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Attachment Ligands of Viable *Toxoplasma gondii* Induce Soluble Immunosuppressive Factors in Human Monocytes

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Previous studies have demonstrated that surface antigen proteins, in particular SAG-1, of *Toxoplasma gondii* are important to this parasite as attachment ligands for the host cell. An in vitro assay was developed to test whether these ligands and other secretory proteins are involved in the immune response of human cells to toxoplasma. Human monocytes were infected with tachyzoites in the presence of antiparasite antibodies, and their effect on mitogen-induced lymphoproliferation was examined. The presence of antibody to either parasite-excreted proteins (MIC-1 and MIC-2) or surface proteins (SAG-1 and SAG-2) during infection neutralized the marked decrease seen in mitogen-induced lymphoproliferation in the presence of infected monocytes. Conversely, antibodies to other secreted proteins (ROP-1) and cytoplasmic molecules had no effect on parasite-induced, monocyte-mediated downregulation. Fluorescence microscope analysis detected microneme and surface antigen proteins on the monocyte cell surface during infection. These results suggest that microneme and surface antigen proteins trigger monocytes to downregulate mitogen-induced lymphoproliferation.

Toxoplasmosis is the most common central nervous system infection in patients with AIDS. As many as one-third of all AIDS patients suffer from symptomatic disease, usually manifested as acute meningoencephalitis (10). The causative agent, *Toxoplasma gondii*, is an obligate intracellular parasite that is ingested by the host and invades the host intestinal cells. Parasite replication is dependent upon infection of host cells by tachyzoites. During the initial stage of infection, tachyzoites attach to and then actively penetrate the host cells to become intracellular. Tachyzoite ligands known to play a role in attachment include the tachyzoite-specific major surface antigens, SAG-1 (9, 15, 16), SAG-2 (9), and SAG-3 (3), and the microneme proteins. Micronemes are small apical organelles containing specific proteins that are found in variable amounts in the invasive stages of all Apicomplexa. Micronemes are present in the tachyzoite stage of *T. gondii*, and three microneme proteins, MIC-1, MIC-2, and MIC-3, have been identified (7). MIC-2 is released from the micronemes during the attachment phase of infection and is believed to insert as an extracellular transmembrane protein at the apical pole of the tachyzoite plasma membrane during the process of invasion. It has been suggested that this protein attaches to host receptors and is capped before formation of the parasitophorous vacuole and released from the posterior end of the parasite (2). MIC-1 has homology to the thrombospondin-related adhesive proteins of *Plasmodium knowlesi* that have been shown to bind to human hepatocytes (17), but the function of MIC-1 and MIC-3 in *T. gondii* is unknown.

We have reported that lymphocytes show a marked decrease in mitogen-induced lymphoproliferation in the presence of human peripheral blood monocytes that have been incubated in vitro with tachyzoites (4). The downregulatory effect on lymphoproliferation was observed when monocytes were infected with either viable parasites or parasites that had been irradiated, which rendered them infective but unable to replicate. Neither heat-killed tachyzoites nor soluble parasite antigen could induce this immune system downregulatory response. Since microneme proteins are secreted only by viable parasites and since tachyzoite surface proteins can be heat denatured and rendered nonimmunogenic, these proteins may be the parasite ligands that trigger the release of the soluble factor(s) involved in immune system downregulation by the parasite. To investigate these hypotheses, we infected monocytes with tachyzoites in the presence of anti-MIC and anti-SAG antibodies and examined the effect on the proliferative response of lymphocytes to mitogen. These studies suggest that the tachyzoite proteins MIC-1, MIC-2, SAG-1, and SAG-2 can function as ligands that trigger host cell signaling events culminating in immune system downregulation.

MATERIALS AND METHODS

Isolation of human monocytes and lymphocytes. Cells were obtained from healthy toxoplasma-seronegative volunteers by cytophoresis. Mononuclear cells were separated from whole blood by using Ficoll-Hypaque (Winthrop Laboratories, New York, N.Y.), and monocytes were 80 to 90% enriched by aggregation as described previously (8). Platelets were removed from monocytes by washing twice in Versene buffer (0.2 g of EDTA per liter in phosphate-buffered saline [PBS]). Enriched monocytes were resuspended in medium (RPMI 1640 containing 25 mM HEPES buffer with L-glutamine) (Gibco Laboratories, Grand Island, N.Y.) supplemented with gentamicin sulfate (50 μg/ml [United States Biochemical Corp., Cleveland, Ohio]) and 10% (vol/vol) heat-inactivated (56°C for 30 min) fetal bovine serum (endotoxin-low [HyClone Laboratories, Inc., Logan, Utah]) and cultured overnight in tissue culture plates or on 12-mm-diameter glass coverslips (100,000 monocytes/coverslip). Under these conditions, monocytes in tissue culture plates remain nonadherent. Cytocentrifuge (Shandon Lipshaw, Pittsburgh, Pa.) preparations of monocytes (100,000 cells; 700 rpm for 5 min) were stained with Diff-Quik (Baxter Healthcare Corp., Miami, Fla.), and the percentages of monocytes, lymphocytes, and neutrophils were determined by microscopy. The remaining lymphocytes, enriched more than 90%, were resuspended at a density of 5×10^6/ml in medium and cultured in tissue culture flasks until their use in assays 48 h later. Special care was taken to ensure endotoxin-free conditions in all experiments, as measured by the *Limulus* amebocyte assay (Associates of Cape Cod, Falmouth, Mass.).

Parasites. *T. gondii* PLK was passaged in human fibroblasts maintained in minimal essential medium (alpha modification; Gibco Laboratories) containing antibiotic-antimycotic solution (Gibco Laboratories) and isolated as described previously (11). Briefly, infected fibroblasts were scraped, forcibly passed through a 27-gauge needle, and centrifuged at $50 \times g$ for 4 min to pellet large host cell debris. The supernatant was centrifuged at $900 \times g$ for 10 min to pellet the parasites, which were then resuspended in medium. The PLK strain is derived from the cloned P strain (Me49) and is regularly passaged in human foreskin fibroblasts in our laboratory.

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Monocyte triggering and lymphoproliferation assay. Monocytes were incubated for 18 h with parasites in the presence or absence of antiparasite antibodies. Antibodies (10 μg/ml for purified immunoglobulin G (IgG); 1:200 dilution for monoclonal antibody ascites) were added to monocytes 5 min before the addition of parasites. Parasites and antibodies were not washed off, since monocytes remained nonadherent throughout each experiment. Since naive lymphocytes respond poorly to specific antigen, the proliferative response of lymphocytes to mitogen was examined. Lymphocytes (150,000/well) and mitogen (2 μg of phytohemagglutinin per ml [Pharmacia Biotech Inc., Piscataway, N.J.]) were added to monocytes (2×10^4/well in a 96-well tissue culture plate) that had been incubated for 18 h with or without parasites. After a 24-h incubation with mitogen, the cultures were pulsed for a further 24 h with [^{3}H]thymidine (0.5 μCi per well for a 96-well plate; 2 μCi per well for 24-well plate; 70 to 90 Ci/mmol [DuPont NEN, Boston, Mass.]). The cells were harvested onto a glass filter with an automated cell harvester, and the isotope incorporation was measured by liquid scintillation counting. The mean value for three replicate cultures was determined and used in calculations of lymphoproliferation. Lymphoproliferation is expressed as a percentage of the response with uninfected monocytes (Δcpm of lymphocytes incubated with monocytes plus parasites/Δcpm of lymphocytes incubated with monocytes plus medium) × 100, where Δcpm is the mean counts per minute in response to mitogen minus the mean counts per minute for unstimulated cultures.

Light microscopy. Monocytes (5×10^5/500 μl in wells of a 24-well tissue culture plate) and parasites were incubated together for 18 h. Antibody was added to monocytes 5 min before the addition of parasites. Triplicate cytospin preparations (10^5 cells; 700 rpm for 5 min) were made for each sample. The cells were fixed and stained with Diff-Quik, and the number of parasites per monocyte was counted with a Zeiss Axiophot microscope. At least 200 cells were counted for each cytospin. The results are expressed as a cumulative percentage of total monocytes.

Fluorescence microscopy. Monocytes adherent to a glass coverslip were incubated with tachyzoites at a monocyte-to-tachyzoite ratio of 1:25 for 1 h at 37°C. Every 5 min, cells were washed extensively with ice-cold PBS to remove extracellular parasites. To determine the binding of parasite proteins to the monocyte cell surface, cells were incubated on ice for 45 min with primary antibodies. Either a mixture of anti-SAG-1 IgG (1 μg/ml) and anti-SAG-2 ascites (1:400 dilution), a mixture of anti-MIC-1, anti-MIC-2, and anti-MIC-3 ascites (1:400 dilution of each ascites), or isotype control antibodies were used, in the presence of human IgG (400 μg/ml) to block nonspecific binding of antibodies to monocyte FcγRI. The cells were washed with PBS–0.1% bovine serum albumin (BSA) and then incubated on ice for 45 min with a fluorescein isothiocyanate (FITC)-conjugated secondary antibody (1:50 final concentration [Caltag Laboratories Inc., South San Francisco, Calif.]). To stain cell nuclei, cells were incubated for 5 min on ice with propidium iodide in permeabilization buffer (0.3 μg of propidium iodide per ml in PBS–0.5% saponin [Sigma Chemical Co., St. Louis, Mo.]). Finally, the cells were fixed with PBS containing 2.5% formaldehyde (ultrapure, electron microscopy grade [Polysciences, Inc., Warrington, Pa.]) and 0.01% glutaraldehyde (ultrapure, electron microscopy grade [Polysciences, Inc.]) for 10 min at room temperature. The cells were examined at 1,250× magnification with a Zeiss Axiophot epifluorescence microscope equipped with a 100× 1.3 N.A. Plan Neofluar objective. Simultaneous examination of red-green fluorescence was possible by using a dual-bandpass filter (Omega Optical, Brattleboro, Vt.; green: excitation 490/20 nm, emission 528/24 nm; red: excitation 576/32 nm, emission 633/42 nm). Images were recorded onto Kodak Elite 200 ASA slide film and then onto prints.

Flow cytometry. Freshly isolated parasites (2×10^7/2 ml) were overlaid on a Percoll gradient (5 ml of 1.04-g/ml Percoll [Pharmacia Biotech Inc.], and pelleted by centrifugation (1,000 × *g* for 10 min), leaving fibroblast debris above the Percoll gradient. The parasites (10^6) were then incubated for 45 min at 4°C with antibodies [rabbit anti-tachyzoite IgG, anti-tachyzoite F(ab′)$_2$, anti-SAG-1, and anti-low-density lipoprotein IgG, 1-μg/ml final concentration; mouse anti-SAG-2 ascites and P3 ascites, 1:200 dilution] in 50 μl of RPMI 1640 supplemented with 0.1% BSA (Sigma Chemical Co.) in 96-well flat-bottom tissue culture plates (Costar Corp., Cambridge, Mass.). After the plates were washed three times with PBS–0.1% BSA, the parasites were incubated for a further 45 min at 4°C in 60 μl of a 1:40 dilution (vol/vol) of FITC-conjugated anti-species IgG diluted in PBS–0.1% BSA. After the plates were washed three times, more than 10,000 parasites per sample were analyzed in a FACScan flow cytometer (Becton Dickinson, San Jose, Calif.).

Antibodies. Mouse anti-MIC-1 (T10 1F7), anti-MIC-2 (T3 4A11), anti-MIC-3 (T4 2F3) ascites, anti-SAG-2 ascites, anti-p97 IgG, anti-ROP-1 IgG, and anti-SAG-1 IgG were isolated and characterized as described previously (1, 5, 11, 12, 14, 18, 19). Rabbit anti-toxoplasma IgG and anti-toxoplasma F(ab′)$_2$ fragments were made in our laboratory. Rabbit anti-low density lipoprotein (anti-LDL), a nonspecific rabbit polyclonal antibody was a generous gift of P. Morganelli, Veterans Administration Hospital, White River Junction, Vt. P3, a nonspecific ascites control, was made in our laboratory from a hybridoma provided by the American Type Culture Collection. FITC-conjugated F(ab′)$_2$ fragments of affinity-isolated anti-mouse IgG were obtained from Caltag Laboratories, and FITC-conjugated F(ab′)$_2$ fragments of affinity-isolated anti-rabbit IgG were from Pierce, Rockford, Ill.

Statistics. Statistics for cpm data were analyzed by one-way analysis of variance with log-transformed data (6).

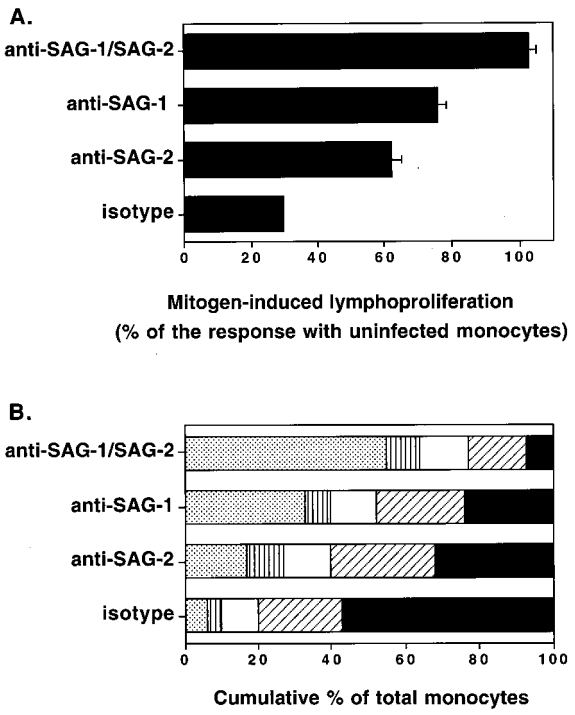

FIG. 1. Anti-SAG antibodies block the effect of infected monocytes on lymphoproliferation and cause a decrease in the number of monocytes that become infected with toxoplasmas. (A) Freshly isolated monocytes were incubated for 18 h with antibodies and parasites at a monocyte-to-parasite ratio of 1:2, and then lymphocytes and mitogen were added for a further 48 h. Lymphoproliferation was determined as the mean and standard deviation of triplicate determinations of cpm, and the results are expressed as a percentage of the response with uninfected monocytes. (B) Monocytes were incubated for 18 h with antibodies and parasites at a monocyte-to-parasite ratio of 1:2, and then cytospin preparations were made for microscopy. The results are reported as the cumulative percentage of cells that were uninfected (▤) or infected with one (▥), two (□), four (▨), or eight (■) tachyzoites per vacuole and are representative of three donors.

RESULTS

Anti-SAG-1 and anti-SAG-2 block the release of inhibitory soluble factors from monocytes, and inhibit parasite uptake into monocytes. When monocytes are infected with *T. gondii*, soluble factors are released that markedly decrease mitogen-induced lymphoproliferation; this response is parasite dose-dependent (4). To determine whether parasite surface antigens may trigger the monocyte signal transduction pathway(s) leading to the release of these soluble factors, monocytes were incubated with tachyzoites in the presence or absence of anti-SAG antibodies. After an 18-h infection, the time at which soluble-factor release is maximal (4), lymphocytes and mitogen were added to infected monocytes. When lymphocytes were incubated with uninfected monocytes, mitogen-induced lymphoproliferation was maximal and is identical to that seen in the absence of monocytes. In contrast, when lymphocytes were incubated with monocytes infected with tachyzoites at a 1:1 ratio, mitogen-induced lymphoproliferation was only 30% of maximal. Identical results were obtained when an isotype-matched control antibody was present during monocyte infection (Fig. 1A). With monocytes infected in the presence of anti-SAG-1 or anti-SAG-2 antibodies, mitogen-induced lymphoproliferation was 76 or 62%, respectively, of that measured in the presence of uninfected monocytes (Fig. 1A). When monocytes were infected in the presence of both anti-SAG-1 and anti-SAG-2, the decrease in lymphoproliferation was

blocked completely. The antibodies alone had no effect on mitogen-induced lymphoproliferation (data not shown). An alternative explanation for our results is that engagement of monocyte Fcγ receptors by immune complexes formed between the antibodies and their ligands may signal a pathway that decreases soluble factor release. This is unlikely since an identical response occurred in the presence of anti-tachyzoite $F(ab')_2$ fragments and anti-tachyzoite IgG (data not shown). Tachyzoites were also incubated with the antibodies at the same concentrations used for monocyte infection experiments, stained, and examined with a FACScan apparatus. Each of the specific antibodies directed at SAG-1, SAG-2, or whole parasites bound to tachyzoites with a mean fluorescence intensity of >200, whereas the isotype-matched control antibody had an intensity of <20, showing that antiparasite antibodies recognize tachyzoite cell surface antigens (data not shown).

In the absence of attachment, host cell surface receptors leading to soluble-factor release may not be triggered. To determine the effect of anti-SAG antibodies on attachment, we also determined tachyzoite uptake and replication. The maximal permissiveness of monocytes for tachyzoite uptake and replication was seen when monocytes were infected in the presence of an isotype-matched control antibody. After an 18-h infection, 6% of all monocytes were uninfected, 4% contained a parasitophorous vacuole with one tachyzoite, 10% contained a vacuole with two tachyzoites, 23% contained a vacuole with four tachyzoites, and 57% contained a vacuole with eight tachyzoites (Fig. 1B). The effect of either antibody reduced the level of infectivity of the monocytes compared to the irrelevant antibody control. When these antibodies were combined, the blocking effect was enhanced (Fig. 1B). For example, after an 18-h infection in the presence of both anti-SAG-1 and anti-SAG-2, 55% of all monocytes were uninfected, 9% contained a parasitophorous vacuole with one tachyzoite, 13% contained a vacuole with two tachyzoites, 16% contained a vacuole with four tachyzoites, and 7% contained a vacuole with eight tachyzoites. Although the majority of the monocytes (55%) were uninfected following treatment with both anti-SAG-1 and anti-SAG-2, the decrease in lymphoproliferation was ablated.

Anti-MIC-1 and anti-MIC-2 block the release of inhibitory soluble factors from monocytes and inhibit parasite uptake into monocytes. To determine whether parasite microneme antigens may trigger the monocyte signal transduction pathway(s) leading to the release of inhibitory soluble factors, monocytes were incubated with tachyzoites for 18 h in the presence of anti-MIC antibodies and then added to a lymphocyte proliferation assay in response to mitogen. When lymphocytes were incubated with monocytes infected with tachyzoites in the presence of an isotype-matched control antibody, mitogen-induced lymphoproliferation was 40% of the lymphoproliferation in the presence of uninfected monocytes (Fig. 2A). When either anti-MIC-1 or anti-MIC-2 antibody was added to monocytes before the addition of parasites, the decrease in mitogen-induced lymphoproliferation was ablated. With anti-MIC-3, mitogen-induced lymphoproliferation was 66% of the level seen with uninfected monocytes. To determine if this was a ligand-specific event, antibodies to another secreted protein, ROP-1, or cytoplasmic protein, p97, were evaluated. Neither anti-ROP-1 nor anti-p97 demonstrated an effect on neutralizing the downregulatory response (data not shown). The effect of anti-MIC antibody on parasite internalization and replication was further evaluated. As shown in Fig. 2B, by 18 h after infection in the presence of anti-MIC-2, 46% of all monocytes were uninfected, 24% of the monocytes contained a parasitophorous vacuole with one tachyzoite, 17% contained a vacuole with two tachyzoites, 11% contained a vacuole with four tachyzoites, and 2% contained a vacuole with eight tachyzoites. Baseline 18-h infection in the presence of an isotype-matched control antibody was 10% of all monocytes uninfected, 9% of all monocytes containing a parasitophorous vacuole with one tachyzoite, 12% containing a vacuole with two tachyzoites, 21% containing a vacuole with four tachyzoites, and 48% containing a vacuole with eight tachyzoites. The blocking effect of the antibodies was concentration dependent (data not shown).

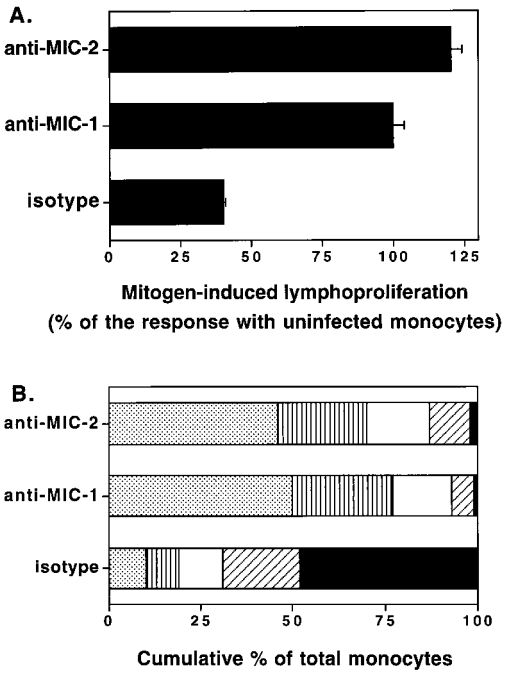

FIG. 2. Anti-MIC antibodies block the effect of infected monocytes on lymphoproliferation and cause a decrease in the number of monocytes that become infected with toxoplasmas. (A) Freshly isolated monocytes were incubated for 18 h with antibodies and parasites at a monocyte-to-parasite ratio of 1:2, and then lymphocytes and mitogen were added for a further 48 h. Lymphoproliferation was determined as the mean and standard deviation of triplicate determinations of cpm, and the results are expressed as a percentage of the response with uninfected monocytes. (B) Monocytes were incubated for 18 h with antibodies and parasites at a monocyte-to-parasite ratio of 1:2, and then cytospin preparations were made for microscopy. The results are reported as the cumulative percentage of cells that were uninfected (▤) or infected with one (▥), two (□), four (▨), or eight (■) tachyzoites per vacuole and are representative of three donors.

To determine whether the blocking effect of the antibodies was additive for the different parasite ligands, combinations of anti-SAG and anti-MIC antibodies were assayed. As was seen when single antibodies were used, incubation of monocytes and either anti-SAG-1 or anti-SAG-2 in the presence of anti-MIC-2 resulted in the reversal of the downregulatory effect compared to isotype-matched irrelevant control antibody (data not shown). However, the combination of these antibodies inhibited parasite uptake and replication more than did single antibodies alone (compare Fig. 3 with Fig. 1B and 2B). Mitogen-induced lymphoproliferation assayed in the absence of monocytes was not affected by the presence of any antitoxoplasma antibody (data not shown). These results suggest that anti-SAG and anti-MIC antibodies block the interaction of parasite ligands with monocyte receptors.

It is possible that a critical level of infection of monocytes is required to stimulate soluble-factor release. However, when monocytes were infected with fewer parasites, resulting in a 15% monocyte infection level, mitogen-induced lymphoprolif-

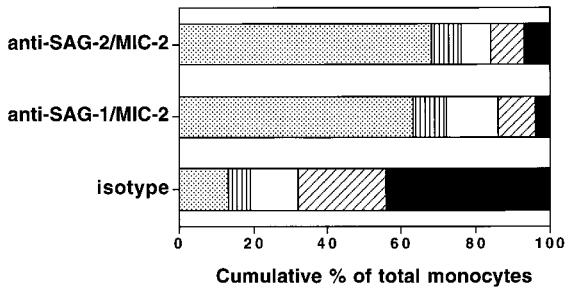

FIG. 3. Anti-SAG and anti-MIC antibodies cause a decrease in the number of monocytes that become infected with toxoplasmas. Monocytes were incubated for 18 h with antibodies and parasites at a monocyte-to-parasite ratio of 1:2, and then cytospin preparations were made for microscopy. The results are reported as the cumulative percentage of cells that were uninfected (▨), or infected with one (■), two (□), four (▨), or eight (■) tachyzoites per vacuole and are representative of three donors.

eration remained suppressed (68% of the response compared to uninfected monocytes), indicating that a 50% or better threshold of infection for induction of a downregulatory response was not required.

SAG and MIC are deposited on the surface of monocytes during infection. The binding of SAG and MIC proteins to monocyte cell surface receptors during infection was evaluated. Monocytes were infected with tachyzoites at a high multiplicity of infection (1:25), and unfixed monocytes were surface stained with mixtures of anti-SAG, anti-MIC, or isotype-matched control antibodies followed by FITC-conjugated secondary antibodies for detection by fluorescence microscopy. After a 15-min incubation with tachyzoites, the time required for parasites to settle onto cells, fluorescent antibody to SAG was observed in discrete patches on the surface of 10 to 15% of the monocytes. The greatest binding appeared to occur close to where a tachyzoite was attached (Fig. 4A). In contrast, only background binding of isotype control antibodies to infected monocytes was noted (Fig. 4B). For anti-MIC antibodies, the kinetics of binding to the surface of 10 to 15% of monocytes was similar. In general, fewer patches were observed compared to the situation for anti-SAG antibodies. We found that anti-SAG antibody binding could be competitively inhibited by soluble tachyzoite antigen and that the antibody did not bind to uninfected monocytes (data not shown). These results show that the interaction between infected monocytes and antibody is specific.

DISCUSSION

Our results show that there are specific parasite proteins, in particular the surface antigens SAG-1 and SAG-2 as well as the secreted microneme proteins, that are essential to the stimulation of human monocytes. Antibodies to these specific parasite ligands can block the production of a soluble factor, produced by human monocytes when infected with tachyzoites, that mediates a decrease in mitogen-induced lymphocyte proliferation (4).

The sequence of events during the process of active penetration includes attachment via the major surface ligand, SAG-1, followed by the immediate release of microneme proteins. Upon entry into the host cell, the parasitophorous vacuole forms as the parasite begins to actively penetrate the host cell (2). In our present studies, when SAG-1 and SAG-2 were blocked together, soluble-factor release was abrogated while the number of monocytes infected was reduced from 94 to 45%. We show, both here and previously (4), that release of soluble factors from infected monocytes persists below a 45% level of infection. It is unlikely that the remaining infected monocytes represent a population that had phagocytosed tachyzoites, since the majority of these cells contain replicating tachyzoites and fusion of tachyzoite-containing phagosomes with endosomes would result in phagosome acidification, an event known to cause parasite death (20). These results suggest that SAG-1 and SAG-2 are critical ligands in soluble-factor triggering but that other surface ligands may be involved in attachment. In this regard, a family of SAG-1-like surface antigens, SRS (SAG-related sequence), have recently been described (13). Blocking either MIC-1 or MIC-2 also abrogates the release of soluble factors from monocytes and inhibits parasite internalization. MIC-3 has a minor effect on both processes. An alternative explanation for our results is that engagement of monocyte Fcγ receptors by immune complexes formed between the antibodies and their ligands may signal a pathway that decreases soluble-factor release. This is unlikely since an identical response occurred in the presence of anti-tachyzoite $F(ab')_2$ fragments and anti-tachyzoite IgG (data not shown). Our results with anti-SAG-1, anti-SAG-2, and anti-MIC-3 may be explained if these antibodies were not present

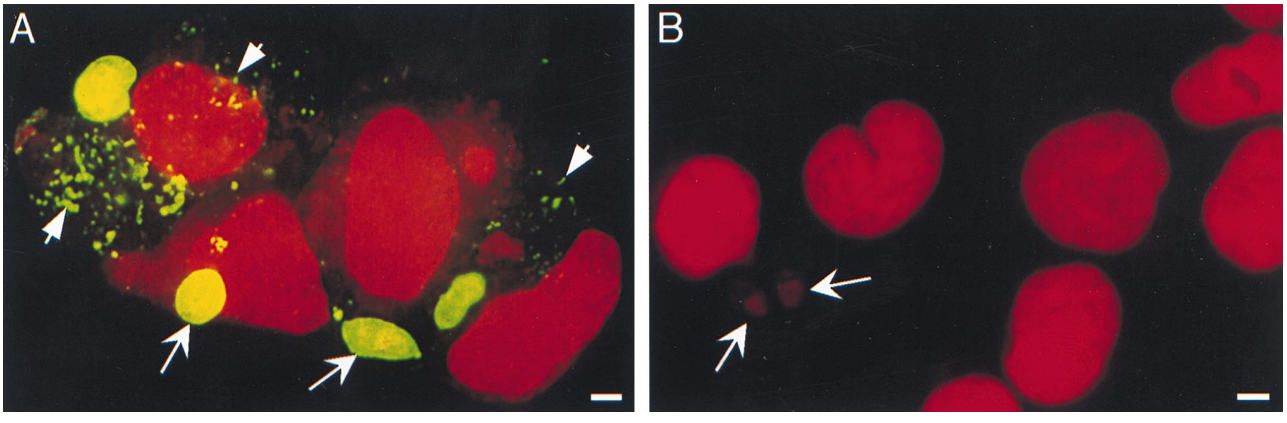

FIG. 4. Anti-SAG antibodies bind to the surface of infected monocytes. Adherent monocytes were incubated with tachyzoites at a monocyte-to-tachyzoite ratio of 1:25, and every 5 min unfixed monocytes were stained with mixtures of anti-SAG-1 and anti-SAG-2 antibodies (A) or isotype-matched control antibodies (B) followed by a FITC-conjugated secondary antibody and propidium iodide (in permeabilization buffer) for visualization by fluorescence microscopy. Anti-SAG but not control antibodies bound to the surface of tachyzoites (arrows) and in discrete patches to the surface of monocytes (arrowheads). Propidium iodide stains monocyte and parasite nuclei red. Bar, 2 μm. The results are representative of two donors.

in saturating concentrations. This is unlikely for SAG-1 and SAG-2, since FACScan analysis shows that the antibodies were present at two- to fivefold the concentration required for maximal fluorescence (data not shown).

These results suggest a new role for microneme and surface antigen proteins, namely, that they are parasite ligands that engage host cell receptors that trigger the pathway(s) resulting in the release of soluble factors. In support of this concept, microneme and surface antigen proteins were detected by fluorescence microscopy on the surface of monocytes following infection, suggesting that these proteins may be shed from the parasite during uptake. This would imply that receptor-ligand binding events are not confined to the immediate area of parasite attachment but may occur over a greater surface area of the host cell membrane. Since blocking either SAG ligands or MIC ligands abrogates soluble-factor release, it is possible that there is a single receptor for SAG or MIC ligands that requires the binding of both ligands before signal transduction occurs. Alternatively, it is possible that there are at least two receptors that form a complex, which also requires both ligands to be bound before signal transduction occurs. Studies to identify the monocyte receptors for SAG-1 or the microneme proteins are under way.

In summary, we suggest that SAG-1, SAG-2, MIC-1, and MIC-2 are parasite ligands that engage the monocyte receptor(s) that triggers the signal transduction pathway(s) leading to stimulation of the monocytes. Hence, only viable, intact parasites that can release microneme proteins following attachment of surface antigen proteins to monocytes can provide this trigger. This explains our previous results showing that monocytes incubated with heat-denatured parasites had no effect on mitogen-induced lymphoproliferation although live and irradiated parasites, both of which can release microneme proteins upon attachment, were effective triggers (4).

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