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Laminin Enhances Binding of *Toxoplasma gondii* Tachyzoites to J774 Murine Macrophage Cells

G. C. FURTADO,[1] M. SLOWIK,[1] H. K. KLEINMAN,[2] AND K. A. JOINER[1]*

Section of Infectious Diseases, Yale University School of Medicine, New Haven, Connecticut 06510-8056,[1] and Laboratory of Developmental Biology, National Institute of Dental Research, Bethesda, Maryland 20892[2]

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We investigated the effects of the extracellular matrix proteins laminin and fibronectin on the attachment of tachyzoites of *Toxoplasma gondii* to the murine macrophage cell line J774. Laminin but not fibronectin increased parasite attachment to J774 cells in a dose-dependent fashion. Cyclic YIGSR, a laminin-derived peptide which inhibits laminin binding to the 32/67-kDa laminin-binding protein on host cells, blocked the laminin-mediated enhancement of parasite attachment. An antiserum to the 32/67-kDa laminin-binding protein also inhibited binding of parasites to J774 cells. These results, in conjunction with our previous observations (G. C. Furtado, F. L. Collins, and K. A. Joiner, submitted for publication), demonstrate that tachyzoites bearing surface laminin bind to multiple laminin receptors in attaching to different target cells.

Tachyzoites of *Toxoplasma gondii* attach to and invade essentially all nucleated cells. The ligands and receptors mediating initial attachment are therefore likely to be varied, ubiquitously distributed, or both. We recently found that tachyzoites harvested from either tissue culture or mice bind to the β1 integrin laminin receptor, α6β1, on Chinese hamster ovary (CHO) cells and human foreskin fibroblasts (HFF) (5a). We also demonstrated that parasites harvested from either tissue culture or mice are coated with laminin and that parasite-bound laminin mediates attachment to α6β1. It is not clear whether tachyzoites attach to α6β1 or to other receptors on different mammalian cells.

A variety of laminin receptors have been described. The widely distributed 32/67-kDa laminin-binding proteins (32/67-kDa LBP) were the first laminin receptors to be identified (15, 18, 21). Although both 32- and 67-kDa molecules bind to laminin, molecular characterization of the 67-kDa molecule remains incomplete (29). Four members of the β1 integrin family (α1β1, α2β1, α3β1, and α6β1) (6, 9, 14, 25, 26) and one member of the β3 integrin family (αVβ3) (13) function as laminin receptors on different cell types. Additional laminin receptors include the 55/65-kDa chicken and rat muscle LBP (8), the 35-kDa Mac-2 galactose-binding molecule on murine macrophages (30), and a 110- to 120-kDa receptor on neuronal cells (3).

Laminin is the most abundant glycoprotein in basement membranes. It consists of three large polypeptide chains, B1, B2, and A, held together in a cruciform structure (reviewed in references 12 and 19). Laminin interactions with cell surface molecules and receptors promote cell-cell adhesion, migration, and cell differentiation (reviewed in reference 19). Seven synthetic peptides derived from the laminin sequence have been identified as active sites for cell binding (reviewed in reference 31). The YIGSR peptide from the short arm of the B1 chain inhibits binding (7) of laminin to the 32/67-kDa LBP (15, 18, 21, 29). More recently, the PA22-2 peptide from the carboxyl end of the long arm of the A chain, containing the sequence IKVAV, has been reported to bind the 67-kDa LBP as well as molecules of 36, 45, and 80 kDa on hepatocytes (2). RGD-containing peptides do not generally inhibit the interaction of laminin with cells (4, 6), and the RGD site is reported to be cryptic in intact laminin (1). Although the relationship among these various molecules is unknown, the results imply that binding of laminin to the LBP group of receptors can be mediated by peptides from different sites on the laminin molecule.

We show in this paper that laminin augments attachment of *T. gondii* to J774 cells, and we demonstrate that the laminin-mediated augmentation is mediated in part by the 32/67-kDa LBP. These results, in conjunction with our previous observations (5a), demonstrate that tachyzoites of *T. gondii* recognize multiple laminin receptors in attaching to different target cells.

MATERIALS AND METHODS

Buffers and reagents. Hanks balanced salt solution, pH 7.4, 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 per ml was used to harvest tachyzoites from mice.

Laminin was isolated from the Engelbret-Holm-Swarm tumor as described elsewhere (28). Human fibronectin was a generous gift from Alex Kurosky, Galveston, Tex. The peptides cyclic YIGSR, YIGSK, cyclic RGDS, and GRGESP were synthesized in an automated model 430A synthesizer (Applied Biosystems, Inc., Foster City, Calif.) as described before (7). All the peptides gave a single major peak when analyzed by high-pressure liquid chromatography on a C18 reverse-phase column. All the peptides were resuspended in Hanks balanced salt solution buffer and used at a final concentration of 300 μg/ml.

Anti-32/67-kDa LBP antiserum was a generous gift from Yoshihiko Yamada, Bethesda, Md. A β-galactosidase fusion protein containing the nearly full-length LBP was expressed by using the pEX vector, and rabbits were immunized with purified inclusion bodies (2). The structures of laminin, peptide sequences within laminin, and selected receptor-binding sites are shown in Fig. 1.

Cell culture. All cells were cultured at 37°C under a 5% CO_2 atmosphere in 75-cm^2 tissue culture flasks (Corning, Corning, N.Y.) and passed when confluent. The J774 mac-

* Corresponding author.

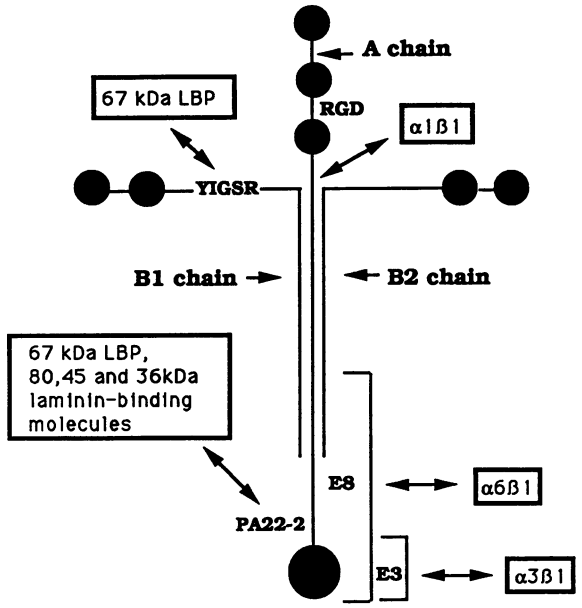

FIG. 1. Structure of laminin. Schematic drawing of laminin showing locations of the peptide sequences YIGSR (7), RGD, and PA22-2 (IKVAV) (27). Cell attachment sites and receptors are indicated in the boxes. The 32/67-kDa LBP is referred to in the figure as 67 kDa LBP. α6β1 and α3β1 integrin receptors bind to the proteolytic fragments E8 and E3, respectively. Globular domains predicted by the laminin sequence and observed by electron microscopy are shown by filled circles.

rophage cell line was maintained in RPMI medium containing 10% heat-inactivated fetal bovine serum (GIBCO BRL, Gaithersburg, Md.). CHO cells were maintained in minimal essential medium-alpha (MEM-α) (GIBCO BRL) supplemented with 2 mM L-glutamine, 3.5% fetal bovine serum, 100 U of penicillin G potassium per ml, and 200 μg of streptomycin sulfate per ml. Human foreskin fibroblasts (ATCC CRL-1521) were grown in supplemented MEM-α containing 10% fetal calf serum.

For use in experiments, cells were detached from tissue culture flasks with 0.04% trypsin and 0.05% EDTA, washed, and added to sterile 13-mm round coverslips (Fisher Scientific, Pittsburgh, Pa.) individually placed in a 24-well tissue culture plate. J774 cells were plated at 5×10^4 cells per coverslip, and CHO cells were plated at 10^4 cells per coverslip. After 1 h of incubation at 37°C, the coverslips were washed once to remove nonadherent cells and then incubated overnight at 37°C. Prior to 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 BALB/c mice by closed peritoneal lavage 2 days after intraperitoneal inoculation of 1×10^7 to 2×10^7 organisms or from infected human fibroblast monolayers as previously described (5, 11). Intact host cells were pelleted by centrifugation at 80 × *g* for 8 min at 4°C. Free tachyzoites from the supernatant fraction were then pelleted by centrifugation at 1,250 × *g* for 8 min at 4°C, suspended in assay buffer, and counted. Viability was assessed by trypan blue exclusion. Preparations were used only if parasites were more than 95% viable by trypan blue exclusion and contained fewer than 1% contaminating host cells.

Immunofluorescence. CHO and J774 cells previously plated on coverslips were fixed with 4% paraformaldehyde diluted in phosphate-buffered saline (PBS) for 30 min at room temperature. Free aldehyde groups were then blocked with 50 mM ammonium sulfate for 30 min at room temperature. Coverslips were incubated for 30 min with 10% goat serum, and then either rabbit anti-32/67-kDa LBP or normal rabbit serum was added to cells at a 1:50 dilution for 1 h at room temperature. Coverslips were washed three times with PBS buffer, and a 1:100 dilution of fluorescein-conjugated goat anti-rabbit immunoglobulin G (Zymed Laboratories, South San Francisco, Calif.) was added to the cells for 1 h at room temperature. After the cells were washed three times with PBS, the slides were mounted in Mowiol (Calbiochem). Coverslips were photographed with a Nikon Microphot-FXA fluorescence microscope (Nikon, New York, N.Y.) using Tri-X Pan 400 film (Kodak, Rochester, N.Y.).

Attachment assay. Tachyzoites resuspended in MEM-α containing 0.1% bovine serum albumin were added to J774 and CHO cells previously plated on coverslips to achieve final parasite/cell ratios of 5:1 and 10:1, respectively. The coverslips containing the J774 and CHO cells were incubated for 50 and 120 min, respectively, 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 Leukostat (Fisher Scientific) according to the manufacturer's instruction. The coverslips were mounted on slides with Mowiol (Calbiochem), and the parasites and infected cells were counted by 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. Only results from ii are shown, since the conclusions were identical regardless of which parameter was analyzed. No attempt was made to distinguish between attached and internalized parasites.

Effects of laminin and fibronectin on tachyzoite attachment. Tachyzoites in MEM-α medium were incubated for 1 h at 37°C either with 25 μg of fibronectin per ml or with various concentrations of laminin. The entire mixture was then added to J774 cells plated previously on coverslips, and incubation proceeded for an additional 50 min at 37°C. Cells were than washed three times, fixed with absolute methanol, and stained as described above.

To test the effects of specific peptides in blocking laminin-dependent attachment, tachyzoites were incubated with 300 μg of cyclic YIGSR, YIGSK, cyclic RGDS, or GRGESP in MEM-α for 20 min at 37°C. Following incubation with peptide or buffer, laminin (25 μg/ml) was added to the samples, and incubation proceeded for an additional 50 min at 37°C. Finally, without being washed, the parasites were added to CHO or J774 cells on coverslips at a 10:1 parasite/cell ratio. Incubation was continued at 37°C for either 50 min (J774 cells) or 2 h (CHO cells), and coverslips were processed as detailed above.

Effect of antibodies to 32/67-kDa LBP on tachyzoite attachment. The effect of anti-32/67-kDa LBP antibodies on parasite attachment to cells was examined. J774 cells were incubated with 1:10, 1:50, and 1:250 dilutions of anti-32/67-kDa LBP antiserum in MEM-α containing 4% normal rabbit serum for 30 min at 37°C. Tachyzoites were incubated for 30 min at 37°C with either buffer or laminin at 25 μg/ml, washed, and suspended in MEM-α–4% normal rabbit serum. Parasites were added to J774 cells at a parasite/cell ratio of 10:1 in the continuous presence of anti-32/67-kDa LBP antiserum. Incubation proceeded for an additional 1 h at

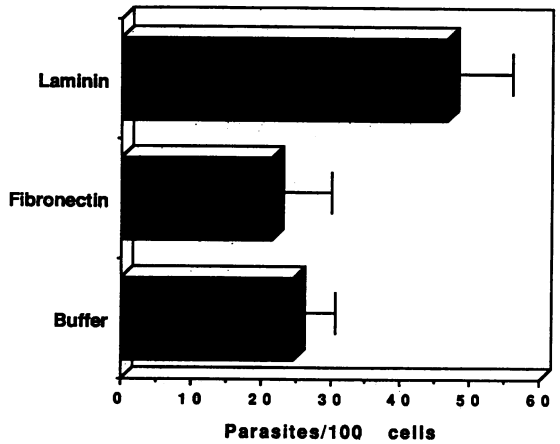

FIG. 2. Effects of laminin and fibronectin on *T. gondii* attachment to J774 cells. Tachyzoites of *T. gondii* were coated with 25 μg of laminin or fibronectin per ml or with buffer alone prior to addition to J774 cells. Coverslips were processed, and the number of parasites per 100 cells was determined as described in Materials and Methods. The results represent the mean ± standard deviation for two or three experiments, each one done on duplicate coverslips. At least 300 cells were counted for each coverslip.

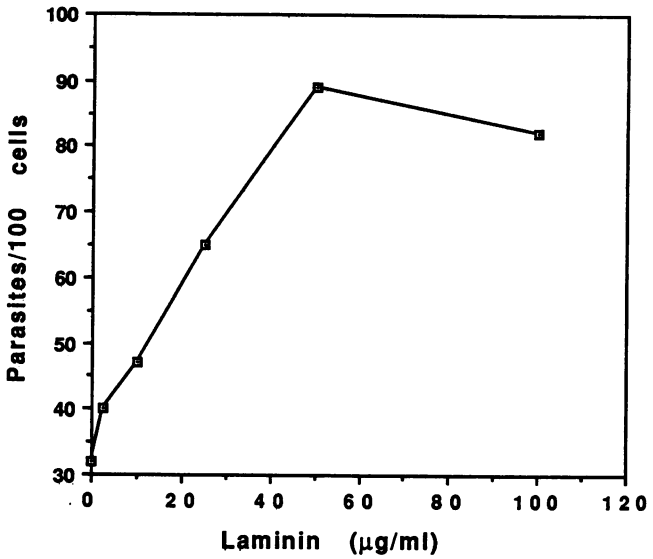

FIG. 3. Dose response effect of laminin on the attachment of *T. gondii* to J774 cells. Tachyzoites were coated for 50 min at 37°C with different concentrations of laminin prior to addition to J774 cells. Infection proceeded for an additional 50 min at 37°C. Coverslips were processed as described in Materials and Methods. All experiments were done on duplicate coverslips; at least 300 cells were counted on each coverslip. Laminin augmented parasite attachment by 2.5- to 3-fold in all experiments (n = 3), only one of which is shown.

37°C. Coverslips were fixed and stained as described above, and the number of parasites per 100 cells was determined.

RESULTS

Laminin promotes *T. gondii* attachment to J774 cells. Laminin and fibronectin were tested for their capacities to augment *T. gondii* attachment to monolayers of J774 cells. Tachyzoite attachment was substantially enhanced when laminin at 25 μg/ml was added to the parasites prior to addition to cells (Fig. 2). The enhancement was dose dependent, and a half-maximal effect was achieved at an approximate concentration of 25 μg/ml (Fig. 3). A concentration of 25 μg/ml was used for all subsequent experiments. Fibronectin at 25 μg/ml showed no effect on parasite attachment to cells (Fig. 2). Similar results were observed with CHO cells and HFF (5a).

Pentapeptide YIGSR inhibits laminin-mediated enhancement of *T. gondii* attachment to J774 cells but not to CHO cells. In order to demonstrate the specific nature of the laminin enhancement and to identify the regions of laminin that might be involved in this process, we tested various biologically active laminin-derived peptides (Fig. 1) for their abilities to block the attachment of tachyzoites to cells. Addition of cyclic YIGSR (7), a peptide sequence within the B1 chain of laminin, almost totally abrogated the laminin enhancement of tachyzoite attachment to J774 cells (Fig. 4A). No inhibition was observed with CHO cells (Fig. 4B). On the other hand, addition of cyclic RGDS (22) did not inhibit laminin enhancement with either J774 (Fig. 4A) or CHO (Fig. 4B) cells. Control peptide GRGESP (4) or YIGSK (7) also resulted in no significant inhibition (Fig. 4A). Addition of the peptides in the absence of exogenous laminin did not inhibit parasite attachment in comparison to addition of buffer (not shown). Since laminin binding via the YIGSR sequence has been found to involve an interaction with the 32/67-kDa LBP (7), we sought to determine if a similar cell surface protein was involved in laminin-mediated tachyzoite attachment to J774 cells.

Antibody to 32/67-kDa LBP blocks binding of laminin-coated and native *T. gondii* to J774 cells. We measured the ability of anti-32/67-kDa LBP antiserum to inhibit parasite attachment to J774 cells. Addition of 32/67-kDa LBP antiserum blocked binding of laminin-coated tachyzoites to J774 cells in a dose-dependent fashion (Fig. 5A). Anti-32/67-kDa LBP also blocked attachment of native parasites to J774 cells (Fig. 5B), consistent with the presence of laminin on native organisms (5a).

32/67-kDa LBP differs in expression and distribution on J774 and CHO cells. The expression of 32/67-kDa LBP on J774 and CHO cells was examined by immunofluorescence. The 32/67-kDa LBP staining is more intense and localized to the cell perimeter in J774 cells (Fig. 6A) compared with the weak immunofluorescence pattern of the 32/67-kDa LBP staining in CHO cells (Fig. 6B). Additional analysis of the staining pattern by confocal microscopy confirmed that 32/67-kDa LBP had a diffuse cytoplasmic localization in CHO cells. Hence, the pattern of immunofluorescence staining for 32/67-kDa LBP in J774 and CHO cells correlates with the level of blocking of parasite attachment by the YIGSR peptide and the anti-32/67-kDa LBP antibody.

DISCUSSION

Tachyzoites of *T. gondii* can invade all nucleated cells, but little is known about the ligands and receptors involved. In the present report, we demonstrate that laminin, but not fibronectin, enhances the attachment of tachyzoites of *T. gondii* to J774 cells. In contrast to CHO cells, however, laminin-augmented binding to J774 cells is blocked by the synthetic peptide YIGSR and by antibody to 32/67-kDa LBP, implicating the 32/67-kDa LBP molecule in parasite attachment.

The inability of the cyclic YIGSR peptide to inhibit laminin-mediated enhancement to below baseline levels

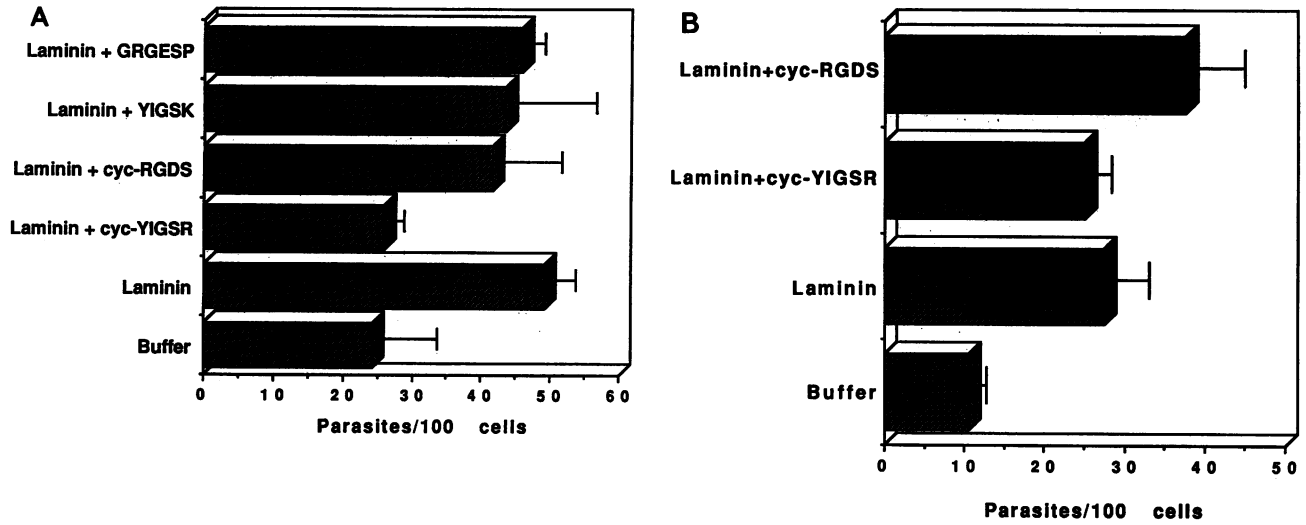

FIG. 4. Effects of laminin peptides on *T. gondii* attachment to J774 and CHO cells. Parasites were coated with 300 μg of cyclic YIGSR or cyclic RGDS per ml or with the control peptides YIGSK and GRGESP prior to incubation with buffer containing 25 μg of laminin per ml. All the mixtures were then added to J774 cells (A) or CHO cells (B) for an additional incubation of 50 or 120 min, respectively, at 37°C. Coverslips were processed, and the number of parasites per 100 cells was determined as described in Materials and Methods. The results represent the mean ± standard deviation for two or three experiments, each one done on duplicate coverslips. At least 300 cells were counted for each coverslip.

(Fig. 4) suggests either that receptors other than 32/67-kDa LBP are involved in parasite binding to J774 cells or that laminin-coated tachyzoites bind to the cell 32/67-kDa LBP via a YIGSR-independent site. Support for the former postulate derives from the incomplete inhibition of parasite attachment with anti-32/67-kDa LBP antiserum (Fig. 5), although the extent of inhibition with this antiserum may have been artifactually low (see below). Macrophages express at least three different laminin binding molecules, the 32/67-kDa LBP (10, 20), the α6β1 integrin receptor (20), and the Mac-2 antigen (30). In resting cells, both 32/67-kDa LBP (20) and Mac-2 are major surface components (30). Phorbol myristate acetate-stimulated but not resting mouse peritoneal macrophages attach to laminin predominantly via α6β1, an event associated with cytoskeletal anchoring of the α6β1 receptor and with phosphorylation of the cytoplasmic domain of the α6 but not the β1 subunit (23). In our experiments, neither antibodies to the α6 or β1 chains of the integrin family nor antibodies to Mac-2 blocked parasite attachment to unstimulated J774 cells (data not shown). Thus, additional receptors mediating attachment are likely to be identified.

Anti-32/67-kDa LBP antiserum recognizes laminin-binding molecules on *T. gondii* (5b), potentially confounding the results shown in Fig. 5. Nonetheless, it is unlikely that the anti-32/67-kDa LBP antiserum inhibited attachment by binding to the parasites, since similar results were observed whether or not excess anti-32/67-kDa LBP antiserum was

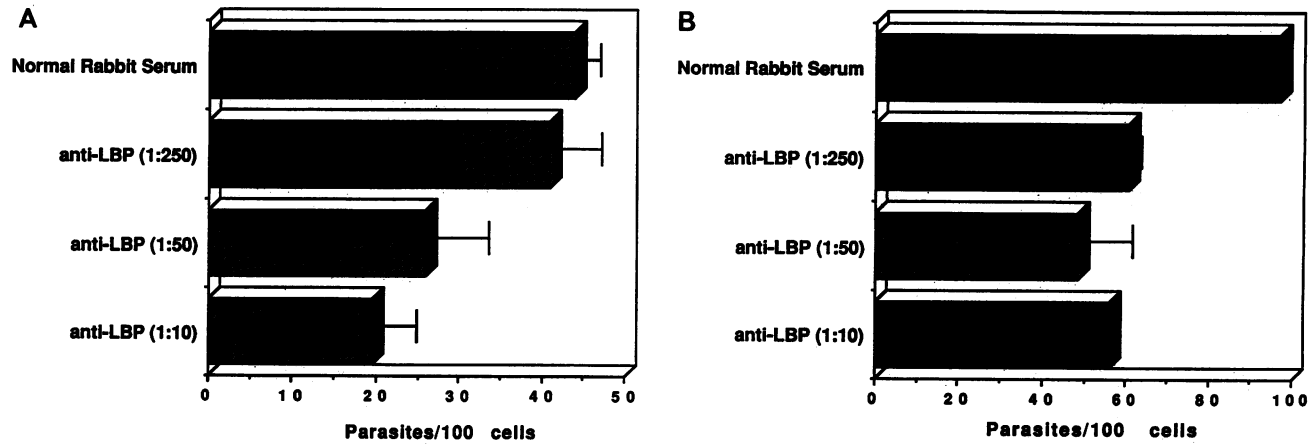

FIG. 5. Inhibition of tachyzoite binding to J774 cells with anti-32/67-kDa LBP antibodies. Target cells were incubated with anti-32/67-kDa LBP antiserum at 1:10, 1:50, and 1:250 dilutions in MEM-α buffer containing normal rabbit serum for 30 min at 37°C. The normal rabbit serum did not recognize tachyzoites by either immunofluoresence or immunoblotting. Native tachyzoites (B) or tachyzoites previously coated with 25 μg of laminin per ml (A) were added to the J774 cells, and incubation proceeded for 50 min at 37°C. Coverslips were processed, and the number of parasites per 100 cells was determined as described in Materials and Methods. The results represent the mean ± standard deviation for two or three experiments, each one done on duplicate coverslips. At least 300 cells were counted for each coverslip.

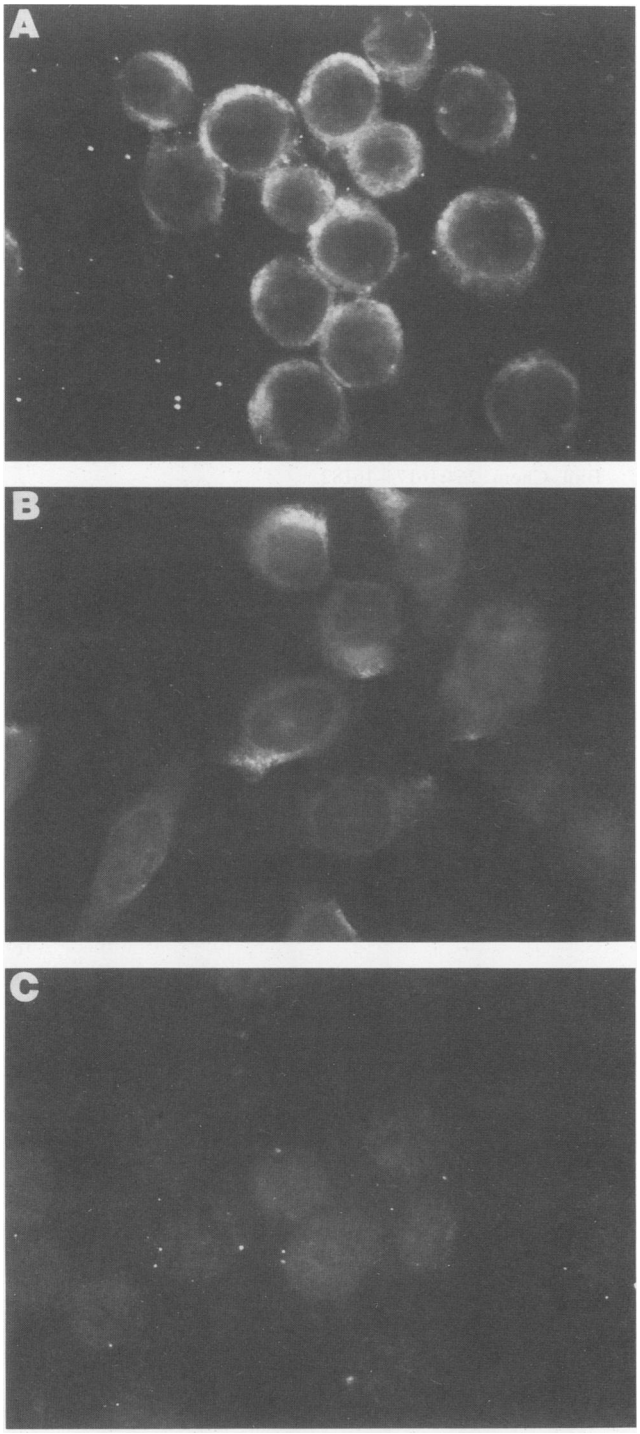

FIG. 6. Distribution of 32/67-kDa LBP on J774 and CHO cells. J774 and CHO cells were plated on coverslips for 16 h and stained by immunofluorescence with antibodies to 32/67-kDa LBP. Intense surface staining for 32/67-kDa LBP was observed in J774 cells (A), whereas minimal staining was observed in CHO cells (B). Negative staining was seen with the normal rabbit serum (C).

washed from the J774 cells prior to addition of parasites. Rather, the extent of attachment inhibition due to blockade of the 32/67-kDa LBP molecule was probably an underestimate, since parasites may have been opsonized by the antiserum and attachment may have thereby been artifactually increased. Insufficient antiserum was available to prepare $F(ab')_2$ or Fab fragments of anti-32/67-kDa LBP immunoglobulin G, which would have allowed a more definitive approach to this issue.

Laminin receptors have been identified on a variety of organisms, including *Trichomonas vaginalis* (24) and *Staphylococcus aureus* (17). Although the receptors in these two organisms vary greatly in molecular weight (118 kDa in *T. vaginalis* and 55 kDa in *S. aureus*), they are both recognized by a monoclonal antibody (1.H12) to the *S. aureus* receptor. This antibody immunoprecipitates a 67-kDa protein from mouse melanoma cells, suggested to be 32/67-kDa LBP (16, 17). It is not known whether laminin binding to *T. vaginalis* and *S. aureus* is mediated by the YIGSR domain of the molecule. We have previously shown that laminin binds to tachyzoites in a specific and saturable manner and that addition of the YIGSR peptide does not block this interaction (5a). Identification of the laminin domains which bind to tachyzoites and of the laminin domains which mediate subsequent cell binding will be necessary to fully characterize the participation of laminin in the process of *T. gondii* attachment to cells.

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REFERENCES

1. **Aumailley, M., M. Gerl, A. Sonnenberg, R. Deutzmann, and R. Timpl.** 1990. Identification of the Arg-Gly-Asp sequence in laminin A chain as a latent cell-binding site being exposed in fragment P1. FEBS Lett. **262:**82–86.
2. **Clement, B., B. Segui-Real, P. Savagner, H. K. Kleinman, and Y. Yamada.** 1990. Hepatocyte attachment to laminin is mediated through multiple receptors. J. Cell Biol. **110:**185–192.
3. **Douville, P. J., W. J. Harvey, and S. Carbonetto.** 1988. Isolation and partial characterization of high affinity laminin receptors in neural cells. J. Biol. Chem **263:**14964–14969.
4. **Elices, M. J., L. A. Urry, and M. E. Hemler.** 1991. Receptor functions for the integrin VLA-3: fibronectin, collagen, and laminin binding are differentially influenced by arg-gly-asp peptide and by divalent cations. J. Cell Biol. **112:**169–181.
5. **Fuhrman, S. A., and K. A. Joiner.** 1989. Complement activation and resistance to killing by tachyzoites of Toxoplasma gondii. J. Immunol. **142:**940–947.

5a. **Furtado, G. C., F. L. Collins, and K. A. Joiner.** Submitted for publication.

5b. **Furtado, G. C., S. A. Fuhrman, J. F. Dubremetz, and K. A. Joiner.** Submitted for publication.

6. **Gehlsen, K. R., L. Dillner, E. Engvall, and E. Rouslahti.** 1988. The human laminin receptor is a member of the integrin family of cell adhesion receptors. Science **241:**1228–1229.
7. **Graf, J., Y. Iwamoto, M. Sasaki, G. R. Martin, H. K. Kleinman, F. A. Robey, and Y. Yamada.** 1987. Identification of an amino acid sequence in laminin mediating cell attachment, chemotaxis, and receptor binding. Cell **48:**989.
8. **Hall, D. E., K. A. Frazer, C. H. Byron, and L. F. Reichardt.** 1988. Isolation and characterization of a laminin-binding protein from rat and chick muscle. J. Cell Biol. **107:**687–697.
9. **Hall, D. E., L. F. Reichardt, E. Crowley, B. Holley, H. Moezzi, A. Sonnenberg, and C. H. Damsky.** 1990. The a1/β1 and a6/β1 integrin heterodimers mediate cell attachment to distinct sites on laminin. J. Cell Biol. **110:**2175–2184.
10. **Huard, T. K., H. L. Malinoff, and M. S. Wicha.** 1986. Macrophages express a plasma membrane receptor for basement membrane laminin. Am. J. Pathol. **123:**365–370.
11. **Joiner, K. A., S. A. Fuhrman, H. Mietinnen, L. L. Kasper, and**

I. Mellman. 1990. Toxoplasma gondii: fusion competence of parasitophorous vacuoles in Fc receptor transfected fibroblasts. Science **249:**641–646.
12. **Kleinman, H. K., and B. S. Weeks.** 1989. Laminin: structure, functions and receptors. Curr. Opinion Cell Biol. **1:**964–967.
13. **Kramer, R. H., Y.-F. Cheng, and R. Clyman.** 1990. Human microvascular endothelial cells use β1 and β3 integrin receptor complexes to attach to laminin. J. Cell Biol. **111:**1233–1243.
14. **Languino, L. R., K. R. Gehlsen, E. Wayner, W. G. Carter, E. Engvall, and E. Ruoslahti.** 1989. Endothelial cells use alpha-2/beta-1 integrin as a laminin receptor. J. Cell Biol. **109:**2455–2462.
15. **Lesot, H., U. Kuhl, and K. von der Mark.** 1983. Isolation of a laminin binding protein from muscle cell membranes. EMBO J. **2:**45–50.
16. **Lopes, J. D., G. F. A. Da-Mota, C. R. W. Carneiro, L. Gomes, F. Costa-e-Filho, and R. R. Bretani.** 1988. Evolutionary conservation of laminin binding proteins. Braz. J. Med. Res. **21:**1269–1273.
17. **Lopes, J. D., M. dos Resi, and R. R. Bretani.** 1985. Presence of laminin receptors in Staphylococcus aureus. Science **229:**275–277.
18. **Malinof, H. L., and M. S. Wicha.** 1983. Isolation of a cell surface receptor protein for laminin from murine fibrosarcoma cells. J. Cell Biol. **96:**1475–1479.
19. **Martin, G. R.** 1987. Laminin and other basement membrane components. Annu. Rev. Cell Biol. **3:**57–85.
20. **Mercurio, A. M., and L. M. Shaw.** 1988. Macrophage interactions with laminin: PMA selectively induces the adherence and spreading of mouse macrophages on a laminin substratum. J. Cell Biol. **107:**1873–1880.
21. **Rao, C. N., S. H. Barsky, V. P. Terranova, and L. A. Liotta.** 1983. Isolation of a tumor cell laminin receptor. Biochem. Biophys. Res. Commun. **111:**804–808.
22. **Ruoslahti, E., and M. D. Pierschbacher.** 1087. New perspectives in cell adhesion: RGD and integrins. Science **238:**491–497.
23. **Shaw, L. M., J. M. Messier, and A. M. Mercurio.** 1990. The activation dependent adhesion of macrophages to laminin involves cytoskeletal anchoring and phosphorylation of the a6β1 integrin. J. Cell Biol. **110:**2167–2174.
24. **Silva-Filho, E., W. deSouza, and J. D. Lopes.** 1988. Presence of laminin binding proteins in trichomonads and their role in adhesion. Proc. Natl. Acad. Sci. USA **85:**8042.
25. **Sonnenberg, A., C. J. T. Linders, P. W. Modderman, C. H. Damsky, M. Aumailley, and R. Timpl.** 1990. Integrin recognition of different cell-binding fragments of laminin (P1, E3, E8) and evidence that α6β1 but not α6β4 functions as a major receptor for fragment E8. J. Cell Biol. **110:**2145–2155.
26. **Sonnenberg, A., P. W. Modderman, and F. Hogervorst.** 1988. Laminin receptor on platelets is the integrin VLA-6. Nature (London) **336:**487–489.
27. **Tashiro, K., G. S. Sephel, B. Weeks, M. Sasaki, G. R. Martin, H. K. Kleinman, and Y. Yamada.** 1989. A synthetic peptide containing the IKVAV sequence from the A chain of laminin mediates cell attachment, migration and neurite outgrowth. J. Biol. Chem. **264:**16174–16182.
28. **Timpl, R., H. Rohde, P. G. Robey, S. I. Rennard, J.-M. Foidart, and G. R. Martin.** 1979. Laminin-a glycoprotein from basement membranes. J. Biol Chem. **254:**9933–9937.
29. **Wewer, U. M., L. A. Liotta, G. A. Jaye, G. A. Ricca, W. N. Drohan, A. P. Claysmith, C. N. Rao, P. Wirth, J. E. Coligan, R. Albrechtsen, M. Mudryj, and M. E. Sobel.** 1986. Altered levels of laminin receptor mRNA in various human carcinoma cells that have different abilities to bind laminin. Proc. Natl. Acad. Sci. USA **83:**7137–7141.
30. **Woo, H. J., L. M. Shaw, J. M. Messier, and A. M. Mercurio.** 1990. The major non-integrin laminin binding protein of macrophages is identical to carbohydrate binding protein 35 (Mac-2). J. Biol. Chem. **265:**17097–17099.
31. **Yamada, K. M.** 1991. Adhesive recognition sequences. J. Biol. Chem. **266:**12809–12812.