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Ingestion of *Giardia lamblia* Trophozoites by Human Mononuclear Phagocytes

DAVID R. HILL[1]* AND RICHARD D. PEARSON[2]

Division of Infectious Diseases, Department of Medicine, University of Connecticut School of Medicine, Farmington, Connecticut 06032[1] and Divisions of Geographic Medicine and Infectious Diseases, Department of Medicine, University of Virginia School of Medicine, Charlottesville, Virginia 22908[2]

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Mononuclear phagocytes may be important effector cells against *Giardia lamblia*. Human monocyte-derived macrophages were incubated with *G. lamblia* trophozoites in 13% heat-inactivated autologous serum. At a *G. lamblia*/macrophage ratio of 1:1, the number of trophozoites ingested per 100 macrophages ranged from 1 to 12 at 0.5 h and increased for all donors (n = 6) to 10 to 92 at 8 h. Ingestion was confirmed by electron microscopy. Increasing the parasite/phagocyte ratio to 5:1 increased the percentage of macrophages with adherent but not ingested trophozoites. Incubating *Giardia* cells and macrophages with 20% immune serum increased ingestion of parasites eightfold, indicating that anti-*G. lamblia* antibody can enhance ingestion. Both phase-contrast microscopy and electron microscopy documented trophozoite destruction within macrophages. Ingestion of parasites elicited an oxidative burst as measured by luminol-enhanced chemiluminescence. In vitro, *Giardia* trophozoites were killed by $\geq 5 \times 10^{-5}$ M H_2O_2. Fusion of lysosomes with parasite-containing phagosomes was suggested by acridine orange-stained preparations. Human macrophages have the capacity to ingest *Giardia* trophozoites and to kill intracellular parasites, possibly by oxidative microbicidal mechanisms.

Giardia lamblia is an important cause of debilitating diarrhea throughout the world. In the United States, *G. lamblia* is both the most common enteric parasite and is the leading identified cause of waterborne outbreaks of diarrhea (3, 4). Because of its importance, efforts have been made to identify aspects of host immunity which play a role in protection from disease and clearance of the parasite. Epidemiologic studies do suggest that partially protective immunity develops. The age-related prevalence of *G. lamblia* in areas where the parasite is endemic is higher among younger age groups (15, 24), and lower rates of symptomatic disease may occur in long-term residents of these areas than among nonimmune visitors (13). In the murine model of *Giardia muris* infection, immunocompetent mice spontaneously clear their infection and become resistant to rechallenge (29).

Both humoral and cellular mechanisms of immunity are likely to be important. Athymic nude mice are unable to clear their infection until they have been immunologically reconstituted with either immune or nonimmune lymphoid cells (28). It is possible that, after reconstitution, these mice are able to clear their infection because they have been supplied with helper-inducer T cells which are required in the production of intestinal antibodies (7). Intestinal anti-*Giardia* immunoglobulin G and secretory immunoglobulin A have been implicated both in protection against and clearance of *Giardia* sp. (2, 9, 32).

Another component of the cellular immune system are mononuclear phagocytes, cells with an important role both as effector cells (22) and as immune regulating cells (34). Previous workers have documented spontaneous cytotoxicity of human peripheral blood monocytes for *G. lamblia* trophozoites (31); however, the methodology and results of this work have recently been questioned (1). Peritoneal macrophages from both rabbits and mice have been shown to ingest trophozoites in the presence of *Giardia* sp.-immune serum (14, 27). Owen and colleagues, in transmission electron micrographs of Peyer's patches from *G. muris*-infected mice, demonstrated macrophage pseudopod penetration between columnar epithelial cells with engulfment of trophozoites (23). They also found lymphocyte rosetting around macrophages which had ingested *G. muris*, suggesting an important antigen-processing function for Peyer's patch macrophages.

In an effort to examine the role of these important cells, further, the present study examined the interaction of human monocyte-derived macrophages with *G. lamblia* trophozoites. We demonstrate that human macrophages ingest trophozoites and that interaction of macrophages with *G. lamblia* is associated with both an oxidative respiratory burst and intracellular destruction of trophozoites.

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MATERIALS AND METHODS

Parasites. *G. lamblia* trophozoites, Portland 1 isolate (18), were used for all studies. They were axenically cultivated in filter-sterilized Diamond modified Trypticase (BBL Microbiology Systems, Cockeysville, Md.)-Panmede (Paines and Byrne Ltd., Greenford, England)-serum medium (35). Ten percent (vol/vol) heat-inactivated (ΔHI; 56°C for 30 min) fetal bovine serum (GIBCO, Grand Island, N.Y.) replaced horse serum, and 3% (vol/vol) National Collection of Type Cultures vitamin mixture no. 108 (GIBCO) was used. Reduced oxygen tension was maintained by adding 0.1% (wt/vol) L-cysteine hydrochloride and placing 16 ml of culture medium in tightly capped borosilicate tubes (16 by 125 mm).

After 2 to 5 days of culture, *G. lamblia* trophozoites were harvested by cooling the culture tubes to 4°C and then sedimenting the parasites at 250 × g for 5 min. Trophozoites were washed four times in Dulbecco phosphate-buffered saline (pH 7.4) before use in all studies. Trophozoites were

* Corresponding author.

suspended in medium 199 (GIBCO) for the macrophage ingestion assay.

Mononuclear cells and sera. Mononuclear cells were obtained by Ficoll-sodium diatrizoate (Histopaque; Sigma Chemical Co., St. Louis, Mo.) sedimentation of peripheral blood from healthy adult donors with no clinical history of giardiasis. (Informed consent was obtained from all donors before blood was drawn. The experimental protocol was approved by, and performed in accordance with guidelines prepared by, the Committees on Human Experimentation at the Universities of Connecticut and Virginia.) Cells were suspended in medium 199, and then approximately 5×10^6 mononuclear cells in 0.5 ml were layered onto 22-mm^2 glass cover slips and incubated at 37°C in 5% CO_2–95% room air. After 2 h, the cover slips were flooded with 1.5 ml of medium 199 containing penicillin (100 U/ml), gentamicin (50 μg/ml), and 20% autologous human serum. Nonadherent cells were removed after 24 h by gentle washing; the cover slip preparations were then flooded with fresh medium containing 13% autologous serum and antibiotics. Cell monolayers were used for study on day 5 after being washed in medium 199. Each cover slip contained 1.5×10^5 to 5.0×10^5 adherent cells, of which greater than 98% were nonspecific esterase stain positive, indicating that they were macrophages (37).

Sera obtained from donors were used immediately or stored at −70°C. The assay to detect a lethal effect of serum on *Giardia* trophozoites was performed exactly as described previously (11). Trophozoites were incubated in 20% fresh human serum and assayed for survival after 1 h at 37°C. The anti-*G. lamblia* antibody titer of the serum was determined by immunofluorescence by the method of Visvesvara et al. (36).

Macrophage ingestion assay. *G. lamblia* trophozoites were added to washed-macrophage (Mϕ) cover slip preparations at a 1:1 or 5:1 parasite-phagocyte ratio in medium 199 with 13% autologous ΔHI serum. The serum was ΔHI to prevent killing of trophozoites by complement-mediated mechanisms (11). After 0.5, 1, 2, 4, and 8 h, the cover slip preparations were stained with acridine orange (5 μg/ml) to aid in the distinction of intracellular from extracellular parasites and to examine macrophage lysosomes (26). In duplicate Mϕ cover slip preparations, adherent and apparently intracellular trophozoites were enumerated by phase-contrast microscopy and fluorescence microscopy using an Olympus BHT microscope which was fitted with reflected-light fluorescence. Photomicrographs were taken after 1 or 2 h of incubation. In one assay, ingestion of trophozoites in the presence of ΔHI *G. lamblia*-immune human serum obtained from a patient with documented giardiasis was compared with ingestion with nonimmune autologous serum.

Electron microscopy. Some Mϕ cover slip preparations which had been incubated with *Giardia* trophozoites for 3 h were processed for electron microscopy. After 10 Mϕ cover slip preparations were cooled for 2 h at 4°C, cells were removed by a rubber policeman and then fixed in 2% glutaraldehyde, followed by osmium tetroxide. After dehydration in serial alcohol washes, specimens were Epon embedded, and then sections were examined for the presence of intracellular trophozoites.

Assay of oxidative response. The oxidative response of the mononuclear phagocytes was measured quantitatively by luminol (5-amino-2,3-dihydro-1,4-phthalazinedione [Sigma])-enhanced chemiluminescence. Mϕ cover slip preparations (15 mm^2) containing approximately 10^5 adherent cells were placed with 1 ml of medium 199 containing 13% ΔHI autologous serum in dark-adapted 20-ml liquid scintillation vials. Washed *G. lamblia* trophozoites were then added in a parasite-to-phagocyte ratio of 3:1, and the mixture was incubated at 37°C. After 0.5, 1, and 2 h, chemiluminescence was measured at 15-min intervals for 1 h in a standard liquid scintillation counter (model LS-250; Beckman Instruments, Inc., Fullerton, Calif.) at ambient temperature. The counter was operating with a single photomultiplier tube activated out of coincidence mode as described by Meshnick and Eaton (17). The light response was enhanced by addition of luminol (1.25×10^{-6} M) to each vial immediately before the reading. As a control for ingestion of particles by macrophages, separate cover slip preparations were assayed for a chemiluminescence response after incubation for 15 min with 1 mg of zymosan (Sigma) per ml. The zymosan was opsonized with complement by incubation in 10% fresh autologous serum for 30 min at 37°C. Each condition was studied with duplicate cover slip preparations, and the results were averaged. The results are presented as counts per minute per 10^5 Mϕs. The counts per minute from control Mϕ cover slip preparations without stimuli were subtracted from the counts per minute obtained from Mϕ cover slips with *G. lamblia* or zymosan added.

Microbicidal assay. The susceptibility of *G. lamblia* trophozoites to a product of the oxidative burst, hydrogen peroxide (H_2O_2), was assessed by incubating 4×10^5 washed

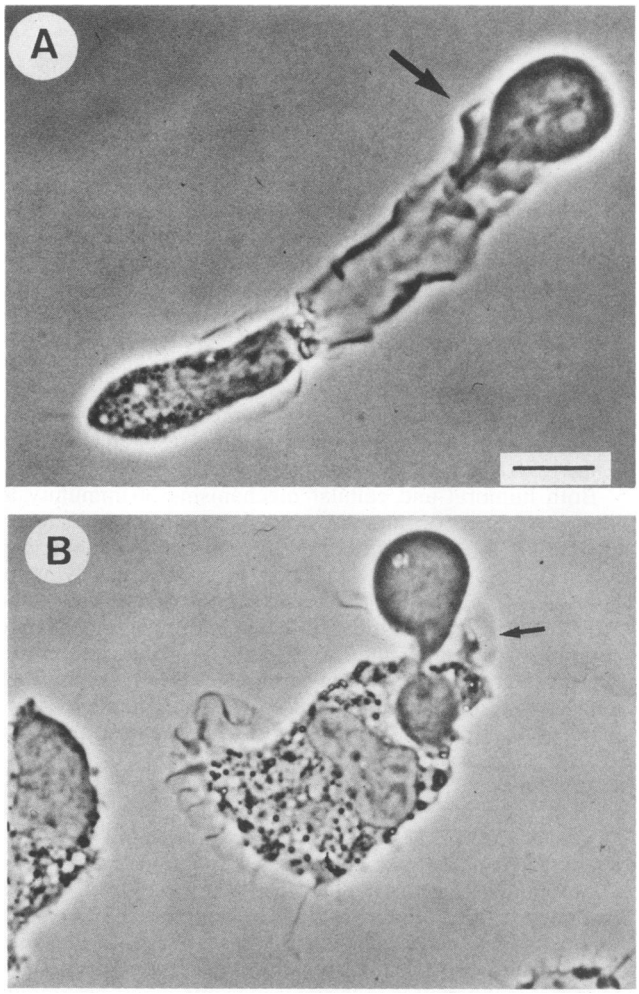

FIG. 1. Attachment and partial ingestion of *Giardia* trophozoites by human macrophages. (A) A macrophage pseudopod (arrow) extends around the posterior flagella of a trophozoite. (B) Partial ingestion has occurred with another cell (arrow). Bar, 10 μm.

trophozoites with duplicate serial dilutions of H_2O_2 ranging from 10^{-3} to 10^{-7} M in 0.2 ml of Hanks balanced salt solution. Lactoperoxidase (Sigma), 50 (mU/ml) and KI (0.05 mM), which served as an oxidizable halide, were added to some suspensions to study a peroxidase-mediated antimicrobial system analogous to the one present in immature macrophages. After 2 h at 37°C, the percentage of dead parasites was determined. The percent dead is the percentage of morphologically disrupted parasites after subtraction of the number of dead control parasites incubated in Hanks balanced salt solution alone. Less than 15% of control parasites died.

Statistical analysis. Comparisons between groups were evaluated with a paired *t* test or Student's two-tailed *t* test for independent means.

RESULTS

Ingestion of *G. lamblia* by human macrophages. Attachment and partial ingestion of *G. lamblia* trophozoites by human macrophages was documented by phase-contrast photomicrographs (Fig. 1A and B). Figure 2 demonstrates the intracellular location of two *G. lamblia* trophozoites by phase-contrast microscopy. When viewed under fluorescence microscopy, the phagosomes containing the *Giardia* trophozoites were green, suggesting that phagosome-lysosome fusion had not yet occurred (8, 26). The intracellular location of *G. lamblia* was further confirmed by the electron micrograph of a Mϕ with an ingested trophozoite (Fig. 3).

Adherence to but not ingestion of *G. lamblia* by macrophages was dependent on the *G. lamblia*/Mϕ ratio. Using cells from one donor, we demonstrated (Figure 4) that there was no difference in the percentage of macrophages with ingested trophozoites between the 1:1 and 5:1 incubation ratios, whereas at the 5:1 ratio there was a 3- to 14-fold increase ($P < 0.005$) in the percentage of macrophages with adherent *G. lamblia*. The ability to saturate ingestion but not adherence further supports the hypothesis that ingestion of trophozoites occurred (33).

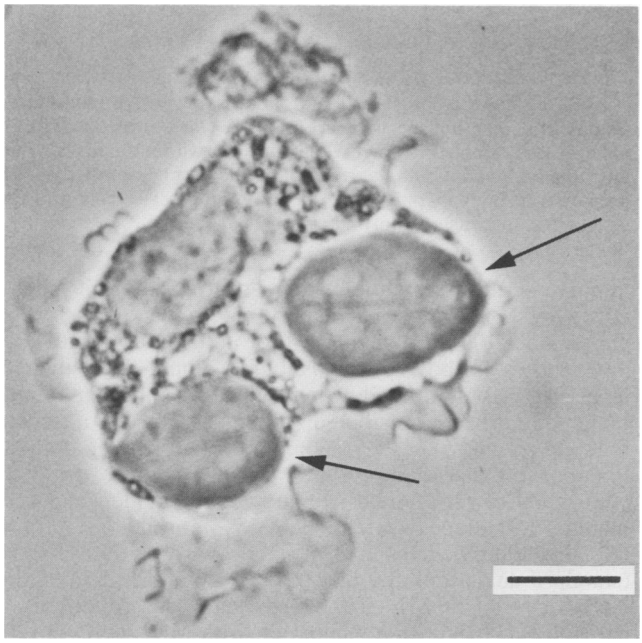

FIG. 2. Phase-contrast photomicrograph of *Giardia* trophozoites ingested by a human macrophage. Two trophozoites (arrows) are seen within a macrophage. Bar, 10 μm.

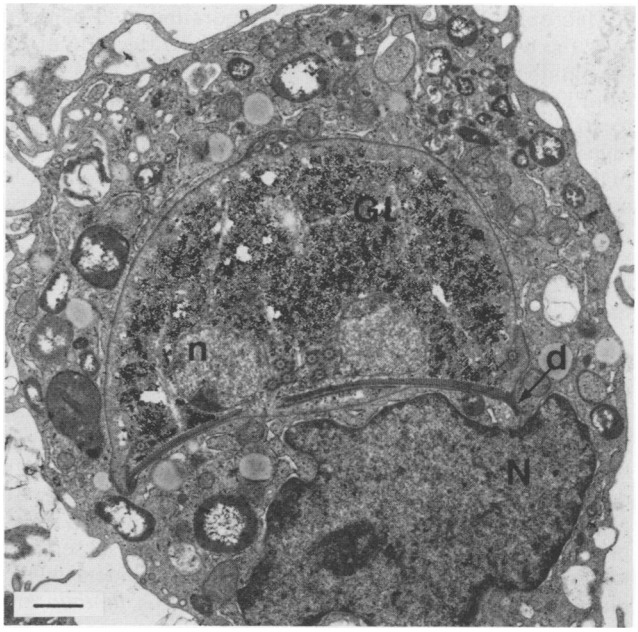

FIG. 3. Transmission electron micrograph of a macrophage with an ingested *Giardia* trophozoite. Macrophages were incubated with trophozoites for 3 h at 37°C with 13% ΔHI autologous serum. Abbreviations: GL, *Giardia* trophozoite; n, *Giardia* nucleus; d, disk; N, macrophage nucleus. Bar, 1 μm.

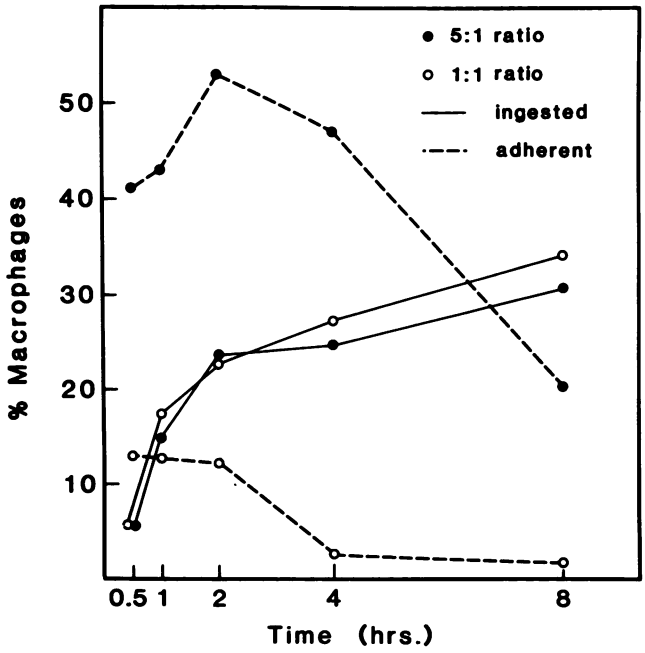

FIG. 4. Adherence to and ingestion by human macrophages at different parasite/phagocyte ratios. At a ratio of 5:1, there was a significantly greater percentage of macrophages with adherent parasites than at the 1:1 ratio ($P < 0.005$). The percentage of macrophages with ingested *G. lamblia* at the 5:1 ratio did not significantly differ from that at the 1:1 ratio. Parasites were incubated with duplicate Mϕ cover slip preparations at 37°C with 13% ΔHI autologous serum and observed under phase-contrast microscopy.

Macrophages from each of six donors ingested parasites over the 8-h assay (Fig. 5). At a *G. lamblia*/Mϕ ratio of 1:1, the number of trophozoites ingested per 100 Mϕs ranged from 1 to 12 at 0.5 h (five donors) and increased for all donors to 10 to 92 at 8 h. From 1 to 11% of the macrophages had ingested trophozoites at 0.5 h, and 13 to 60% of the macrophages had ingested trophozoites at 8 h. As a control for ingestion by macrophages, the particulate stimulus opsonized zymosan (0.5 mg/ml) was added to cells of one donor, 8% of which had ingested trophozoites after 2 h. Of these donor cells on control cover slips, 96% ingested zymosan, indicating that all of the cells were capable of ingestion.

The variation in the abilities of macrophages from different donors to ingest *G. lamblia* (Fig. 5) correlated ($r = 0.88$) with the ability of the autologous fresh serum to kill *G. lamblia* trophozoites in a serum killing assay (11). Five donors whose Mϕs ingested 10 to 42 parasites per 100 Mϕs in ΔHI sera at 8 h had sera that killed only 8 to 19% of giardias, whereas the donor whose macrophages ingested 92 trophozoites per 100 Mϕs had serum that killed 76% of trophozoites. We have previously demonstrated that the ability of fresh serum to kill *G. lamblia* occurs via a complement-dependent mechanism (11) and correlates closely with anti-*G. lamblia* antibody titers (unpublished data).

In another experiment to examine further the role of antibody, cells from one donor were incubated with ΔHI autologous serum (anti-*G. lamblia* antibody titer, 1:4), ΔHI *G. lamblia*-immune human serum (antibody titer, 1:128), or 1% bovine serum albumin alone (Table 1). The presence of immune serum substantially increased ingestion of trophozoites by Mϕs by approximately eightfold after 4 h: 26.2 giardias per 100 Mϕs versus 3.3 for autologous serum ($P < 0.02$) and 4.8 for bovine serum albumin alone. This enhanced ingestion was confirmed when cells from another nonimmune donor were incubated with giardias and a second immune serum. This suggests an important role of antibody in enhancing ingestion.

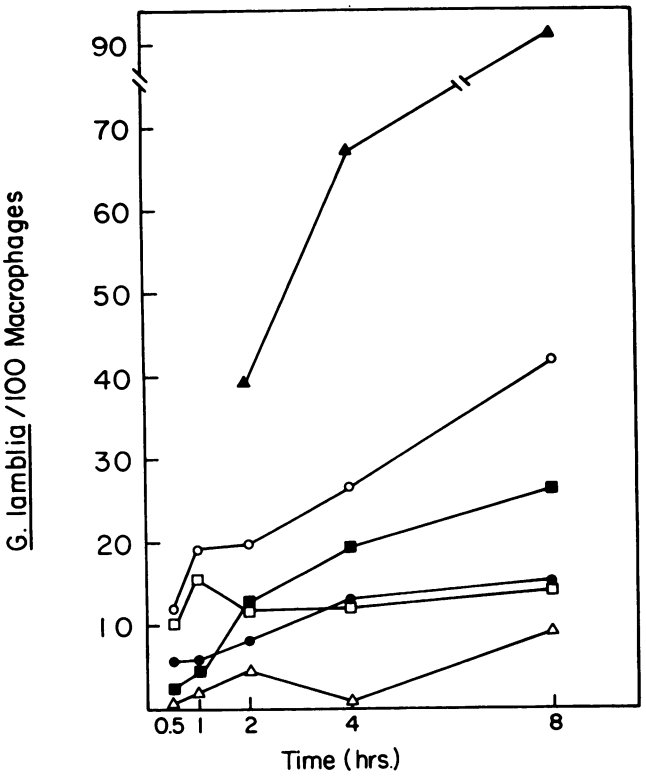

FIG. 5. Ingestion of *Giardia* trophozoites by macrophages from six donors. Cells from all of the donors ingested increasing numbers of trophozoites over time. Mϕ cover slip preparations were incubated with *G. lamblia* trophozoites in a 1:1 ratio at 37°C with 13% ΔHI autologous serum. Cells were observed under phase-contrast microscopy at 0.5, 1, 2, 4, and 8 h. Each point represents the mean of duplicate cover slip preparations.

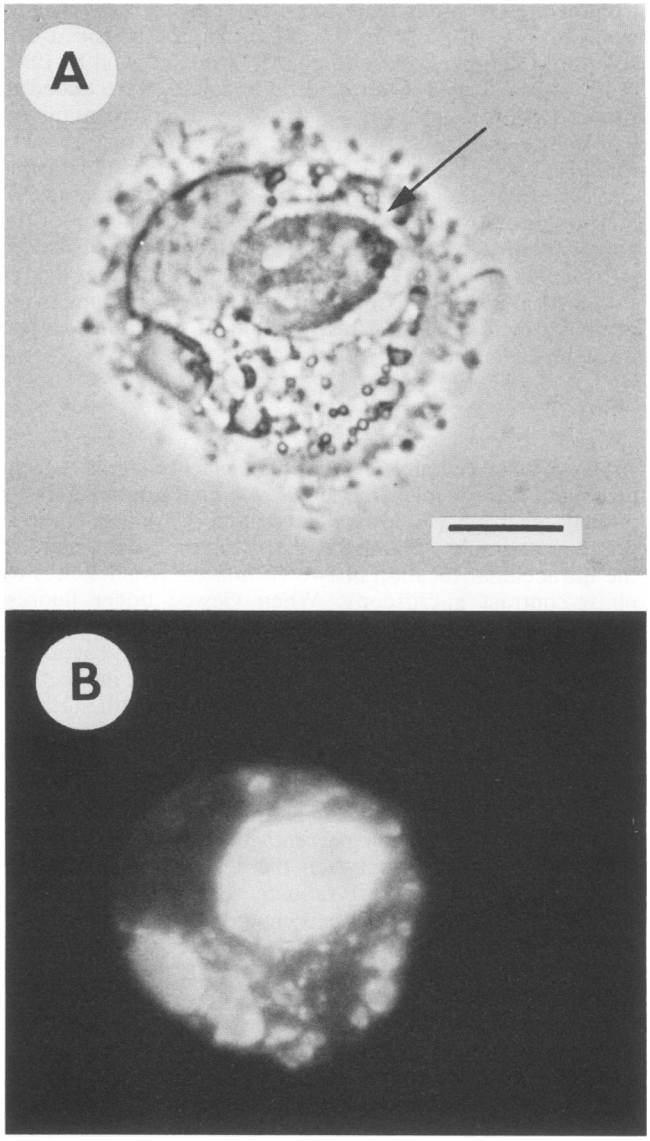

FIG. 6. Phase-contrast (A) and fluorescence (B) photomicrographs of a macrophage with an ingested *Giardia* trophozoite. In panel A, the trophozoite (arrow) can be seen within the macrophage and appears pycnotic and shrunken. In the acridine orange-stained preparation (B) the trophozoite stained brightly, suggesting phagosome-lysosome fusion. Bar, 10 μm.

Survival of *G. lamblia* trophozoites incubated at 4°C in medium 199 alone was excellent; by morphologic criteria (12), including flagellar motility, survival was 95.4 ± 1.5% (standard deviation) at 2 h, 93.2 ± 2.1% at 4 h, and 91.6 ± 2.6% ($n = 4$) at 8 h. To approximate the conditions of parasite survival during incubation with Mϕs more closely, trophozoites also were incubated in medium 199 with 13% ΔHI human serum at 37°C in 5% CO_2–95% room air. Their survival under these conditions was comparable to that in

TABLE 1. Ingestion of *G. lamblia* by human macrophages in the presence of autologous or immune serum

Serum (concn [%])	No. of *G. lamblia*/100 Mϕ at[a]:	
	2 h[b]	4 h[b]
Autologous (20)[c]	2.9	3.3
Immune (20)[d]	16.6[e]	26.2[e]
Bovine serum albumin (1)	2.5	4.8

[a] The data represent the means of duplicate cover slip preparations.
[b] Time after incubation of *G. lamblia* trophozoites with human monocyte-derived macrophages.
[c] Anti-*G. lamblia* antibody titer, 1:4.
[d] Anti-*G. lamblia* antibody titer, 1:128.
[e] $P < 0.02$ compared with autologous serum.

medium 199 alone, except at 8 h: survival was 90.0 ± 3.1% at 2 h, 87.6 ± 4.9% at 4 h, and 45.3 ± 27.6% ($n = 3$) at 8 h. We have demonstrated that morphologic criteria correlate closely with survival by subculture (12). Trophozoites were incubated with Mϕs at a 1:1 ratio, assuring that live trophozoites would come into contact with Mϕs. Finally, both trophozoite movement and flagellar motility could often be documented when parasites were within macrophages. This indicates that macrophages bound to and ingested viable parasites.

Intracellular trophozoite destruction. Figure 6A demonstrates a trophozoite within a phagosome of a Mϕ. When this same cell was viewed under fluorescence microscopy (Fig. 6B), the parasite was orange, suggesting fusion of the phagosome with the lysosome (8). The trophozoite also was shrunken and pycnotic, indicating partial digestion. Over the 8-h experiment, it was noted that trophozoites within Mϕs became progressively shrunken and distorted, with loss of normal morphology. By electron microscopy (Fig. 7), ingested trophozoites demonstrated disintegration, vacuolization, and loss of nuclear integrity.

Oxidative response. Macrophage interaction with *G. lamblia* was associated with an oxidative response as measured by the chemiluminescence assay. Figure 8 demonstrates the luminol-enhanced chemiluminescence response of macrophages incubated with *G. lamblia* trophozoites for 0.5, 1, and 2 h. A mean peak response of $5.4 \times 10^4 \pm 1.4 \times 10^4$ (standard error of the mean [SEM]) cpm occurred at 1 h. This response was 13.0 ± 3.2% (SEM) (range, 3.7 to 22.3%) of the concurrently determined chemiluminescence response to opsonized zymosan.

Susceptiblity to H_2O_2. Fig. 9 demonstrates that increasing percentages of trophozoites were killed with increasing concentrations of H_2O_2; over 93% of trophozoites were dead after they were incubated with 5×10^{-5} M H_2O_2, and 100% were killed at higher concentrations of H_2O_2. Killing occurred at lower concentrations of H_2O_2 when lactoperoxidase and KI were added; 51.7 ± 23.0% (SEM) of trophozoites were killed at 5×10^{-6} M, and 56.7 ± 12.7% were killed at 1×10^{-5} M ($P < 0.01$ compared with H_2O_2 alone).

DISCUSSION

We have demonstrated that human peripheral blood macrophages from multiple donors adhere to and ingest *G. lamblia* trophozoites; ingestion was documented by both phase-contrast microscopy and electron microscopy.

The role of anti-*G. lamblia* antibody in enhancing ingestion is suggested by several factors. (i) When macrophages were incubated in autologous serum which had a low anti-

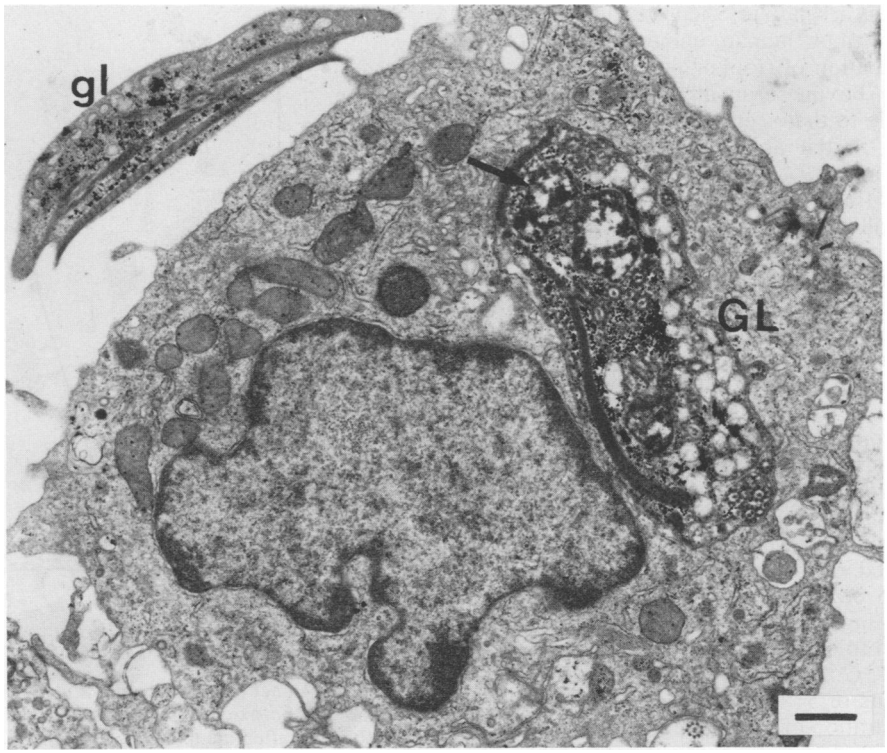

FIG. 7. Transmission electron micrograph of a macrophage with a degenerated trophozoite (GL), indicating parasite death. There was nuclear disintegration (arrow) and extensive cytoplasmic vacuolization. An intact trophozoite (gl) can be seen outside of the macrophage. Bar, 1 μm.

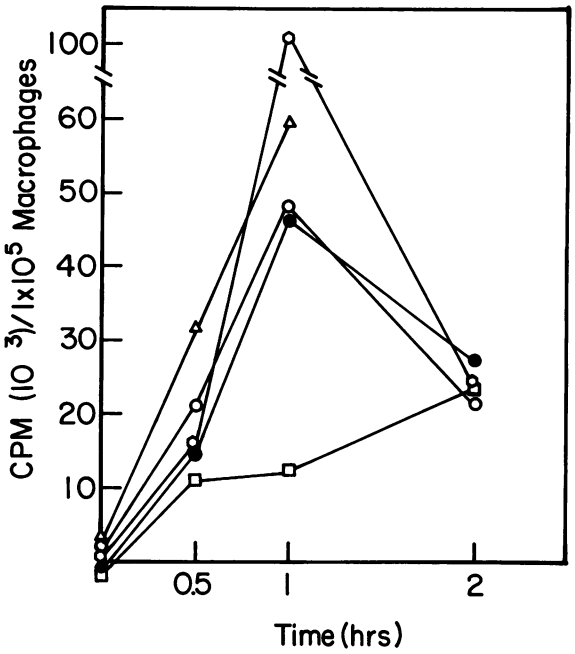

FIG. 8. Chemiluminescence response of Mϕ cover slip preparations from five donors incubated with *G. lamblia* trophozoites at a 3:1 ratio in the presence of 13% ΔHI autologous serum. The response was enhanced by luminol (1.25×10^{-6} M) and measured in counts per minute; counts from control cover slips without giardias were subtracted. The maximum response at 1 h of cells incubated with giardias was 13.0 ± 3.2% (SEM; range, 3.7 to 22.3%) of the concurrently determined response to opsonized zymosan (data not shown).

body titer against *G. lamblia* (1:4 by immunofluorescent-antibody assay), the number of trophozoites ingested per 100 Mϕs was eightfold less at 4 h than when the same cells were incubated in heterologous human immune serum (1:128 antibody titer). Incubation of trophozoites and Mϕs in the absence of serum (1% bovine serum albumin) resulted in low ingestion, comparable to that seen with nonimmune serum.

(ii) The variation in the ability of macrophages from different donors to ingest *G. lamblia* correlated with the ability of autologous fresh serum to kill *G. lamblia* trophozoites in a serum killing assay (11). We have previously demonstrated that the ability of fresh serum to kill trophozoites occurs via activation of the classical pathway (11) and correlates closely with anti-*G. lamblia* antibody titers (unpublished data). These findings further suggest that antibody plays an important role as an opsonin for the ingestion of trophozoites by macrophages (14, 27).

The mechanism by which macrophage interaction with *G. lamblia* resulted in trophozoite destruction was investigated. Because oxidative microbicidal mechanisms are important in monocytes and macrophages, these were studied. Association of *G. lamblia* with macrophages produced an oxidative burst. The lower oxidative response of cells incubated with trophozoites compared with those incubated with opsonized zymosan is likely to be due to the smaller percentage of macrophages which ingested parasites. When survival of trophozoites exposed to one product of the oxidative burst, H_2O_2, was examined, *G. lamblia* were found to be susceptible to concentrations of 5×10^{-5} M and greater. This is similar to the concentration of 10^{-4} M required for complete killing of *Leishmania donovani* promastigotes (25). The contrast, *G. lamblia* is more sensitive to the lethal effect of H_2O_2 than are either *Trypanosoma cruzi* epimastigotes, which require 3×10^{-4} M H_2O_2 for killing (21), or *Toxoplasma gondii* cells, more than 40% of which survive a concentration as high as 10^{-2} M (19).

The enhanced killing of *G. lamblia* with the addition of lactoperoxidase and KI suggests that immature macrophages and monocytes may be even more effective in oxidative killing of *G. lamblia* than are mature macrophages, which tend to have decreased levels of myeloperoxidase (20).

Oxygen-independent mechanisms may also be important for the intracellular destruction of *G. lamblia*. These include a marked increase in acidity of the phagosome after ingestion of particles and emptying of numerous digestive enzymes, including lysozyme, into the phagosome upon lysosomal fusion (6). Phagosome-lysosome fusion was suggested by acridine orange staining of macrophages with ingested trophozoites.

Trophozoites which were ingested by Mϕs underwent progressive morphologic destruction, as shown by both phase-contrast microscopy and electron microscopy. There is good evidence that most trophozoites ingested by Mϕs at 4 h and earlier were alive at the time of adherence and ingestion. This avoided a potential problem raised with the original study by Smith and colleagues in which they examined the cytotoxicity of peripheral blood mononuclear cells to *G. lamblia* in an 18-h assay (31). Aggarwal and Nash subsequently found spontaneously high death of trophozoites under conditions (aerobic) optimal for mononuclear cells but detrimental to microaerophilic *G. lamblia*, therefore negating the contribution of killing of trophozoites by mononuclear cells at 18 h (1). Our study examined the interaction of trophozoites with cells at earlier time points (0.5, 1, 2, 4, and 8 h), when there was excellent trophozoite survival through 4 h. Additionally, trophozoites were incubated with Mϕs at a 1:1 ratio, assuring that live trophozoites would come into contact with Mϕs, and trophozoite and flagellar

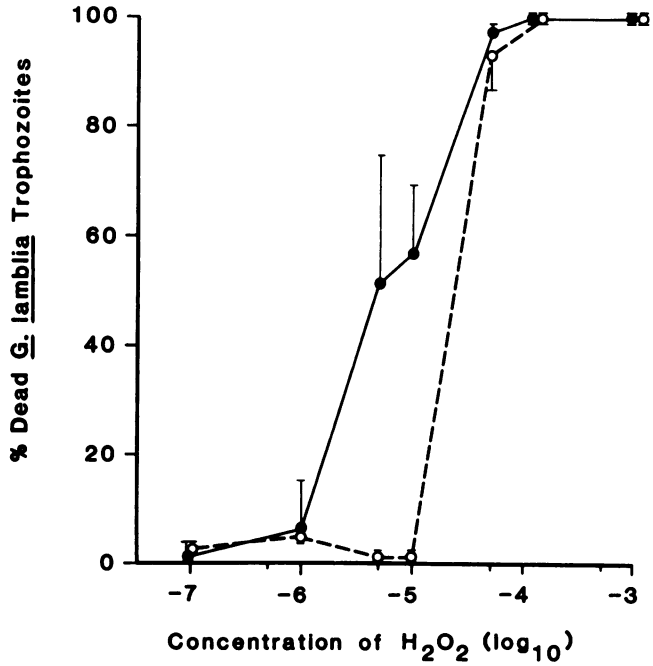

FIG. 9. Effect of hydrogen peroxide with (●) and without (○) 50 mU of lactoperoxidase per ml and 0.05 mM KI on *G. lamblia* trophozoites after incubation at 37°C for 2 h. Killing was significantly increased ($P < 0.01$) by addition of lactoperoxidase and KI at H_2O_2 concentrations of 5×10^{-6} and 1×10^{-5} M. Each point represents the mean, and bars represent the SEM ($n = 3$).

movements within macrophages were frequently documented.

This study, along with those which have examined animal peritoneal macrophages (14, 27), documents the ability of human macrophages to ingest *Giardia* trophozoites. The role of mononuclear phagocytes in clinical infection with *Giardia* sp. is not yet determined. *Giardia* sp. are primarily luminal agents; their invasion of the submucosa has been occasionally documented (30). Macrophages are in the minority when leukocytes harvested from the intestinal lumen are examined in a murine model of giardiasis (10). Although there may be relatively few macrophages in normal intestinal mucosa (16), histopathology of the intestine in some cases of human giardiasis has demonstrated marked mucosal flattening with intense mononuclear cell infiltration into the mucosa, to which macrophages may be contributors (5). Electron microscopy of Peyer's patches from mice with *G. muris* infection has documented in vivo ingestion of trophozoites (23). The ability to ingest this parasite allows macrophages to play a role as effector cells and to have the potential to participate as antigen-processing cells. Further study in this area will help to define the multiple important factors in the host immune response in giardiasis.

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