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Binding of the Extracellular Matrix Component Entactin to *Candida albicans*

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We have investigated the interaction between *Candida albicans* and entactin, a recently characterized glycoprotein present in the extracellular matrix, especially in the basement membrane. Organisms of both the yeast and the hyphal morphologies of the fungus had the ability to bind recombinant entactin, as detected by an indirect immunofluorescence assay. Material present in the 2-mercaptoethanol cell wall extracts from both *C. albicans* growth forms was capable of binding to immobilized recombinant entactin in a dose-dependent manner. Binding to entactin was approximately twice that observed for laminin. Binding of an extract component(s) to entactin was partially inhibited by an Arg-Gly-Asp-Ser peptide. A polyclonal antientactin antiserum, as well as a pooled antiserum preparation raised against components present in different *C. albicans* cell wall extracts, completely or almost completely abolished binding. The existence of morphology-specific receptor-like molecules which bind to different domains of the entactin molecule was ruled out in a competition binding assay. The entactin-binding material(s) in the cell wall also displayed some ability to bind laminin and fibronectin, since preadsorption in the presence of these extracellular matrix components resulted in reduction of binding to entactin. Moieties with a molecular mass of approximately 25, 44, and 65 kDa present in the 2-mercaptoethanol cell wall extracts from both blastoconidia and germ tubes were detected in a ligand affinity blotting experiment as having the ability to bind entactin. Interactions between *C. albicans* and entactin could be important in mediating adhesion of the fungus to the host tissues and may play a role in the establishment of the disseminated form of the disease.

Candida albicans is a dimorphic fungus which is a component of the normal flora as well as an opportunistic pathogen. Adhesion of *C. albicans* to host cells and tissues is the initial step leading to colonization as a commensal and establishment of infection as a pathogen. Attachment involves the interaction between complementary molecules present in both the parasite and the host surfaces. In recent years, substantial progress towards a better understanding of this phenomenon has been achieved, and a number of molecules with receptor-like characteristics have been described for *C. albicans* (5, 6). Most of these molecules are glyco(manno)proteins present in the external part of the fungal cell wall and are known as adhesins displaying properties similar to lectins or integrins, recognizing either fucosyl glycosides or arginine-glycine-aspartic acid (RGD)-containing peptides in the host cells and tissues (5). Most of these molecules are considered to be virulence factors (16, 35). This repertoire of adhesins may confer on *C. albicans* a great degree of plasticity and adaptability in its interaction with the host. This may be an important factor which allows *C. albicans* to maintain a commensal relationship with and to take advantage of a host with impaired defenses and undergo a transition to pathogenic interaction.

Adhesins identified so far for *C. albicans* include receptors for complement fragments iC3b and C3d (1, 7, 20, 21), laminin (3, 40), fibrinogen (2, 11), fibronectin (27, 34, 47), and different extracellular matrix (ECM) components, including collagen IV (32–35), moieties that mediate binding to plastic (50), and a component(s) responsible for cell surface hydrophobicity (24, 56, 39); a unique adhesion system involving mannan moieties by which the fungus binds to macrophages in the spleen and lymph node (13, 22, 28, 29, 38); and an extracellular adhesin which mediates attachment to epithelial cells (15, 48). Some of these adhesion molecules have been described to be differentially expressed depending on the morphology of the fungus, but others are found at the cell surfaces of both yeast cells and germ tubes. The existence of multiple biological activities has been suggested for some of these molecules (3, 34, 49). Also, the presence of different receptor-like molecules that bind to the same ligand has been reported (3, 34, 40). Some of these ligands are found in subendothelial ECM, and the fungus may adhere better to ECM than it does to uninjured endothelium (33). The binding to these ligands may involve the RGD sequence (20, 34) associated with integrin family ligand-receptor interactions. Binding of iC3b may also be mediated through an RGD sequence (20). An important role for RGD-mediated interactions is indicated by a protective role for an RGD-containing peptide in experimental infection (35). Once *C. albicans* has bound to exposed ECM or other noncommensal sites, dissemination of the microorganism may occur, resulting in metastatic *Candida* infections.

A novel adhesion molecule, entactin (also called nidogen), has been described as an integral and ubiquitous component of the basement membrane, and its tissue distribution has been studied (31). Entactin is a 150-kDa sulfated glycoprotein which contains the cell attachment-promoting sequence RGD, known to be recognized by many integrins. It forms a tight stoichiometric complex with laminin; interacts with itself and with type IV collagen, fibronectin, and fibrinogen; supports cell adhesion; and binds calcium ions (12, 54–56).

We have studied the interactions between *C. albicans* and entactin using both the intact microbe and cell wall extracts. We have used indirect immunofluorescence techniques to demonstrate the presence of entactin receptor-like molecules

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on both morphological forms of *C. albicans*. Characteristics of extract components that bind to immobilized entactin have been examined, and components binding entactin have been identified by ligand affinity blotting.

MATERIALS AND METHODS

Organism and culture conditions. *C. albicans* 3153A was employed throughout this study and was maintained by subculturing weekly on Sabouraud dextrose medium with 1.5% agar. Cultures were grown as previously reported (8, 9) in minimal medium supplemented with amino acids described by Lee et al. (37). Yeasts were grown at 22 to 24°C with shaking to stationary phase. Stationary-phase yeast cells were harvested, washed in water, and maintained at 4°C for 1 to 3 days before germ tubes were induced at 37°C in the same medium as yeast growth.

Indirect immunofluorescence assay. Germ tubes or yeast cells were harvested, washed twice with distilled water, and resuspended at approximately 10^6 cells per ml in phosphate-buffered saline (pH 7.4) (PBS) containing 0.5 mg of mouse recombinant entactin (Upstate Biotechnology Inc., Lake Placid, N.Y.) per ml. After incubation for 2 h at 37°C with gentle agitation in a gyratory incubator shaker, the cells were washed four times with PBS, resuspended in a 1:50 dilution of rabbit antientactin antiserum (Upstate Biotechnology Inc.) in PBS plus 1% bovine serum albumin (BSA), and incubated for 1 h at 37°C. The antientactin antiserum had been previously preadsorbed with *C. albicans* cells (yeast cells or germ tubes) to eliminate nonspecific reactivity. The cells were washed again as described above, resuspended in a 1-to-10 dilution of fluorescein-conjugated goat anti-rabbit antiserum in PBS–1% BSA (Boehringer Mannheim, Indianapolis, Ind.), and incubated for 1 h at 37°C with gentle agitation. Finally, the cells were washed again four times with PBS and resuspended in a small volume (0.1 ml) of PBS. Drops of these suspensions were placed on the wells of microslides and examined with a Nikon Labophot equipped for epifluorescence. Images of the *C. albicans*-entactin interaction were obtained by conventional photography or with a cooled (−45°C) charge-coupled device camera (Star 1 camera and Star1/Macintosh Image Acquisition Software; Photometrics Ltd., Tucson, Ariz.). The digital image was converted to a tagged image format file, processed for brightness and contrast (Adobe Photoshop; Adobe Systems Inc., Mountain View, Calif.), scaled (imgworks; Silicon Graphics Inc., Mountain View, Calif.), and printed (Codonics [Middleburg, Ohio] printer).

In this study we have used recombinant entactin from a commercial source. Previous reports have characterized the recombinant material. The amino acid sequence from the purified recombinant protein is identical to that of the native entactin and exhibits structural and functional properties reflecting those of the naturally occurring molecule, which include promotion of cell attachment in an RGD-dependent manner (12, 51). Western blot (immunoblot) analysis indicates that the recombinant entactin used is free of fibronectin and laminin and cell attachment to this recombinant entactin is inhibited only by antientactin antibodies and not by antifibronectin or antilaminin antibodies, indicating the absence of fibronectin and laminin in the recombinant entactin preparation (12).

Preparation of cell wall extracts from untreated and surface biotinylated cells. In vivo labeling with biotin was performed basically as previously described (10). Fungal cells, either yeast cells or germ tubes, were collected by centrifugation, washed once with 100 mM phosphate buffer, pH 8, and resuspended in 1/10 the original culture volume in the same buffer containing 1 mg of *N*-hydroxysuccinimidobiotin (Sigma Chemical Co., St. Louis, Mo.) per ml. *N*-Hydroxysuccinimidobiotin was previously dissolved in dimethyl sulfoxide. After incubation for 1 h at 22°C in a shaking water bath, the cells were recovered, washed four times with 50 mM phosphate buffer, pH 6, and then washed once with 10 mM phosphate buffer, pH 7.4. Biotinylated cells were chemically extracted as described below.

Cell wall extracts were prepared from intact cells by treatment with 2-mercaptoethanol (βME) as described before (8, 10). Nontreated cells were harvested and washed with water. Organisms were resuspended in 1/10 the original culture volume in ammonium carbonate buffer containing 1% (vol/vol) βME and incubated for 30 min at 37°C in a rotary incubator shaker. After treatment, the cells were sedimented, and the supernatant fluid was recovered, dialyzed against distilled water (four changes) for 48 h at 4°C, and concentrated by freeze-drying. Zymolyase extracts were prepared as previously described by incubating the βME-extracted cells with zymolyase (8). After incubation the supernatant was dialyzed and lyophilized. The total sugar contents in the different samples were determined by the method of Dubois et al. (18) with mannose as the standard.

Preparation of anti-cell wall antisera. Cell wall extracts were obtained from yeast cells and germ tubes as described above. Antiserum was prepared for each extract. Ten milligrams (based on sugar content) of extract in 500 μl of saline was mixed with 500 μl of Ribi adjuvant reconstituted in saline (Ribi ImmunoChem Research, Inc., Hamilton, Mont.) and injected subcutaneously into a New Zealand White rabbit. Immunization was repeated at least four times at 3-week intervals. Serum (20 ml) was obtained 10 days after each immunization. Antibody was partially purified by precipitation with 40% ammonium sulfate. The precipitate was recovered by centrifugation, suspended in 10 mM Tris, pH 7.4, and dialyzed against the same buffer containing 2 mM sodium azide. For these experiments pooled antiserum was prepared by combining equal volumes of antiserum prepared against each of the four antigen preparations. The presence of anti-*Candida* antibody in the different sera was determined in an enzyme-linked immunosorbent assay (ELISA) with immunizing antigen, indirect immunofluorescence, and immunoblot with immunizing antigen.

Binding of extracted cell wall moieties to immobilized ECM components. An assay was developed to study the attachment of the material present in the βME extracts from yeast cells and germ tubes to ECM components. Wells of microtiter plates (Immulon IV; Dynatech Laboratories, Inc., Chantilly, Va.) were coated with the ECM component diluted in 100 mM borate buffer, pH 8.2, to the appropriate concentration. After overnight incubation at 4°C, plates were washed twice with PBS and the wells were treated with PBS containing 5% (wt/vol) nonfat milk for 2 h at 37°C. Biotinylated cell wall materials were diluted in PBS–0.05% Tween 20 containing 1% BSA, 1 mM $CaCl_2$, and 1 mM $MgCl_2$; added to the appropriate wells; and incubated for 1 h at 37°C. The unbound material was removed by washing with PBS–0.05% Tween 20. Peroxidase-conjugated ExtrAvidin (Sigma Chemical Co.) at a dilution of 1/3,000 in PBS–0.05% Tween 20 plus 1% BSA was added to the wells, and the plate was incubated for 1 h at 37°C. After the incubation period, the plates were developed by standard procedures (52). The color intensity was determined at 492 nm with an automated plate reader (Bio-Tek Instruments Inc., Winooski, Vt.). Background values of binding to uncoated

wells were subtracted from experimental values. The experiment was repeated three times with similar results.

To determine the ability of antientactin antiserum, anti-cell wall antiserum, RGDS peptide, or unlabeled cell wall extracts to inhibit binding, wells were coated with 2 μg of entactin per well. Antientactin antiserum was diluted in PBS–0.05% Tween 20 with 1% BSA and added to the wells, and the plate was incubated for 1 h at 37°C. The plate was rinsed with PBS, 6 μg (based on sugar) of βME extract of biotinylated yeast cells per well was added to the appropriate wells, and the assay was completed as described above. One hundred percent binding was determined by addition of extract to a control well initially incubated with buffer but no antiserum. To determine the ability of pooled anti-cell wall antiserum to inhibit binding, 12 μg of biotinylated extract was incubated in a microcentrifuge tube with increasing amounts of antiserum in a volume of 200 μl for 1 h at 37°C. At the end of the incubation 100 μl was placed in an entactin-coated well and the assay continued as described above. One hundred percent binding was determined by addition of 100 μl from a tube which received buffer but no antiserum. To determine the ability of the RGDS peptide (Sigma Chemical Co.) to inhibit binding, the previous experiment was repeated with various amounts of peptide rather than pooled antiserum added to the microcentrifuge tube. To determine the ability of unlabeled extracts to inhibit binding of extracts from biotinylated cells, different amounts (based on sugar) of unlabeled βME extract were added to the wells and the plate was incubated for 1 h at 37°C. At the end of the incubation 2 μg of the appropriate βME extract of biotinylated cells was added and the incubation continued for 1 h. Subsequently, the assay was completed as described above. One hundred percent binding was determined by binding of labeled extract to a control well not receiving unlabeled extract in the first incubation. Each of the inhibition experiments was repeated twice with similar results.

To determine the depletion of entactin-binding material by preincubation with laminin or fibronectin, the wells of a microtiter plate were coated with 20 μg of laminin (Sigma Chemical Co.) or fibronectin (Sigma Chemical Co.). Six micrograms of biotinylated extract was added to each well, and the plate was incubated for 1 h at 37°C. The unbound material was removed and transferred to a well previously coated with 2 μg of entactin, and the assay was completed as described above. One hundred percent binding was determined by material preincubated in an uncoated well. The experiment was performed twice in duplicate.

Electrophoresis, immunoblotting, and ligand affinity blotting. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed basically as described by Laemmli (36) with minor modifications by using the miniblot apparatus and precast 4 to 15% polyacrylamide minigels (Bio-Rad Laboratories, Richmond, Calif.) and following the manufacturer's instructions. Sixty micrograms of material (based on sugar) was loaded in each well for determination of biotinylated proteins, and 150 μg was loaded for determination of entactin binding. Electrophoretic transfer to nitrocellulose paper was performed basically as described previously (8) by using a semidry electroblotter at 0.8 mA/cm^2 for 1 h. Approximate sizes of components were determined by linear regression from molecular weight standards (Life Technologies Inc., Gaithersburg, Md.) run on each gel.

The nitrocellulose membranes were treated with 3% BSA in 10 mM Tris buffer, pH 7.4, containing 0.9% NaCl (TBS buffer) for 1 h at 37°C. After being washed once with TBS containing 0.05% Tween 20 (TBST), the nitrocellulose sheets were incubated with peroxidase-conjugated ExtrAvidin at a 1:3,000 dilution in TBST plus 1% BSA. After incubation for 1 h at room temperature with agitation, blots were washed four times with TBST. Reactive bands were developed with H_2O_2 and 4-chloro-1-naphthol (23).

For ligand affinity binding the nitrocellulose membranes were blocked with 4% BSA in TBS for 2 h at 37°C, rinsed with TBS, and then incubated with agitation for 4 h at room temperature in 10 mM phosphate buffer, pH 7.4, containing 1% (wt/vol) nonfat dry milk, 1 mM $CaCl_2$, 1 mM $MgCl_2$, 0.05% Tween 20, and 50 μg of entactin per ml. The filters were washed four times for 10 min with TBST and were incubated with a 1-to-2,000 dilution of rabbit antientactin antiserum in TBST plus 1% BSA. After 3 h at room temperature with agitation, the filters were washed as described above and incubated in the presence of peroxidase-conjugated antirabbit antiserum (Cappel; Organon Teknika Corporation, Durham, N.C.) at a 1/1,000 dilution in TBST–1% BSA for 1 h at room temperature with agitation. The filters then were washed and reactive bands were developed as described above. The specificity of the reaction was assessed by omitting incubation with entactin.

RESULTS

Binding of entactin to *C. albicans* cells. The ability of *C. albicans* to bind entactin was first determined by an indirect immunofluorescence assay. Organisms were recovered from a culture induced to form germ tubes. After incubation with entactin, most of the fluorescent organisms were germ tubes and fluorescence was localized primarily to the hyphal extension (Fig. 1A to C). Fluorescence was dependent on the reaction of the cells with entactin, since no fluorescence was observed when entactin was not present in the assay. Although *C. albicans* germ tubes have a tendency to aggregate by nature, this effect appeared to be strongly enhanced after incubation in the presence of entactin. This phenomenon could be due to the self-agglutinating nature of the ECM protein (51). Fluorescence appeared to be heterogeneously distributed along the hyphal elements, as has been described previously for fibrinogen binding (11, 43). No fluorescence was observed on the surface of the parent blastocondium from which the germ tubes emerged (Fig. 1A). However, a small percentage of nongerminated blastoconidia present in the preparations showed fluorescence (Fig. 1D and E). Also a small percentage (about 10%) of yeast cells grown at 24°C bound entactin (Fig. 1F and G). In general, a punctate or patchy pattern rather than a homogeneous fluorescence pattern was observed. Some aggregation of cells in treated, compared with control (no entactin), samples was observed, even though minimal fluorescence was observed on some of the cells in the clump (Fig. 1F).

Binding of extracted cell wall components to immobilized entactin and laminin. The ability of extracted cell wall components to bind entactin was examined in an assay developed for that purpose. Entactin was immobilized in wells of a microtiter plate and incubated with βME cell wall extract obtained from surface biotinylated intact organisms. As shown in Fig. 2, materials present in the βME extracts from both *C. albicans* morphological phases bound to entactin and laminin with a dose response. Results demonstrated that, in our assay conditions and at similar quantities of extract, binding to entactin was approximately double that observed in the case of laminin. Cell wall material extracted from blastoconidia had a higher binding to the basement membrane components than the materials obtained from germ tubes. The specificity of the interaction was further supported by the fact that an antientactin antiserum, as well as a pooled polyclonal antiserum

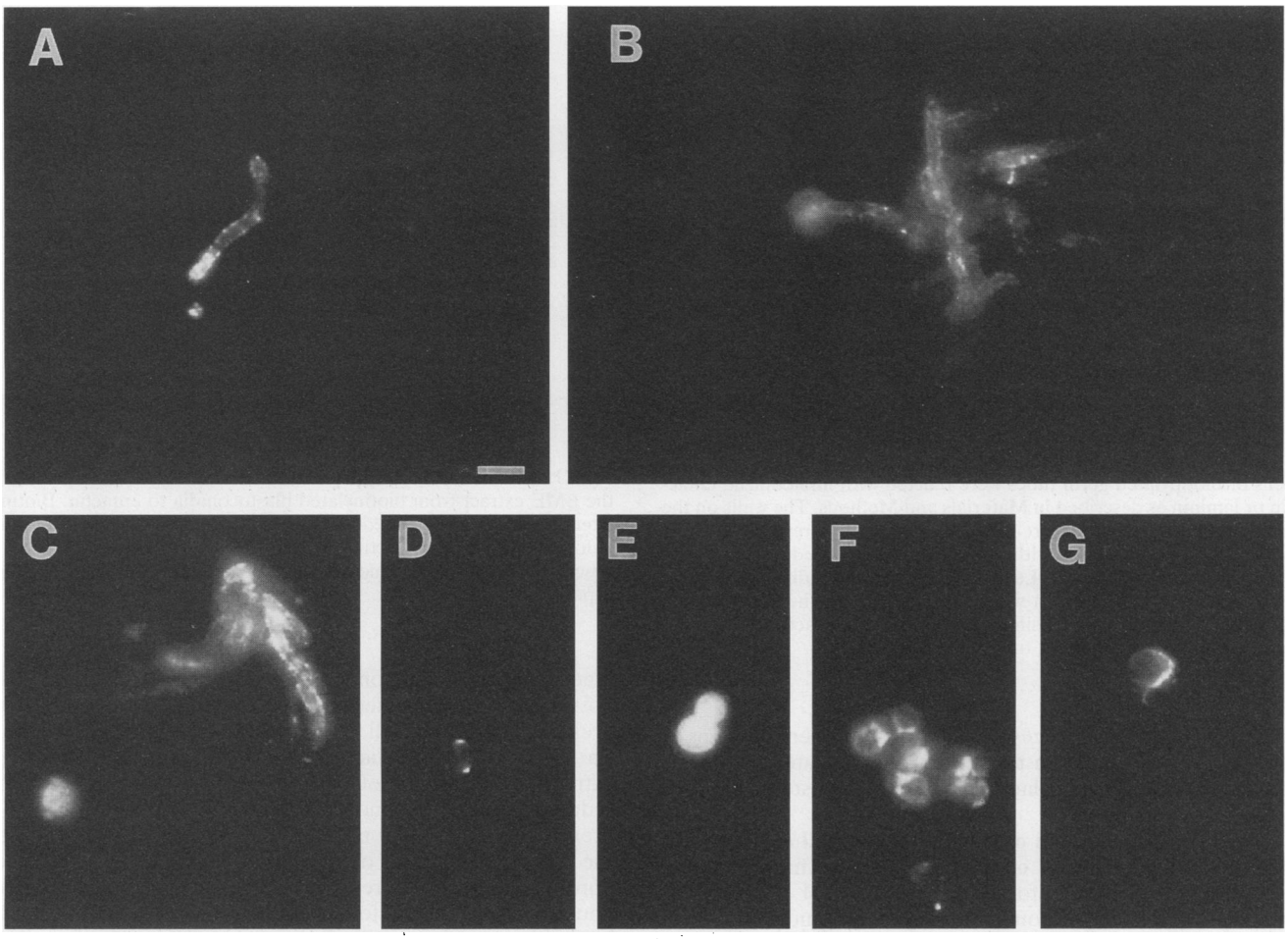

FIG. 1. Binding of entactin to *C. albicans* germ tubes and blastoconidia detected by indirect immunofluorescence. Organisms were incubated with entactin, rabbit antientactin antiserum and fluorescein isothiocyanate-conjugated goat anti-rabbit immunoglobulin as described in Materials and Methods. Digital microscopic images were obtained and processed as described in Materials and Methods. Most germ tubes were fluorescent, with binding localized primarily to the hyphal extension (A to C), although a few yeast cells were also positive (D and E). Some yeast cells in a yeast culture also bound entactin (F and G), and positive cells were also frequently found in clumps (F). Generally, fluorescence was heterogeneously distributed on the surface (A and D). Bar, 10 μm.

raised against materials present in different *C. albicans* cell wall extracts (βME and zymolyase extracts from both growth forms of the fungus), was capable of completely or almost completely abolishing the binding to entactin in a modified attachment assay also in a dose-dependent manner (Fig. 3).

Role of RGD sequence in mediating binding of entactin. The amino acid sequence in the entactin molecule contains the integrin recognition RGD motif, and the attachment of different types of cells to entactin has previously been described to be mediated, in part, by this peptide sequence (12, 56). Also, attachment of *C. albicans* yeast cells to other ECM components has been reported to take place in an RGD-dependent fashion (34). To investigate the contribution of the RGD sequence to binding of cell wall materials to entactin, we determined the ability of the synthetic peptide RGDS to inhibit binding to entactin of a component(s) in the yeast cell extract. Different concentrations of RGDS peptide were incubated with the cell wall extracts prior to addition to binding assay (Fig. 4). RGDS peptide showed an inhibitory effect which appeared to reach nearly 50% maximum inhibition at 400 μg/ml. Thus binding of *C. albicans* cell wall components to recombinant entactin is partially mediated by RGD. However, the failure to completely inhibit binding suggested that other entactin sequences could also be involved in interaction with cell wall components.

Competition between entactin-binding moieties from yeast cells and germ tubes. If, as suggested by the previous experiment, other domains of entactin serve as binding sites for *Candida* proteins, the site(s) recognized by the binding protein(s) from yeast cells may differ from the site(s) recognized by the binding protein(s) from germ tubes. To assess this possibility, a modified binding assay in which different amounts of nonlabeled cell wall material from one morphological form of *C. albicans* would compete with a fixed amount of biotin-labeled material from the other form was designed (Fig. 5). Each unlabeled extract was able to successfully abolish binding of the labeled component(s) of the other form. When a similar competition experiment was carried out with binding to laminin, the observations were different. Competition with unlabeled material from germ tubes decreased binding of biotinylated blastoconidium extract to laminin by about 10%, while the unlabeled blastoconidium extract decreased binding of the biotinylated germ tube extract by about 45%. Thus, the possibility of the existence of morphology-specific receptor-

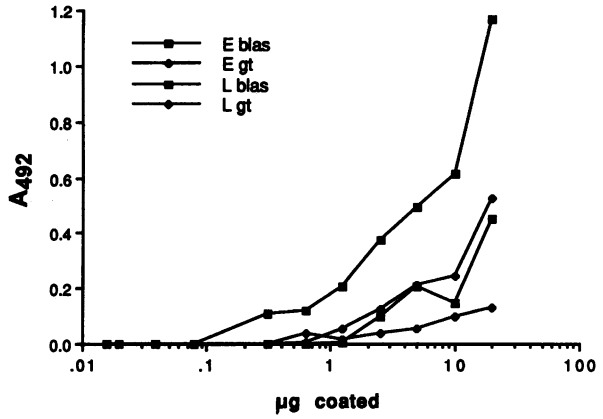

FIG. 2. Binding of extracted cell wall material to immobilized entactin and laminin. βME cell wall extracts from biotinylated yeast cells or biotinylated germ tubes were reacted with immobilized entactin or laminin as described in Materials and Methods. The wells on the plate were coated with increasing amounts of the respective ECM component and incubated with 6 μg of the biotinylated material from either morphological form. Levels of nonspecific binding (binding to plastic in a noncoated well) were subtracted from the experimental values. The data are from a single experiment. E, entactin; L, laminin; blas, blastoconidia; gt, germ tubes.

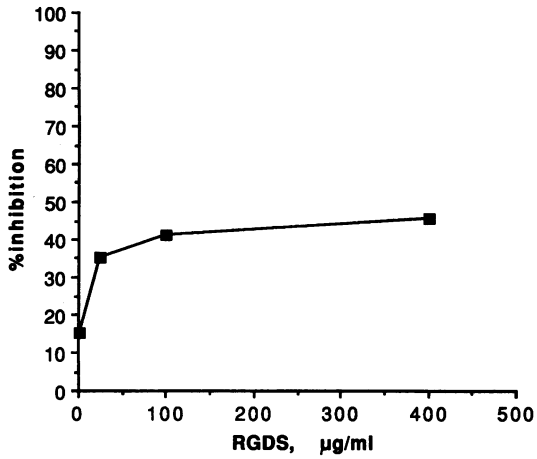

FIG. 4. Effect of RGDS peptide on binding of materials present in the βME extract from biotinylated blastoconidia to entactin. Biotinylated extract was incubated with various amounts of RGDS peptide before addition to entactin-coated wells of a microtiter plate as described in Materials and Methods. The data are from a single experiment.

like molecules in *C. albicans* which bind to different domains of the recombinant entactin molecule was eliminated, while the similar experiment with laminin binding suggested form-specific differences.

Effect of preincubation of the fungal cell wall extracts with laminin and fibronectin on binding to entactin. The RGD recognition sequence is found in several ECM proteins (including laminin and fibronectin). This RGD motif has been shown to mediate binding of *C. albicans* both to host tissues and to purified ECM components. Thus, since the binding of entactin to a cell wall component(s) was in part through an RGDS site, it might be possible for the same component to bind other ECM components containing the RGD motif. This possibility was examined by determining the potential for preincubation of the cell wall extracts with laminin or fibronectin to remove the entactin-binding component(s) from the extract. Preincubation of the yeast βME extract with laminin reduced binding to immobilized entactin by about 28%, while preincubation with fibronectin reduced binding by 34%. When the extent of binding of biotinylated extract to laminin or fibronectin was monitored, almost three-times-higher levels of bound material were found for laminin compared with fibronectin. However, the effect of this absorption on subsequent binding to entactin was similar.

Identification of cell wall components with the ability to

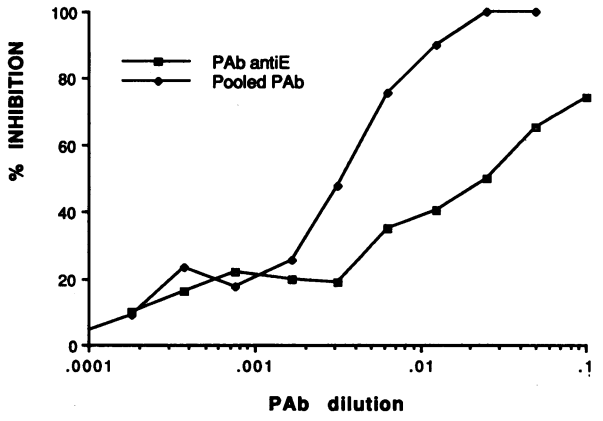

FIG. 3. Effects of antientactin (antiE) antiserum (polyclonal antibody [PAb]) and pooled anti-cell wall antiserum on binding of βME extract from blastoconidia to entactin. Antientactin antibody was preincubated with immobilized entactin prior to addition of βME extract of biotinylated yeast cells as described in Materials and Methods. Pooled antiserum against the *C. albicans* cell wall was preincubated with βME extract of biotinylated yeast cells before addition to entactin. The data are from a single experiment.

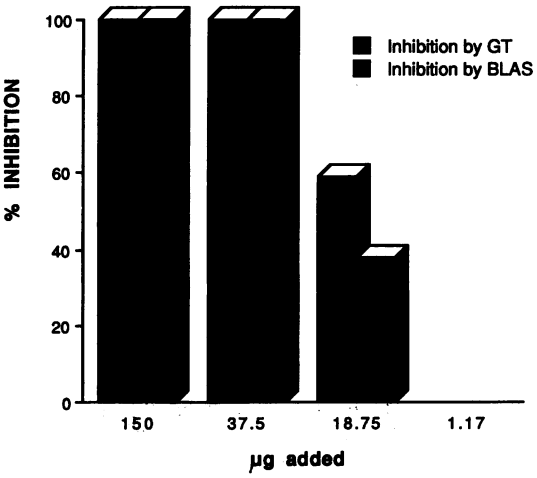

FIG. 5. Competition for binding to entactin between extracts from yeast cells (blastoconidia [BLAS]) and germ tubes (GT). Various amounts of βME extracts from nonbiotinylated yeast cells or germ tubes were incubated in entactin-coated wells of a microtiter plate before addition of βME extract of biotinylated cells of the other growth form, and the subsequent amount of biotinylated material bound was determined. The data are from a single experiment.

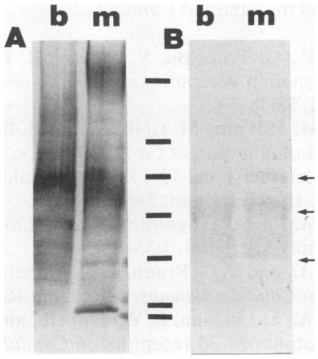

FIG. 6. Identification of the extract component(s) with the ability to bind entactin by ligand affinity blotting. Biotinylated cell wall components released by βME treatment (βME extracts) from yeast cells (lanes b) and germ tubes (lanes m) were separated by SDS-PAGE on 4 to 15% gradient gels and then transferred to nitrocellulose membranes as described in Materials and Methods. The migration of the following molecular mass standards in the same gel is indicated between the panels (from the bottom): 15, 18, 28, 43, 70, 105, and 200 kDa. In panel A the biotinylated proteins were detected with peroxidase-conjugated ExtrAvidin. In panel B the components that bound entactin were determined by sequential incubation with entactin, antientactin antiserum, and peroxidase-conjugated anti-rabbit antiserum. Three components in the cell wall extracts from both *C. albicans* morphological forms with apparent molecular masses of 25, 44, and 65 kDa are indicated (arrows).

bind entactin. Ligand affinity binding to separated components of cell wall extracts was used to identify the component(s) of the extracts able to bind entactin. Biotinylated cell wall components present in the βME extracts from both yeast cells and germ tubes of *C. albicans* were separated by SDS-PAGE under reducing conditions and transferred to nitrocellulose membranes. The distribution of moieties within the extract was detected with peroxidase-conjugated ExtrAvidin. Reactive biotinylated components were distributed from 14 to >500 kDa, as has been previously described (Fig. 6A) (10). Entactin binding to three major components of molecular masses of approximately 25, 45, and 65 kDa which were present in extracts from both growth forms of the fungus was detected (Fig. 6B). This was a specific reaction, as indicated by the absence of bands when the reaction was performed in the absence of entactin with the antientactin antiserum alone (data not shown).

DISCUSSION

Attachment of fungal cells to host cells and tissues is mediated by complementary molecules exposed on both surfaces (5, 6). Among these interactions are those between *C. albicans* and components of the ECM which are postulated to be more important in infection, particularly disseminated disease, than in normal colonization (3, 5, 16, 27, 33, 35, 40, 47). Entactin is a component of the basement membrane, where it plays a number of biological functions in the normal host (14). It has been shown to mediate attachment of epithelial as well as other normal and tumor cells (12, 17, 46, 56). As has been described for binding of some other ligands (11, 30), both yeast cells and germ tubes were able to bind entactin. However, greater fluorescence intensity was consistently detected along the hyphal elements in a pattern of heterogeneous distribution (Fig. 1). This heterogeneous binding pattern is similar to that previously shown for fibrinogen binding (11, 40). However, it is not known whether the components binding entactin and fibrinogen are concentrated at the same or different sites on the surface.

Cell wall materials from both *C. albicans* yeast cells and *C. albicans* germ tubes bound to immobilized entactin in a dose-dependent manner (Fig. 2). Although the proportion of organisms binding entactin in yeast cultures was less than that in germ tube cultures, the binding of biotinylated extracted material was greater with yeast cell wall extracts than that with germ tube cell wall extracts. The source of this seeming difference is not clear, but three possibilities are (i) greater extraction of the binding components from yeast cells than that from germ tubes, (ii) differences in biotinylation of the binding components in the two forms which affect the response in the avidin-based ELISA, and (iii) a combination of these two. As noted below, subsequent characterization of the binding components from the two forms suggested that they were qualitatively similar.

As shown for different types of mammalian cells (12), attachment of *C. albicans* cell wall components to entactin was in part mediated by the integrin recognition RGD peptide, since in our experimental conditions the peptide RGDS could inhibit the binding by approximately 50% (Fig. 4). A single RGD sequence is present in the central domain of entactin (19, 41). RGD-dependent binding of *C. albicans* blastoconidia to a number of ECM components has been previously reported (34, 35). This result suggested that *C. albicans* binding to entactin may be mediated by one of the members of the integrin receptor family. The presence of integrin-like molecules in *C. albicans* has been demonstrated (20, 21, 42). The inability of RGDS peptide to completely inhibit binding suggested that entactin may contain additional binding sites for *C. albicans*. Also, the fact that recombinant entactin lacks the sugar moieties of the original glycoprotein found in its native state opens the possibility that these sugar moieties also contain recognition motifs for *C. albicans*, since molecules with lectin-like properties capable of recognizing sugar moieties on host cells have been described for the surface of the fungus (4–6, 15, 48). The specificity of the interaction between *C. albicans* cell wall components and entactin was further demonstrated by the ability of antientactin antiserum, as well as pooled polyclonal antiserum raised against *C. albicans* cell wall components, to completely or almost completely abolish binding (Fig. 3). Since unlabeled extracts from one form were able to completely inhibit binding of labeled material from the other form (Fig. 5), binding components in each form must compete for the same binding sites on entactin. Thus, it is unlikely that there are morphology-specific receptors that bind to different independent domains of the recombinant entactin. The similar experiment with laminin suggested some morphological specificity in the binding sites on laminin recognized by components in the extract. This is consistent with previous reports suggesting the presence of a form-specific laminin-binding component in yeast cells and germ tubes (3, 40).

The amount of bound biotinylated yeast cell wall extract was greatest for entactin, followed by laminin, and was least to fibronectin. This difference may arise from three sources: the number of binding sites available in a given amount of ECM component, the proportion of cell wall components binding to the ligand, and the extent of biotinylation of the component(s) binding to each ECM ligand. The laminin molecule (composed of three different subunits) with a size of approximately 800 kDa is about five times larger than the 150-kDa entactin, and both ECM components contain a single RGD sequence (19, 41, 45). Fibronectin, with a molecular mass of about 550 kDa, has two polypeptides which contain a cell attachment domain

with the RGD motif (44). Preadsorption of the fungal cell wall extracts in the presence of either laminin or fibronectin resulted in lower levels of attachment to entactin, suggesting that these ECM components may share, to some extent, the same candida receptor-like molecule. However, it seems unlikely that they completely share the same binding site. Different kinds of laminin receptors have been shown to exist in *C. albicans* (3, 40). This observation agrees with what has been shown for mammalian cells, for which the $\alpha_3\beta_1$ integrin has been demonstrated to be the receptor for entactin (17), and the promiscuous nature of this integrin, which has the ability to bind to laminin, fibronectin, and collagen, has also been demonstrated (26, 53). Since cell wall components of *C. albicans* can bind laminin, fibronectin, and entactin at least partially through an RGD-mediated site and compete for binding to a component(s) (34, 35) (Fig. 4), this organism may contain an equivalent of mammalian receptor. In support of this possibility, a moiety reacting with antibody to the C-terminal region of the integrin family β1 chain has been found in the plasma membrane of *C. albicans* (42). As yet, it has not been demonstrated that this moiety forms a complex with a ligand-binding subunit or spans the cell wall to the surface, at which at least some, if not all, of the integrin-like adhesive interactions may occur.

Three components, 25, 44, and 65 kDa, were identified in the βME extract of yeast cells (Fig. 6). Bands of similar sizes were detected in the germ tube extract. These bands may represent different stages of modification of the same entactin-binding protein or different binding proteins that may recognize the same or different sites on entactin. As noted above, the extract of one form was able to compete effectively with the extract of the other form for binding to entactin. Together, these observations suggest that the entactin-binding moieties are the same in both forms. However, the relationship of these molecules to some other receptor-like molecules previously described for *C. albicans* within the same molecular weight range remains unclear.

In conclusion, we have described and studied the characteristics of the interaction between *C. albicans* and entactin, thus expanding the number of components of the ECM with which the organism may interact. As proposed for binding to other components of the ECM, binding to entactin may facilitate adhesion of *C. albicans* to tissue sites and initiation of infection in which ECM may be exposed normally or as a result of disease or insult to the host. Since entactin has the ability to interact with other ECM components, the binding of *C. albicans* to entactin or another ECM component could facilitate binding to other ECM components and increase the security of microbial adhesion at the site of exposed ECM. To our knowledge, this is also the first report suggesting a role for entactin in interaction with nonhost moieties, which adds an additional dimension to the biological functions played by this ECM component.

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