Molecular Mimicry in *Candida albicans*

Role of an Integrin Analogue in Adhesion of the Yeast to Human Endothelium

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Abstract

Hematogenous infection with the yeast *Candida albicans* now occurs with increasing frequency in the neonate, the immunocompromised patient, and the hyperglycemic or hyperalimented host. Yeast-phase *C. albicans* expresses a protein that is antigenically and structurally related to CD11b/CD18, a member of the β_2 integrins and a well-characterized adhesin for mammalian neutrophils. Both the neutrophil protein and its analogue in *C. albicans* have an identical affinity for the C3 ligand iC3b, and both proteins are significantly increased in expression at 37°C. Given these several similarities, we therefore studied the role of the integrin analogue on *C. albicans* in the adhesion of the yeast to human umbilical vein endothelium (HUVE).

After growth of *C. albicans* in 20 mM D-glucose, as opposed to 20 mM L-glutamate, flow cytometric analysis with monoclonal antibodies recognizing the α-subunit of CD11b/CD18 demonstrated a 25.0% increase in mean channel fluorescence (range 18.4–31.8%), as well as an increased percentage of yeasts fluorescing ($P < 0.02$). This increased intensity of fluorescence, which corresponds to increased expression of the integrin analogue, also correlated with a significant increase of 30–80% in adhesion of glucose-grown *C. albicans* to HUVE ($P < 0.02$). Blockade of the integrin analogue on *C. albicans* by monoclonal antibodies recognizing adhesive epitopes on neutrophil CD11b/CD18 inhibited glucose-enhanced adhesion of *C. albicans* to HUVE. Incubation of glucose-grown *C. albicans* with saturating concentrations of purified human iC3b, the ligand for CD11b/CD18, reduced adhesion of the yeast to HUVE by 49.7%, whereas BSA in equimolar concentration had no effect ($P < 0.001$). These results identify a glucose-responsive integrin analogue on *C. albicans* as one of possibly several cellular structures that mediate adhesion of the yeast to human endothelium. (*J. Clin. Invest.* 1991. 87:1896–1902.) Key words: *Candida albicans* • integrin • adhesion

Introduction

The yeast *Candida albicans*, a normal inhabitant of the human gastrointestinal tract, has emerged as a leading pathogen in the neonate, the immunocompromised patient, and the hyperglycemic or hyperalimented host (1–3). Invasive disease usually arises from endogenous sources (4, 5); in animal models of invasive disease, pathogenicity of candidal strains in vivo correlates directly with yeast adhesion in vitro (6). To understand whether the ability of the yeast to adhere to host tissues or to components of extracellular matrix is fundamental to the pathogenesis of invasive candidal infection, the mechanisms of this process must be further clarified.

Several investigations have examined factors in the yeast and in the host that influence adhesion of *C. albicans* (for review see reference 7). All studies concur that viable *C. albicans* adhere more actively than do heat- or ultraviolet-killed yeasts (8–11). Transformation from the yeast phase to the mycelial phase is also correlated with increased adhesion (7, 10). Elevated concentrations of glucose in saliva or vaginal fluids dramatically increase the ability of candida to adhere or to replicate actively at local tissue sites (12, 13); indeed, in one study, increased concentrations of salivary glucose in diabetic patients were the single most important influence upon candidal replication (12).

Current information indicates that adhesion moieties on the yeast are expressed on the cell surface (8–11, 14–15), are most likely glycoproteins because of their sensitivity to proteolytic enzymes and reducing agents (8–11), and typically contain some mannan (14, 16). Although the use of nonstandardized matrices such as swabbed specimens from oral or vaginal mucosa has led to widely varying results, the development of reproducible assays that quantitate adhesion of yeast to well-characterized cell lines is an important first step in defining these mechanisms (17).

We have recently described a surface protein on yeast-phase *C. albicans* that shares antigenic and structural homology with the α-subunit of the leukocyte adhesion glycoprotein CD11b/CD18, also known as Mo1, Mac-1, the iC3b receptor, or complement receptor type 3 (CR3); CD11b/CD18 is found on mammalian neutrophils, monocytes, and macrophages (18–21). MAbs that recognize the α subunit of CD11b/CD18 bind specifically to yeast- or hyphal-phase *C. albicans*, while isotype controls do not (18–19, 22). Increases in ambient temperature to 37°C result in increased expression of CD11b/CD18 in the neutrophil and of its analogue in *C. albicans* (18, 23–24); in the yeast, this temperature-dependent change is also accompanied by hyphal transformation. In addition, C3 fragment iC3b, which serves as the ligand for the neutrophil receptor, binds saturably and reversibly to *C. albicans* with a K_a virtually identical to that established for its interaction with CD11b/CD18 on the neutrophil (18, 25); thus, the iC3b-binding protein in *C. albicans* can be classified as a true receptor.

CD11b/CD18, the iC3b receptor in the neutrophil, consists of a β subunit of M_r 95 kD, common to all members of the leukocyte adhesion glycoprotein family, and a specific α subunit of M_r 165 kD (20). Immunoprecipitation or Western blotting of cellular extracts of *C. albicans* with MAbs recognizing

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the α-subunit of CD11b/CD18 detects a single band of M_r 130–165 kD (19, 24). In humans, genetic absence of the β subunit of this protein in neutrophils and monocytes results in an adhesion defect that impairs chemotactic and phagocytic functions of the neutrophil and predisposes these patients to recurrent infections (21, 26). Because of the structural and antigenic similarities between the candidal protein and CD11b/CD18, we assessed the role of the candidal protein in the adhesion of the yeast to cultured human endothelium.

Methods

Isolation and labeling of Candida albicans. Strains of *Candida albicans* used in these experiments were isolated in pure culture from blood or urine of infected patients and were identified in the Clinical Microbiology Laboratory of the University of Minnesota Hospital by morphology, germ tube expression, and multiple assimilation substrates on a yeast biochemical card (Vitek Automicrobic System, Cleveland, OH). Yeasts were grown to exponential phase in Sabouraud's broth (Difco Laboratories, Inc., Detroit, MI) as determined by colony counts and spectrophotometric measurement of OD at 420 nm and were then aliquoted and frozen at −70°C until use.

Before each experiment, an aliquot of *C. albicans* was grown overnight at 24°C in a defined medium (27) containing albumin, amino acids, and the indicated carbon source (20 mM D-glucose or 20 mM L-glutamate). The following morning, yeast cells were pelleted and washed, and 50 μl of the pellet was inoculated into 5 ml of defined medium in which the methionine concentration had been reduced from 1.2 mM to 8.7 μM. 3 μl of [^{35}S]methionine (Amersham Corp., Arlington Heights, IL; sp act > 800 Ci/mmol) were then added, and the organisms were grown to exponential phase (~ 4 h at 24°C). Organisms were washed extensively to remove free label and then diluted to an absorbance of 0.5 at 420 nm, which corresponded to a concentration of 5×10^7 organisms/ml.

Fluorescent microscopy and flow cytometry. Methods used to quantitate the expression of the iC3b receptor (integrin analogue) on *C. albicans* have been previously described by our laboratory (18, 19). Briefly, 100 μl of yeast-phase organisms at 5×10^7 CFU/ml were incubated in suspension with MAbs that recognize α chain epitopes of CD11b/CD18 on human neutrophils. Anti-Mo1-94, an IgM MAb obtained from Coulter Immunology, Hialeah, FL, was used over a range of concentrations from 200 to 1,200 μg/ml, as specified. Three separate lots were employed in the experiments reported in this paper. OKM1, an IgG_{2b} MAb, was purchased as a hybridoma from the American Type Culture Collection, Rockville, MD (ATCC CRL 8026) and was purified from supernatant by affinity chromatography on Protein A Sepharose CL4B (Pharmacia, Inc., Piscataway, NJ). SDS-PAGE under reducing conditions verified the presence of heavy and light chains at 50 kD and 25 kD, respectively. OKM1 was used in flow cytometric analyses over a range of concentrations from 1,200 to 6,500 μg/ml. MAb 44 (IgG2a) and MAb 17 (IgM), the kind gifts of Dr. Robert Todd (University of Michigan, Ann Arbor, MI), were used at the recommended dilution of 1:50 unless otherwise specified; their concentrations ranged from 2 to 4 mg of immunoglobulin/ml. The final vol of incubation was 50 μl. We have previously established that the yeast form of *C. albicans* does not exhibit specific fluorescence on flow cytometry after incubation with MAb directed against complement receptors type 1 (CR1) or type 2 (CR2); moreover, isotype controls such as mouse IgM (Coulter) do not bind specifically to *C. albicans*, as assessed by flow cytometry or fluorescent microscopy (18, 19, 22).

After a 30-min incubation on ice with murine MAb, yeasts were centrifuged and washed three times with PBS/0.02% azide. Yeasts were then incubated on ice for 30 min with a 1:20 dilution of goat $F(ab')_2$ antibody to mouse IgM/IgG conjugated to fluorescein isothiocyanate (Tago Inc., Burlingame, CA). For fluorescent microscopy, 2×10^4 CFU were incubated in suspension with anti-Mo1-94 or mouse IgM as described above and adhered to microscope slides coated with 0.6% agarose; phase contrast and immunofluorescent microscopy were performed on a fluorescent microscope (Laborlux 12; E. Leitz, Inc., Rockleigh, NJ) at ×400 (Fig. 1).

Flow cytometry was performed on a FACS® IV (Becton-Dickinson Immunocytometry Systems, Mountain View, CA) as previously described (18, 19). For experiments with *C. albicans*, an argon laser (488 nm) was used with a DF 530/30 filter; forward and right angle (90°) light scatter was used to scale for size in the fluorescent population. The machine was calibrated linearly with yellow-green fluorescinated carboxylated microspheres of 1.33 μm in diameter (Polysciences, Inc., Warrington, PA); coefficient of variation (background fluorescence) for fluoresceinated microspheres was < 3%. For experimental samples, linear amplifiers were charted channel by channel for 256 channels. Fluorescence of control samples, in which the primary antibody was omitted, was then subtracted channel by channel to give the value designated as specific fluorescence. A positive yeast cell exhibited an increase in fluorescence intensity above autofluorescence, while the signal from a negative cell was no greater than that of the autofluorescent control. For quantitation, we have reported both the mean channel fluorescence and the percent of each sample of 20,000 organisms that exhibited specific fluorescence for the desired MAb, after subtraction of nonspecific autofluorescence exhibited in the absence of the primary antibody.

Endothelial cell cultures. Human umbilical vein endothelial cells (HUVE)[1] were harvested from umbilical cords by collagenase digestion as previously described (28) and exhibited factor VIII antigen and Weibel-Palade bodies. Primary endothelial cells were plated in 24-well plates (Costar Data Packaging Corp., Cambridge, MA), grown to confluent monolayers in Medium 199 with Ryan's growth supplement (Life Technologies, Inc., Grand Island, NY), and used in the adhesion assay.

Adhesion assay. C. albicans grown in the presence of 20 mM D-glucose or equimolar L-glutamate and labeled with [^{35}S]methionine were washed extensively in PBS and resuspended in HBSS with calcium and magnesium, to which was added 1% BSA ($HBSS^{++}$/BSA). Yeast cells were then held at 4°C to prevent germination before incubation with HUVE. 5×10^5 CFU were added to confluent monolayers of HUVE in a 24-well plate in a final vol of 250 μl and incubated for 1 h at 37°C. Following the incubation period the wells were washed three times with $HBSS^{++}$/BSA to remove nonadherent yeasts; these were counted in a scintillation counter (LS230; Beckman Instruments, Inc., Palo Alto, CA). Adherent cells were removed with a commercial preparation of trypsin/EDTA (Life Technologies, Inc.), solubilized in 0.5 M NaOH, and counted. Loss of yeast cells was < 10% of total cpm. Total adhesion was calculated from the following formula:

$$\left[\frac{cpm_{(\text{adherent yeasts})}}{cpm_{(\text{total yeasts added})}}\right] \times 100.$$

Specific adhesion was calculated as the difference between total adhesion and nonspecific adhesion, the latter measured in the presence of 100-fold excess unlabeled *C. albicans*. Adhesion was also verified microscopically; yeast phase was maintained throughout, and agglutination was not observed, either by direct vision or by microscopy.

Inhibition of adhesion with MAb. C. albicans were grown in the defined medium plus 20 mM D-glucose as described above and were then incubated in suspension with 300 μg/ml anti-Mo1-94, 300 μg/ml MAb 44 (IgG_{2a}), or 300 μg/ml MAb 17 (IgM) for 30 min at 4°. Yeasts were then pelleted and used in the adhesion assay as described above.

Purification of iC3b. iC3b was isolated after proteolysis of purified human C3 according to our published methods (25). Briefly, human C3 was isolated from fresh plasma after affinity chromatography on Sepharose L-lysine, ion exchange chromatography on DEAE-Sephacel, and sizing chromatography on Sepharose CL-6B, according to the method of Tack et al. (29). Possible trace contaminants, C5, IgG, or

1. *Abbreviation used in this paper:* HUVE, human umbilical vein endothelium.

IgA, were removed by passage of purified C3 over CNBr-Sepharose 4B (Pharmacia) that had been coupled to antibodies to C5, IgG, and IgA.

Antibodies to human complement proteins H and I were isolated from goat antisera, the kind gift of Dr. Brian F. Tack (Scripps Clinic, LaJolla, CA), after caprylic acid precipitation (30) and coupled to CNBr Sepharose 4B (Pharmacia) for use in affinity purification of Factors H and I from fresh human serum. Factors H and I were eluted from their respective affinity columns with 3 M KSCN/150 mM NaCl/100 mM Tris, pH 8.0. Purified Factor H yielded a single band of 150 kD on 10% SDS-PAGE under reducing conditions; Factor I yielded a single band of 105 kD.

After cleavage of C3 to C3b by 0.15% wt/wt L-1-tosylamide-2-phenylethyl chloromethyl ketone trypsin (Millipore Continental Water Systems, Bedford, MA) at 37°C, the reaction was halted by a 20-fold molar excess of soybean trypsin inhibitor (Worthington Biochemical Corp., Freehold, NJ). After precipitation with 15% wt/vol polyethylene glycol (Sigma Chemical Co., St. Louis, MO), the protein was then resuspended in 0.05 M Na acetate/1.0 M NaCl, pH 5.6, and chromatographed on a 2.5 × 100-cm Sephacryl S-300 column (Pharmacia). Fractions containing monomeric C3b by SDS-PAGE analysis were pooled, concentrated by ultrafiltration, and dialyzed into PBS at 4°C. Monomeric C3b (2.0 mg/ml) was then incubated for 18 h at 37°C with 50 μg/ml Factor H and 20 μg/ml Factor I in PBS that had been made 1 mM in PMSF and magnesium acetate (Sigma). H and I were removed by passage over their respective affinity columns, and iC3b was concentrated and dialyzed against PBS. Neither uncleaved C3b nor oligomeric forms of C3b or iC3b were detected on analysis by SDS-PAGE, which demonstrated three bands of 75 kD, 67 kD, and 39 kD under reducing conditions.

Inhibition of adhesion with iC3b. Purified human iC3b in a concentration of 1.5 mg/ml was incubated on a rotator with 2.5×10^6 [^{35}S]-methionine-labeled, glucose-grown *C. albicans* for 45 minutes at 37°C in 50 μl PBS; 1.0×10^7 unlabeled yeast cells of the test strain were incubated with 6.0 mg/ml of purified iC3b in 50 μl PBS under the identical conditions. These concentrations of iC3b are at least 100-fold in excess of that required to saturate 2×10^6 receptors. As a control, labeled and unlabeled *C. albicans* were incubated with BSA (Sigma) in equimolar concentration under identical conditions. iC3b- and BSA-treated yeast cells were then used in the adhesion assay as described above, except for the following modifications: 2.5×10^4 [^{35}S]methionine-labeled organisms in 50 μl HBSS^{++}/1% BSA were incubated with HUVE monolayers in the presence and absence of 2.5×10^6 unlabeled organisms. Final vol for incubation was 200 μl. Duration of the incubation and experimental conditions of temperature, washing, and release were identical to those described above in the adhesion assay.

Statistics. Results, which are expressed as mean±standard error unless otherwise noted, were analyzed using the Student's *t* test for paired samples when comparing expression of the iC3b receptor and adhesion of glucose- and glutamate-grown organisms; the Student's *t* test for unpaired samples was used for inhibition studies. $P < 0.05$ was considered significant.

Results

Fluorescent microscopy of C. albicans. Fig. 1 displays a population of yeast cells in phase contrast microscopy (*A*) and after incubation with anti-Mo1-94 (*B*); fluorescence is uniform, circumferential, and consistent with a surface-borne antigen. Yeasts of the identical strain (*C*) do not exhibit immunofluorescence after incubation with the isotype control mouse IgM (*D*).

Expression of the integrin analogue after growth of C. albicans in the presence of glucose or glutamate. The use of increasing concentrations of MAb anti-Mo1-94 resulted in a linear

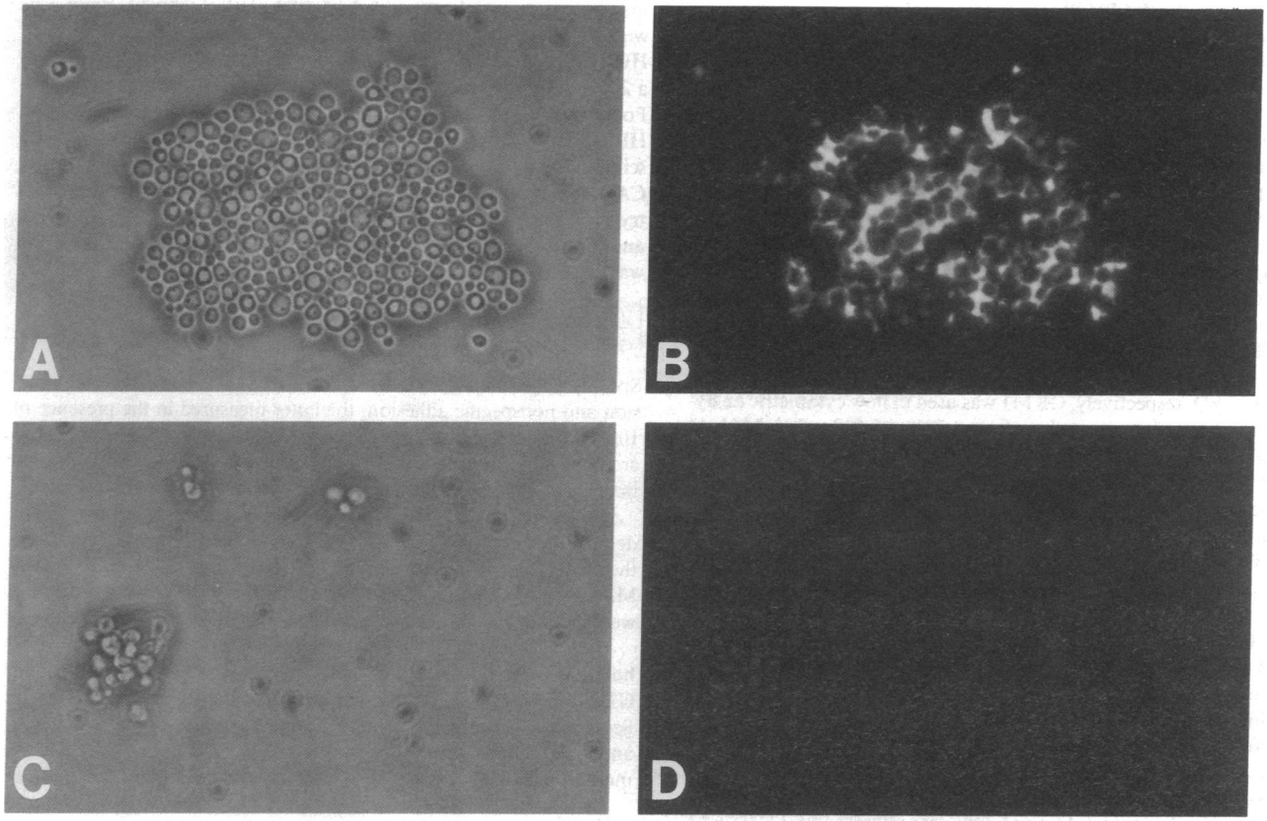

Figure 1. Phase contrast (*A, C*) and immunofluorescence microscopy (*B, D*) of a blood isolate of *C. albicans* after incubation with anti-Mo1-94 (*B*) or the isotype control MsIgM (*D*).

Table I. Relationship of Monoclonal Antibody Concentration to Percent Yeasts Fluorescing

Concentration	Percent yeasts fluorescing	
	Anti-Mo1-94 (Lot 1)	Anti-Mo1-94 (Lot 2)
200 μg/ml	13.2%	24.1%
600 μg/ml	43.5%	71.9%
1000 μg/ml	52.3%	ND

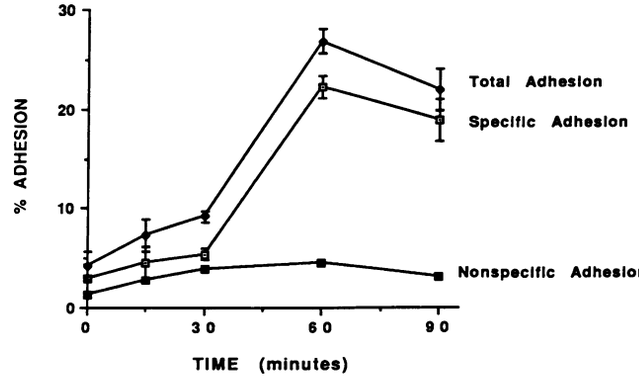

Figure 2. Time course of adhesion of *C. albicans* (blood isolate) to human umbilical vein endothelium.

increase in the percentage of yeasts fluorescing at a given concentration of MAb (Table I). In Table II, flow cytometry with two different concentrations of anti-Mo1-94 and OKM1 was used to compare the expression of the Mo1 analogue in yeast-phase *C. albicans* after growth in D-glucose or L-glutamate. At both low and high concentrations of anti-Mo1-94 (400 μg/ml and 1,200 μg/ml) and OKM1 (1,200 μg/ml and 6,500 μg/ml), both the percentage of yeast cells fluorescing and the mean channel fluorescence were significantly increased for glucose-grown *C. albicans* as compared to glutamate-grown organisms. For example, with anti-Mo1-94 at 400 μg/ml, the percentage of yeasts fluorescing increased from 2.8±1.3 after growth in L-glutamate to 15.8±3.3 after growth in D-glucose ($P = 0.004$); similarly, the mean channel fluorescence increased from 201.8±1.7 to 267.4±22.5 under these same conditions ($P = 0.008$). A similar increase in percentage of yeasts fluorescing (from 7.0±2.2 after growth in L-glutamate to 22.9±5.0 after growth in D-glucose; $P = 0.008$) and in mean channel fluorescence (from 224.8±8.9 to 302.1±29.5, respectively; $P = 0.013$) was observed with anti-Mo1-94 at high concentration (1,200 μg/ml). For OKM1 at high concentration, these increases were even greater, with an eightfold rise in percentage of yeasts fluorescing (4.3±1.6 after growth in L-glutamate to 33.3±2.1 after growth in D-glucose, $P < 0.001$) and a 65.8% increase in mean channel fluorescence (208.4±9.6 after growth in L-glutamate to 345.0±7.0 after growth in D-glucose; $P < 0.001$). These changes were highly significant at each comparison.

Time course of adhesion of C. albicans. Fig. 2 graphs the adhesion of a blood isolate of *C. albicans* as a function of time. Specific adhesion, calculated as the difference between total adhesion and nonspecific adhesion, was maximal at 60 min, which was therefore chosen as the incubation time for subsequent experiments.

Effect of glucose on receptor expression and adhesion in C. albicans. As shown in the top panel of Fig. 3, growth of a blood isolate of *C. albicans* in 20 mM D-glucose (*hatched bars*) was associated with a 31.2% increase in mean channel fluorescence for anti-Mo1-94, as compared to growth of the identical organism in 20 mM L-glutamate (*solid bars*) (144.4±4.2 for glucose vs. 109.9±11.8 for glutamate; $P = 0.013$, $n = 7$). Thus, expression of the integrin analogue was increased on each individual yeast after growth in the presence of D-glucose. A statistically significant increase of 18.5% in mean channel fluorescence was also observed for a urinary isolate after growth in D-glucose as opposed to L-glutamate (148.4±19.5 for glucose vs. 125.2±22.7 for glutamate; $P = 0.049$, $n = 7$).

The lower panel of Fig. 3 compares the specific adhesion of the blood isolate after growth in 20 mM D-glucose (*hatched bars*) vs. 20 mM L-glutamate (*solid bars*) as carbon source. Specific adhesion of the blood isolate to the endothelial monolayer increased by 80% after growth in 20 mM D-glucose as opposed to 20 mM L-glutamate (36.1±4.3% vs. 20.3±2.8%, $P = 0.013$, $n = 7$). Specific adhesion of the urinary isolate increased by 28.9% after growth in D-glucose as opposed to L-glutamate (49.4±6.2% vs. 38.3±5.6%; $P = 0.003$, $n = 7$) (data not shown). The glucose-dependent increase in expression of the integrin analogue on *C. albicans* thus correlated directly with glucose-enhanced adhesion of both isolates.

Table II. Increase in Expression of the Integrin Analogue after Growth in Glucose

	No MAb		Anti-Mo1				OKM1			
			Low (400 μg/ml)		High (1,200 μg/ml)		Low (1,200 μg/ml)		High (6,500 μg/ml)	
	%	*Mean channel fluorescence*	%	*Mean channel fluorescence*	%	*Mean channel fluorescence*	%	*Mean channel fluorescence*	%	*Mean channel fluorescence*
Glutamate*	1.0±0.2	187.5±3.3	2.8±1.3	201.8±1.7	7.0±2.2	224.8±8.9	0.8±0.3	191.6±6.2	4.3±1.6	208.4±9.6
Glucose*	1.4±0.9	196.3±13.5	15.8±3.3	267.4±22.5	22.9±5.0	302.1±29.5	10.6±4.9	247.8±9.2	33.2±2.1	345.0±7.0
P value:										
glucose vs. glutamate:	0.466	0.338	0.026	0.001	<0.001	<0.001	0.004	0.008	0.008	0.013

* Results show mean±standard deviation for $n = 4$ experiments in each column.

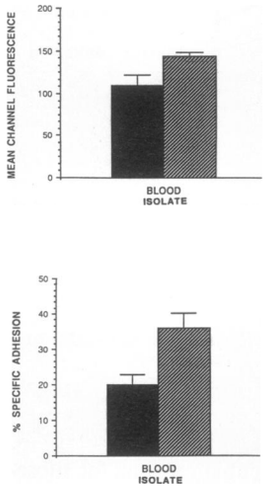

Figure 3. (Upper panel) Mean channel fluorescence for the Mo1 analogue on a blood isolate of *C. albicans* after growth in 20 mM L-glutamate (*solid bars*) vs. 20 mM D-glucose (*hatched bars*), as quantitated by flow cytometry with anti-Mo1-94. Expression of the Mo1-like protein is significantly increased for organisms grown in 20 mM D-glucose ($P = 0.013$). Results show the mean±standard error for $n = 7$ experiments. (Lower panel) Adhesion of *C. albicans* to HUVE after growth in 20 mM L-glutamate (*solid bars*) vs. 20 mM D-glucose (*hatched bars*). Adhesion is significantly increased after growth in D-glucose ($P = 0.013$ for a blood isolate). Results show the mean±standard error for $n = 7$ experiments. ■, 20 mM L-glutamate; ▨, 20 mM D-glucose.

Effect of anti-CD11b/CD18 MAbs on adhesion of C. albicans. As shown in Table III, MAbs that recognize epitopes on the α-subunit of CD11b/CD18 on the mammalian neutrophil bound to blood and urinary isolates of *C. albicans* in a manner independent of isotype. Fig. 4 displays the ability of several MAbs to inhibit the glucose-enhanced adhesion of *C. albicans* to HUVE. The IgM MAb anti-Mo1-94 had no effect on the specific adhesion of *C. albicans* to endothelium, even when its concentration was increased threefold; this MAb has been shown by others to be less effective in blocking adhesion-dependent functions in the neutrophil (31). In contrast, MAb 44 (IgG_{2a}) and MAb 17 (IgM), both known to inhibit CD11b/CD18-mediated adhesion of neutrophils (31), inhibited the glucose-enhanced adhesion of *C. albicans* to HUVE by 44% and 71%, respectively. These results show that the inhibition of *C. albicans* adhesion is epitope-specific and not isotype-specific.

Inhibition of adhesion of C. albicans to HUVE with iC3b. Untreated *C. albicans* demonstrated specific adhesion of 28.7±4.2%, while preincubation of yeast cells with BSA did not significantly decrease adhesion (26.5±3.1%, $P > 0.05$). However, after incubation of yeast cells with iC3b, the ligand for the Mo1 analogue in *C. albicans,* only 14.3±2.9% of yeast cells adhered to HUVE. Thus, blockade of the integrin analogue on *C. albicans* with its specific ligand decreased adhesion by 49.7%

Table III. Specific Binding of Anti-Mo1 Monoclonal Antibodies to Candida albicans

Yeast	MAb	Isotype	Percent yeasts fluorescing*
Blood isolate	Anti-Mo1-94	IgM	40.3±13.3
	17	IgM	31.6±11.3
	44	IgG_{2a}	17.7±10.0
Urinary isolate	Anti-Mo1-94		38.3±13.3
	17		29.5±11.8
	44		14.8±4.1

* Results display mean±standard error for $n = 3$ experiments.

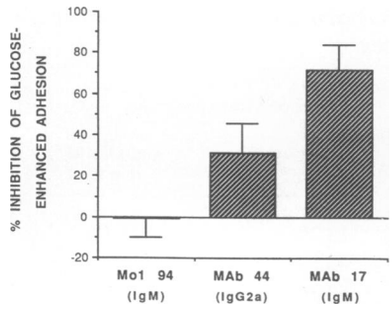

Figure 4. Inhibition of glucose-enhanced adhesion by anti-Mo1 monoclonal antibodies anti-Mo1-94, MAb 44, and MAb 17. Results show the mean±standard error for $n = 4$ experiments.

(Fig. 5). This change was highly statistically significant ($P < 0.001$). These results indicate that a substantial proportion of the adhesion of *C. albicans* to HUVE is mediated by the integrin analogue and can be inhibited by blockade with its specific ligand.

Discussion

The results of these experiments demonstrate that adhesion of the yeast *Candida albicans* to cultured human endothelium is mediated at least in part by a protein on the yeast that shares antigenic, structural, and functional characteristics with the α-subunit of the leukocyte adhesion glycoprotein CD11b/CD18 (Mac-1, Mo1, or CR3), also known as the iC3b receptor of neutrophils and macrophages.

Our previous experiments have identified ~ 2×10^6 iC3b binding sites per yeast (18), which exceeds by nearly 100-fold the density of the analogous protein on the neutrophil (25). The percentage of yeast cells that fluoresce, as well as the mean channel fluorescence per individual cell, is thus dependent on the concentration of MAb employed in flow cytometry (Table I). Both parameters will increase when increasing concentrations of MAbs are employed.

Growth of *C. albicans* in 20 mM D-glucose significantly increased the expression of the integrin analogue in yeast, as assessed by flow cytometry with the MAbs anti-Mo1-94 and OKM1. As shown in Table II, flow cytometry with two different MAbs demonstrated a four- to eightfold increase in the percentage of yeasts fluorescing and a 30–65% increase in mean channel fluorescence for yeast cells grown in the presence of D-glucose, as opposed to L-glutamate. These observations were confirmed for both high and low concentrations of each MAb and were statistically significant at each experimental point.

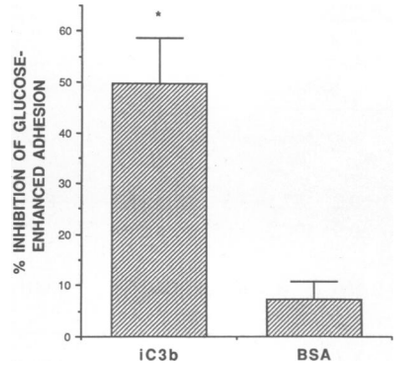

Figure 5. Inhibition of glucose-enhanced adhesion by purified human iC3b, with BSA as control. Results show the mean±standard error for $n = 3$ experiments. Inhibition by iC3b was highly significant, with $*P = 0.001$.

Mean channel fluorescence provides perhaps the best measure of the density of the integrin analogue per individual yeast cell. As shown in Fig. 3, enhancement of the expression of this protein after growth of *C. albicans* in D-glucose (*top panel*) correlated with increased yeast adhesion to HUVE (*bottom panel*). This effect also occurred with a urinary isolate of *C. albicans*. In absolute terms, adhesion of the blood isolate rose from 20% to 36% and that of the urinary isolate from 38% to 49% after growth in D-glucose. These observations suggest that specific adhesion is dependent not only upon the expression of the integrin analogue on the yeast surface, but also upon the availability of binding sites on the endothelial cell or in the subendothelial matrix.

The endothelial cell and its surrounding matrix display several potential ligands that may serve to attach the integrin analogue on *C. albicans*. Synthesis of C3 by endothelial cells is well described (32); the degradation of this protein by serum or cellular proteinases may provide a local source of iC3b at the endothelial surface that serves to attach *C. albicans* to endothelium. Because iC3b contains the sequence arginine-glycine-aspartic acid (RGD), this tri-peptide may be important in mediating candidal adhesion, as it is for integrin-mediated adhesion on mammalian cells (33, 34). Alternatively, RGD-containing subendothelial matrix components, such as fibronectin or laminin, may promote adhesion in vivo. For example, exposure of the subendothelial matrix by pharmacologic contraction of cultured endothelial cells increased the adhesion of *C. albicans* (35).

Additional support for the involvement of the candidal integrin in adhesion derives from the recent description of an avirulent mutant of *C. albicans*, that has reduced binding of iC3b-coated, but not C3d-coated, sheep erythrocytes (36). However, neither the binding of anti-Mo1-94 nor adhesion to human endothelial cells was studied with this mutant.

Preliminary results indicate that ICAM-1, an endothelial surface molecule, may not be involved, because upregulation of ICAM-1 by activation of HUVE with tumor necrosis factor did not increase yeast adhesion (Hostetter, M. K., and G. M. Vercellotti, unpublished observations). This latter observation is supported by the recent report that CD11b/CD18 on neutrophils fails to recognize ICAM-1 on HUVE (37).

Three MAbs directed against specific epitopes of the α subunit of CD11b/CD18 were next examined for their effects on yeast adhesion. All MAbs bound specifically to *C. albicans*, but only two, MAb 17 (IgG_{2a}) and MAb 44 (IgM), inhibited adhesion of the yeast to HUVE, while the third, anti-Mo1-94 (IgM), did not. MAbs 17 and 44 have been shown to inhibit integrin-mediated adhesion of mammalian neutrophils as well as the rosetting of iC3b-coated sheep erythrocytes, while anti-Mo1-94 is much less potent in this regard (31, 38, 39). Thus, only those MAbs that recognize iC3b-binding epitopes on CD11b/CD18 are able to inhibit candidal adhesion. The failure of MAb 44 to inhibit glucose-enhanced adhesion to the same degree as MAb 17 may be related to differing affinities for the candidal protein among antibodies of different isotypes. However, inhibition of glucose-enhanced adhesion of yeasts to HUVE depends on recognition of adhesive epitopes on a yeast protein that is structurally and functionally akin to the leukocyte integrin CD11b/CD18; because inhibition occurred with both IgG and IgM MAbs, these results cannot be attributed to MAb isotype or to steric effects of IgM MAbs.

Lastly, the substantial inhibition of adhesion after preincubation of yeast cells with iC3b, an RGD-containing ligand specific for CD11b/CD18, provides more definitive evidence of the participation of the integrin analog in yeast adhesion. Clearly, our results do not exclude a role for other surface interactions that may also be implicated in candidal adhesion; for example, candidal surface structures capable of interacting with fibronectin, fibrinogen, or laminin may account for that portion of adhesion that could not be inhibited by iC3b (40). Nonetheless, our results indicate that the integrin analogue in *C. albicans* is responsible for nearly 50% of the adhesion of this yeast to HUVE.

The presence of an integrin-like protein in *C. albicans* has been shown in separate studies to subvert opsonization and to inhibit phagocytosis of the yeast, by virtue of its competition with the iC3b receptor on the neutrophil (18, 19). This effect may be a fortuitous consequence of its mimicry of a phagocytic receptor. The experiments of this paper establish a more fundamental function: to allow the yeast to adhere to host tissues and thereby gain a foothold for potential invasion. Because of the morbidity of *C. albicans* infections of the urinary tract, gastrointestinal tract, integument, and vascular tree, the role of the integrin analogue in adhesion of the yeast to epithelial and mucosal surfaces, or even to synthetic polymers such as silastic, should be thoroughly investigated. Blockade of the initial point of contact between yeast and host by inhibition of adhesion might well avert invasive candidal infections.

Inhibition of adhesion by MAbs 44 and 17 indicates not only a remarkable degree of homology between adhesive epitopes of yeast and neutrophil proteins, but also an intriguing phylogenetic similarity. In other studies, polyclonal antibodies to the conserved cytoplasmic domain of the integrin β_1 subunit detected cross-reactive proteins on *C. albicans* (41), while other work has shown that numerous MAb directed against the β_2 class of integrins, the leukocyte adhesion glycoproteins, also recognized candidal proteins (18, 19, 22). Thus, the yeast protein that we have identified may well be a precursor of mammalian integrins. A definitive statement of phylogenetic relationship must await sequencing of the entire protein, which is well underway.

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