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Laminin Receptors on *Candida albicans* Germ Tubes

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Recent evidence for the role of laminin in cell adhesion and in the pathogenesis of several bacterial infections has led us to investigate the existence of receptors for this extracellular matrix component in *Candida albicans*. At first, immunofluorescence demonstrated the presence of laminin-binding sites at the surface of germ tubes. Electron microscopy confirmed this result and permitted precise localization of the binding sites on the outermost fibrillar layer of the germ tube cell wall. By using ^{125}I-radiolabeled laminin, the binding was shown to be saturable and specific, hence demonstrating characteristics of true receptors. Analysis of the data by the Scatchard equation indicated that there were about 8,000 binding sites per cell, with a dissociation constant (K_d) of 1.3×10^{-9} M. Binding was inhibited by prior heating or trypsinization of cells. Furthermore, of the different proteins and carbohydrates tested in competition experiments, only fibrinogen greatly reduced the laminin binding. Finally, dithiothreitol and iodoacetamide treatment of germ tubes allowed us to identify the laminin receptors through analysis of this extract by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and Western immunoblotting. Two components, of 68 kilodaltons and a doublet of 60 and 62 kilodaltons, were detected. Thus, *C. albicans* possesses germ tube-specific surface receptors for laminin which could mediate its attachment to basement membranes and so contribute to the establishment of candidiasis.

Candida albicans is an opportunistic fungus which can parasitize various tissues. Several processes implicated in the invasion of the host have been described for this dimorphic fungus (35). Propagation of the infection may take place by contiguity from mucocutaneous lesions. Likewise, contaminated catheters may allow entry of the yeast. After vascular dissemination, extravasation can occur, which requires the adherence of the yeast to the subendothelial basement membrane exposed by mechanical rupture or by enzymatic lysis of the endothelium (24, 41). Thus, adherence to epithelial or endothelial cells represents a crucial step in the development of candidiasis. A prerequisite for this phenomenon is the expression of complementary molecules on both the yeast and the host cell surfaces. However, the candidal adhesins and their cellular receptors have not yet been identified (11, 40). Indirect evidence (8, 9, 42, 44) suggests the presence of lectins specific to L-fucose, D-mannose, or *N*-acetyl-D-glucosamine in *C. albicans*. In vitro studies have shown that some of the host proteins having adhesive properties could be recognized by the yeast: iC3b (12, 15, 18) and C3d (6, 12, 18), like fibrinogen (2, 3, 36, 53) and fibronectin (7, 45), have been proposed as host cell receptors.

Recent attention has been focused on the role of laminin, a major component of basement membranes (50), in cell adhesion. This multifunctional glycoprotein promotes the adhesion of various eucaryotic cell types in vitro. Specific laminin receptors have been found on cells that normally interact with basement membranes (56), as well as on cells that extravasate, such as metastatic tumor cells (1, 4, 17, 33, 38, 54), granulocytes (5), lymphocytes (5), and macrophages (19). Furthermore, some pathogenic microorganisms such as *Escherichia coli* (46), *Staphylococcus aureus* (31, 34), *Treponema pallidum* (14), some bacteria associated with periodontal diseases (55), and streptococci (47, 48) recognize laminin through specific surface receptors.

In the present study we used immunofluorescence and electron microscopy to demonstrate the presence of laminin receptors on *C. albicans* germ tubes, a morphological stage that initiates the filamentous forms encountered in the invasive process. Binding characteristics and biochemical analysis of the receptors are also described.

MATERIALS AND METHODS

Organism and culture conditions. The study was performed with *C. albicans* 1066 serotype A (2), originally isolated from a patient with septicemia and cloned by limit dilution. Blastoconidia were prepared by subcultures in synthetic Lee medium (28) modified as previously described (52). After a 48-h incubation at 22°C with constant shaking, cells in the stationary phase were collected by centrifugation (5 min at 3,500 × *g*) and washed twice in distilled water. Germ tubes were obtained by inoculation of the blastoconidia into medium 199 (pH 6.7; Biochrom, Angoulème, France) and incubation at 37°C for 2.5 h. After washing, fungal elements were counted with a hemacytometer and adjusted to 10^7 cells per ml in 0.15 M phosphate-buffered saline (PBS) (pH 7.2).

Laminin iodination. Laminin, isolated from a mouse Englebreth-Holm-Swarm (EHS) sarcoma tumor (50), was a gift from M. Vigny (Institut National de la Santé et de la Recherche Médicale U 118, Paris, France). Laminin was labeled with ^{125}I (specific activity, 14.3 Ci/mg; Amersham Corp., Arlington Heights, Ill.) by the chloramine-T method (20). Free iodine was removed by gel filtration on a Sepharose G-25 column (model PD-10; Pharmacia Fine Chemicals, Uppsala, Sweden) previously equilibrated in PBS and saturated with 1 ml of 1% bovine serum albumin (BSA) solution in PBS.

Immunofluorescence and electron microscopy. Two procedures were used to visualize the binding of laminin to *C.*

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albicans. In both cases, 10^7 pelleted organisms were suspended in 100 μl of laminin solution at 1 mg/ml in PBS and incubated for 30 min at 37°C with constant shaking. Then, the cells were washed three times in PBS and incubated in 100 μl of rabbit anti-laminin immune serum (kindly given by M. Vigny) at a 1:100 dilution in PBS containing 0.5% BSA. After 30 min at 37°C, the cells were washed in PBS and treated as follows. For the immunofluorescence assay, the cells were incubated for 30 min at 37°C in 100 μl of fluorescein isothiocyanate-conjugated goat anti-rabbit immunoglobulin G antibodies (Biosys, Compiègne, France) at a 1:100 dilution in PBS supplemented with BSA. After several washings, cells were dropped onto glass slides, air dried, mounted in glycerol-PBS (9:1), and observed under a Nikon microscope equipped for epifluorescence. For transmission electron microscopy, protein A-sensitized gold particles (diameter, 10 nm) (53) at a 1:10 dilution in PBS were added to the cells for 30 min. Fixation was performed for 1 h with 2.5% (vol/vol) glutaraldehyde buffered at pH 7.4 with 0.1 M sodium cacodylate. Then the cells were postfixed for 1 h in 1% (vol/vol) osmium tetroxide in the same buffer, dehydrated in alcohol, and embedded in Epon. Finally, sections stained with uranyl acetate were examined on a 100 CX JEOL microscope.

Control experiments were performed by omitting laminin or by using a rabbit anti-human fibrinogen immune serum (at a 1:50 dilution in PBS containing 0.5% BSA; Diagnostica Stago, Asnières, France) instead of anti-laminin antiserum.

^{125}I-laminin-binding assay. Quantification of the binding was carried out by the method of Kronvall et al. (25). Unless otherwise stated, duplicate 200-μl samples of the germ tube suspension (10^7/ml) were centrifuged and the resulting pellets were suspended in 100 μl of radiolabeled laminin (2 μg/ml in PBS; specific activity, 1 mCi/mg). Then, 100 μl of PBS or inhibiting compound solution in PBS was added to the tubes. After incubation for 30 min at 37°C with constant shaking, the cells were washed four times in PBS and the radioactivity in the sediment was measured in a gamma spectrometer (Wallac 1274; LKB, Turku, Finland). Radioactivity from the incubation mixture containing no cells was considered to be background and was subtracted. Moreover, the plastic tubes used in the experiments were precoated by overnight incubation at 4°C with 1% BSA in PBS to minimize nonspecific binding of protein to the walls of the tubes.

Saturability was studied by using various amounts of ^{125}I-laminin ranging from 20 ng to 10 μg in a final volume of 200 μl. Competitive binding was determined in the presence of a 100-fold molar excess of unlabeled ligand. Results were analyzed by using the Scatchard equation (43) and by assuming that all the laminin molecules bound to the yeast surface via one combining site.

Heat treatment and trypsinization of the cells. For some experiments, samples of the germ tube suspension were first heated at 80°C for different periods ranging from 10 to 60 min. The suspensions were then cooled on ice and used for the binding assay. In other experiments, the fungal suspension was pretreated for 30 min at 37°C with increasing amounts (100 μl containing 2.5 to 250 μg/ml) of trypsin solution in PBS (type III-S; Sigma Chemical Co., St. Louis, Mo.). The reaction was then stopped by addition of egg white trypsin inhibitor (100 μl at 1 mg/ml in PBS; Serva, Heidelberg, Federal Republic of Germany). Finally, the cells were washed in PBS and used for the binding assay.

Inhibition experiments. To test the specificity of the laminin receptors, we performed competition experiments by incubating the germ tubes with a constant concentration of ^{125}I-laminin in the presence of a 100-fold molar excess of cold proteins such as BSA, fibronectin (Flow Laboratories, Irvine, Scotland), and human fibrinogen (Kabi Vitrum, Stockholm, Sweden), further purified to be free of fibronectin (13) or laminin (10).

Other inhibition experiments were performed with various sugars or derivatives (at a final concentration of 4 to 100 mM in PBS) including glucose, galactose, mannose, fucose, *N*-acetyl-D-glucosamine, and *N,N'*-diacetylchitobiose. Cell wall mannans of *C. albicans* blastoconidia, extracted by the method of Peat et al. (37), were also tested at a final concentration of 5 mg/ml.

Preparation of germ tube cell wall extract. Cell wall components were extracted by dithiothreitol and iodoacetamide treatment of germ tubes (52). The total protein content of the extract was estimated by the method of Lowry et al. (32) with BSA as a standard.

SDS-PAGE. Samples were dissolved in a buffer containing 62.5 mM Tris hydrochloride (pH 6.8), 2% (wt/vol) sodium dodecyl sulfate (SDS), 10% (wt/vol) glycerol, and 5% (vol/vol) 2-mercaptoethanol and boiled for 2 min. Then they were applied to 1.5-mm-thick slab gels of 12.5% polyacrylamide with a 3% polyacrylamide stacking gel and electrophoresed as described by Laemmli (26). The gels were stained with Coomassie brilliant blue R250, and apparent molecular masses were interpolated from the migration of phosphorylase *b* (94 kilodaltons [kDa]), BSA (67 kDa), hen egg albumin (43 kDa), carbonic anhydrase (30 kDa), soybean trypsin inhibitor (20 kDa), and α-lactalbumin (14.4 kDa).

Western immunoblotting. After completion of electrophoresis, proteins were electrophoretically transferred to a membrane (pore size, 0.45 μm; Millipore Corp., Bedford, Mass.) at 0.25 A for 2 h in 25 mM Tris–192 mM glycine (pH 8.3) buffer containing 20% methanol (51). The efficiency of the transfer was checked by Coomassie brilliant blue staining of the transferred gel and by transfer and amido black staining of the molecular mass standards. Other transfers were blocked with 10% (wt/vol) nonfat dry milk in PBS for 1 h at 60°C, and the membranes were washed three times (10 min per wash) in PBS containing 0.05% Tween 20 (PBST) and incubated for 30 min at 37°C with laminin solution (50 μg/ml) in PBST supplemented with 1% BSA (PBST-BSA). After being washed three times (10 min per wash) in PBST, the nitrocellulose sheets were incubated with rabbit anti-laminin immune serum at a 1:100 dilution in PBST-BSA. After 30 min at 37°C, blots were washed as described above, incubated for 30 min at 37°C with alkaline phosphatase-conjugated goat anti-rabbit immunoglobulin G F(ab')$_2$ (at a 1:1,000 dilution in PBST-BSA; Sigma), and washed again in PBST. Finally, bands were detected by using the Nitro Blue Tetrazolium and 5-bromo-4-chloro-3-indolyl phosphate substrate system (27).

The specificity of the reaction was assessed by omitting laminin or by using an irrelevant immune serum (rabbit anti-human fibrinogen serum at a 1:50 dilution in PBST-BSA).

RESULTS

Visualization of the binding of laminin to *C. albicans*. The immunofluorescence assay indicated that nongerminating blastoconidia of *C. albicans* did not interact with soluble laminin (data not shown). On the contrary, germ tubes exhibited a strong and homogeneous fluorescence on all their cell wall surfaces, whereas the mother cells were not labeled (Fig. 1a and b). Germ tubes producing mycelium (3-h-old

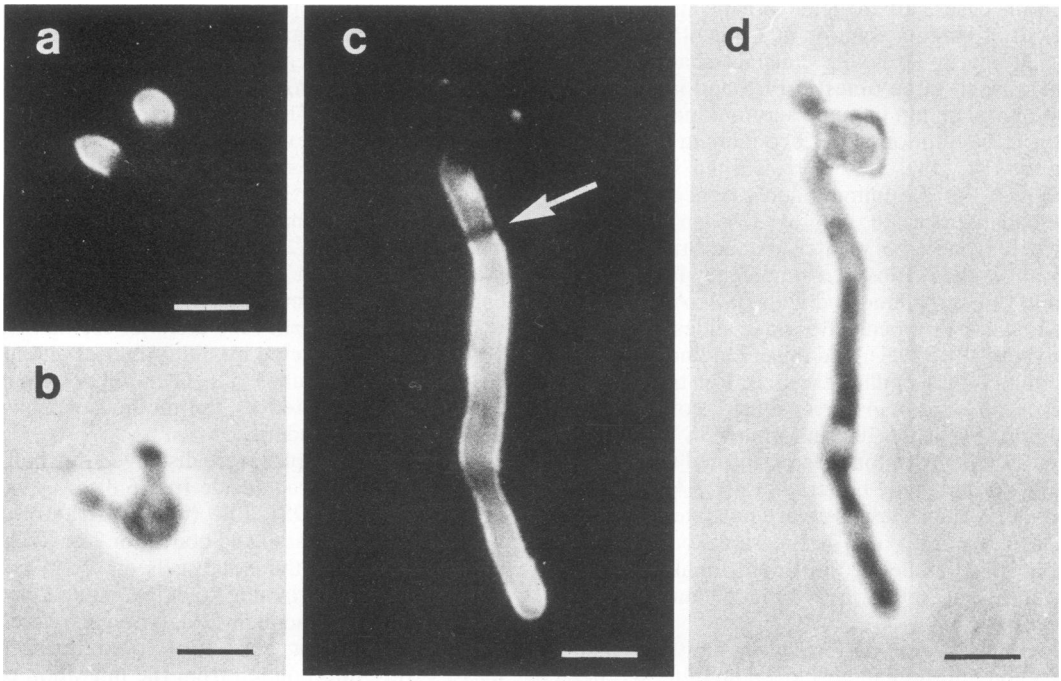

FIG. 1. Visualization by immunofluorescence of the binding of laminin to *C. albicans* germ tubes (a) and germ tubes producing mycelium (c). Note the strong and homogeneous fluorescence at the hyphal surface. On the contrary, septa (arrow), as mother cells visualized by phase-contrast microscopy (b and d), were not labeled. Bars, 5 μm.

germ tubes) also bound laminin intensely, but their septa were not labeled (Fig. 1c and d).

This binding pattern was confirmed and revealed in more detail by transmission immunoelectron microscopy, which showed that the labeling was distributed over the outermost fibrillar layer of the germ tube cell wall (Fig. 2a and b). Interestingly, no labeling could be seen at the germ tube emergence, in the area of rearrangement of the cell wall

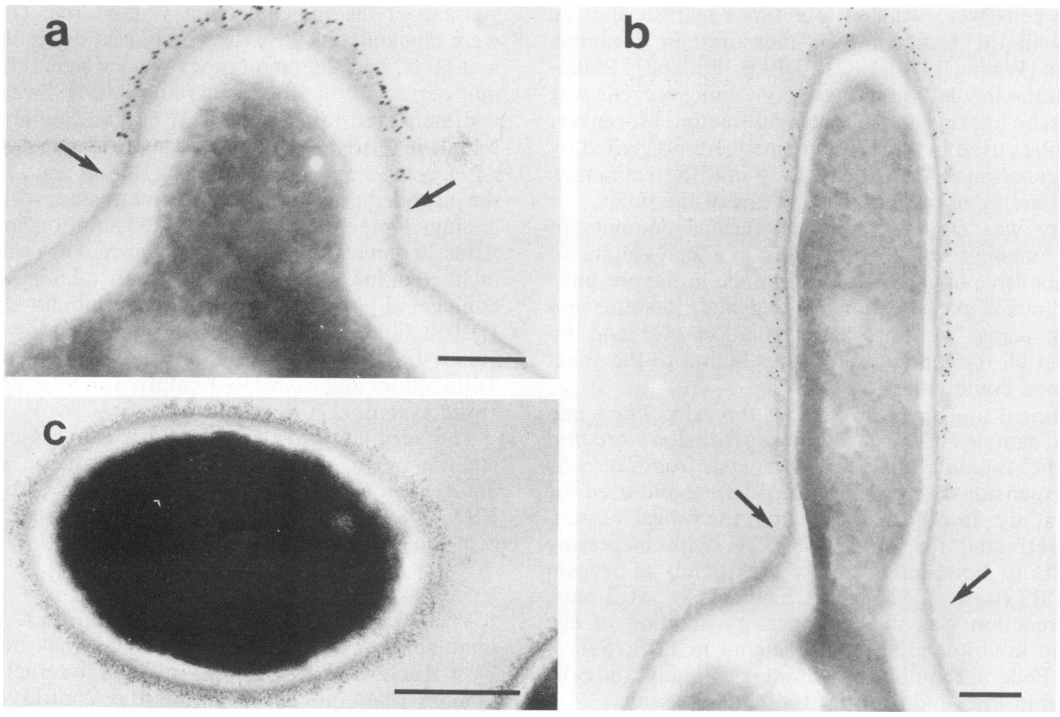

FIG. 2. Immunoelectron microscopy detection of laminin binding. Labeling was associated with the outermost fibrillar layer of the hyphal cell wall (a and b), and no particles were detected at the germ tube emergence (arrows). (c) Control experiment without laminin: cross-section of a germ tube showing the absence of any labeling. Bars, 0.5 μm.

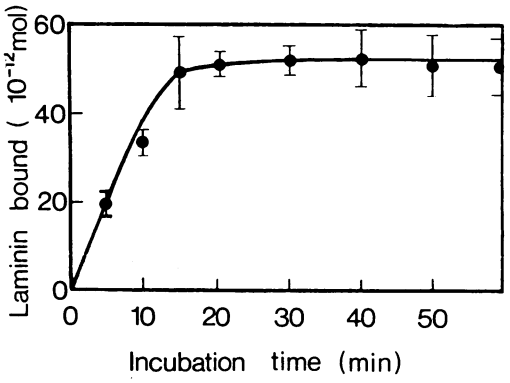

FIG. 3. Kinetics for laminin binding to 150-min-old *C. albicans* germ tubes. After incubation, the amount of ^{125}I-laminin associated with the fungal cells was determined as indicated in the text. Background values were subtracted, and mean values of duplicate experiments were calculated. Bars represent standard error.

components. For the two procedures, control experiments yielded negative results (Fig. 2c).

Quantitative study of the binding. Since immunofluorescence and electron microscopy showed that binding of laminin to *C. albicans* was associated with germination, germ tubes were selected for a further examination of the binding characteristics.

^{125}I-labeled laminin bound to the germ tubes rapidly and in a time-dependent manner, reaching saturation within 20 min (Fig. 3). The amount of bound protein remained constant when incubation was prolonged for up to 1 h (Fig. 3). Therefore, in subsequent experiments, counts were performed after a 30-min incubation, which was the length of time necessary to achieve the equilibrium.

Laminin-binding sites on the germ tube cell wall presented characteristics of receptors. First, dose-response experiments showed that germ tubes could be saturated with labeled ligand, suggesting that this interaction involved a limited number of receptors (Fig. 4). A maximum of 13 ng of laminin bound to 10^6 germ tubes of *C. albicans* 1066. Second, competitive experiments demonstrated the specificity of the interaction. Nonspecific binding, determined as

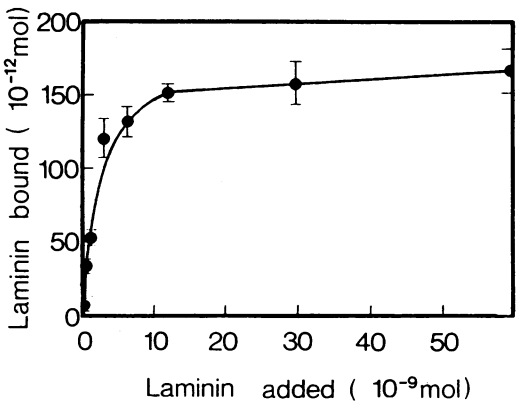

FIG. 4. Saturability of the candidal receptors for laminin. Germ tubes were incubated for 30 min in the presence of increasing concentrations of labeled laminin. Background values were determined for each concentration of laminin added and were subtracted. Finally, mean values of duplicate experiments were calculated. Bars represent standard error.

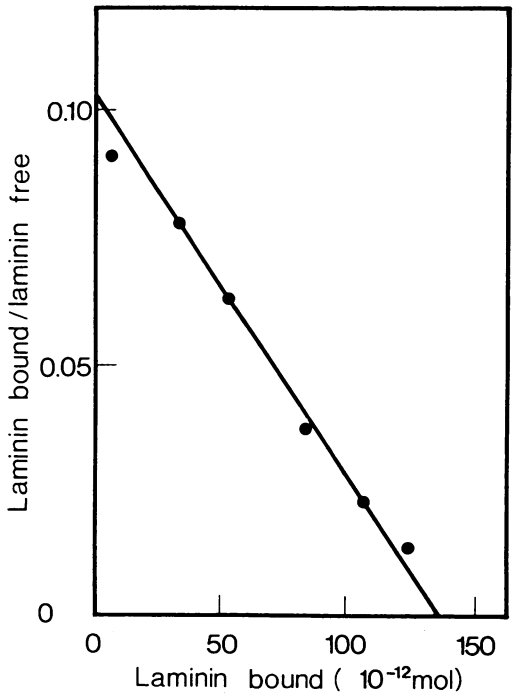

FIG. 5. Scatchard plot analysis of the data presented in Fig. 4 after subtraction of the nonspecific binding determined as indicated in the text.

the residual binding in the presence of a 100-fold excess of unlabeled ligand, was dependent upon the concentration of laminin added: for low concentrations of ^{125}I-laminin, nonspecific binding represented only 0 to 8% of the total labeled laminin binding, whereas it was 17 to 25% of the total binding for high concentrations.

The Scatchard equation was used to plot and analyze the data. By assuming a molecular mass of 850 kDa for laminin (50) and assuming that all binding sites were occupied at saturation, we calculated the average number of receptors per cell to be about 8,000 (Fig. 5); moreover, a single dissociation constant (K_d) was calculated (1.3×10^{-9} M), suggesting that germ tubes possessed only one class of receptor sites.

Heat and trypsin sensitivity of the binding sites. To specify the biochemical nature of the binding sites, we heated germ tubes at 80°C for different periods and then analyzed their abilities to bind ^{125}I-laminin. The binding capacity decreased dramatically for cells heated at 80°C for 10 min and was abolished after a 20-min heating (Table 1). In addition, the sensitivity of the binding sites to trypsin was studied. Curiously, treatment of the cells with a 2.5-μg/ml trypsin solution increased their binding capacity. However, increasing the concentration of the trypsin solution resulted in the degradation of the laminin-binding components: a complete loss of binding was observed when a concentration of 50 μg/ml was used, which confirmed the protein nature of the laminin receptors (Table 1).

Influence of potential inhibitors on laminin binding to *C. albicans*. Selected carbohydrates or polysaccharides, which are constituents of the laminin sugar chains or of the candidal cell wall, were studied as potential inhibitors of the laminin binding. Coincubation of the cells with radiolabeled laminin and different carbohydrate solutions failed to inhibit the binding (Table 2). Moreover, it was curiously enhanced

TABLE 1. Influence of prior heat or trypsin treatments of the germ tubes on laminin binding[a]

Treatment	Binding relative to the control (%)[b]
None	100
80°C, 10 min	9
80°C, 20 min	0
80°C, 30 min	0
80°C, 60 min	0
Trypsin (2.5 μg/ml)	124
Trypsin (25 μg/ml)	95
Trypsin (50 μg/ml)	0
Trypsin (250 μg/ml)	0

[a] Germ tubes were heated at 80°C for different periods or pretreated for 30 min at 37°C with increasing amounts of trypsin. After the suspensions had been cooled on ice or trypsin inhibitor had been added, cells were washed and incubated with ^{125}I-laminin.

[b] Results correspond to mean values of duplicate experiments after subtraction of the background.

by glucose or by mannans extracted from the cell wall of blastoconidia.

The specificity of the binding was studied by direct-competition experiments in which ^{125}I-laminin was added to the organisms together with a 100-fold molar excess of unlabeled proteins. Compared with cold laminin, none of the proteins tested showed similar inhibitory effects (Table 2). Indeed, BSA and fibronectin did not affect the binding. However, ^{125}I-laminin binding was reduced (ca. 57% inhibition) in the presence of purified human fibrinogen.

Identification of the laminin receptors. Since fungal fibrils promote the binding of laminin to the cell surface, material isolated from the outermost layers of the germ tube cell wall must contain the laminin receptors. In this study, cell wall components of germ tubes were extracted by dithiothreitol-iodoacetamide treatment. When analyzed by SDS-polyacrylamide gel electrophoresis (PAGE) under reducing conditions on a 12.5% polyacrylamide slab gel and then stained with Coomassie blue, this extract was seen to contain about 30 polypeptide chains with molecular masses ranging from 90 to 14 kDa (Fig. 6, lane 1). Among these proteins or polypeptides, only two components were revealed by Western blotting and incubation of the blots with laminin (Fig. 6, lane 2). One of them, of approximately 68 kDa, seemed to be one of the major components of the germ tube extract, and the other was present as a doublet of 60 and 62 kDa. No bands were detected for control experiments, attesting the specificity of the reaction (Fig. 6, lanes 3 and 4).

TABLE 2. Influence of potential inhibitors on laminin binding to *C. albicans* germ tubes[a]

Sugar or protein tested	Binding relative to the control (%)[b]
None	100
Glucose (100 mM)	144
Galactose (100 mM)	117
Mannose (100 mM)	106
Fucose (100 mM)	103
N-Acetyl-D-glucosamine (16 mM)	80
N,N'-Diacetylchitobiose (4 mM)	108
Mannans (5 mg/ml)	134
BSA (0.12 μM)	87
Fibronectin (0.12 μM)	97
Laminin (0.12 μM)	0
Fibrinogen (0.12 μM)	43

[a] Germ tubes (2×10^6) were incubated for 30 min with a mixture (200 μl) of ^{125}I-laminin (1.2 nM) and various oligosaccharides or proteins (at concentrations indicated in parentheses).

[b] Results correspond to mean values of duplicate experiments after subtraction of background values for the same mixtures containing no cells.

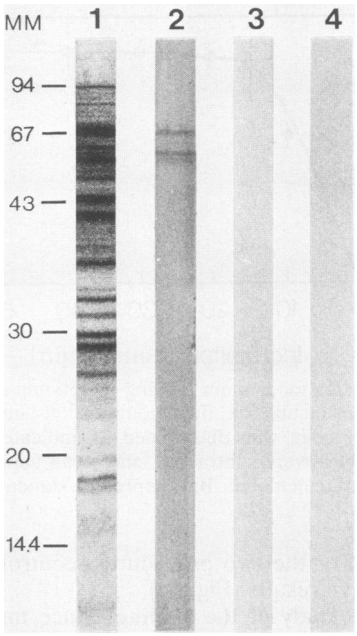

FIG. 6. SDS-PAGE and Western blotting analysis of the candidal receptors for laminin. A germ tube extract (80 μg of proteins per lane) was applied to a 12.5% polyacrylamide slab gel and then transferred to nitrocellulose. Blots were sequentially treated with laminin, rabbit anti-laminin serum, and alkaline phosphatase-conjugated goat anti-rabbit immunoglobulin G F(ab')$_2$. Finally, the reaction was developed by the method of Leary et al. (27). Two components, of 68 kDa and a doublet of 60 and 62 kDa, were revealed (lane 2). No bands were detected when laminin was omitted (lane 3) or when an irrelevant immune serum was used (lane 4). Lane 1 shows Coomassie blue staining of the germ tube extract. Molecular masses (MM) of the standard proteins (in kilodaltons) are indicated on the left.

DISCUSSION

Adherence of *C. albicans* to basement membrane underlining epithelia and endothelia has received particular attention during the last few years. Klotz et al., using cultured bovine endothelial cells, demonstrated that adherence took place at intercellular junctions (23) and at sites of the basement membranelike matrix exposed secondary to cellular contraction (24). Likewise, Klotz showed that clinically important yeasts adhered more strongly to purified extracellular matrix than to confluent cells (22). Basement membranes are normally unexposed in vivo, except in fenestrated endothelia such as those in kidney glomeruli, a frequent site of systemic candidiasis (35). However, they can be exposed after tissue damage, and it has been shown with corneal cells that injury greatly enhances the adhesion of *C. albicans* (39). In this work, we have demonstrated the interaction of the fungus with laminin, a major glycoprotein of basement membranes.

Evidence for this interaction was first supported by immunofluorescence. As described for fibrinogen (2, 36, 53), albumin (36), and transferrin (36), labeling was restricted to germ tubes which initiate the filamentous parasitic phase.

Other studies also reported evidence for a greater adherence of germinated than nongerminated *C. albicans* to epithelial (21) or endothelial (41) cells. This suggests that attachment correlates with the presence of extracellular matrix component receptors on the germ tube surface. The location of the binding sites was further specified by electron microscopy. Labeling appeared to be associated with the outermost fibrillar layer of the germ tube cell wall, whose development in vivo represents one of the most intriguing characteristics of the fungal cell.

Binding sites at the germ tube surface seemed to possess characteristics of receptors. Binding was saturable and specific; Scatchard plot analysis of the data showed about 8,000 binding sites per cell, and a K_d of 1.3×10^{-9} M. Taking into account the difference in size between germ tubes and mammalian cells, these values are similar to those described for the binding of laminin to metastatic tumor cells (1, 33, 49).

The thermolability of binding sites and their sensitivity to trypsin suggested a protein nature. Furthermore, the results of competitive assays do not provide support for a lectin-mediated interaction, since various oligosaccharides tested failed to demonstrate binding inhibition. At least, among the host proteins which link to *C. albicans*, it appeared that BSA and fibronectin recognized some candidal receptors different from those for laminin; fibronectin was reported to bind to the cell wall surface of blastoconidia (45). On the contrary, purified human fibrinogen greatly reduced the binding of laminin to *C. albicans*. This suggests that binding of fibrinogen leads to a steric hindrance of the laminin receptors or that binding of these two host proteins to germ tubes implies the same receptors.

Dithiothreitol-iodoacetamide treatment of germ tubes, resulting in the release of fibrils from the outer cell wall layers (52), allowed us to identify the candidal receptors for laminin. After SDS-PAGE and Western blotting, two components were revealed by incubation of blots with laminin. The first had a molecular mass of 68 kDa and was therefore identical or similar to the laminin receptors previously described on different cell types: mammalian tumor cells (1, 4, 17, 33, 38, 54), skeletal muscle cells (29), endothelial cells (56), and neuronal cells (16). The second component consisted of a doublet of 60 and 62 kDa, which could correspond to a degradation product of the 68-kDa component, as described for laminin receptors on murine EHS tumor cells (17).

These two components could be related to some previously described *C. albicans* protein. Glycosylated proteins of 70 and 62 kDa were isolated by Calderone et al. using affinity chromatography on C3d-thiol Sepharose (6). These proteins, present in pseudohyphal- but not in yeast-phase extracts, were then separated by high-performance liquid chromatography, allowing the identification of the C3d receptor of *C. albicans* as a 60-kDa protein (30). In addition, we previously described some fibrillar adhesins responsible for the adherence of *C. albicans* germ tubes to plastic (52). SDS-PAGE analysis of this material revealed four proteins, two of which (68 and 60 kDa) seem to play the major role in the adhesion process.

These observations concerning C3d binding, added to the fact that fibrinogen inhibited the binding of laminin, led us to investigate the presence of receptors for these two molecules on the dithiothreitol extract. The same components of 68 and 60 kDa were detected (data not shown), demonstrating multiple biological activities. However, the molecular mechanism of recognition of these unrelated host proteins by the same candidal receptors remains unclear and needs further investigations.

In conclusion, identification of these receptors and evidence for their multifunctional nature constitute new information for the understanding of the mechanisms that allow the fungus to colonize host tissues.

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LITERATURE CITED

1. **Barsky, S. H., C. N. Rao, D. Hyams, and L. A. Liotta.** 1984. Characterization of a laminin receptor from human breast carcinoma tissue. Breast Cancer Res. Treat. **4**:181–188.
2. **Bouali, A., R. Robert, G. Tronchin, and J. M. Senet.** 1986. Binding of human fibrinogen to *Candida albicans in vitro*: a preliminary study. J. Med. Vet. Mycol. **24**:345–348.
3. **Bouali, A., R. Robert, G. Tronchin, and J. M. Senet.** 1987. Characterization of binding of human fibrinogen to the surface of germ-tubes and mycelium of *Candida albicans*. J. Gen. Microbiol. **133**:545–551.
4. **Brown, S. S., H. L. Malinoff, and M. S. Wicha.** 1983. Connectin: cell surface protein that binds both laminin and actin. Proc. Natl. Acad. Sci. USA **80**:5927–5930.
5. **Bryant, G., C. N. Rao, M. Brentani, W. Martins, J. D. Lopes, S. E. Martin, L. A. Liotta, and E. Schiffman.** 1987. A role for the laminin receptor in leukocyte chemotaxis. J. Leukocyte Biol. **41**:220–227.
6. **Calderone, R. A., L. Linehan, E. Wadsworth, and A. L. Sandberg.** 1988. Identification of C3d receptors on *Candida albicans*. Infect. Immun. **56**:252–258.
7. **Calderone, R. A., and W. M. Scheld.** 1987. Role of fibronectin in the pathogenesis of candidal infections. Rev. Infect. Dis. **9**(Suppl. 4):400–403.
8. **Centeno, A., C. P. Davis, M. S. Cohen, and M. Warren.** 1983. Modulation of *Candida albicans* attachment to human epithelial cells by bacteria and carbohydrates. Infect. Immun. **39**:1354–1360.
9. **Critchley, I. A., and L. J. Douglas.** 1987. Role of glycosides as epithelial cell receptors for *Candida albicans*. J. Gen. Microbiol. **133**:637–643.
10. **Del Rosso, M., R. Cappelletti, M. Viti, S. Vannuchi, and V. Chiarugi.** 1981. Binding of the basement-membrane glycoprotein laminin to glycosaminoglycans. An affinity chromatography study. Biochem. J. **199**:699–704.
11. **Douglas, L. J.** 1987. Adhesion of *Candida* species to epithelial surfaces. Crit. Rev. Microbiol. **15**:27–43.
12. **Edwards, J. E., T. A. Gaither, J. J. O'Shea, D. Rotrosen, T. J. Lawley, S. A. Wright, M. M. Frank, and I. Green.** 1986. Expression of specific binding sites on *Candida* with functional and antigenic characteristics of human complement receptors. J. Immunol. **137**:3577–3583.
13. **Engwall, E., and E. Ruoslathi.** 1987. Binding of soluble form of fibroblast surface protein, fibronectin, to collagen. Int. J. Cancer **20**:1–5.
14. **Fitzgerald, T. J., L. A. Repesh, D. R. Blanco, and J. N. Miller.** 1984. Attachment of *Treponema pallidum* to fibronectin, laminin, collagen IV and collagen I, and blockage of attachment by immune rabbit IgG. Br. J. Vener. Dis. **60**:357–363.
15. **Gilmore, B. J., E. M. Retsinas, J. S. Lorenz, and M. K. Hostetter.** 1988. An iC3b receptor on *Candida albicans*: structure, function and correlates for pathogenicity. J. Infect. Dis. **157**:38–46.
16. **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**:989–996.
17. **Graf, J., R. C. Ogle, F. A. Robey, M. Sasaki, G. R. Martin, Y. Yamada, and H. K. Kleinman.** 1987. A pentapeptide from the

laminin B1 chain mediates cell adhesion and binds the 67000 laminin receptor. Biochemistry **26**:6896–6900.
18. **Heidenreich, F., and M. P. Dierich.** 1985. *Candida albicans* and *Candida stellatoidea*, in contrast to other *Candida* species, bind iC3b and C3d but not C3b. Infect. Immun. **50**:598–600.
19. **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.
20. **Hunter, W. H., and F. C. Greenwood.** 1962. Preparation of iodine-131 labelled human growth hormone of high specific activity. Nature (London) **194**:495–496.
21. **Kimura, L. H., and N. N. Pearsall.** 1978. Adherence of *Candida albicans* to human buccal epithelial cells. Infect. Immun. **21**: 64–68.
22. **Klotz, S. A.** 1987. The adherence of *Candida* yeasts to human and bovine vascular endothelium and subendothelial extracellular matrix. FEMS Microbiol. Lett. **48**:201–205.
23. **Klotz, S. A., D. J. Drutz, J. L. Harrison, and M. Huppert.** 1983. Adherence and penetration of vascular endothelium by *Candida* yeasts. Infect. Immun. **42**:374–384.
24. **Klotz, S. A., and R. D. Maca.** 1988. Endothelial cell contraction increases *Candida* adherence to exposed extracellular matrix. Infect. Immun. **56**:2494–2498.
25. **Kronvall, G., E. B. Myhre, L. Bjorck, and J. Berggard.** 1978. Binding of aggregated human β_2-microglobulin in surface protein structure in group A, C, and G streptococci. Infect. Immun. **22**:136–142.
26. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227**:680–685.
27. **Leary, J. J., D. J. Brigati, and D. C. Ward.** 1983. Rapid and sensitive colorimetric method for visualizing biotin-labeled DNA probes hybridized to DNA or RNA immobilized on nitrocellulose: bioblots. Proc. Natl. Acad. Sci. USA **80**:4045–4049.
28. **Lee, K. L., H. R. Buckley, and C. C. Campbell.** 1975. An amino acid liquid synthetic medium for the development of mycelial and yeast forms of *Candida albicans*. Sabouraudia **13**:148–153.
29. **Lesot, H., U. Kühl, and K. Von der Mark.** 1983. Isolation of a laminin binding protein from muscle cell membranes. EMBO J. **2**:861–865.
30. **Linehan, L., E. Wadsworth, and R. A. Calderone.** 1988. *Candida albicans* C3d receptor, isolated by using a monoclonal antibody. Infect. Immun. **56**:1981–1986.
31. **Lopes, J. D., M. Dos Reis, and R. R. Brentani.** 1985. Presence of laminin receptors in *Staphylococcus aureus*. Science **229**:275–277.
32. **Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall.** 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193**:265–275.
33. **Malinoff, 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.
34. **Mota, G. F., C. R. W. Carneiro, L. Gomes, and J. D. Lopes.** 1988. Monoclonal antibodies to *Staphylococcus aureus* laminin-binding proteins cross-react with mammalian cells. Infect. Immun. **56**:1580–1584.
35. **Odds, F. C.** 1979. *Candida* and candidosis. Leicester University Press, Leicester, England.
36. **Page, S., and F. C. Odds.** 1988. Binding of plasma proteins to *Candida* species *in vitro*. J. Gen. Microbiol. **134**:2693–2702.
37. **Peat, S., W. J. Whelam, and T. E. Edwards.** 1961. Polysaccharides of baker's yeast. IV. Mannan. J. Chem. Soc. **1**:29–34.
38. **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.
39. **Rao, N. A., D. W. Riggio, J. M. Delmage, A. J. Calandra, S. Evans, and W. Lewis.** 1985. Adherence of *Candida* to corneal surface. Curr. Eye Res. **4**:851–856.
40. **Rotrosen, D., R. A. Calderone, and J. E. Edwards.** 1986. Adherence of *Candida* species to host tissues and plastic surfaces. Rev. Infect. Dis. **8**:73–85.
41. **Rotrosen, D., J. E. Edwards, T. R. Gibson, J. C. Moore, A. H. Cohen, and I. Green.** 1985. Adherence of *Candida* to cultured vascular endothelial cells: mechanisms of attachment and endothelial cell penetration. J. Infect. Dis. **152**:1264–1274.
42. **Sandin, R. L., A. L. Rogers, R. J. Patterson, and E. S. Beneke.** 1982. Evidence for mannose-mediated adherence of *Candida albicans* to human buccal cells in vitro. Infect. Immun. **35**: 79–85.
43. **Scatchard, G.** 1949. The attractions of proteins for small molecules and ions. Ann. N.Y. Acad. Sci. **51**:660–672.
44. **Segal, E., N. Lehrer, and I. Ofek.** 1982. Adherence of *Candida albicans* to human vaginal epithelial cells: inhibition by amino sugars. Exp. Cell Biol. **50**:13–17.
45. **Skerl, K. G., R. A. Calderone, E. Segal, T. Sreevalsan, and W. M. Scheld.** 1984. *In vitro* binding of *Candida albicans* yeast cells to human fibronectin. Can. J. Microbiol. **30**:221–227.
46. **Speziale, P., M. Höök, T. Waldstrom, and R. Timpl.** 1982. Binding of the basement membrane laminin to *Escherichia coli*. FEBS Lett. **146**:55–58.
47. **Switalski, L. M., H. Murchison, R. Timpl, R. Curtiss III, and M. Höök.** 1987. Binding of laminin to oral and endocarditis strains of viridans streptococci. J. Bacteriol. **169**:1095–1101.
48. **Switalski, L. M., P. Speziale, M. Höök, T. Waldstrom, and R. Timpl.** 1984. Binding of *Streptococcus pyogenes* to laminin. J. Biol. Chem. **259**:3734–3738.
49. **Terranova, V. P., C. N. Rao, T. Kalebic, I. M. Margulies, and L. A. Liotta.** 1983. Laminin receptors on human breast carcinoma cells. Proc. Natl. Acad. Sci. USA **80**:444–448.
50. **Timpl, R., H. Rohde, P. Gehron-Robey, S. I. Rennard, J. M. Foidart, and G. Martin.** 1979. Laminin—a glycoprotein from basement membranes. J. Biol. Chem. **254**:9933–9937.
51. **Towbin, H., T. Staehelin, and J. Gordon.** 1979. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. Proc. Natl. Acad. Sci. USA **76**:4350–4354.
52. **Tronchin, G., J. P. Bouchara, R. Robert, and J. M. Senet.** 1988. Adherence of *Candida albicans* germ tubes to plastic: ultrastructure and molecular studies of fibrillar adhesins. Infect. Immun. **56**:1987–1993.
53. **Tronchin, G., R. Robert, A. Bouali, and J. M. Senet.** 1987. Immunocytochemical localization of *in vitro* binding of human fibrinogen to *Candida albicans* germ tube and mycelium. Ann. Inst. Pasteur Microbiol. **138**:177–187.
54. **Wever, U. M., L. A. Liotta, M. 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.
55. **Winkler, J. R., S. R. John, R. H. Kramer, C. I. Hoover, and P. A. Murray.** 1987. Attachment of oral bacteria to a basement-membrane-like matrix and to purified matrix proteins. Infect. Immun. **55**:2721–2726.
56. **Yannariello-Brown, J., U. M. Wever, L. A. Liotta, and J. A. Madri.** 1988. Distribution of a 69-kDa laminin-binding protein in aortic and microvascular endothelial cells: modulation during cell attachment, spreading and migration. J. Cell Biol. **106**: 1773–1786.