11-1995

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INFECTION AND IMMUNITY, Nov. 1995, p. 4290–4294 Vol. 63, No. 11
0019-9567/95/$04.00+0

Survival of Immunoglobulin G-Opsonized *Toxoplasma gondii* in Nonadherent Human Monocytes

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Received 6 June 1995/Returned for modification 4 July 1995/Accepted 17 August 1995

***Toxoplasma gondii* is a protozoan parasite that is able to penetrate human monocytes by either passive uptake during phagocytosis or active penetration. It is expected that immunoglobulin G (IgG) opsonization will target the parasite to macrophage Fcγ receptors for phagocytic processing and subsequent degradation. Antibody-opsonized *T. gondii* tachyzoites were used to infect nonadherent and adherent human monocytes obtained from the peripheral blood of seronegative individuals. The infected monocytes were evaluated for the presence of intracellular parasites and the degree of parasiticidal activity. A marked difference in both the numbers of infected macrophages and numbers of parasites per 100 macrophages was observed in the nonadherent cells when compared with those of the adherent cell population. When macrophage Fcγ receptors were down-modulated, opsonized tachyzoites retained their ability to penetrate the host cell at a rate similar to that observed for unopsonized parasites. These results suggest that antibody opsonization of *T. gondii* does not prevent active penetration of human monocytes by the parasite and, furthermore, has little effect on intracellular replication of the parasite.**

Toxoplasma gondii is a protozoan parasite that is able to infect all mammalian cells (18), either by active penetration into nonphagocytic host cells or by phagocytosis into phagocytic host cells. During the process of active penetration, the parasite invaginates the host cell membrane to form a parasitophorous vacuole that is separated from the host cell cytoplasm by a membrane perhaps derived from the host cell itself (23). This parasitophorous vacuole fails to fuse with either host endosomes or lysosomes and allows for uninterrupted replication of the parasite (23). Alternatively, in phagocytic cells such as monocyte-derived macrophages, *T. gondii* can also gain access by being phagocytosed. Binding of antibody-opsonized parasites to the host cell Fcγ receptors (FcγR) triggers phagocytosis, and the parasite is internalized within a phagosome that subsequently fuses with host endosomes, resulting in acidification, and lysosomes, resulting in parasite degradation (10, 18, 23). Previous studies have shown that unopsonized tachyzoites are susceptible to killing by fresh monocytes (14, 25), whereas the less-activated monocyte-derived macrophage is more permissive, allowing intracellular parasites to replicate (1). Antibody sensitizes the parasite to killing or stasis by both types of human phagocytes (1, 26). More recently, it has been shown by a [^{3}H]uracil uptake assay that no significant differences in parasite replication occurs in human monocytes or macrophages infected with either opsonized or unopsonized *T. gondii* (20).

In this report, we demonstrate, by use of an enriched population of nonadherent, unactivated human monocytes derived from seronegative individuals, that antibody opsonization fails to enhance intracellular killing of the parasite and, moreover, fails to block intracellular parasite replication. Furthermore, we have observed that opsonized parasites readily gain access to the intracellular environment of phagocytic cells independent of FcγR binding and, once invasion occurs, replicate equally well as unopsonized parasites.

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MATERIALS AND METHODS

Reagents. RPMI 1640 was obtained from JRH Biosciences, Lenexa, Kans.; Ficoll-Hypaque was from Winthrop Laboratories, New York, N.Y.; endotoxin-low fetal bovine serum FBS (0.5 endotoxin unit per ml) was from HyClone Laboratories, Inc., Logan, Utah; minimal essential medium, antibiotic-antimycotic solution, EDTA buffer, and HEPES (*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid) were from Gibco Laboratories, Grand Island, N.Y.; gentamycin sulfate was from the United States Biochemical Corp., Cleveland, Ohio; and Diff-Quik was from Baxter Healthcare Corp., Miami, Fla. VECTASHIELD was supplied by Vector Laboratories, Inc., Burlingame, Calif. Glutaraldehyde (50%, aqueous solution EM grade) was obtained from Ted Pella, Inc., Redding, Calif. Ox erythrocytes (ox-E) were obtained from Micropure Medical Inc., White Bear Lake, N. Mex. Rabbit antiserum to bovine erythrocytes was supplied by Organon Teknika Corp., Durham, N.C.; fluorescein-conjugated, ImmunoPure goat anti-rabbit immunoglobulin G (IgG), F(ab′)$_2$ fragment specific, was supplied by Pierce, Rockford, Ill.; anti-FcγRI monoclonal antibody (MAb) 22 and anti-FcγRII MAb IV.3 were generously provided by Medarex, Inc., Annandale, N.J.; a rabbit polyclonal IgG against *T. gondii* SAG-1 (p30; the major cell surface antigen) was made and purified in our laboratory; and a rabbit polyclonal IgG against low-density lipoprotein (LDL) was generously supplied by Peter Morganelli, VA Medical Center, White River Junction, Vt.

Effector cells. Human monocytes were isolated from cytophoresis packs obtained from healthy *Toxoplasma*-seronegative volunteers. Mononuclear cells were separated from whole blood with Ficoll-Hypaque, and monocytes were enriched 80 to 90% by aggregation at 4°C (7). Platelets were removed from enriched monocytes by washing with EDTA buffer (0.2 g of EDTA per liter in phosphate-buffered saline [PBS]). Cytocentrifuge preparations of purified cells were made (70,000 cells at 700 rpm for 5 min; Cytospin 3 cytocentrifuge [Shandon Lipshaw, Pittsburgh, Pa.]) and stained with Diff-Quik, and the percentages of lymphocytes, monocytes, and neutrophils or eosinophils were determined. Nonadherent enriched monocytes were cultured overnight in Teflon beakers in RPMI 1640 containing 20 mM HEPES, 50 μg of gentamycin sulfate per ml, and 10% heat-inactivated FBS (medium). The following day, these 1-day-old enriched monocyte-derived macrophages (macs) were washed and resuspended in medium, and 500,000 cells per 500 μl were added to wells of 24-well plates. Macs (200,000/100 μl of medium without FBS) were added in a drop to a 12-mm-diameter glass coverslip in a 24-well plate and incubated for 30 min at 37°C for adherence to occur. Nonadherent cells were washed away, and adherent cells were incubated in 500 μl of medium containing 10% FBS. Special care was taken to ensure endotoxin-free conditions in all of the experiments.

Parasites. The RH and ptg strains of *T. gondii* were maintained by serial passage in confluent cultures of human fibroblast monolayers as described previously (11). Infected fibroblasts were scraped and then forcibly extruded through a 27-gauge needle. After a low-speed spin to pellet large fibroblast

debris, tachyzoites were pelleted by centrifugation at 900 × *g* for 10 min and resuspended in medium. Mouse intraperitoneal parasites of the RH strain were obtained from the peritoneal exudate of BALB/c mice 48 h after intraperitoneal inoculation of parasites, as described by McLeod and Remington (15). Briefly, diluted peritoneal exudate was forcibly extruded through a 27-gauge needle twice to release parasites from host cells and centrifuged at 900 × *g* for 10 min. The pellet was resuspended and passed twice through a 3.0-μm-pore-size Nuclepore polycarbonate membrane filter (Costar Corp., Cambridge, Mass.) to separate parasites from host cell debris. Parasites were used within 2 h of harvesting.

Parasite opsonization. An initial comparison of opsonization at 4, 25, and 37°C showed no difference in the distribution of fluorescence on the surface of the parasite, and parasites were routinely opsonized at room temperature. Tachyzoites were incubated in medium containing 10 μg of rabbit polyclonal anti-SAG-1 IgG per ml, in medium containing 10 μg rabbit polyclonal anti-LDL IgG per ml, or in medium alone, for 30 min at room temperature, with rotation. Rabbit IgG binds to human monocyte FcγR with an affinity as high as that of homologous IgG (27). Opsonization was confirmed for each experiment by adding fluorescein isothiocyanate (FITC)-conjugated secondary antibody to a cytocentrifuge preparation (70,000 parasites; 500 rpm for 5 min) of treated *T. gondii*. Only parasites incubated with anti-SAG-1 IgG fluoresced. Parasites were not washed before being added to macs because centrifugation caused anti-SAG-1-opsonized parasites to agglutinate (our unpublished observations).

Mac-tachyzoite interactions and discrimination of intracellular from extracellular parasites. Parasites were added to nonadherent or adherent macs in tissue culture plates at a parasite/mac ratio of 4:1, and the plates were incubated at 37°C for either 2 or 18 h. At each time point, the plates were placed on ice to halt parasite uptake, and extracellular parasites were labeled with FITC-conjugated anti-SAG-1 IgG. After incubation at 4°C for 20 min, cytocentrifuge preparations of nonadherent cells were made (70,000 macs; 750 rpm for 5 min), slides or coverslips were fixed in methanol for 15 min at room temperature, and then propidium iodide (0.5 μg/ml) was added for an additional 5 min in the dark. The slides or coverslips were washed with water and air dried, and cells were examined by dual-color fluorescence microscopy. In these preparations, nuclei of macs and of both intracellular and extracellular *T. gondii* parasites stain red, whereas the cell surfaces of extracellular parasites stain green.

Down-regulation of FcγR. Antibody-coated coverslips were prepared as described previously (16, 28). Briefly, 12-mm-diameter glass coverslips were treated with 0.1 mg of poly-L-lysine per ml at room temperature for 30 min, washed, and then treated with 2% glutaraldehyde (in PBS) at room temperature for 15 min. After extensive washing, coverslips were treated overnight at 4°C with a mixture of MAbs 22 (anti-FcγRI) and IV.3 (anti-FcγRII) each at a concentration of 100 μg/ml. On the following day, the coverslips were washed, incubated with 1 mg of bovine serum albumin per ml in 0.1 M glycine for 30 min at room temperature, and washed again. Macs (5×10^5) were allowed to adhere and spread on coverslips for 60 to 90 min at 37°C before the addition of either parasites or opsonized ox-E (see below). The latter were used as a positive control for modulation of host FcγR from the apical surface of the monocyte.

Mac-erythrocyte interactions. Ox-E were washed twice with medium and opsonized at a subagglutinating dilution of antibody (1:600) for 30 min at 37°C. Opsonization was confirmed for each experiment by adding FITC-conjugated secondary antibody to cytocentrifuged cells (70,000 ox-E; 750 rpm for 5 min). Opsonized ox-E were not washed before being added to macs, to parallel the conditions used with opsonized tachyzoites. Opsonized ox-E were added to adherent macs in a plate at a mac/ox-E ratio of 1:20. After a 10-min incubation at 37°C, the plate was centrifuged at 150 × *g* for 5 min to increase the contact between the cells. After a further 20-min incubation at 37°C, the coverslips were dipped in distilled water to lyse extracellular erythrocytes and stained with Diff-Quik for determination of phagocytosis of opsonized ox-E.

Microscopy. A Zeiss Axiophot microscope equipped for epifluorescence, with a FITC-tetramethyl rhodamine isothiocyanate filter set (excitation, 5395; emission, 5422; beamsplitter, 5262; Chroma Technology Corp., Brattleboro, Vt.), was used at ×1,000 magnification. At least 200 macs were counted when more than 20% of the macs were infected, and 500 macs were counted for infection rates of less than 20%. Only parasites with a normal morphology were counted. The percentage of infection was measured as the number of macs with intracellular parasites. Replication was estimated by calculating the mean number of intracellular tachyzoites per infected mac after 2 and 18 h of infection. The number of intracellular *T. gondii* parasites per 100 macs was also determined to assess survival and reinfection (22). The mean and standard deviation are expressed for evaluation of three or more cytocentrifuge preparations from each well. A Bio-Rad MRC 1000 confocal microscope with a krypton-argon laser confocal imaging system was also used.

RESULTS

Discrimination of intracellular from extracellular parasites. Dual-color fluorescent studies of infected cell cultures were performed to distinguish intracellular from extracellular parasites. Infected cells were incubated on ice with anti-SAG-1 IgG and then with FITC-labeled secondary antibody; fixation and the addition of propidium iodide followed, as described in Materials and Methods. Free extracellular tachyzoites and parasites attached to the host cell membrane displayed a pattern of bright green fluorescence over the entire tachyzoite membrane surface, whereas intracellular parasites were not stained green. Nuclei of both intracellular and extracellular parasites as well as host cells could be readily distinguished (red stain). This technique was especially beneficial at the 2-h postinfection time point, when the distinction between intracellular, extracellular, and attached parasites was most difficult.

TABLE 1. Replication of opsonized and unopsonized parasites in nonadherent human macs

Time after infection (h)	Parasite pretreatment	% Macs infected[a]	Intracellular parasites/infected mac[a]	Intracellular parasites/100 macs[a]
2	Unopsonized	63 ± 5	2.8 ± 0.3	180 ± 28
	Anti-LDL IgG	55 ± 6	3.1 ± 0.4	172 ± 36
	Anti-SAG-1 IgG	59 ± 9	3.2 ± 0.2	185 ± 35
18	Unopsonized	71 ± 6	9.1 ± 0.9	644 ± 11
	Anti-LDL IgG	62 ± 4	7.9 ± 0.9	492 ± 80
	Anti-SAG-1 IgG	62 ± 3	8.5 ± 1.8	529 ± 100

[a] Parasites were added at a 4:1 parasite/mac ratio. Values are the means ± standard deviations of three different slides and are representative of three different donors.

Antibody-opsonized *T. gondii* replicates within human macs. Tachyzoites were pretreated with either anti-SAG-1 IgG, anti-LDL IgG (as a nonspecific antibody control), or medium and then added to nonadherent macs. As shown in Table 1, at 2 h postinfection, both antibody-treated and unopsonized parasites were internalized. There was no significant difference for antibody-treated or unopsonized parasites in either the number of macs infected (range, 55 to 63%), the number of intracellular parasites per mac (range, 2.8 to 3.2), or the number of intracellular parasites per 100 macs (range, 172 to 185). At 18 h postinfection, slightly more macs were infected (range, 62 to 71%), perhaps as a result of reinfection, and this was true whether parasites were opsonized or unopsonized. A nominal difference was observed in the number of intracellular parasites between the antibody-opsonized (2.9-fold increase) and unopsonized (3.6-fold increase) groups. Similar results were obtained when parasites were opsonized with serum (heat-inactivated anti-SAG-1 or anti-whole parasite serum) and when the monocyte-like cell line THP-1 was used as the effector cell (data not shown). Hence, opsonization had no effect on curtailing parasite entry into nonadherent human monocyte-derived macrophages, and although some opsonized parasites may be killed, most are able to replicate at a rate similar to that of unopsonized parasites. These results suggest that not only unopsonized but also opsonized parasites enter macrophages in intracellular vacuoles that do not fuse with the host endocytic network, enabling most parasites to escape acidification and digestion.

Opsonized parasites retain antibody following invasion. IgG-opsonized parasites may evade FcγR-mediated phagocytosis and concomitant endosome-lysosome fusion events by removal of anti-SAG-1 IgG before, or at the time of, invasion (6). To determine whether antibody was removed before invasion, *T. gondii* was opsonized with FITC-conjugated anti-SAG-1 IgG for 30 min at room temperature. Homogeneous fluorescence was observed over the entire parasite surface, and there were no patches or caps suggesting removal of antibody. To determine whether antibody was still detectable on parasites that had just become intracellular, host cells were incu-

bated with either unopsonized or anti-SAG-1-opsonized parasites for 30 min at 37°C, fixed, permeabilized, and stained with FITC-conjugated secondary antibody. Confocal microscopy showed fluorescent opsonized, but not unopsonized, intracellular parasites (data not shown). These results suggest that opsonized tachyzoites do not cap and remove their antibody coat extracellularly and, moreover, that antibody remains detectable on recently invaded, intracellular parasites; therefore, ligation of parasites to FcγR was possible. A minor loss of antibody during internalization may have occurred since the amount of antibody on extracellular and intracellular parasites was not quantified.

Mouse peritoneal- and tissue culture-derived parasites replicate equally in nonadherent human macs. Previous studies reporting that human monocytes and macrophages killed opsonized parasites used mouse peritoneum-derived tachyzoites (1, 2, 14, 26), which exhibit differential infectivity in vitro depending on the time of harvest (8, 13). In our studies, tissue culture-derived tachyzoites were used to prevent contamination with any mouse-derived peritoneal or serum components that would increase cidal activity. Experiments comparing the source of parasites were carried out, and unopsonized and opsonized parasites replicated similarly whether parasites were fibroblast or mouse peritoneum derived (data not shown). Therefore, the parasite source did not alter the microbicidal outcome of infection of human macs with opsonized parasites.

Host cell adherence determines the magnitude of parasite infection. Adherent macrophages have been used in previous studies showing killing of opsonized tachyzoites (1, 26). In the present study, to avoid inadvertent activation of monocytes, cells were cultured under nonadherent conditions. To determine whether host cell adherence may affect the outcome of parasite uptake, opsonized and unopsonized parasites were incubated with either adherent or nonadherent macs. As shown in Fig. 1, macrophage adherence markedly compromised both internalization and replication of tachyzoites. At 2 h postinfection, twice as many nonadherent macs than adherent macs were infected with a similar number of intracellular unopsonized parasites (1.5 versus 1.1), although with twice as many opsonized tachyzoites (3.1 versus 1.3), perhaps as a result of antibody-induced aggregation. At 21.5 h postinfection, there was reinfection of nonadherent macs and unopsonized parasites had undergone two doublings per infected mac. This replication and reinfection was reflected in the nearly ninefold increase in the number of parasites per 100 macs. For nonadherent macs incubated with opsonized parasites, there was less reinfection than there was for unopsonized parasites, and parasites underwent a single doubling, resulting in a threefold increase in the number of parasites per 100 macs. Hence, a combination of parasite killing, stasis, and replication may have occurred when opsonized parasites infected nonadherent macs, whereas replication appears predominant in nonadherent macs infected with unopsonized parasites. This is in contrast to the results in Table 1, where perhaps as a result of donor variability, nonadherent macs from one donor supported the replication of opsonized parasites to a greater extent than the other donor. Nevertheless, both donors supported the replication of opsonized parasites. At 21.5 h postinfection, there was no reinfection of adherent macs, although a doubling of both unopsonized and opsonized parasites had occurred. Hence, adherent macs were markedly less permissive to parasites than nonadherent macs. For both adherent and nonadherent macs, opsonization did not target parasites exclusively to the host's endocytic network, resulting in parasite death; rather, most opsonized parasites were able to replicate.

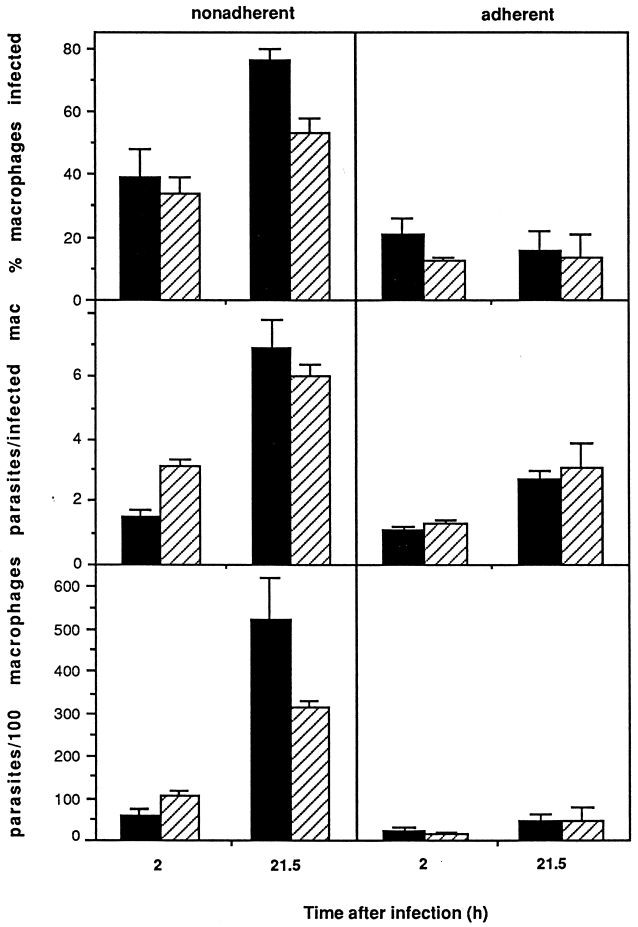

FIG. 1. Uptake and replication of unopsonized (■) and anti-SAG-1-opsonized (▨) tachyzoites compared in nonadherent and adherent macs. Parasites were incubated with host cells at a 2:1 parasite/mac ratio for 2 or 21.5 h. Values are the means ± standard deviations of three slides and are representative of one donor.

Opsonized parasites can enter human macs whose FcγR have been down-modulated. Since opsonized *T. gondii* parasites were able to enter and replicate within macs, it was important to evaluate whether host FcγR were required for uptake of IgG-coated parasites. To investigate this, FcγRI and FcγRII were modulated from the apical to the basal surface of the mac by ligation to anti-FcγRI and anti-FcγRII MAb-coated coverslips. Adherence of macs to anti-FcγRI and -FcγRII MAbs almost completely blocked their ability to phagocytose antibody-treated ox-E (99% reduction) (Table 2). This demonstrates that down-modulation of FcγRI and -II markedly reduces the opportunity for IgG-coated particles to be ligated. Parasites were opsonized with anti-SAG-1 IgG, nonspecific IgG, or medium and then added to the macs. Two hours postinfection, parasite uptake was compared between adherent FcγR-modulated and unmodulated macs. There was no significant difference between unopsonized and opsonized parasites in either the number of macs infected (range, 42 to 48%) or the number of parasites per mac (range, 1.9 to 2.0) following down-modulation of host FcγRI and FcγRII (Table 2). A similar result was seen in macs that were not modulated by antibody adherence. Interestingly, more macs were infected when FcγRI and -II were down-modulated. Hence, IgG-opsonized parasites can become intracellular in macrophages that

TABLE 2. Internalization of opsonized and unopsonized parasites compared in unmodulated macs and in macs with modulated FcγRI and -II

Parasite pretreatment	% Macs with particles[a]	Intracellular parasites/ infected mac[a]	Intracellular parasites/ 100 macs[a]
FcγRI and -RII unmodulated			
Unopsonized	28 ± 9	1.8 ± 0.4	53 ± 29
Anti-LDL IgG	26 ± 8	1.6 ± 0.3	43 ± 20
Anti-SAG-1 IgG	28 ± 9	1.6 ± 0.3	47 ± 22
Opsonized ox-E[b]	85 ± 2	ND[c]	ND
FcγRI and -RII down-modulated			
Unopsonized	42 ± 1	1.9 ± 0.1	78 ± 1
Anti-LDL IgG	48 ± 7	2.0 ± 0.2	95 ± 13
Anti-SAG-1 IgG	45 ± 4	1.9 ± 0.3	86 ± 16
Opsonized ox-E[b]	1	ND	ND

[a] Parasites were added to macs at a 4:1 parasite/mac ratio. The values are means ± standard deviations of three coverslips and are representative of three different donors.

[b] Opsonized ox-E were used to confirm down-modulation of host cell FcγRI and -II.

[c] ND, not done.

are unable to carry out FcγR-mediated phagocytosis, implicating active penetration, rather than phagocytosis, as the mechanism of entry of opsonized parasites into the FcγR down-modulated phagocytic host cell.

DISCUSSION

These studies suggest that antibody opsonization does not sensitize *T. gondii* to killing by 1-day-old human monocyte-derived macrophages. Further, antibody-opsonized parasites are able to enter host cells and replicate nearly as well as unopsonized tachyzoites. These observations differ in several respects from those of previous reports demonstrating either inhibition of growth or killing of *T. gondii* by monocytes and macrophages. The role of macrophages in the killing of *T. gondii* was first reported more than 20 years ago (21). In that study, it was demonstrated that activation of mouse peritoneal macrophages increased toxoplasmacidal activity, whereas resident macrophages were permissive. If, however, the parasites were antibody opsonized, the resident murine peritoneal macrophages were able to kill the tachyzoite (2, 9, 26). In human monocyte-derived macrophages, the story is less clear. Short-term cultured monocytes (<24 h) were able to destroy unopsonized parasites (14, 25), whereas human monocyte-derived macrophages cultured for 7 to 10 days were less able to kill these intracellular parasites. Antibody-treated parasites were not killed by human monocyte-derived macrophages but were unable to replicate (2).

When phagocytosis is triggered by ligation of FcγR with IgG-coated particles, a phagosome that fuses first with early endosomes and then with late compartments is formed (4). Endosome fusion results in acidification of the phagosome, and lysosomal fusion results in further degradation of the opsonized particle. Hence, ligation of FcγR by IgG-opsonized tachyzoites is expected to trigger a phagocytic signal, resulting in internalization of the parasite into a phagosome; this phagosome would be expected to fuse subsequently with endosomes, become acidified, and then fuse with lysosomes, resulting in parasite degradation. In a study in which nonphagocytic murine cells were transfected with FcγRII and infected with opsonized *T. gondii*, acidification of the phagosome and fusion with lysosomes was indeed observed (10). Our results, however, indicate that the majority of opsonized parasites replicated as efficiently as unopsonized tachyzoites, in populations of fresh (<24-h), nonadherent, enriched human monocytes. It was possible that *T. gondii* parasites may have evaded FcγR-mediated phagocytosis by preclearing ligand from their surface during the entry process into host cells (6). Our results, however, indicate that surface SAG-1 remained reactive to antibody after invasion, suggesting that not all ligand is stripped upon entry. Furthermore, these antibody-coated parasites continued to replicate within the host cell. It was also possible that the presence of unbound antibody blocked phagocytosis of tachyzoites by host cells. However, opsonized ox-E, which have no alternative mechanism of uptake other than phagocytosis, were phagocytized by 85% of the macs in the presence of unbound rabbit polyclonal antibody, and the same result was seen when unbound antibody was washed off (data not shown). This result suggests that the presence of unbound antibody does not block phagocytosis of opsonized particles by human macs.

The most reasonable explanation for the differences between our observations and those previously reported lies in the experimental conditions. In our study, to avoid inadvertent activation of monocytes, freshly isolated, enriched cells were cultured under endotoxin-free and nonadherent conditions. The macs were then infected with tachyzoites, and after the appropriate incubation period, a sample of cells was cytocentrifuged and prepared for microscopy. This cytospin preparation of nonadherent cells differs from the monocyte population used in previous studies (2, 26). In those experiments, monocytes were adhered to glass coverslips, washed free of the nonadherent population, and then infected. Previous studies have reported that monocytes are heterogeneous in their ability to adhere and that the adherent population may be an unrepresentative population of monocytes (5, 19). Nonadherent monocytes are functionally less activated than their adherent counterparts. Adherent, but not nonadherent, cells selectively express genes for interleukin-1β, superoxide dismutase, and interleukin-8 (24); for macrophage colony-stimulating factor (12), they release monocyte chemoattractant protein 1 (29), and their spontaneous release of superoxide anion is also greater (19). In our studies, nonadherent macs were more permissive than adherent macs to parasite uptake and replication, reflecting this less-activated state. Monocytes adherent to fibronectin have been reported to be 14-fold more actively phagocytic than nonadherent cells (19). In our studies, nonadherent host cells may have been less phagocytically active, and parasites, both opsonized and unopsonized, were able to enter by an alternative mechanism. It is very difficult to distinguish a phagosome from a parasitophorous vacuole 2 h postinfection. However, since the parasites readily underwent replication once inside the monocyte, it would suggest that they entered via a pathway that produces a nonfusigenic parasitophorous vacuole, such as active penetration. Although antibody opsonization of the parasite may allow for closer contact with the phagocyte through ligation to FcγR, the process of phagocytosis requires a longer period of time (minutes) than that required by active penetration (seconds). It is possible that the parasites initially attach to the host via FcγR, and the majority actively penetrate the host membrane before they are phagocytosed, going on to replicate, whereas the remainder are phagocytosed and killed.

Our results with adherent FcγR-modulated macs lead us to the same conclusion. In the absence of FcγRI and -II, and hence FcγR-mediated phagocytosis, no difference in the numbers of macs infected or the numbers of parasites per infected

mac was noted between IgG-coated and uncoated parasites. This observation would suggest that internalization of antibody-treated parasites may be through active penetration. Interestingly, more macs were infected when FcγRI and -II were down-modulated. Perhaps in a cell where FcγR are down-modulated, IgG-opsonized parasites do not trigger FcγR-mediated gross cytoskeletal changes that might make the host cell less accessible to the actively penetrating parasite.

Our findings show that most internalized *T. gondii* parasites are able to survive and replicate in a population of enriched nonadherent macs. Parasite replication is independent of antibody opsonization, and uptake of opsonized parasites can occur by a non-FcγR-mediated phagocytic event. These observations are in contrast to previous results from our laboratory, in which anti-SAG-1 blocked the attachment of tachyzoites to human fibroblasts and mouse enterocytes (17). The cells used in these previous studies lacked FcγR. The present study used monocyte-derived macrophages that express FcγR. It is possible that anti-SAG-1-opsonized parasites bind to host cell FcγR by the exposed Fc region of IgG and that this attachment event triggers active penetration. However, since anti-SAG-1-opsonized parasites can enter macs whose FcγR are down-modulated, it is also possible that parasite antigens other than SAG-1 may bind to receptors that are specific to macs, again triggering active penetration.

These observations provide a potential mechanism to explain how the parasite is able to disseminate in the infected host. Following infection of the gut epithelial cells, survival of the parasite is dependent upon successful transit to other organs of the body, in particular, cells of the central nervous system and muscle. To accomplish this, the parasite must find "a mode of transportation," and the nonadherent monocyte in the capillaries of the villi is an excellent candidate. When circulating, nonadherent monocytes become parasitized, the normal phagocytic mechanisms are not activated because the parasite gains entry via active penetration. Once inside, the parasite is able to survive whether opsonized or not and be deposited in tissue through which the monocytes transit.

ACKNOWLEDGMENTS

This work was supported by NIH grants AI 19613 and AI 30000.

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Editor: S. H. E. Kaufmann