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# Roles of CR3 and Mannose Receptors in the Attachment and Ingestion of *Leishmania donovani* by Human Mononuclear Phagocytes

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***Leishmania donovani* is an obligate intracellular parasite of mammalian macrophages. Two macrophage receptors, the mannose-fucose receptor (MFR) and the receptor for complement component C3bi, CR3, were examined for their roles in the attachment and ingestion of *L. donovani* by human monocyte-derived macrophages. Two monoclonal antibodies which bind to the human CR3, anti-Mo1 and anti-Mac-1, inhibited both attachment and ingestion of *L. donovani* promastigotes after preincubation with human monocyte-derived macrophages; attachment was inhibited by 40 and 62% by anti-Mo1 and anti-Mac-1, respectively, and ingestion was inhibited by 34 and 51% by anti-Mo1 and anti-Mac-1, respectively. The interaction between promastigotes and CR3 may not have involved the C3bi-binding site on CR3, however, because a monoclonal antibody which exhibits specificity for this site, OKM10, inhibited promastigote attachment by only 18%. In contrast, OKM1, which is believed to react with the alternate lectinlike binding site on CR3, inhibited ingestion by 65%. MFR activity was inhibited using the soluble MFR ligands, mannan and mannosylated bovine serum albumin, which also inhibited promastigote attachment by 40 and 37%, respectively. The simultaneous inhibition of both CR3 (by anti-Mac-1) and the MFR (by either mannan or mannosylated bovine serum albumin) resulted in a greater decrease in promastigote attachment than inhibition of either receptor alone. Additionally, the reduction of MFR activity by allowing macrophages to adhere to a mannan-coated surface followed by the addition of anti-CR3 antibodies resulted in an 81% inhibition of promastigote ingestion, a greater decrease than was obtained by manipulation of either receptor alone. The results suggest that the MFR and CR3 independently participate in the attachment and ingestion of *L. donovani* promastigotes by human macrophages.**

Much of the developing world is affected by one or more forms of leishmaniasis, a group of diseases caused by trypanosomatid protozoa of the genus *Leishmania*. At the onset of visceral leishmaniasis, the causative protozoan, *Leishmania donovani*, is introduced into the skin of a susceptible mammal by a phlebotomine sandfly. Once in the skin, the parasite is ingested by a mononuclear phagocyte and converts from its extracellular promastigote stage to its intracellular amastigote stage. The parasite then disseminates throughout the reticuloendothelial system, where it survives and multiplies as an obligate intracellular parasite of macrophages. Without treatment, symptomatic disease follows a chronic progressive course, usually resulting in death of the host (21, 31).

Histologic studies have revealed that *L. donovani* can be found intracellularly in both neutrophils and mononuclear phagocytes soon after the inoculation of promastigotes into the skin. However, long-term survival of the parasite occurs solely in macrophages (43a). In vitro studies have underlined the importance of the macrophage in providing a protective environment for the parasite. *L. donovani* is killed in vitro by human neutrophils, and it is lysed by normal human serum (28, 29). It can survive and proliferate, however, in human monocyte-derived macrophages (31). Thus, for the disease to be initiated, the promastigote must enter a mononuclear phagocyte efficiently and evade these potentially lethal humoral and cellular factors.

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The interaction between macrophage membranes and leishmania promastigotes exhibits characteristics of a receptor-ligand interaction, such as specificity, saturability, and competitive inhibition (17). Previous investigations have examined the roles of several receptors on the macrophage surface which may participate in promastigote ingestion. These include a receptor found on human and rodent macrophages which recognizes mannose-, fucose-, or *N*-acetylglucosamine-terminal glycoconjugates and is called the mannose-fucose receptor (MFR) (37–39). Inhibitors of the MFR inhibit *L. donovani* promastigote ingestion by murine macrophages (3, 11) and both ingestion and attachment of promastigotes to human monocyte-derived macrophages (45). Studies have also identified the macrophage receptor for the complement breakdown product C3bi, called the third complement receptor or CR3, as a potential participant in the ingestion of *Leishmania* promastigotes by murine macrophages (3, 24). The initial step of parasite attachment to the macrophage CR3, however, has not been separately assessed.

The aim of the present study was to investigate three questions which arise from these findings. First, is CR3 on human, as opposed to murine, macrophages important in the ingestion of *L. donovani*? Second, does CR3 affect both the attachment and the ingestion of promastigotes? And finally, do CR3 and the MFR cooperate in promoting the attachment of promastigotes to human macrophages? The ultimate goal of such studies is to elucidate the means by which this important pathogen establishes itself in the human host.

## MATERIALS AND METHODS

**Parasites.** *L. donovani* (Sudanese strain S-3) was maintained by serial intracardiac injection of amastigotes into golden hamsters. Spleens were removed from heavily infected hamsters, homogenized in a tissue grinder, and suspended in 20 to 30 ml of phosphate-buffered saline, pH 7.4 (PBS). After low-speed centrifugation to remove splenic debris (150 × *g*, 20 min), amastigotes were washed twice in PBS (1,200 × *g*, 15 min). Parasites were suspended in a modified minimal essential medium (GIBCO Laboratories, Grand Island, N.Y.) with 10% heat-inactivated fetal bovine serum (56°C, 30 min; GIBCO), penicillin (100 U/ml), and gentamicin (50 μg/ml) (Sigma Chemical Co., St. Louis, Mo.) (28). After 3 days, amastigotes converted to promastigotes. Promastigotes in stationary-growth phase were used for experiments because of their greater infectivity compared with log-phase parasites (39, 40). Thus, cultures were used 7 days after their last day of passage, when the parasites had stabilized at a concentration of approximately $0.5 \times 10^7$ to $1 \times 10^7$/ml. Experiments were performed with parasites within 15 days after their isolation from hamster spleens.

**Mononuclear phagocytes.** Mononuclear cells were separated from the peripheral blood of normal human volunteers by Ficoll-sodium diatrizoate sedimentation. Monocytes were separated from lymphocytes by adherence to 12-mm-diameter glass cover slips. Monocytes were maintained in 13% autologous serum in medium 199 with 10 mM HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid; GIBCO), penicillin, and gentamicin for 5 days at 37°C and 5% $CO_2$ and used for experiments as monocyte-derived macrophages.

Macrophage suspensions were prepared as described above, and the mononuclear cell layer was allowed to adhere to 75-mm-diameter plastic petri dishes in 10% heat-inactivated fetal bovine serum in medium 199 for 2 h and then carefully released from petri dishes with a sterile rubber policeman. Macrophage suspensions were maintained for 5 days in medium 199 with 26% autologous serum, 10 mM HEPES, and antibiotics in Teflon vials (36 by 27 mm; Scientific Specialties Services, Inc., Randallstown, Md.).

**Ingestion of parasites by monocyte-derived macrophages.** *Leishmanias* spp. are ingested by mononuclear phagocytes in the absence of exogenous serum factors (5, 8–10, 30, 51, 53). The serum-independent ingestion of *L. donovani* was assessed with promastigotes in stationary-growth phase that were washed twice by centrifugation (1,200 × *g*, 15 min) and suspended with macrophage monolayers at a 12:1 parasite/macrophage ratio in Hanks balanced salt solution (GIBCO). After 30 min of incubation at 37°C, slides were blow-dried, fixed with methanol, and stained with Diff-Quik (American Scientific Products, McGaw Park, Ill). The number of intracellular parasites per 100 mononuclear phagocytes and the percentage of macrophages infected were enumerated by light microscopy; at least 200 cells were examined. During experiments with ligand-coated cover slips, monolayers were stained with acridine orange (10 μg/ml) in PBS (Sigma), and the degree of infection was assessed with a microscope with phase and epifluorescence optics.

The degree of mononuclear phagocyte infection was assessed in the presence of ligands of the MFR or monoclonal antibodies against CR3, as potential inhibitors of these receptors. MFR ligands included mannosylated bovine serum albumin (BSA) at 20 μg/ml (E-Y Laboratories, San Mateo, Calif.) and mannan at 2.5 mg/ml (approximately $3.125 \times 10^{-6}$ M) (Sigma). Monoclonal antibodies directed against CR3 were anti-Mol (clone 94; Coulter Immunology, Hialeah, Fla.), monoclonal antibody to the murine Mac-1 antigen (M1/70), and OKM1 (American Type Culture Collection, Rockville, Md.); anti-Mac-2 antigen (M3/38) (Hybritech, San Diego, Calif.) was used as a control. Monoclonal antibody OKM10, which is directed toward the C3bi-binding site on CR3 (49), was kindly provided by Gideon Goldstein of Ortho Pharmaceuticals. Antibodies were added to macrophages at 4 μg/$2.5 \times 10^5$ macrophages in 350 μl of Hanks balanced salt solution. Phagocytes were preincubated with receptor ligands or monoclonal antibodies for 30 min before the addition of parasites.

**Attachment of parasites to monocyte-derived macrophages.** Assays of attachment were performed as described by Wyler (51). Promastigotes were incubated with monocyte monolayers as described above, in the presence of the microfilament inhibitor cytochalasin D at 10 μg/ml (Sigma). After 30 min, monolayers were processed as described above.

Data for macrophage infection or attachment experiments are expressed as the means ± the standard errors for four experiments, with duplicate conditions in each experiment. Statistical analysis was by using Student's paired *t* test.

**Macrophage adherence to ligand-coated surfaces.** Glass cover slips were coated with ligands as described by Michl et al. (22) and modified by Sung et al. (40). Round glass cover slips (12-mm diameter) were incubated in 24-well plates with 0.1 mg of poly-L-lysine ($M_r$, 30,000 to 70,000) per ml in PBS for 45 min, rinsed with PBS, and overlaid with a mixture of 10 mg of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride per ml and 50 mg of either mannan or BSA per ml in PBS (reagents from Sigma). After overnight incubation at 4°C, cover slips were rinsed and sterilized for 15 min under UV light. Five-day-old monocyte-derived macrophages in suspension were washed twice in medium 199 by centrifugation at 4°C (350 × *g*, 15 min), adjusted to $5 \times 10^6$ cells per ml, and plated onto the coated cover slips. After 1 h the macrophages were overlaid with 15% heat-inactivated fetal calf serum in medium 199, and after 2 h, they were rinsed and used for infection experiments.

**Assays of MFR activity.** MFR activity in macrophages adherent to mannan- or BSA-coated surfaces was documented by using the binding assay of Stahl and Gordon (37). Mannosylated BSA was labeled with $Na^{125}I$ by using a solid-phase chloroglycoluril (13). $^{125}I$-labeled mannosylated BSA (5 μg) was added to $2.5 \times 10^5$ macrophages on ligand-coated cover slips. After 60 min at 4°C, cells were lysed in 1% sodium dodecyl sulfate, and binding was assessed as $^{125}I$ activity. Nonspecific binding was defined as the binding of $^{125}I$-labeled mannosylated BSA to macrophages in the presence of mannan (2.5 mg/ml).

MFR activity was also assessed in the presence of monoclonal anti-CR3 antibodies. Five-day-old monocyte-derived macrophages were suspended at $10^7$ cells per ml in Hanks balanced salt solution with 15% fetal calf serum, penicillin, and gentamicin, and 100 μl of the suspension was carefully layered over 100 μl of a mixture of two parts silicon oil (Accumetric, Elizabethtown, Ky.) and one part mineral oil (Sigma) in a microfuge tube at 4°C. Macrophages were incubated with or without monoclonal antibodies, and control cells contained mannan at 2.5 mg/ml to determine nonspecific binding. After 15 to 30 min, 4 μg of $^{125}I$-labeled mannosylated BSA was added to each tube. The macrophage mixtures were incubated at 4°C for 60 min, and then the cells were spun through the oil mixture in a microfuge (13,000 × *g*, 6 min). Pellets were cut from the tubes with a scalpel and assayed for $^{125}I$ activity. MFR activity was

calculated as the binding of $^{125}$I-labeled mannosylated BSA to experimental cells minus nonspecific binding to cells in the presence of mannan (18, 39).

**Preparation of complement-coated erythrocytes.** Sheep erythrocytes ($E^s$) in Alsever solution were sensitized with immunoglobulin M ($E^sA$) and exposed to R3 serum (human serum which has been partially depleted of C3 by exposure to zymosan) as previously described (43). All steps were performed in DGVB (gelatin-Veronal buffer with dextrose), except the final washes, which were performed in 0.04 M EDTA in gelatin-Veronal buffer. $E^sAC43b$ were exposed to 20% heat-inactivated human serum in DGVB, as a source of factors H and I, for 17 min at 37°C, washed three times by centrifugation (300 × *g*, 10 min), and suspended to 1% (vol/vol) in DGVB to give $E^sAC43bi$. $E^sAC43bi$ were incubated for 30 min with monocyte-derived macrophages in DGVB at 37°C, and the extent of rosetting was assessed (43).

## RESULTS

**Inhibition of promastigote ingestion by anti-CR3 antibodies.** We examined the serum-independent ingestion of promastigotes by human macrophages in the absence or presence of monoclonal antibodies directed toward different epitopes on CR3 in order to assess the role of CR3 in ingestion. Five-day-old human monocyte-derived macrophages ingested an average of 102.0 parasites per 100 macrophages on the monolayer (Fig. 1). Murine monoclonal antibody to human Mol antigen, which binds to CR3 on human phagocytes (33, 41, 42, 49), reduced the number of promastigotes ingested by 34%, compared with control cells ($P < 0.04$). Rat monoclonal antibody to murine Mac-1 antigen, which binds to murine CR3 but cross-reacts with the human receptor (1), inhibited promastigote ingestion by 51%. In contrast, OKM10, which may block the C3bi-binding site on CR3 of human mononuclear phagocytes (49), did not significantly alter promastigote ingestion. Antibody to murine Mac-2 antigen, an unrelated epitope on murine macrophages, also did not affect parasite ingestion.

**Inhibition of promastigote attachment to macrophages by monoclonal anti-CR3 antibodies.** The process of phagocytosis involves first the attachment of a particle to the surface of a phagocyte, followed by internalization. Because leishmania promastigotes bind poorly to macrophages at 4°C, it was not feasible to use low temperature as a means of studying attachment without internalization. As such, cytochalasin D, a microfilament inhibitor which allows promastigotes to attach to macrophages but prevents their phagocytosis and which does not interfere with glucose transport (16, 51). During these studies, a mean of 49.2 promastigotes attached per 100 monocyte-derived macrophages in control cells (Fig. 2). Monoclonal anti-Mol and anti-Mac-1 antibodies inhibited promastigote attachment by 40% ($P < 0.006$) and 62% ($P < 0.002$), respectively. Experiments with various concentrations of anti-Mac-1 revealed that the inhibitory effects of this monoclonal antibody were detectable at 0.2 μg/2.5 × $10^5$ macrophages and saturating at 4.0 μg/2.5 × $10^5$ macrophages. OKM1, a monoclonal antibody which reacts with a different epitope on CR3, inhibited promastigote ingestion by 65% at 4 μg/ml (46.7 ± 9.3 to 16.2 ± 1.0 parasites per 100 macrophages). OKM10 inhibited promastigote attachment by 18% ($P < 0.05$), making this antibody a considerably less potent inhibitor than either of the former monoclonal antibodies. The OKM10-induced inhibition was not statistically significant when calculated for percentage of macrophages infected. As with parasite ingestion, antibody to Mac-2 antigen had no effect on promastigote attachment. The inhibition patterns shown in Fig. 1 and 2 were consistent in all experiments, i.e., inhibitions due to the antibodies were anti-Mac-1 > anti-Mol > OKM10.

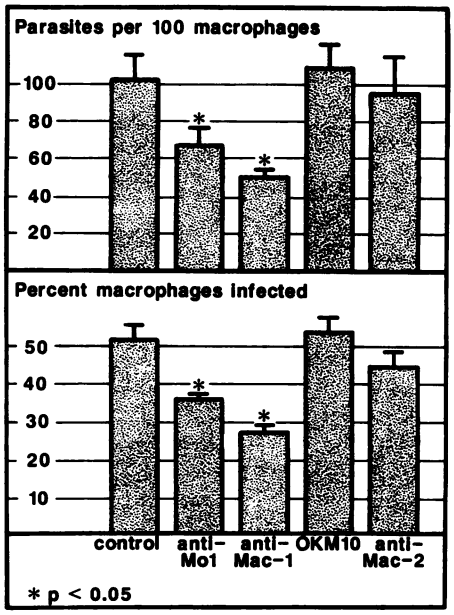


FIG. 1. Ingestion of *L. donovani* promastigotes by human monocyte-derived macrophages after preincubation of macrophages with monoclonal antibodies (4 μg/2.5 × $10^5$ cells). Anti-Mol and OKM10 bind to different epitopes on the human monocyte or macrophage CR3. Anti-Mac-1 is directed toward CR3 on murine macrophages and cross-reacts with the human receptor. Anti-Mac-2 is directed toward an unrelated antigen on murine macrophages. Macrophages were exposed to promastigotes for 30 min, and the degree of infection was assessed microscopically.

The binding of monoclonal antibodies to CR3 was verified by a rosetting assay of C3bi-coated $E^s$ to human monocyte-derived macrophages. $E^sAC43bi$ were prepared from $E^sAC43b$ cells; thus some rosetting occurred because of binding of unconverted C3b to the first complement receptor, CR1 (33). Anti-CR3 antibodies diminished only that portion of rosetting caused by CR3 binding. Of the control macrophages, 93% formed rosettes with $E^sAC43bi$, and the percentage of cells rosetted decreased by 39, 31, and 37% in the presence of anti-Mol, anti-Mac-1, and OKM10, respectively. Controls revealed that 33.9% of macrophages formed rosettes with $E^sAC43b$ not exposed to factors H or I (CR1 activity), and 14.1% formed rosettes with $E^sA$ alone (nonspecific binding).

**Simultaneous inhibition of the MFR and CR3.** Previous studies have revealed that ligands of the MFR on human macrophages inhibit both the ingestion and attachment of promastigotes to monocyte-derived macrophages (45). We questioned whether the inhibitory effects of MFR ligands and antibodies directed toward CR3 might be additive. Anti-Mac-1 inhibited promastigote attachment to monocyte-derived macrophages by 62% ($P < 0.002$), and mannan, a ligand of the MFR, inhibited attachment by 40% ($P < 0.03$) when it was in solution (Fig. 3). Anti-Mac-1 and mannan together resulted in a 79% inhibition of promastigote attachment, which was greater than the inhibitory effects of either mannan alone ($P < 0.005$) or anti-Mac-1 alone ($P < 0.05$). Similar results were obtained with another MFR ligand, synthetically mannosylated BSA, which decreased attach-

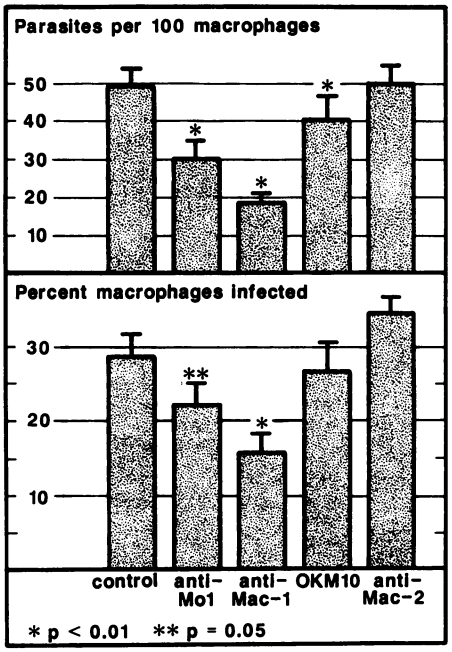


FIG. 2. Attachment of *L. donovani* promastigotes to human monocyte-derived macrophages as assessed with cytochalasin D to prevent internalization. Macrophages were preincubated with monoclonal antibodies (4 μg/2.5 × $10^5$ cells) for 15 to 30 min before exposure to parasites for 30 min. The specificities of monoclonal antibodies are as described in the legend to Fig. 1. The degree of attachment was assessed microscopically.

ment by 32% when used alone at 20 μg/ml ($P < 0.04$) and by 74% when combined with antibody to Mac-1 ($P < 0.01$ when compared with mannosylated BSA; $P < 0.05$ when compared with anti-Mac-1).

**Promastigote ingestion by macrophages on ligand-coated cover slips.** Macrophages adherent to surfaces coated with the MFR ligand, mannan, exhibited a 93.9% reduction in MFR activity, when compared with control cells on a BSA-coated surface; 1.27 ± 0.18 ng of $^{125}$I-labeled mannosylated BSA bound to macrophages on cover slips coated with BSA (2.5 × $10^5$ macrophages per cover slip), compared with 0.0768 ± 0.0119 ng that bound to macrophages cultured on mannan at 4°C. The ingestion of *L. donovani* promastigotes by these mannan-adherent macrophages is presented in Fig. 4. Similar to results shown in Fig. 1, the results shown in Fig. 4 indicate that either anti-Mo1 or anti-Mac-1 decreased parasite ingestion by control macrophages cultured on BSA by 59% ($P < 0.05$) or 67% ($P < 0.005$), respectively, compared with cells without antibodies. Monocyte-derived macrophages adherent to a mannan-coated surface exhibited a significant decrease in promastigote ingestion (35%), compared with control macrophages cultured on BSA ($P < 0.005$). The addition of monoclonal anti-Mo1 antibodies to macrophages cultured on mannan resulted in an 81% inhibition of promastigote ingestion, which was significantly greater than the inhibitory effects of culturing macrophages on mannan without antibodies ($P < 0.05$) or of exposing control (BSA) macrophages to anti-Mo1 ($P < 0.01$). Similarly, promastigote ingestion was inhibited by 81% in macrophages adherent to mannan exposed to anti-Mac-1, compared with macrophages on mannan ($P < 0.05$) and with control macrophages exposed to anti-Mac-1 ($P < 0.025$).

Monoclonal anti-CR3 antibodies did not interfere with the binding of mannosylated BSA to the MFR, since $10^5$ macrophages in suspension bound 0.229 ng of $^{125}$I-labeled mannosylated BSA, and this binding was not significantly altered by anti-Mo1 or by anti-Mac-1 (0.217 or 0.227 ng of protein, respectively).

## DISCUSSION

*L. donovani* is an obligate intracellular parasite of mammalian mononuclear phagocytes. In vitro studies have suggested that *L. donovani* promastigotes enter macrophages by attaching to surface receptors (17). The attachment of the parasite then triggers both the phagocytosis of the organism and an oxidative response by the mononuclear phagocyte (28, 31, 34). Thus, macrophage receptors must be able to both bind to the parasite and initiate the subsequent cellular events induced by the organism.

Previous reports have suggested that several macrophage receptors participate in the serum-independent ingestion of *Leishmania* promastigotes by macrophages. CR3, the receptor for the complement breakdown component C3bi, has been implicated in the ingestion of *L. major* (25) and *L. donovani* promastigotes (3, 11) by murine macrophages, even in the absence of serum. However, the step of attachment to CR3 has not been examined separately from the entire process of phagocytosis. The present study demonstrates that CR3 participates in the ingestion of *L. donovani* promastigotes by human monocyte-derived macrophages and that this participation probably occurs at the step of parasite attachment. The monoclonal antibodies used as inhibitors of CR3 activity, anti-Mo1, anti-Mac-1, OKM1, and OKM10, bind to identical proteins on sodium dodecyl sulfate-polyacrylamide gel electrophoresis; however, they probably recognize different epitopes on CR3 (1, 41, 42). In particular, Wright and colleagues have suggested that OKM10, the monoclonal antibody which exhibited the least

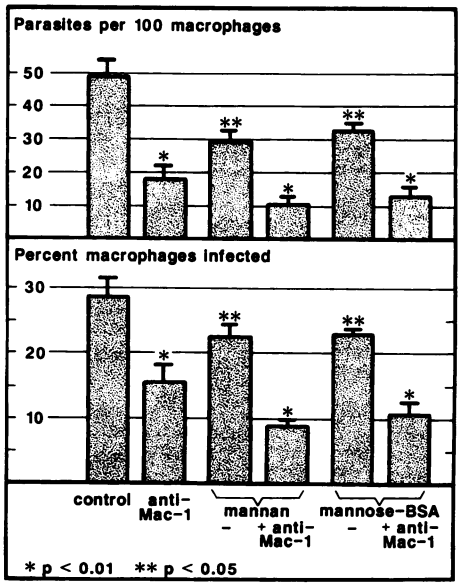


FIG. 3. Attachment of *L. donovani* promastigotes to human monocyte-derived macrophages after preincubation of macrophages with a ligand of the MFR (mannan [2.5 ng/ml] or mannosylated BSA [mannose-BSA; 20 μg/ml]), with or without the monoclonal anti-CR3 antibody, anti-Mac-1 (4 μg/2.5 × $10^5$ cells). Attachment was assessed after 30 min of exposure to promastigotes. Cytochalasin D was present throughout the experiment to prevent the internalization of parasites.

inhibition of promastigote attachment in our assay, reacts with the C3bi-binding site on human monocyte-derived macrophages (49). Ross et al. have suggested that CR3 on human neutrophils exhibits two distinct binding sites: one for particle-bound C3bi which does not trigger the release of superoxide and another with lectinlike characteristics which binds unopsonized zymosan, which does signal oxidative function, and which is recognized by the monoclonal antibody OKM1 (32). OKM1 was a potent inhibitor of parasite attachment in the present study, supporting the hypothesis that promastigotes may bind directly to the lectinlike site. However, *L. enriettii* and *L. major* fix C3 on their surfaces (23), and Wozencraft et al. have recently demonstrated that *L. donovani* promastigotes incubated with murine macrophages in serum-free medium are opsonized locally with C3 (46). These observations lend credence to the theory that opsonized parasites may bind to the C3bi site. The actual identity of the CR3 binding site for leishmania will require further clarification.

Receptors for C3b or C3bi on resting macrophages avidly bind ligand in the form of C3b- ($E^s$-C3b) or C3bi-coated $E^s$, but do not stimulate phagocytosis of the macrophages (47, 50). However, several receptors modulate the activities of these receptors for C3 components (14, 47, 48). For instance, after ligation of fibronectin receptors, human macrophages or neutrophils ingest $E^s$-C3b or $E^s$-C3bi, but they do not release $H_2O_2$ (50). Promastigotes stimulate both phagocytosis and a respiratory burst when they contact macrophages (27), thus it seems unlikely that promastigotes bind to CR3 alone. The macrophage MFR, which is involved in *L. donovani* promastigote attachment to macrophages (43), can stimulate both phagocytosis and an oxidative burst (2). It is possible that CR3 and the MFR cooperate or modulate one another during promastigote ingestion.

The MFR and CR3 had additive effects on promastigote attachment to human macrophages, as the present study shows both by using soluble inhibitors of the receptors and by modulating MFR activity. The latter was achieved by culturing macrophages on cover slips coated with a ligand of the MFR, resulting in a 93.9% reduction in MFR activity. Presumably, this reduction occurred because receptors diffused laterally through the phagocyte membrane to form complexes with the ligand on the ventral surface of the cell, exposing a membrane depleted of MFR activity to the overlying medium (22). When the MFR was occupied in such a manner, promastigote ingestion decreased, and the addition of anti-CR3 antibodies resulted in still further inhibition of parasite ingestion. It therefore seems unlikely that soluble mannan exerted its effect by sterically hindering the binding of parasites to CR3. Blackwell et al. studied CR3 and the MFR on murine macrophages and found inhibition of *L. donovani* ingestion by inhibitors of each receptor. These researchers did not, however, find additive effects of the two receptors, a finding which raised the question of whether the receptors must link to form one large "receptor complex" before ingestion of promastigotes, or whether the receptors exert their effects through the activation of a common final pathway (3, 11). In contrast, the data presented here suggest that this is not the case with human macrophages and that CR3 and the MFR act independently in promoting the attachment of promastigotes to human cells.

Other receptors appear to participate in the ingestion of leishmania promastigotes as well. Wyler et al. suggested that *L. major* promastigotes coated with fibronectin bind to fibronectin receptors (52). Mosser et al. showed that *L. major* promastigote ingestion is 50% inhibited by blocking

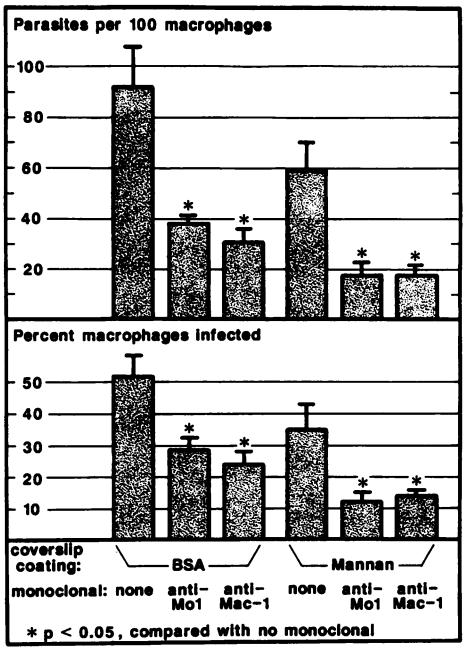


FIG. 4. Decreased MFR activity of macrophages adherent to a cover slip coated with the MFR ligand, mannan. Control macrophages, adherent to BSA-coated cover slips, retained their MFR activity. Antibodies to CR3, anti-Mo1 or anti-Mac-1, were incubated with the cover slip-adherent macrophages for 15 to 30 min before the addition of *L. donovani* promastigotes (4 μg of antibody per 2.5 × $10^5$ macrophages). After 30 min, the ingestion of promastigotes was assessed microscopically.

receptors for advanced glycosylation end products (25). The situation is complicated by the fact that *Leishmania* spp. differ in surface glycosylation, as determined by lectin binding (12, 17, 44), and also in surface antigens, as evidenced by species-specific monoclonal antibodies (18). Thus, findings with one species of *Leishmania* may not be directly applicable to others.

The identity of a ligand(s) on promastigotes for macrophage receptors has led several groups to isolate a mannose-containing glycoprotein with an $M_r$ of approximately 63,000 to 65,000 from several species of *Leishmania* (4, 6, 7, from several species of *Leishmania* 19, 20, 34). K.-P. Chang and colleagues isolated such a glycoprotein from *L. mexicana amazonensis*, which they call gp63, that inhibits the binding of promastigotes to murine macrophages (6, 7). Russell and Wilhelm documented a 30 to 35% inhibition of promastigote ingestion by macrophages with Fab fragments of monoclonal antibody to a *L. major* gp63 (34). A mannose-containing attachment glycoprotein obviously represents a potential ligand of the MFR, a theory supported by the finding of Russell and Wilhelm that the attachment of liposomes containing gp63 to macrophages is inhibited by greater than 90% by the MFR ligand, mannan (34). Handman and Goding reported a different ligand isolated from *L. major* promastigotes, a galactose-containing lipophosphoglycan (LPG) which is involved in promastigote binding to murine macrophages (15). It is now clear that other species of *Leishmania* contain a similar LPG. The specific interactions of gp63 and LPG with macrophage receptors have yet to be clarified.

The present study provides evidence for the simultaneous attachment of *L. donovani* promastigotes to two receptors on human monocyte-derived macrophages. Both receptors,

CR3 and the MFR, are parts of the normal macrophage mechanisms the macrophage for clearing microbes or glycoproteins from human tissues or serum. By attaching to these receptors, the parasite attains the protective intracellular environment of the macrophage, where it is able to survive in the host. Whether and how the two receptors cooperate to allow the ingestion and survival of parasites within the macrophage are questions that remain to be answered.

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