings have a number of implications. They may help to explain why healthy individuals have innate resistance to *T. gondii* but are susceptible to disease caused by *T. cruzi*. It has been recently demonstrated that human macrophages are microbicidal against *T. gondii* because of a nonoxidative killing mechanism (2, 3). Our data imply that whatever the nature of the nonoxidative microbicidal mechanisms against *T. gondii*, when *T. cruzi* infects human macrophages, the same mechanisms which result in killing of *T. gondii* either are not induced or are insufficient to have microbicidal activity against the intracellular (amastigote-form) trypanosome.

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