Effect of Monoclonal Antibodies on Phagocytosis and Killing of Toxoplasma gondii by Normal Macrophages

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Treatment of intact toxoplasma tachyzoites with individual mouse monoclonal antibodies to toxoplasma which are directed against individual membrane-associated antigenic determinants facilitated the phagocytosis of toxoplasma and also prepared the toxoplasma for intracellular destruction by nonelicited mouse peritoneal macrophages. In instances in which the organisms survived intracellularly, their multiplication was significantly reduced. Such monoclonal antibodies should be useful in further elucidating the role of antibody in resistance to toxoplasma and other facultative and obligate intracellular organisms.

The role of cell-mediated immunity in resistance to infection by Toxoplasma gondii has been studied extensively in animals (4, 9, 14, 17) and in humans (2, 19, 20). Although macrophages appear to play a central role in the expression of this cell-mediated immunity, the contribution of antibody in limiting initial infection and reinfection with toxoplasma also has been shown to be of importance (10, 11, 16). In particular, various studies have shown that exposure of extracellular toxoplasma to antibodies to toxoplasma predisposes the organism to intracellular inhibition and destruction by nonimmune macrophages which ordinarily support the growth of toxoplasma (1, 8, 15, 16, 20). The recent isolation and characterization of mouse monoclonal antibodies to toxoplasma which are directed against single membrane-associated antigens of the organism (5, 6) has provided a means to study further the role of antibody in controlling infection by toxoplasma. The present study was designed to assess in vitro the effect of these monoclonal antibodies to toxoplasma on toxoplasma infection of normal macrophages.

MATERIALS AND METHODS

Toxoplasma infection. Chronic infection with the C37 strain of T. gondii was accomplished as previously described (6) in 8-week-old Swiss Webster female mice (Simonsen Laboratories, Gilroy, Calif.).

Preparation of monoclonal antibodies. The isolation and characterization of monoclonal antibodies to toxoplasma have been described previously by Handman et al. (5, 6). All monoclonal antibodies used in this study were derived from serum of BALB/c mice, and all are directed against the membrane antigens of toxoplasma tachyzoites. Figure 1 shows a representative example of polyacrylamide gel electrophoresis patterns obtained in our immunochemical analyses of toxoplasma membrane antigens, using monoclonal antibodies.

Collection of sera. Sera from C37 mice, infected 6 to 8 months earlier, were pooled and stored at −20°C. This serum, referred to as immune serum, had a titer of 1:4,096 in the Sabin-Feldman dye test. A pool of sera from age-, sex-, and weight-matched normal uninfected mice was stored in the same manner and had a dye test titer of <1:4 (no measurable neutralizing antibody). The mouse monoclonal antibodies to toxoplasma used in these experiments were selected from those previously isolated and characterized by Handman and Remington (6) and included 3E6 (immunoglobulin G subclass 2), 2G11 (immunoglobulin G subclass 2), and 1E11 (immunoglobulin G subclass 3). These monoclonal antibodies are referred to as MC1, MC2, and MC3, respectively. All monoclonal sera had dye test titers of 1:16,384.

Preparation of macrophage cultures. Normal, nonelicited mouse peritoneal macrophages were harvested as previously described (13). Peritoneal exudate cells (2 × 106) in 0.5 ml of medium 199 (M199; GIBCO Laboratories, Grand Island, N.Y.) containing penicillin (100 U/ml) and streptomycin (100 µg/ml) plus 20% fetal calf serum (M199-FCS) were seeded in duplicate chambers in four-chamber tissue culture slides (LabTek Products, Naperville, Ill.). The cultures were incubated for 2 h at 37°C in an atmosphere containing 5% CO2. They were then washed with warm saline to remove nonadherent cells, and the resultant monolayers were reincubated in fresh M199-FCS overnight (16 to 18 h).

Preparation of toxoplasma. Purified preparations of toxoplasma tachyzoites of the RH strain were obtained from the peritoneal fluid of 2-day infected mice, as previously described (20). Viability was greater than 95% for all experiments, as assessed by exclusion of 0.025% trypan blue in saline both before and after incubation with the control and test sera. Tachyzoites (1.6 × 105) were then placed into individual plastic tubes (12 by 75 mm; Becton Dickinson and Co., Oxnard, Calif.) containing one of the following: normal serum, immune serum, or one of the monoclonal antibodies. This resulted in a final serum concentration of 50% in a total volume of 1 ml. The tubes were then capped and incubated for 30 min at 37°C.
with continuous mixing, using a LabTek mixer (Ames; LabTek, Inc., Westmont, Ill.) at 18 cycles/min. After the 30-min treatment period, tachyzoites were centrifuged at 550 × g for 10 min at 4°C and then washed with 10 ml of fresh M199 to remove any residual antibody. The concentration of organisms in each tube was then adjusted to 4 × 10⁴/ml with 4 ml of fresh M199-FCS. All sera used, including FCS, were heat-inactivated at 60°C for 30 min before use to remove any accessory factor (3).

**Infection of macrophage monolayers.** Macrophage monolayers that had been prepared the previous day were washed with warm saline and infected with 2 × 10⁶ tachyzoites in a volume of 0.5 ml. The organisms had been previously incubated with either normal serum, immune serum, or one of the monoclonal antibodies. After incubation for 1 h at 37°C, the monolayers were washed thoroughly with warm saline to remove extracellular organisms and then reconstituted with 1 ml of M199-FCS per well and reincubated for varying periods of time. Slides were fixed in cold 0.4% aminoacridine hydrochloride in 50% ethanol and stained with Giemsa stain. Monolayers were then examined by light microscopy and evaluated as previously described (2) for intracellular infection, multiple infection with toxoplasma in individual macrophages, and intracellular multiplication of the organism.

Results were compared for statistical significance between control and test groups by chi-square analysis.

**RESULTS**

The experimental and statistical data presented are from one experiment of six performed in which the three monoclonal antibodies MC1
(3E6), MC2 (2G11), and MC3 (1E11) were run in parallel. The experimental and statistical results were similar in each.

When analyzed in terms of the percentage of macrophages with both viable and nonviable intracellular toxoplasma at 2 h after infection, those monolayers challenged with tachyzoites that had been incubated with any of the monoclonal antibodies had significantly higher numbers of infected macrophages than did controls (Fig. 2). Interestingly, at that same time after infection, there was a higher number of nonviable-appearing intracellular organisms and a lower number of viable-appearing intracellular organisms in all monolayers challenged with toxoplasma treated with antibody compared with monolayers challenged with organisms treated with normal serum (Fig. 2). Also, treatment of tachyzoites with the monoclonal antibodies MC1 and MC3 consistently resulted in higher numbers of intracellular organisms than did treatment with immune serum or monoclonal antibody MC2 (Fig. 2). By 18 h after infection, the number of macrophages with intracellular organisms was significantly less in all antibody-treated groups than in controls (Fig. 2). The number of organisms per macrophage was increased significantly in all antibody-treated groups (Fig. 3). Interestingly, treatment of the organisms with monoclonal antibody resulted in significantly higher rates of multiple infection than did treatment with immune serum (Fig. 3).

Once intracellular, those tachyzoites able to persist and, by 18 h after infection, to undergo more than one division or more than three divisions were significantly reduced in all antibody-treated groups (Fig. 4).

**DISCUSSION**

The results of this study confirm and extend previous observations on antibody-dependent killing of toxoplasma tachyzoites by normal mouse peritoneal macrophages (1, 8, 20). Significant enhancement of inhibition and killing of intracellular toxoplasma was noted not only with organisms treated with immune serum but also with those treated with monoclonal antibodies. As judged by staining characteristics and morphology (2), the greater number of macrophages with nonviable-appearing tachyzoites at 2 h in all of the monoclonal antibody-treated groups paralleled or exceeded that in the immune serum-treated group. These results suggest that significant killing of toxoplasma had already taken place early after initial infection with organisms pretreated with the monoclonal antibodies. Support for this conclusion comes from our observation that the numbers of macrophages with viable-appearing intracellular toxoplasma were significantly reduced in all antibody-treated groups by 2 h after infection. With time, significant reductions occurred in the percentage of macrophages with intracellular organisms in all antibody-treated groups. This reduction has previously been shown to be due to destruction of the intracellular organisms rather than to release of the cells from the monolayers or to release of intracellular toxoplasma (12, 20). These results coincide with previous observations on the effect of immune serum (1, 8) and suggest that the mechanism of recognition and killing after treatment with the three mono-
clonal antibodies is the same as that after treatment of toxoplasma with immune serum.

Our results indicate that phagocytosis of toxoplasma was enhanced in macrophages in all three monoclonal antibody-treated groups. Although phagocytosis in monoclonal antibody-treated groups was significantly greater than that in the immune serum-treated controls, the difference may have been artificial since the levels of the various specific toxoplasma antibodies in the monoclonal antibody preparations may have differed from the levels in the immune serum.

Even though the majority of antibody-coated toxoplasma underwent significant destruction by normal macrophages, some were able to persist. The subsequent ability of these survivors to multiply intracellularly was significantly inhibited. This inhibition of multiplication with early killing has also been observed in other models (7, 20).

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LITERATURE CITED


