Evidence for $\alpha v\beta 3$ and $\alpha v\beta 5$ Integrin-like Vitronectin (VN) Receptors in Candida albicans and Their Involvement in Yeast Cell Adhesion to VN

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The expression of integrin vitronectin (VN) receptors on Candida albicans yeasts and their involvement in the adhesion to VN were investigated. By immunofluorescence and cytofluorimetric analysis, several antibodies directed against human $\alpha v\beta 3$, $\beta 5$, $\alpha v\beta 3$, or $\alpha v\beta 5$ integrin positively stained C. albicans yeasts. Biochemical analysis on yeast lysates with anti-human $\alpha v$, $\beta 3$, or $\beta 5$ antibody revealed molecular species of 130, 110, 100, and 84 kDa. The 130-kDa band was identified as $\alpha v$, whereas the doublet of 110/100 kDa and the 84-kDa band likely correspond to the $\beta 3$ and $\beta 5$ subunits, respectively. Some 48%–54% of Candida yeasts specifically adhered to VN, and this binding was strongly inhibited by anti-human $\alpha v$, $\beta 3$, $\alpha v\beta 3$, and $\alpha v\beta 5$ antibodies and by RGD- but not RGE-containing peptides. In addition, VN inhibited C. albicans adherence to a human endothelial cell line. Thus, C. albicans in the yeast phase expresses VN receptors antigenically related to the vertebrate $\alpha v\beta 3$ and $\alpha v\beta 5$ integrins, which mediate its adhesion to VN.

Candida albicans is an opportunistic pathogen capable of causing serious diseases in the immunocompromised host [1, 2]. Secondary candidal infection frequently occurs in patients undergoing immunosuppressive therapy for organ transplantation or chemo- or radiotherapy for tumor load reduction [3–5]. In addition, persons with primary immunodeficiencies or AIDS often succumb to persistent candidal infections [6, 7]. The hallmark of disseminated candidiasis is the presence of metastatic sites of infection throughout the body [8]. These sites presumably are established after yeast adherence to the endothelial basement membrane or subendothelial extracellular matrix (ECM). Therefore, adherence to ECM components represents a crucial step in the development of candidiasis. Because C. albicans strains can bind to ECM proteins, and ECM correlates with the rank order of their relative pathogenicity; it is considered to be a significant virulent factor for these fungi [9–11].

Vitronectin (VN), or serum-spreading factor (S-protein), is a multifunctional adhesive glycoprotein found in the circulation and in tissues that include smooth and skeletal muscles and vascular wall and renal tissue [12, 13]. Several lines of evidence suggest that VN provides a unique regulatory link between cell adhesion, humoral immune defense mechanisms, and cell invasion [13]. A role for VN in the microbial invasion is suggested by several experimental observations. Specific interactions of VN with streptococci, staphylococci, and Escherichia coli– and VN-mediated adherence of streptococci to the luminal side of cultured endothelial cells and to epithelial cells have been reported [14–18]. In addition, Pneumocystis carinii–specific binding to VN, which mediates organism attachment to cultured lung epithelial cells, has been observed [19]. In fungi, there is evidence that C. albicans yeast cells specifically bind to soluble VN and that this interaction mediates fungus adherence to the NR8383 macrophage cell line [20, 21]. A band of 70–80 kDa was found by immunoblot analysis with an anti-human VN receptor (VNR) polyclonal antibody in Saccharomyces cerevisiae [22].

Among the molecules involved in ECM cell adhesion, integrins are a superfamily of highly conserved heterodimers composed of noncovalently linked $\alpha$ and $\beta$ subunits that mediate cell-matrix and cell-cell interactions in embryogenesis, hemostasis, wound healing, tumor invasion, immune response, and inflammation [23–25]. At least 4 integrin receptors ($\alpha v\beta 1$, $\alpha v\beta 3$, $\alpha IIb\beta 3$, and $\alpha v\beta 5$) specifically bind to VN. In the $\beta 3$ integrin subfamily, $\alpha v\beta 3$ and $\alpha IIb\beta 3$ (platelet membrane glycoprotein IIb-IIIa) receptors show a large spectrum of ligand recognition (i.e., VN, fibronectin [FN], fibrinogen, von Willebrand factor); $\alpha v\beta 1$ has a more restricted ligand specificity (i.e., FN or VN), and $\alpha v\beta 5$ recognizes only VN [26]. The $\alpha$-containing integrins
bind to their respective ligands through a consensus recognition site, RGD, which is present within the primary amino acid sequence of many adhesive proteins [27].

The integrin receptors are evolutionarily conserved receptors whose expression is not restricted to mammalian cells. Several investigators have found surface proteins in Candida species that are antigenically, structurally, and functionally related to receptors belonging to the integrin superfamily. A specific cross-reactive protein with a size similar to the β1 integrin was detected in C. albicans by immunoblotting with an antibody to the conserved cytoplasmic domain of this subunit [28]. Moreover, an α5β1 integrin receptor that mediates the adhesion of C. albicans and less pathogenic Candida species to FN has been reported [29, 30], and in Candida tropicalis a membrane protein of 125 ± 25 kDa that is antigenically related to the β1 subunit of the vertebrate α5β1 FN receptor has been found [31]. In addition, the expression of a receptor antigenically identical or similar to the mammalian cell iC3b receptor that belongs to the β2 integrin family [32] has been described in C. albicans [33–35]. At least an α chain similar to the human neutrophil αM has been identified both in C. albicans blastocandidia [36] and pseudomyceae [37]. In addition, a gene (αINT1) encoding for a surface protein of 188 kDa (Int1p) that is structurally related to αM and αX chains of the β2 integrin family has been cloned in C. albicans blastospores [38]. Destruction of the INT1 gene in C. albicans suppresses hyphal growth, adhesion to epithelial cells, and virulence in mice, suggesting a relevant role for integrin-related receptors in C. albicans pathogenicity [39].

Although C. albicans binds to VN [20, 21], the molecular mechanisms mediating this adhesive interaction have been poorly elucidated. We undertook these experiments to identify putative integrin-like VNRs in C. albicans yeast cells and to assess their involvement in microorganism adherence to VN.

Methods

Yeast strains and antibodies. C. albicans microorganisms from 5 clinical isolates (CA type 2, 8, 12, 16, 17) identified by the API 20C system were provided by A. Cassone (Istituto Superiore di Sanità, Rome). Yeasts were cultured in Sabouraud’s dextrose agar (Becton Dickinson Microbiology Systems, Cockeysville, MD), frozen at −80°C in small volumes in Sabouraud’s dextrose broth containing 5% glycerol, and subcultured bimonthly in Sabouraud’s dextrose agar. For assays, a loopful of yeast cells was removed from the agar slant, washed twice with cold PBS by centrifugation at 1500 g, and grown to midexponential phase at 24°C in Sabouraud’s broth. Growth curves were determined spectrophotometrically at 420 nm and by simultaneous counts. All isolates were tested for their ability to adhere to VN and were found to be equivalent (data not shown). Therefore, results for a single isolate are presented.

The following mouse monoclonal antibodies (MAbs) were used: anti-human αv, clone LM142 (IgG); anti-human αvβ3, clone LM609 (IgG1); anti-human αvβ5, clone P1F6 (IgG1; Chemicon International, Temecula, CA); anti-human β1, clone 4B4 (IgG1; Coulter Immunology, Hialeah, FL); anti-human β3, clone SZ.21 (IgG1); anti-human αv, clone VNR139 (IgG1; Gibco BRL, Grand Island, NY); anti-human α2, clone P1E6 (IgG1; Telios, San Diego); and anti-human VN, clone E08 (IgG1; Sigma, St. Louis). A MAb to a cell-surface glucomannoprotein (GMP) constituent of C. albicans clone AF1 (IgM) was provided by A. Cassone [40].

The following rabbit polyclonal antibodies were used: anti-human β3 integrin (Chemicon); anti-human VNR (Calbiochem-Novacalbiochem, San Diego); an antisem directed to the carboxy-terminal domain of the human αV integrin (gift of Guido Tarone, University of Turin, Italy) [41]; antibody to a COOH-terminal sequence of the human β5 integrin (Bioline Diagnostic, Turin, Italy); and anti-human VN (Gibco BRL).

We used affinity-purified rabbit anti-mouse IgG (Zymed Laboratories, San Francisco) as a negative control in immunoprecipitation studies and a horseradish peroxidase (HRP)-conjugated goat anti-rabbit antibody (Amersham Life Sciences, Amersham, UK) in immunoblotting. For immunofluorescence studies, we used purified fluorescein isothiocyanate (FITC)-conjugated goat F(ab’)2 fragment of anti-mouse (GaM) and anti-rabbit IgG (GaR; purchased from Cappell-Organon Teknika, Turnhout, Belgium) and purified FITC-conjugated swine F(ab’)2 fragment of anti-rabbit IgG (SaR; Dako, Copenhagen).

Adhesive proteins and peptides. Human plasma FN and VN were purchased from Gibco and Telios Pharmaceuticals, respectively. Substrate preparations were 95% pure by SDS-PAGE. Bovine serum albumin (BSA) was purchased from Sigma. We purchased GRGDSP and GRGESP (Gibco) and GRGD peptides (Bachem Biochimica, Heidelberg, Germany).

Immunofluorescence and flow cytometry. Aliquots of 10⁶ C. albicans yeasts were incubated with 50 μL of the first antibody properly diluted (1:25), for 30 min at 4°C, and then washed twice with 2 mL of cold PBS. Cells were then incubated with 20 μL of the FITC-conjugated secondary antibody (1:40) for 30 min at 4°C, washed twice with 2 mL of cold PBS, and resuspended in 0.5 mL of PBS. Second-step reagents and irrelevant isotype-matched MAb plus GaM or GaR were used as negative controls. Yeast phase was verified microscopically and was not <97%. The cell populations were analyzed for percentages of positive stained cells over 10,000 events, by FACScan cytometer (Becton Dickinson, Mountain View, CA). Fluorescence intensity is expressed in arbitrary units on a logarithmic scale. In some experiments, C. albicans yeast cells were permeabilized as previously described [42], and immunofluorescence and flow cytometry analyses were done by use of a rabbit polyclonal antibody directed to a COOH-terminal sequence of human β5 integrin subunit (Bioline Diagnostic).

In brief, at 37°C we incubated 10⁶/mL C. albicans yeast cells, suspended in PBS without calcium and magnesium (Hyclone-Europe, Erembodegem, Belgium), with 1% saponin from Quillaja Bark (Sigma) and 1% EtOH (Baker, Deventer, The Netherlands), stirred for 30 min, and then washed twice with cold PBS plus 0.1% saponin. SaR was used as second-step reagent, and normal rabbit serum plus SaR or SaR alone was used as a negative control.

C. albicans metabolic labeling and immunoprecipitation. C. albicans yeasts were grown overnight to midexponential phase in YPD broth (dextrose 2%, yeast extract 0.5%, peptone 2% w/vol)
with vigorous shaking, pelleted, washed three times, and resuspended at 10^8/mL in YPD broth and then incubated at 32°C for 20 min. ([35S]methionine (6.6 μL: specific activity >1000 Ci/mmol [15 mCi/mL]; Amersham) was then added to the yeast cells for 3 h at 32°C. Cells were washed three times in PBS and resuspended in 0.4 mL of RIPA buffer (50 mM TRIS, pH 8.0, 150 mM NaCl, 1% Nonidet P-40, 0.5% deoxycholate, 0.1% SDS, 4 mM phenylmethylsulfonil fluoride; Sigma), to which an equal volume of 500-μm nitric acid–washed glass beads (Sigma) was added. Cell suspensions were then vortexed for 2 min and cooled 1 min on ice; the procedure was repeated 8–10 times. Lysates were centrifuged at 10,000 g for 5 min and immunoprecipitated by a modification of standard procedures [43]. In brief, lysates were precleared for 1 h at 4°C with 150 μL of 20% suspension of protein A-Sepharose CL-4B (Pharmacia, Uppsala, Sweden). Precleared lysates were combined with protein A-Sepharose CL-4B preconjugated with rabbit polyclonal antibodies directed against the β3 (Chemicon), a COOH-terminal peptide of the human αv or β5 integrin subunit (Bioline Diagnostic), a rabbit antiserum recognizing the VNR (Calbiochem), or a rabbit anti-mouse IgG (Zymed) and were incubated overnight at 4°C. Immunocomplexes were then washed four times with washing buffer (50 mM Tris, pH 7.4, 0.25% Triton X-100, 0.1% SDS, 150 mM NaCl, 5 mM EDTA-Na2). Bound proteins were released by boiling in reducing buffer (Tris, EDTA, 10% glycerol, β-mercaptoethanol, SDS, 0.05% bromophenol blue) and centrifuged at 10,000 g for 5 min. Samples were analyzed by SDS-PAGE on 7.5% polyacrylamide gels and were fixed, dried, and exposed to XAR-5 film (Eastman Kodak, Rochester, NY) at −80°C.

Western blotting. Immunoprecipitates from C. albicans yeast lysates were electrotransferred overnight onto polyvinylidene difluoride membranes (Immobilon-P, Millipore, Bedford, MA), at 4°C and 20 V, with Trans-Blot Electrophoretic Transfer Cell (BioRad, Richmond, CA). The membrane was then incubated with a blocking solution (5% BSA, 0.05% Tween 20 in PBS) for 60 min at room temperature and washed four times with washing buffer. Thereafter, the membrane was incubated for 2 h at room temperature with one of the following rabbit polyclonal antibodies: anti-human β3 integrin (P1F6) MAb (Chemicon), a rabbit antiserum recognizing the VNR (Calbiochem), or a rabbit anti-mouse IgG (Zymed) and were incubated overnight at 4°C. Immunocomplexes were then washed four times with washing buffer (50 mM Tris, pH 7.4, 0.25% Triton X-100, 0.1% SDS, 150 mM NaCl, 5 mM EDTA-Na2). Bound proteins were released by boiling in reducing buffer (Tris, EDTA, 10% glycerol, β-mercaptoethanol, SDS, 0.05% bromophenol blue) and centrifuged at 10,000 g for 5 min. Samples were analyzed by SDS-PAGE on 7.5% polyacrylamide gels and were fixed, dried, and exposed to XAR-5 film (Eastman Kodak, Rochester, NY) at −80°C.

Adhesion assay to ECM proteins. Stock preparations of human VN, FN, or BSA were diluted in PBS, pH 7.4, at concentrations of 1 μg/mL (VN) and 10 μg/mL (FN and BSA). Protein solution (100 μL) was distributed in 96-well tissue culture flat-bottomed plates (Costar, Cambridge, MA). After overnight incubation at 4°C, coated plates were washed three times with PBS to remove nonimmobilized protein. C. albicans yeasts were labeled with [3H]glucose (NET-807, glucose D-[6-3H] specific activity 1.29 TBq/mmol, 37 MBq, 35.0 Ci/mmol; Amersham) as previously described [29]. In brief, yeast cells (10^9) were labeled with [3H]glucose (20 μCi) in 1 mL of PBS supplemented with 0.5 mM CaCl2 and MgCl2, for 3 h at room temperature. After labeling, microorganisms were washed twice in cold PBS and resuspended at 5 × 10^8/mL in PBS supplemented with CaCl2 and MgCl2.

Cell suspensions (100 μL) of radiolabeled C. albicans yeasts were allowed to adhere to the protein-coated surface and were incubated for 30 min at 37°C in 5% CO2. Optimal cell concentration was used as determined by dose-response experiments. Adhesion was also verified microscopically. The yeast phase was maintained throughout the adhesion assay, and agglutination was not observed. Unattached cells were removed by extensive washing with warm PBS, and adherent cells were harvested by twice adding 100 μL of bleach for 10 min at 37°C. Bound cells and cells from nonadherent cells plus washes were quantitated in a β scintillation counter. Quadruplicate wells were assayed for each sample.

To rule out the contribution of C. albicans binding to plastic [44] or BSA in the adhesion to VN, assays were always performed in the presence of anti-VN MAb (E08) or a rabbit anti-VN antisera, which maximally inhibited cell binding to VN but did not substantially affect that to BSA or FN. Cell adhesion was calculated (in counts per minute) as follows: adherent cells (cpm)/total cells (cpm) × 100, where total cpm indicates the sum of nonadherent cells, washes, and adherent cell cpm. This value routinely exceeded 95% of total cells. Specific binding to VN was calculated as the difference between the total percent adhesion and the percent adhesion not inhibited by anti-VN antibody (nonspecific adhesion).

Inhibition assay. For inhibition of C. albicans adhesion to VN, we did the binding assay after pretreatment of [3H]glucose-radiolabeled C. albicans yeast cells with anti-human αv (LM-142, VNR 139), β3 (SZ.21), αvβ3 (LM 609), or αvβ5 integrin (P1F6) MAB or anti-human β3 integrin subunit polyclonal antibody or with anti-GMP (AF1) or anti-human α2 (PIE6) as control antibodies. All assays were at 4°C for 30 min or with different concentrations of GRGDSP, GRGDS, and GRGESP peptides at room temperature for 1 h. In some experiments, the binding assay was done in the presence of suboptimal concentrations (which only partially inhibited adhesion) of anti-αvβ3 (1:50) and αvβ5 (1:100) MABs alone or in combination.

Adhesion assay to endothelial cells. Human EA.hy 926 endothelial cell line was maintained in Dulbecco’s MEM (DMEM; Gibco) supplemented with 10% heat-inactivated fetal calf serum (Flow Laboratories, Irvine, UK), 2% hypoxanthine-aminopterin-thymidine, 2 mM HEPES, 2 mM L-glutamine, 100 IU/mL penicillin, and 100 μg/mL streptomycin as previously described [45]. For the adhesion assay, cells (3 × 10^4) were grown to confluence for 24 h at 37°C in 5% CO2 in collagen-coated 96-well tissue culture flat-bottomed microtiter plates (Costar). [3H]glucose-labeled yeast cells (5 × 10^5/well) were then added to confluent monolayers in a final volume of 100 μL of DMEM and incubated for 1 h at 37°C. After the incubation, the wells were washed with warm DMEM to remove nonadherent yeasts. Adherent cells were removed with trypsin/EDTA (Flow Laboratories) solubilized in 0.5 M NaOH for 10 min at 37°C. In some experiments, C. albicans yeast cell adherence to endothelial cells was performed in the presence of VN. Quadruplicate wells were assayed for each sample. Percent cell adhesion was calculated as described above.

Statistical analysis. Statistical analysis (Student’s t test) was performed by the STATPAC computer program. P < .01 was used as the significance criterion.
Results

*C. albicans* yeast cell adhesion to VN: dose-response, time course, and cation dependence. The ability of *C. albicans* yeast phase to bind to immobilized VN was evaluated by incubation of 3H glucose-labeled microorganisms on VN-coated plates for 30 min at 37°C. As shown in figure 1A, optimal adhesion to VN was observed at 1 μg/mL, which was therefore chosen for all subsequent experiments. The ability of *C. albicans* yeast phase to adhere to immobilized VN as a function of time was also investigated and was maximal at 30 min (figure 1B).

Because integrin-mediated binding to ECM components is dependent on divalent cations [32, 46], we tested the effect of different amounts of Mg2⁺ and Ca2⁺ on *C. albicans* yeast adhesion to VN (figure 2). Addition of MgCl₂ and CaCl₂ to the assay increased yeast cell adhesion to VN; Mg2⁺ was more effective than Ca2⁺. Pretreatment of *C. albicans* yeast cells with the chelant agent EDTA completely inhibited the cation-dependent increase (figure 2, insert).

*C. albicans* yeasts specifically bind to VN. To determine whether *C. albicans* yeast cells specifically bind to VN, their ability to adhere to VN-coated plates in the presence of an anti-VN antibody was analyzed. An anti-human plasma VN polyclonal antibody strongly (85%) inhibited *C. albicans* adhesion to VN in a dose-dependent manner. The anti-VN antiserum did not substantially affect the adhesion to FN- or BSA-coated plates (figure 3). Similar results were obtained by use of anti-human VN (E08) MAb (data not shown). Overall, these results indicate that *C. albicans* yeasts specifically adhere to VN.

Expression of VNRs antigenically related to αvβ3 and αvβ5 integrins by *C. albicans* yeast cells. The molecular basis for the binding of *C. albicans* to VN [20, 21] has not been determined. Therefore, we investigated the expression of VN integrin receptors on this organism. *C. albicans* yeasts were stained with a panel of MAbs directed against αv, β3, αvβ3, or αvβ5 or...
with rabbit polyclonal antibodies directed against human β3 or a COOH-terminal peptide of the human β5 integrin subunit and were analyzed by immunofluorescence and flow cytometry. As shown in figure 4, anti-αv, anti-β3, anti-αvβ3, and αvβ5 MAbs as well as the anti-β3 and anti-β5 antisera reacted with yeast cells. As a control for phase-specific antigen expression, we used a MAb directed against a cell-surface glucosamino-protein constituent that positively stains yeast cells [40] and an anti-human β1 integrin MAb that binds to germ tubes but not to yeast cells [29]. Overall, these results suggested that C. albicans on yeast phase expresses VNRs with antigenic similarity to the vertebrate αvβ3 and αvβ5 integrins.

Biochemical characterization of αvβ3 and αvβ5 integrin-like VNRs expressed on C. albicans yeast cells. Immunoprecipitation studies were performed on 35S-labeled microorganism lysates with rabbit polyclonal antibodies directed against the carboxy-terminal domain of the human αv or β5 or against the β3 integrin subunit (figure 5A). Both anti-αv and anti-β3 antisera immunoprecipitated the same set of proteins migrating at 130, 110, or 100 kDa under reducing conditions. The higher band (130 kDa) has a molecular mass consistent with the reduced form (127 kDa) of the human αv integrin subunit, whereas the doublet of 110/100 kDa approximates the mass of the reduced form (105 kDa) of the β3 integrin subunit [47].

Anti-β5 immunoprecipitates contained a major species migrating at 84 kDa and two less intense bands migrating at 130 and 100 kDa, which were also present in anti-αv immunoprecipitates. The 84-kDa protein approximates the molecular weight of the human β5 integrin subunit, which migrates at ~95 kDa when reduced [48, 49]. Immunoprecipitation with rabbit anti-mouse immunoglobulin did not reveal any equivalent band. Anti-human αv, β3, or β5 immunoprecipitates were also probed with the same antibodies, by Western blot analysis (figure 5B).

Immunoblotting with anti-αv antisera detected a band of 130 kDa in all immunoprecipitates, likely corresponding to the αv integrin subunit, and cross-reacts with a band migrating at ~100 kDa in anti-αv immunoprecipitates whose identity is unclear. Blotting with the anti-β3 polyclonal antibody identified the β3 subunit as a 95-kDa band in the anti-β3 immunoprecipitates and a band migrating at ~100 kDa in anti-β3 immunoprecipitates, both likely corresponding to the β3 integrin subunit. The anti-β5 antisera revealed an 84-kDa band in anti-β5 immunoprecipitates and was reactive with a protein migrating with the same electrophoretic mobility in anti-αv immunoprecipitates, likely corresponding to the β5 integrin subunit. In addition, this antisera cross-reacts with a 100-kDa band present in all immunoprecipitates and with the two bands migrating at 95- and 66-kDa in the anti-β3 immunoprecipitate. This cross-reactivity was consistently seen with different antisera and experimental conditions and may be attributable to the use of polyclonal antibodies. (Available MAbs do not work in immunoprecipitation or Western blot analysis of C. albicans lysates.) We also performed immunoprecipitation and Western blot analyses with a rabbit anti-human VNR, which recognizes both human αvβ3/β5 VNRs [50], and we found 2 major bands migrating at 130 and 110 kDa and a broader band of 100 ± 10 kDa (figure 6). Together these results indicate that heterodimers of 130–110/100 and 130/84 kDa, likely corresponding to αvβ3 and αvβ5 integrin VNRs, respectively, expressed on C. albicans yeast cells.

C. albicans yeast cell adhesion to VN is mediated by αvβ3 and αvβ5 integrin-like VNRs. The involvement of αvβ3 and αvβ5 integrin-like VNRs in C. albicans adhesion to VN was investigated by testing the ability of different antibodies directed against αv, β3, or αvβ3, or αvβ5 integrin subunits to inhibit yeast binding to VN (figure 7A). Treatment of C. albicans yeasts with different doses of anti-αv, anti-β3, or anti-αvβ3, or anti-αvβ5 MAbs or a rabbit anti-human β3 polyclonal antibody blocked adhesion of the microorganism to VN in a dose-dependent manner. No inhibition was observed with the anti-GMP or isotype-matched anti-human α2 integrin subunit MAb used as control (data not shown).

To further investigate the role of αvβ3 and αvβ5 integrin-like VNRs in C. albicans yeast cell adhesion to VN, yeasts were allowed to react with a combination of suboptimal concentrations of anti-αvβ3 and anti-αvβ5 MAbs (figure 7B). Both αvβ3
Figure 4. *Candida albicans* yeast cells express vitronectin receptors (VNRs) antigenically related to αvβ3 and αvβ5 integrins. Reactivity of monoclonal antibodies directed against human αv (VNR 139; LM 142), β3 (SZ.21), αvβ3 (LM 609), αvβ5 (P1F6), β1 (4B4) integrin, or anti-glucomannoprotein (AF1) and of polyclonal antibodies recognizing human β3 (A) or COOH-terminal sequence of human β5 (B) with *C. albicans* yeast cells was evaluated by immunofluorescence and flow cytometry. Permeabilized yeasts analyzed with anti-β5 antibody or normal rabbit serum. Dotted areas indicate negative controls. Results are representative of 1 of 5 separate experiments. FITC, fluorescein isothiocyanate.
and αvβ5 contributed to _C. albicans_ yeast cell adhesion to VN, since anti-αvβ3 or anti-αvβ5 alone only partially inhibited cell adhesion to VN, whereas complete inhibition was observed when both MAbs were used simultaneously in the assay. The anti-GMP control MAb failed to inhibit cell adhesion and did not increase the inhibition observed with either anti-integrin MAb.

**Inhibition of _C. albicans_ yeast cell adhesion to VN by RGD-containing peptides.** Studies of mammalian cells show that both αvβ3 and αvβ5 recognize the RGD binding site present in the central region of the VN molecule [26, 27]. Therefore, we performed adhesion assays in the presence of different RGD-containing peptides (figure 8). Pretreatment of _C. albicans_ yeast cells with different doses of GRGDSP and GRGDS peptides markedly inhibited their adhesion to VN in a dose-dependent manner. GRGDSP was more effective than GRGDS peptide at lower doses. No inhibition was observed in the presence of different doses of GRGESP control peptide.

**VN inhibits _C. albicans_ adherence to human endothelial cells.** Several lines of evidence indicate that _C. albicans_ binds to endothelial cells, and the possible involvement of the RGD sequence in this interaction has been suggested [11, 51–56]. In addition, the presence of VN on vascular cell walls has been detected [13]. Therefore, the role of VN in the _C. albicans_ adherence to endothelial cells was evaluated. As a source of endothelial cells, we used the human EA.hy 926 cell line, which is positively stained by the anti-VN E08 MAb, as assessed by flow cytometry (data not shown). Of the ^3H-labeled _C. albicans_ yeast cells, 30%–38% adhered to EA.hy 926 cells, and this ad-
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Inhibition of C. albicans yeast cell adhesion to vitronectin (VN) by RGD-containing peptides. 3H glucose-labeled C. albicans yeast cells were pretreated 1 h at room temperature with different concentrations of GRGDSP, GRGDS, or GRGESP synthetic peptides and were tested for binding to VN (1 μg/mL). % cell adhesion determined after 30 min incubation at 37°C. % specific adhesion to VN was . Results are mean % of inhibition of specific binding of 4 separate experiments. Statistical analysis compared specific binding to VN in presence of GRGDSP with that in presence of either MAb alone (Student’s t test, *).

Discussion

Despite the increasing incidence of candidosis in immunocompromised hosts, little is known about virulence factors such as cell adhesion molecules involved in C. albicans invasion and colonization [57]. Adhesion of C. albicans to ECM components represents a crucial step in the development of candidiasis. In previous studies [20, 21], C. albicans bound to soluble VN in a concentration-dependent manner with 9.8 × 10^4 affinity binding sites per organism and a kDa of 3.5 × 10^-7 M [21]. However, the receptor(s) involved in C. albicans-adhesive interactions with VN remain elusive.

The present study provides the first evidence that C. albicans during yeast phase expresses VNRs that share antigenic homology with the αvβ3 and αvβ5 vertebrate integrins and mediate yeast cell adhesion to VN. Moreover, we found that VN is involved in the interaction of C. albicans with human endothelial cells. αvβ3 and αvβ5 integrin-like VNRs were identified by immunofluorescence, flow cytometry, and biochemical analysis. Cytoskeletal analysis indicates that certain antibodies that recognize human αv, β3, β5, αvβ3, and αvβ5 heterodimers specifically bind to C. albicans yeasts.

Immunoprecipitation and Western blot analyses with anti-human αv, β3, or β5 polyclonal antibodies yielded major bands.
migrating at 130, 110/100, and 84 kDa under reducing conditions. The 130-kDa band was identified as αv, whereas the doublet of 110/100 kDa and the 84-kDa band likely correspond to the β3 and β5 integrin subunits, respectively. These results indicate that, in C. albicans, the αv subunit can associate with β3 and β5 to form αvβ3 and αvβ5 integrin-like receptors with molecular masses of 130/110–100 and 130/84 kDa, respectively. The mass of the αvβ3 receptor is consistent with that of the vertebrate counterpart (127/105 kDa) [47–49]. Conversely, the β5 subunit expressed in C. albicans shows a slightly lower molecular mass than that described for the vertebrate homologue (95 kDa). Proteolytic degradation, the precursor form of the β chain, different glycosylation states, and/or phylogenetic differences may account for the differences in the molecular masses observed for mammalian and fungal receptors.

The αvβ3 and αvβ5 integrin-like VNRs on C. albicans yeast cells are functional and capable of mediating adhesion to immobilized VN. Indeed, 48%–54% of C. albicans yeasts adhered to VN, and this adhesion was specifically blocked by functional MAbs directed against αv, β3, αvβ3, αvβ5, or an anti-β3 polyclonal antibody, indicating that both αvβ3 and αvβ5 integrin-like receptors mediate microorganism adhesion to VN. We also demonstrated the simultaneous involvement of these VNRs in the interaction of C. albicans with VN by the combined use of MAbs directed against the αvβ3 or αvβ5 heterodimers at sub-optimal concentrations in the adhesion assay. The functional role of αvβ3 or αvβ5 integrin-like receptors was further shown by the ability of different peptides containing RGD [27], the specific amino acid sequence recognized on the VN molecule by both αvβ3 and αvβ5 integrin receptors, to markedly inhibit C. albicans yeast cell adhesion to VN. These results are consistent with previous reports showing that preincubation of C. albicans with the synthetic GRGDS (1 mg/mL) peptide causes decreased binding to soluble VN [21].

A distinct role of αvβ3 and αvβ5 integrin VNRs in cell attachment, spread, and migration of mammalian cells has been reported [58]. Cell spread and migration response on VN is mainly mediated by αvβ3, whereas αvβ5 is preferentially involved in cell adhesion. It is unclear whether αvβ3 and αvβ5 integrin-like receptors on C. albicans mediate distinct functions. Preliminary results indicate that their expression is differently regulated during germ-tube transition (data not shown). As C. albicans may exist as budding yeast or filamentous hyphae, depending on environmental conditions, the differential expression of distinct VNRs on C. albicans phases may represent an adaptive response to VN-containing environments.

Of interest, the peculiar distribution of VN in the circulation or as ECM-bound protein in different tissues overlaps C. albicans tissue dissemination [2], thus supporting the hypothesis that the expression of αvβ3 and αvβ5 integrin-like VNRs may be particularly relevant to the microorganism host invasion. C. albicans binds to endothelial cells, and the possible involvement of RGD tripeptide in this interaction has been suggested [11, 51–56]. In addition, in vivo experiments show that an RGD-containing peptide (pepTite-2000) affects the distribution and the fate of intravenously administered C. albicans yeast cells [8]. Nevertheless, the RGD-binding receptors and the RGD-containing adhesive proteins that mediate the binding of fungus to the endothelium remain undefined. Our study indicates that VN may be one of the adhesion molecules recognized by C. albicans on the endothelial cell surface. The possibility that iC3b may also display a similar function has been suggested [54].

C. albicans is frequently involved in infections of intravascular and intraperitoneal catheters [59] or biliary stents [60]. VN is adsorbed to polymeric biomaterials to a much higher degree than FN or human serum albumin, suggesting that C. albicans may colonize polymeric biomaterials by binding to VN.

Adherence of C. albicans to phagocytic cells has been described [61, 62]. VN through interaction of its heparin-binding domain with the fungal β-glucan enhances C. albicans binding to a macrophage cell line [20] and stimulates tumor necrosis factor–α release by macrophages [63]. The presence of integrin-like VNRs on C. albicans suggests that VN-mediated interaction between fungus and phagocytes may also occur via an RGD-mediated pathway.

Taken together, our results indicate an important role for αvβ3 and αvβ5 integrin-like receptors as primary mediators of.
C. albicans yeast phase adhesion to VN. We found direct involvement of VN in C. albicans interaction with human endothelial cells. Inhibition of integrin-mediated fungus adherence to vasculature may be critical to prevention of the dissemination of C. albicans infections [11]. The understanding of the mechanisms mediating C. albicans adherence to host structures could be of potential benefit in the development of new antifungal therapies.

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References