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Laminin on *Toxoplasma gondii* Mediates Parasite Binding to the β1 Integrin Receptor α6β1 on Human Foreskin Fibroblasts and Chinese Hamster Ovary Cells

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We investigated the role of parasite-bound laminin and the host cell β1 integrin receptors for this extracellular matrix protein in *Toxoplasma gondii* binding to fibroblasts. Laminin but not fibronectin was detected on extracellular tachyzoites by immunofluorescence and immunoblotting. Binding of parasites to CHO cells was inhibited by polyclonal antibodies to laminin and by a monoclonal antibody directed against the globular carboxy-terminal portion of the long arm of laminin (at or near the suggested ligand-binding sites for α3β1 and α6β1), but not by a monoclonal antibody directed against the lateral short arms of laminin near the cross region of the molecule. Antibodies to the α6 but not the α2, α3, or α5 chains of the β1 family of integrins blocked parasite attachment to human foreskin fibroblasts and CHO cells. Attachment of *T. gondii* to cells via laminin on the parasite surface and laminin receptors on the mammalian cell is consistent with the capacity of the parasite to invade almost all nucleated cells.

Tachyzoites of *Toxoplasma gondii* are nearly unique in their capacity to invade all nucleated cells. Although this attribute of *T. gondii* tachyzoites has been recognized for nearly 25 years, the ligands and receptors which mediate cell attachment and internalization are poorly understood.

Extracellular matrix proteins are implicated in attachment of a variety of extracellular and intracellular pathogens to tissue sites or host cells of both phagocytic and nonphagocytic origin (reviewed in references 9, 21, 23, and 36). For *Trypanosoma cruzi*, receptors for fibronectin (37), collagen (56), and C1q (42) on the parasite participate in host cell attachment and entry, possibly by binding these extracellular matrix components or collagen-like complement proteins, which then bind to host cell receptors. A similar mechanism is presumed to operate for a variety of extracellular pathogens, including *Staphylococcus aureus* (reviewed in reference 38), *Streptococcus pyogenes* (reviewed in reference 46), *Treponema pallidum* (reviewed in reference 2), and *Entamoeba histolytica* (51). The basement membrane protein laminin is implicated in cell attachment of extracellular pathogens, such as *Treponema denticola* (7), *Trichomonas vaginalis* (45), *S. aureus* (30), and *S. pyogenes* (50) to nonphagocytic target cells. Although microbial components which bind extracellular matrix proteins have been defined in some instances, the host cell receptors which mediate the attachment process are generally unknown.

Direct interactions between microorganisms and integrin receptors (reviewed in references 4, 18, 19, and 43) have been described. *Yersinia pseudotuberculosis* binds to and invades a variety of target cells via the *Yersinia* outer membrane protein invasin, which attaches directly to α1β1, α3β1, α4β1, and α6β1 integrins (22). Intracellular pathogens restricted to macrophages generally bind to complement receptors, including the β2 integrin, CR3 (Cd11b,18), via both complement-dependent and complement-independent mechanisms (reviewed in references 23 and 44). Pathogens such as *Bordetella pertussis* which remain largely extracellular may also attach to macrophages via CR3 (41).

Parasite attachment to the murine macrophage J774 cell line is increased by the addition of exogenous laminin and mediated, in part, by parasite binding to the 32/67-kDa laminin-binding protein (LBP) on J774 cells (11). In contrast, the 32/67-kDa LBP (29, 32, 40, 59) was not implicated in parasite binding to Chinese hamster ovary (CHO) fibroblasts. These earlier experiments did not clarify whether laminin was present on the parasite surface in the absence of added exogenous laminin, nor did they identify receptors on fibroblasts involved in parasite attachment to cells. We report here that host cell laminin on the parasite surface mediates binding of tachyzoites to the laminin receptor α6β1 on human foreskin fibroblasts (HFF) and CHO cells.

MATERIALS AND METHODS

Buffers and reagents. The following buffers were used: Hanks balanced salt solution containing 1 mM $CaCl_2$, 1 mM $MgCl_2$, 1% (wt/vol) glucose, 10 mM HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid; Calbiochem-Behring Diagnostics, La Jolla, Calif.), and 5 U of preservative-free heparin (pH 7.4) per ml (HCMD); 0.05 M Tris–0.15 M NaCl (pH 7.4) (TBS); TBS plus 0.05% Tween 20 (TTBS); extraction buffer containing 1% Triton X-100 in 100 mM NaCl–25 mM Tris–10 mM EDTA (pH 7.5). Where indicated, the following protease inhibitors were used: 25 μM nitrophenyl-*p'*-guanidinobenzoate (Sigma Chemical Co., St. Louis, Mo.), 10 μM leupeptin (Boehringer Mannheim Biochemicals, Indianapolis, Ind.), and 10 μM pepstatin A (Calbiochem).

Laminin was isolated from the Engelbret-Holm-Swarm tumor as described previously (53). Human fibronectin, a generous gift from Alex Kurosky, Galveston, Tex., was purified from plasma by using gelatin-Sepharose affinity chromatography.

Antibodies. Monoclonal antibodies recognizing the β1 chain and selected α chains of the β1 family of integrins were

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generously provided by the indicated investigators: anti-β1 (P4C10, ascites fluid), anti-α2 (P1H5, tissue culture supernatant), anti-α3 (P1B5, tissue culture supernatant), and anti-α5 (P1D6, tissue culture supernatant) were from Elizabeth Wayner, Seattle, Wash.; anti-α2 (12F1, ascites fluid) was from Virgil Woods, San Diego, Calif.; anti-α4 (HP2/1, tissue culture supernatant) was from Francisco Sanchez-Madrid, Madrid, Spain; anti-α5 (B1E5, tissue culture supernatant) and anti-β1 (AIIB2, tissue culture supernatant) were from Caroline Damsky, San Francisco, Calif.; anti-α6 (GOH3, tissue culture supernatant) was from Arnoud Sonnenberg, Amsterdam, The Netherlands. Each of the antibodies used has been shown to block adhesion of cells to one or more extracellular matrix protein ligands (18, 39, 49, 57). Rabbit antiserum to a rat integrin laminin receptor (α3β1) (13) was kindly provided by Kurt Gehlsen and Erkki Rouslahti, La Jolla, Calif. Rabbit antiserum to the human fibronectin receptor (α5β1) was kindly provided by Kenneth Yamada, Bethesda, Md.

As described below, anti-integrin antibodies were tested by immunofluorescence and by immunoprecipitation with surface-iodinated CHO cells, HFF cells, and tachyzoites. All antibodies to integrin chains gave a negative signal by immunofluorescence and immunoprecipitation with tachyzoites of *T. gondii*. Results with CHO cells and HFF cells are described in the text and shown in Fig. 4.

Monoclonal antibodies that recognize the lateral short side arms of human laminin near the cross region (AL-3) and the globule at the C terminus of the A chain (AL-4) of laminin were kindly provided by Amy Skubitz (St. Paul, Minn) (47).

Rabbit antiserum to laminin purified from the Engelbret-Holm-Swarm tumor was kindly provided by Hynda Kleinman, Bethesda, Md. Rabbit antiserum to whole *T. gondii* RH was generously provided by Lloyd Kasper, Hanover, N.H.

Cell lines and cell culture. CHO cells were grown in alpha minimal essential medium (MEM-α) (GIBCO-BRL, Gaithersburg, Md.) supplemented with 2 mM L-glutamine, 3.5% fetal calf serum, 100 U of penicillin G potassium per ml, and 200 μg of streptomycin sulfate per ml. HFF were grown in the same medium containing 10% fetal calf serum. Both CHO and HFF cells were cultured at 37°C under a 5% CO_2 atmosphere, maintained in 75-cm^2 tissue culture flasks (Corning Glass Works, Corning, N.Y.), and passaged when confluent. For use in experiments, cells were detached with a mixture of 0.04% trypsin and 0.05% EDTA, washed, and added to sterile 13-mm round coverslips individually placed in 24-well tissue culture plates. Cells were plated at a concentration of 10^4 cells per coverslip. After a 1-h incubation at 37°C, the coverslips were washed once to remove nonadherent cells and then incubated overnight at 37°C. Before use in parasite attachment assays, the coverslips containing the cells were washed three times in medium without fetal calf serum.

Parasites. Tachyzoites of *T. gondii* RH were harvested from infected human fibroblast monolayers as previously described (10, 24). Intact host cells were pelleted by centrifugation at 80 × *g* for 8 min at 4°C. Free tachyzoites from the supernatant were then pelleted by centrifugation at 1,250 × *g* for 8 min at 4°C, resuspended in MEM-α medium, and counted. Viability was assessed by trypan blue exclusion. Preparations were used only if parasites were greater than 95% viable by trypan blue exclusion and contained fewer than 1% contaminating host cells.

Gel electrophoresis and immunoblotting. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and immunoblotting were performed as described elsewhere (27, 54). Briefly, approximately 150 μg (10^8 parasites) of unlabelled RH strain tachyzoites were solubilized in SDS sample buffer at 100°C, electrophoresed on 5 to 20% gradient gels without lanes, and transblotted onto polyvinylidene difluoride (PVDS) membranes (Immobilon; Millipore Corp., Bedford, Mass.). The membranes were blocked with Tween-Tris-buffered saline (TTBS) buffer containing 3% bovine serum albumin (BSA) overnight at room temperature. Strips were incubated with antibody by incubation for 1 h at room temperature with dilutions of antiserum in TTBS buffer containing 1% BSA. After being washed, strips were developed by incubation with 5 × 10^5 cpm of ^{125}I-protein A (Dupont, NEN Research Products, Boston, Mass.) per ml in TBS buffer. Autoradiography was performed on dried strips with Kodak AR film.

Immunofluorescence. Tachyzoites of *T. gondii* were harvested from tissue culture, washed in PBS buffer, and either air dried and fixed with acetone (for screening of monoclonal antibodies) or fixed with cold absolute methanol or with 3% PBS–paraformaldehyde for 30 min at 4°C for all other experiments. After fixation, parasites were washed three times with cold PBS. For paraformaldehyde-fixed parasites, free aldehyde groups were blocked with 50 mM of ammonium chloride for 20 min at room temperature. Tachyzoites were then washed three times in Tris buffer (20 mM Tris, 150 mM NaCl, 1 mM EDTA, 5 mM NaN_3, pH 7.5) and incubated with primary antibody diluted in Tris buffer containing 0.1% gelatin. After incubation with primary antibody (mouse or rabbit), parasites were washed three times in Tris-gelatin buffer. Fluorescein isothiocyanate-conjugated goat anti-mouse immunoglobulin G (Zymed Laboratories, South San Francisco, Calif.) or fluorescein isothiocyanate-conjugated goat-anti rabbit immunoglobulin G (Zymed) at a dilution of 1:50 was added to the parasites, and incubation proceeded for 30 min at room temperature. After being washed, slides were mounted with Mowiol (Calbiochem) and examined at ×1,500 magnification in a Nikon Microphot FXA fluorescence microscope.

Immunoprecipitation. Anti-integrin antibodies were tested by immunoprecipitation with surface-iodinated CHO cells, HFF cells, and *T. gondii* tachyzoites. CHO cells and HFF cells grown as described above were released from flasks by incubation for 20 to 30 min at 37°C in 10 mM EDTA-PBS; this was followed by scraping. Released CHO and HFF cells were washed twice in PBS and resuspended to 10^7/ml in PBS. Tachyzoites released from HFF cells were harvested as described above and resuspended to 10^8/ml in PBS. Cells and parasites were then surface iodinated with lactoperoxidase by adding 1 mCi of ^{125}INa (Amersham Corp., Arlington Heights, Ill.), 0.4 U of lactoperoxidase (Sigma), and 75 μl of an H_2O_2 solution prepared by diluting 30% stock H_2O_2 1:1,000 in PBS. The mixture was incubated for 4 min and then pulsed again for 3 min with 0.2 U of lactoperoxidase and 7.5 μl of diluted H_2O_2. Cells and parasites were washed three times with PBS.

The washed pellets containing labelled cells (10^7 CHO and HFF cells and 10^8 tachyzoites) were suspended in 1 ml of extraction buffer (1% Triton X-100, 100 mM NaCl, 25 mM Tris, 10 mM EDTA [pH 7.5] containing 25 μM *p*-nitrophenyl-*p'*-guanidinobenzoate (NPGB), 10 μM leupeptin, and 10 μM pepstatin A). Incubation was continued for 2 h at 4°C. The detergent-insoluble residue was removed by centrifugation at 12,500 × *g* for 10 min at 4°C.

For immunoprecipitation, the detergent-soluble supernatant (900 μl) was first precleared by incubation with 50 μl of protein G-Sepharose (Pharmacia) for 2 h at 4°C. To 80 μl of

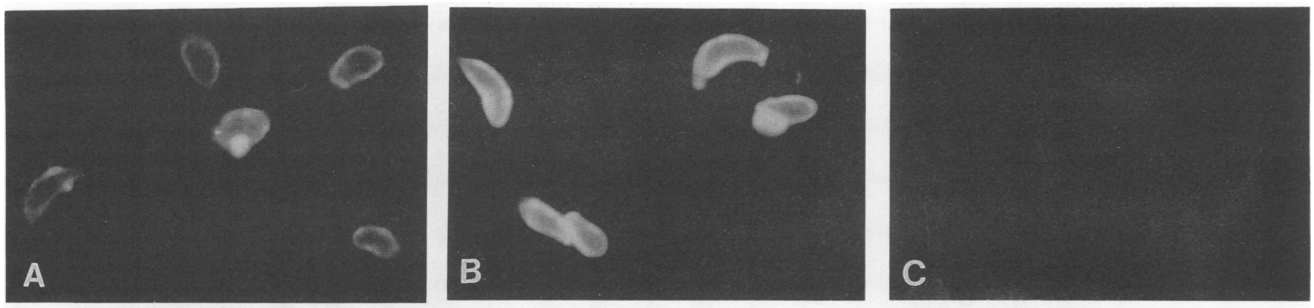

FIG. 1. Detection of laminin on extracellular tachyzoites of *T. gondii* by immunofluorescence. Parasites harvested from tissue culture were stained by immunofluorescence as described in Materials and Methods. (A) Rabbit antilaminin antiserum; (B) rabbit anti-*T. gondii* antiserum; (C) preimmunization serum from the rabbit used for preparation of the rabbit antilaminin antiserum.

precleared detergent-soluble supernatant was added either 80 μl of tissue culture supernatant containing anti-integrin monoclonal antibodies or 2 μl of rabbit anti-integrin antiserum. Mixtures were rotated overnight at 4°C and then added to 15 μl of protein G-Sepharose beads for an additional incubation period of 2 h at 4°C. Protein G-Sepharose beads were washed four times in extraction buffer, and then bound material was released by heating in 50 μl of SDS-sample buffer at 100°C; this was followed by SDS-PAGE as described above.

Attachment assay. Tachyzoites resuspended in MEM-α medium containing 0.1% BSA were added to CHO or HFF cells previously plated on coverslips to achieve a final parasite/cell ratio of 10:1. The coverslips were incubated for 120 min at 37°C in 5% CO_2 and then washed three times with medium to remove unattached parasites. Cells were then fixed with absolute methanol for 5 min and stained with 4,6-diamidino-2-phenyl-indol (DAPI; Sigma) at a final concentration of 10 μg/ml (25). The coverslips were then mounted on glass slides with Mowiol, and the number of parasites and infected cells were counted by fluorescence (DAPI) and phase-contrast microscopy.

The following determinations were made after a population of at least 300 cells was counted on each coverslip: (i) percentage of infected cells, (ii) number of parasites per 100 cells, and (iii) number of parasites per 100 infected cells. The conclusions were identical regardless of which parameter was analyzed. Percent inhibition was calculated based on either an unrelated tissue culture supernatant or an ascites fluid. No attempt was made in these experiments to distinguish between attached and internalized parasites.

Effect of antibodies to laminin on tachyzoite attachment. The effect of antilaminin antibodies on parasite attachment to cells was examined. Tachyzoites harvested from mice or tissue culture were washed with MEM-α–BSA and added at a 10:1 parasite/cell ratio to CHO or HFF cells in the presence of polyclonal or monoclonal antibodies to laminin. Parasite agglutination was excluded by microscopic analysis. Incubation was continued for 2 h at 37°C. Coverslips were processed as described above, and both percentage of infected cells and number of parasites per 100 cells were determined.

Effect of anti-integrin antibodies on tachyzoite attachment. The effect of anti-integrin antibodies on parasite attachment to cells was tested. Parasites harvested from mice or tissue culture were washed with MEM-α–BSA and added at a 10:1 parasite/cell ratio to CHO or HFF cells on coverslips that had been previously incubated for 30 min at 37°C with monoclonal or polyclonal antibodies to the α2, α3, α5, α6, or β1 chain of the β1 integrin family. Monoclonal antibodies from tissue culture supernatant were generally used at a dilution of 1:2 in MEM-α–BSA; monoclonal antibodies from ascites fluid were generally used at a dilution of 1:50 in MEM-α–BSA. Additional dilutions were used to confirm the initial findings. The parasite-cell mixture was incubated at 37°C in a 5% CO_2 atmosphere for an additional 2 h and then washed. In some experiments, the infection was then allowed to proceed for an additional 22 h. Coverslips were fixed and stained as described above, and both the percentage of infected cells and the number of parasites per 100 cells were calculated.

RESULTS

Tachyzoites are coated with laminin but not fibronectin. The presence of laminin on parasites harvested from tissue culture was tested by immunofluorescence. As shown in Fig. 1, parasites were diffusely coated with laminin. Similar results were shown with parasites harvested from mice (data not shown). In contrast, no fibronectin was detected on parasites by immunofluorescence (data not shown).

We also assessed the presence of laminin on parasites using immunoblots. A band consistent with the laminin B1 and B2 chains was observed using polyclonal antiserum to laminin (Fig. 2). No fibronectin was detected on parasites by immunoblotting. Hence, surface laminin on *T. gondii* tachyzoites does not reflect generalized binding of extracellular matrix proteins by the parasite.

Antibodies to laminin inhibit binding of tachyzoites to CHO cells. We sought to determine whether parasite-bound laminin was mediating binding of tachyzoites to target cells. Polyclonal antiserum to laminin inhibited parasite attachment to CHO cells in a dose-dependent fashion (Fig. 3). Monoclonal antibodies to different domains within the laminin molecule were then tested for the capacity to inhibit the binding of parasites to target cells. Monoclonal antibody AL-4, recognizing the globular domain near the carboxy terminus of the cross-like laminin molecule, as well as rabbit antiserum to whole laminin, blocked association of tachyzoites with CHO cells (Fig. 3). Monoclonal antibody AL-3, recognizing a domain on the lateral short arms near the cross, did not inhibit parasite attachment.

Antibodies to α6β1 integrins inhibit parasite attachment to HFF and CHO cells. We then tested the involvement of β1 integrin receptors recognizing laminin in mediating the attachment of tachyzoites to HFF and CHO cells. We had previously shown that 32/67-kDa LBP was not involved in tachyzoite attachment to CHO cells (11).

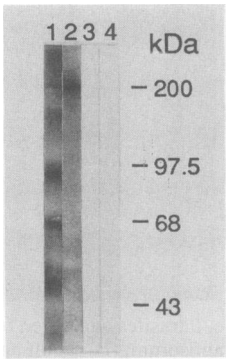

FIG. 2. Detection of laminin on extracellular tachyzoites of *T. gondii* by immunoblotting. Parasites harvested from mouse peritoneal cavities were washed extensively before solubilization for SDS-7.5% PAGE (reducing conditions) and then immunoblotting with anti-*T. gondii* antiserum (lane 1), antilaminin antiserum (lane 2), antifibronectin antiserum (lane 3), and normal rabbit serum (lane 4). The expected migration of the laminin B1 and B2 chains is at approximately M_r 220,000, whereas the laminin A chain migrates at M_r 400,000 and would not enter the gel in this system. Although the band in lane 2 migrates at approximately 205 kDa in this experiment, the more typical migration seen with immunoblotting in other experiments (using both polyclonal and monoclonal antibodies to laminin) was at 220 to 230 kDa.

We first examined the recognition of β1 integrins in HFF and CHO cells by selected monoclonal and polyclonal antibodies. Although most of the monoclonal antibodies gave a positive signal by immunofluorescence with both cell types, the intensity of staining was weak (data not shown). Hence, we examined the antibodies by immunoprecipitation from surface-iodinated cells. Monoclonal antibodies to β1, α2, α3, α5, and α6 immunoprecipitated heterodimeric complexes from HFF (Fig. 4A). Monoclonal antibodies to α3, α5, and α6 but not α2 or β1 recognized the corresponding heterodimeric receptors in CHO cells (Fig. 4B). Rabbit antisera to rat α3β1 and human α5β1 immunoprecipitated the β1 chain in CHO cells.

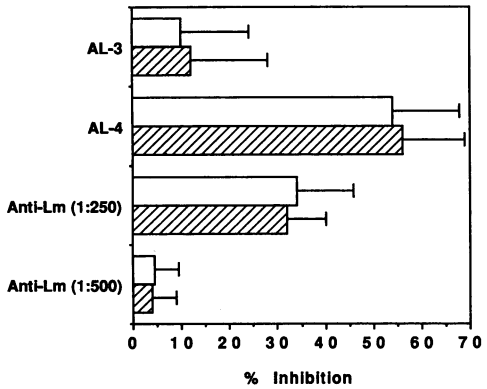

FIG. 3. Inhibition of tachyzoite binding to CHO cells with antibodies to laminin. Target cells were incubated with monoclonal antibody AL-3 or AL-4 at a 1:2 dilution in MEM-α–BSA or with rabbit antilaminin (Lm) immunoglobulin G at 1:250 or 1:500 dilution in the same buffer for 20 min at 37°C. Tachyzoites were washed and added to the cells at a parasite/cell ratio of 10:1, and incubation continued for an additional 120 min at 37°C. Coverslips were processed, and the percentage of infected cells (□) and the number of parasites per 100 cells (▨) were determined as described in Materials and Methods. The results are expressed as percent inhibition based on an unrelated control monoclonal antibody. Results represent the mean ± standard deviation for two or three experiments, each done on duplicate coverslips. At least 300 cells were counted for each coverslip. The percentage of infected cells in control samples ranged from 27 to 42% in separate experiments.

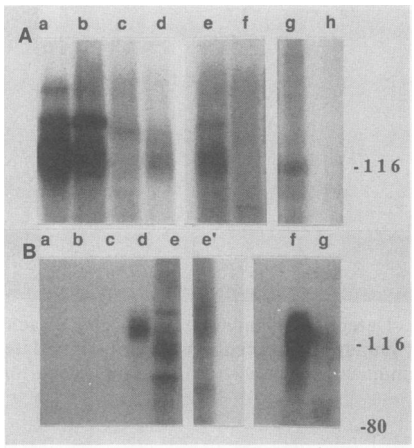

FIG. 4. Immunoprecipitation of β1 integrins from surface-iodinated HFF and CHO cells. Monoclonal and polyclonal antibodies to β1 integrins were used in immunoprecipitation assays with surface-iodinated HFF and CHO cells as described in Materials and Methods. Immunoprecipitates were electrophoresed by SDS-7.5% PAGE. The 116- and 80-kDa molecular size standards are shown. (A) HFF. Lanes: a, anti-β1 (AIIB2); b, anti-α2 (P1H5); c, anti-α4 (HP2/1); d, anti-α5 (B1E5); e, anti-α3 (P1B5); f, control monoclonal antibody; g, anti-α6 (GOH3); h, control monoclonal antibody. As expected, a β chain of M_r 120 was immunoprecipitated with all antibodies, whereas the α chains varied in M_r and in intensity relative to the β chain. (B) CHO cells. Lanes: a, anti-β1 (AIIB2); b, anti-β1 (P4C10); c, anti-α4 (HP2/1); d, anti-α5 (B1E5); e and e', anti-α6 (GOH3); f, rabbit anti-human fibronectin receptor (α5β1); g, rabbit anti-rat laminin receptor (α3β1). The pattern of bands immunoprecipitated with GOH3 varied between experiments (lanes e and e'), suggesting some degradation of the α chain (lane e). No bands were immunoprecipitated from CHO cells with β1 or α4 antibodies. The anti-α3 antibody P1B5- but not the anti-α2 antibody P1H5 immunoprecipitated bands of appropriate M_r in CHO cells (not shown).

Antibodies to α6 but not to α2, α3, or α5 inhibited parasite attachment to HFF (Fig. 5A) and CHO cells (Fig. 5B). The anti-β1 antibody AIIB2 inhibited attachment to HFF but not to CHO cells. The inhibition of attachment with anti-α6 was dose dependent (21 and 25% inhibition of attachment to HFF and CHO cells, respectively, at a 1:10 dilution of GOH3) and was similar when parasites were coated with exogenous laminin (data not shown). The percentage of infected cells and the number of parasites per 100 cells were blocked to a similar extent (Fig. 5).

The lack of attachment inhibition to HFF with α2 and α5 antibodies was independently confirmed with at least two antibodies directed against each receptor. The lack of inhibition with the anti-α2 antibody P1H5 was confirmed with a second antibody directed against α2 (12F1). The lack of inhibition with anti-α5 in both CHO and HFF cells was also observed with two separate antibodies (B1E5 and P1D6), each of which blocks the fibronectin-binding domain of the receptor, as well as with a polyclonal antibody to human α5β1.

Monoclonal antibodies to α3 and a polyclonal antibody to

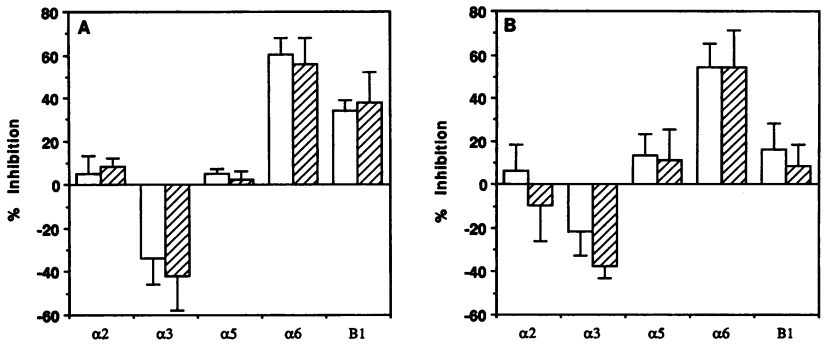

FIG. 5. Inhibition of tachyzoite attachment to HFF and CHO cells by antibodies against the β1 family of integrins. HFF (A) and CHO cells (B) were preincubated with monoclonal antibodies to α2 (P1H5), α3 (P1B5), α5 (B1E5), α6 (GOH3), and β1 (AIIB2) for 20 min before addition to parasites. Results shown are with antibodies (tissue culture supernatants) at a 1:2 dilution. Tachyzoites were added at a 10:1 parasite-to-cell ratio, and infection proceeded for 2 h. Coverslips were processed, and the percentage of infected cells (□) and the number of parasites per 100 cells (▨) were determined as described in Materials and Methods. The results express the percent inhibition relative to results with an unrelated tissue culture supernatant. Results represent the mean ± standard deviation for two or three experiments, each done on duplicate coverslips. At least 300 cells were counted for each coverslip. The percentage of infected cells in control samples ranged from 27 to 36% in separate experiments for CHO cells and varied between 17 and 28% for HFF. The number of parasites per 100 cells in control samples varied from 38 to 52 in CHO cells and from 25 to 48 in HFF.

α3β1 (data not shown) consistently augmented parasite attachment to HFF and CHO cells. Although these data do not directly support a role for α3 in tachyzoite binding, we cannot exclude its possible role in parasite-host cell interaction, since (i) most antibodies to α3 do not efficiently block interaction of cells with laminin, collagen, or fibronectin (8), (ii) selected α- and β1-chain monoclonal antibodies can enhance integrin heterodimer function (3, 34), and (iii) α3β1 binds to a region in the laminin molecule near the recognition site for AL-4 (Fig. 3).

DISCUSSION

These experiments document a role for the integrin α6β1 in mediating attachment of *T. gondii* to fibroblasts. Importantly, the basement membrane protein laminin serves as a molecular bridge between tachyzoites and host cells. The clearest previous documentation of microbial interaction with β1 integrins involves direct binding of the invasin protein from *Y. pseudotuberculosis* to α1β1, α3β1, α4β1, and α6β1 integrins (22).

Many surface membrane proteins that bind laminin have been described including (i) members of the integrin receptor family, consisting of α/β heterodimers, involved in cell-matrix and cell-cell adhesion functions, and (ii) the nonintegrin LBP. Four β1 integrins (α1β1, α2β1, α3β1, and α6β1) and one β3 integrin (αVβ3) are reported to bind laminin (12, 13, 17, 20, 26, 28, 49, 55, 57), although the ligand specificity of α2β1 varies with cell type. The capacity of α6β4 to bind laminin is not resolved (31, 48). The lack of availability of monoclonal antibodies to α1 precluded concurrent analysis of the role of this receptor in parasite binding. We also did not test the participation of αVβ3 in tachyzoite binding to CHO cells or HFF. Finally, parasite binding to receptor domains not blocked by the antireceptor antibodies was not assessed in the current experiments, nor were the receptors involved in parasite binding to other cell types sought. It is likely, therefore, that other receptors for *T. gondii* cell binding will be identified. Nonetheless, these are the first experiments to define a cell surface receptor for *T. gondii* in fibroblasts.

The nonintegrin LBP are not involved in tachyzoite binding to CHO cells. These LBP are a group of ubiquitously distributed molecules of 67, 45, and 32 kDa, the relationship between which and the subcellular localization of which are yet to be completely defined (16, 29, 32, 33, 59). Although our recent data suggest that the 32/67-kDa LBP is involved in parasite attachment to the murine J774 macrophage line (11), this molecule is not involved in parasite attachment to CHO cells. Neither polyclonal rabbit immunoglobulin G directed against the entire recombinant 32-kDa LBP molecule (6), the pentapeptide YIGSR from the laminin B1 chain, reported to mediate binding of laminin to the 32/67-kDa LBP molecule (15), nor antipeptide antibodies to NH_2-terminal or carboxy-terminal portions of the 32-kDa molecule blocked binding of tachyzoites to CHO cells (11).

Domains within laminin implicated in cell attachment are partially defined (1, 5, 12, 14, 17, 47, 48, 52). Carboxy-terminal domains within the A, B1, and B2 chains of laminin, as suggested by studies with laminin fragments, laminin peptides, and antilaminin monoclonal antibodies, mediate binding of laminin to α3β1 and α6β1 integrins, whereas laminin binds to α1β1 and most likely to α2β1 at regions near the cross. Our results with monoclonal antibody AL-4 (Fig. 3) are consistent with binding of parasite-associated laminin to target cell integrins via the carboxy terminus of the laminin molecule. Specific binding to α6β1 may be a function of the orientation of laminin on the parasite surface.

Earlier experiments indicated that exogenous laminin augmented parasite attachment to murine J774 macrophage-like cells (11). We have also found that exogenous laminin but not fibronectin augments tachyzoite binding to CHO cells and HFF and that iodinated laminin binds in saturable fashion to extracellular parasites harvested from tissue culture (10a). Host cell laminin may associate with nonprotein molecules on the parasite surface, since we have been unable to identify any parasite surface proteins to which exogenous laminin binds.

These experiments did not distinguish between receptors involved in parasite attachment and parasite invasion. Productive invasion requires that the parasite attach to cells, either initially or after reorientation, via the specialized apical complex (reviewed in reference 58). A circumferential moving junction that has not been characterized biochemi-

cally forms between parasites and cells, and constriction of the tachyzoite occurs at this site as invasion proceeds. The submembranous cytoskeleton of the target cell is disorganized at the point of parasite entry (35), possibly as a consequence of parasite binding to integrin receptors. As determined by immunofluorescence, the distribution of laminin on extracellular tachyzoites is uniform. If laminin results in indiscriminate, nonoriented binding, the parasite must subsequently be capable of reorienting. Whether redistribution of parasite components occurs during the process of oriented invasion is not known. Regardless of the precise orientation, our results indicate that laminin participates in tachyzoite binding to CHO cells and HFF by serving as a molecular bridge between the parasite and α6β1 on the target cell.

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