INFECTION AND IMMUNITY, Aug. 1992, p. 3087–3091
0019-9567/92/083087-05$02.00/0

Vol. 60, No. 8

Cell Extracts of *Candida albicans* Block Adherence of the Organisms to Endothelial Cells

JOHN E. EDWARDS, JR.,[1,2]* CYNTHIA L. MAYER,[1,2] SCOTT G. FILLER,[1,2] ELSA WADSWORTH,[3] AND RICHARD A. CALDERONE[3]

Division of Adult Infectious Diseases, Department of Medicine, Harbor/UCLA Medical Center, Torrance, California 90509[1]; UCLA School of Medicine, Los Angeles, California 90024[2]; and Department of Microbiology, Schools of Medicine and Dentistry, Georgetown University, Washington, D.C. 20007[3]

Received 13 May 1991/Accepted 30 April 1992

Cell extracts of *Candida albicans* were fractionated by concanavalin A affinity chromatography. Eluted mannosylated proteins (fraction II) and nonbinding, nonmannosylated proteins (fraction I) were collected and assayed directly for inhibition of adherence of *C. albicans* to endothelium. Fraction II blocked blastospore adherence to endothelial cells. Fraction I blocked both blastospore and germ tube adherence to endothelial cells. Monoclonal antibody OKM-1 (anti-CR3) and an anti-*C. albicans* monoclonal antibody, CA-A (anti-CR2), reacted in Western blots with proteins from fraction I, suggesting the presence of the CR2- and CR3-like proteins that have been previously identified on *C. albicans* germ tubes.

Adherence to human host cells is considered the pivotal first step in the pathogenesis of hematogenous infections caused by *Candida* species. However, an understanding of the adherence mechanisms of *Candida* species as related to their outer cellular components is only in the initial stages.

The cell wall of *Candida* species consists of proteins, glucans, mannans, and chitin (4, 15). These components have been evaluated individually for their role as potential adhesins by different investigative groups. Certain evidence points to mannosylated proteins serving as adhesins for many of the cell types studied. (i) preincubation of *Candida* species with concanavalin A (ConA), which binds to and presumably blocks alpha-linked D-mannose residues, decreases adherence (25–28), (ii) mannosylated proteins from culture supernatants inhibit adherence (21), and (iii) tunicamycin, a mannosylated protein synthesis inhibitor, decreases adherence (5, 18). Evidence for proteins serving as adhesins is derived from two types of studies: (i) treatment of *Candida* species with protein-denaturing substances, such as proteolytic enzymes (19, 30) or mercaptoethanol and dithiothreitol (19), decreases adherence; and (ii) glycosidases, lipases, salts, or detergents have little effect (19). Additionally, Segal and Savage (29) found that chitin-soluble extracts reduced the adherence of *Candida albicans* to epithelial cells.

This report continues our investigations into the mechanisms of adherence of *Candida* species to human vascular endothelial cells from the perspective of understanding how *Candida* species escape the intravascular compartment to infect target organs. In the experiments reported here, we studied *Candida* germ tubes as well as blastospores, since increased adherence of the germinated phase to host cells has been shown (16, 24). Additionally, certain molecules on *Candida* germ tubes have characteristics of both complement receptor 2 (CR2) and CR3 found on human host cells (2, 6, 7, 10, 11, 13). The human CR3-like molecules on *Candida* germ tubes have been of particular interest to us, since CR3 promotes the adherence of polymorphonuclear leukocytes to human vascular endothelial cells (6) and may be important in the adherence of *C. albicans* to endothelial cells (12). These receptors may also be present on blastospores of the organism (11).

In the studies described here, we used an approach complementary to that used in previous investigations of *Candida* adherence mechanisms. Extracts of *Candida* hyphae were prepared, separated by column chromatography into either protein or mannosylated protein fractions, and used as competitive inhibitors in adherence assays with endothelial cells and *Candida* organisms. Our results show that blocking of adherence by *Candida* fractions was growth phase specific, indicating that the yeast and germ tube forms have different adhesins.

MATERIALS AND METHODS

Cell extract preparation. *C. albicans* 4918 blastoconidia were collected from an overnight room temperature culture in Phytone peptone broth (BBL, Cockeysville, Md.). The cells were washed, resuspended in phosphate-buffered saline (PBS; pH 6.4), and allowed to germinate in Phytone peptone broth (37°C) for 2 to 4 h. The hyphae were washed and homogenized with a Braun homogenizer as described previously (3). The cell homogenate was dialyzed and lyophilized following ultrafiltration. Extraction was carried out in the presence of 1 mM phenylmethylsulfonyl fluoride (Sigma Chemical Co., St. Louis, Mo.) to inhibit proteolysis (20). Each lyophilized extract was subjected to ConA–Sepharose R-4B (Pharmacia, Uppsala, Sweden) affinity chromatography as described below. Eluted mannosylated proteins (fraction II) from ConA chromatography were collected, as were nonbinding, nonmannosylated proteins (fraction I). Both fractions were assayed directly for inhibition of adherence of *C. albicans* to endothelial cells (see below). Fraction I contained approximately 1.5% carbohydrate, and fraction II contained approximately 55% carbohydrate (both as dry weights).

* Corresponding author.

ConA chromatography. Cell extracts from *C. albicans* were fractionated by ConA chromatography by the following procedure (3). The lyophilized extract from whole hyphal cells (200 mg [dry weight]) was dissolved in PBS and separated on a column (0.7 by 18 cm) at 4°C and a flow rate of 8 ml/h. Two fractions, the flowthrough soluble protein (fraction I) and the bound mannosylated protein complex, which was eluted with 0.01 M phosphate buffer containing 0.2 M α-methylmannoside (fraction II), were dialyzed against PBS or distilled water and lyophilized. These fractions were separated and then tested for blocking of the adherence of the organism to endothelial cells.

SDS-PAGE and Western blotting (immunoblotting). Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and Western blotting were performed by standard procedures (20, 22). Electrophoresis was carried out in a minigel system (Bio-Rad Laboratories, Richmond, Calif.). The total amount of protein loaded per lane was 3 μg for each of the test preparations. Western blotting was performed with either a serum sample from a patient with chronic mucocutaneous candidiasis or a monoclonal antibody (MAb) to either human CR3 (OKM-1) or the *C. albicans* C3d-binding protein (CA-A). The primary antibody was diluted 1:400 for immunoblotting of extracts. Binding of the primary antibody to *C. albicans* antigens was detected with alkaline phosphatase-labeled protein A as described previously (20, 22).

Preparation of cells for adherence assays. *C. albicans* ATCC 36082 (American Type Culture Collection, Rockville, Md.) was maintained on yeast extract (Difco Laboratories, Detroit, Mich.)-glucose-potassium phosphate agar slants at 4°C. Prior to experiments, it was grown in yeast nitrogen base broth (Difco) supplemented with 1% glucose (J. T. Baker Chemical Co., Phillipsburg, N.J.) and 0.15% L-asparagine (Calbiochem, San Diego, Calif.). Organisms were inoculated from the modified agar slants into yeast nitrogen base broth for overnight culturing on a rotating drum at 27°C. An aliquot of the overnight culture was inoculated into fresh yeast nitrogen base broth and incubated. The stationary-phase organisms were sonicated for 3 s (setting no. 4; model 350 sonicator; Branson Sonic Power Co., Danbury, Conn.) to yield a suspension of >90% singlet blastoconidia as previously described (8). Viability (determined by trypan blue exclusion) was unaltered by sonication. The singlet suspension was centrifuged at 700 × *g* (Sorvall RC-3 centrifuge) for 7 min and washed twice with 0.85% NaCl. The cells were resuspended in Dulbecco's PBS, sonicated for 3 s, and counted with a hemacytometer. For the enzyme-linked immunosorbent assay (ELISA) with blastospores, organisms were adjusted to 2×10^6/ml. For the ELISA with germ tubes, blastoconidia were first adjusted to 10^7 in 10 ml of germination medium (0.075% gelatin and 0.15% sucrose in distilled water) and then incubated at 37°C until the germ tube length was approximately equal to the yeast cell diameter. Germ tubes were sonicated for 3 s, counted with a hemacytometer, and adjusted to 6×10^5/ml for the adherence assays.

Endothelial cell monolayers. Human umbilical vein endothelial cells were obtained by use of minor modifications of the method of Jaffe (14). They were grown to confluence from a primary culture in 25-cm^2 tissue culture flasks and split into 96-well microtiter plates (Costar, Van Nuys, Calif.) for the ELISAs. Cells were grown in the presence of extracellular growth factor (Bionetics, Rockville, Md.) at a concentration of 0.12 mg/ml in medium M-199 (GIBCO, Grand Island, N.Y.). The microtiter wells were coated with 0.01 mg of fibronectin (Collaborative Research, Bedford, Mass.) per ml prior to plating of the endothelial cells. Trypsin (0.018% in 0.85% NaCl; Sigma) was used to transfer the cells from primary flasks to the microtiter wells.

ELISA reagents. A single MAb conjugated to alkaline phosphatase was used for the ELISA in a modification of our previously published assay (8). The MAb was an anti-*Candida* mannan immunoglobulin, CDE344.3-AP (Hybritech, San Diego, Calif.), diluted in 1% bovine serum albumin (BSA)–1% Tween 20 in calcium- and magnesium-free PBS (pH 7.35). Blocking buffer against the anti-*Candida* mannan MAb was 1% BSA–0.1% Tween 20 in PBS (pH 7.2).

ELISA. The endothelial cells were washed twice with 37°C sterile PBS by gentle pipetting in and aspirating out with a Pasteur pipette. The microtiter plates were held vertically for both washes. Two controls were used: endothelial cells with antibodies but no *C. albicans* cells and endothelial cells with no antibodies or *C. albicans* cells. One hundred microliters of 37°C PBS was added to the control wells. The wells used for testing of the potential blockers received 50 μl of a 400-μg/ml concentration of a potential blocker or 50 μl of PBS plus 50 μl of a 2× concentration of *C. albicans*. The final concentration of *C. albicans* was 3×10^4 cells per well, and that of the possible blocker was 200 μg/ml. A control column of eight wells containing a potential blocker plus PBS but no *C. albicans* cells was also included. This control served as an indicator of cross-reactivity between the cell wall extract and MAb CDE344.3-AP. Neither fraction I nor fraction II was cross-reactive. All columns (of eight replicates each) were tested in duplicate: one set to be rinsed and one set nonrinsed. The nonrinsed wells were used to determine the optical density (OD) of the total number of organisms added to each well. The microtiter plates were incubated for 30 min (in room air) at 37°C.

After incubation, the *C. albicans* suspension from one set of columns (potential blocker plus PBS, *C. albicans* plus PBS, and potential blocker plus *C. albicans*) was aspirated and the wells were rinsed four times with 37°C PBS (75 μl per rinse). The plates were centrifuged for 10 min at 200 × *g*, and the contents of the nonrinsed wells were aspirated. The cells were fixed for several hours in 45 μl of 2% paraformaldehyde at 4°C.

Following fixation, the wells were washed three times with 50 μl of 1% BSA–0.1% Tween 20. Fifty microliters of 37°C MAb CDE344.3-AP was added to each well. The plates were incubated at 37°C for 60 min. The antibody solution was aspirated, and the wells were rinsed with 125 μl of 1% BSA–0.1% Tween 20. Chromogenic substrate (185 μl) was added to each well, including an extra column of wells used to determine the baseline for the ELISA reader (Titertek Multiskan; Flow Laboratories, McLean, Va.). The plates were incubated for 30 min at room temperature in the dark. One hundred sixty microliters from each well was transferred to each well of a clean microtiter plate. The OD of each well was read at A_{405}. At no time during the adherence assays did the operators know the constituents of any of the fractions being tested.

Statistical methods. The data were analyzed by comparing the ODs of supernatants from the experimental and control wells. These values were corrected for nonspecific binding of the antibody to the endothelial cells and cell wall fractions. In addition, the corrected ODs for all experiments were normalized so that the mean OD of the control wells (which contained endothelial cells and *C. albicans* without cell wall fractions) was 1. In half of the experiments, wells containing known numbers of organisms were run in parallel

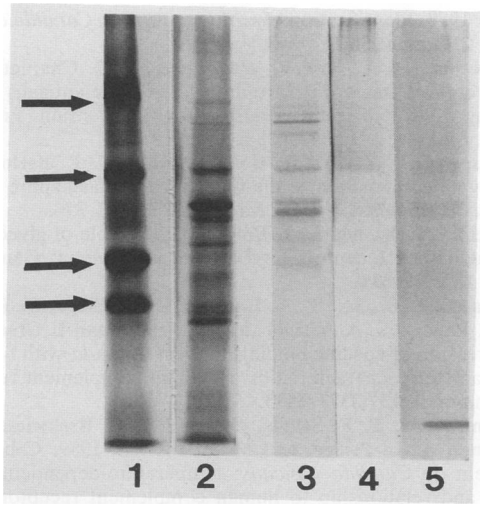

FIG. 1. SDS-PAGE and Western blot analysis of fraction I from *C. albicans*. Lanes: 2, Aurodye stain of fraction I; 3 to 5, fraction I tested with patient serum (lane 3), an MAb to mammalian CR3 (lane 4), and an MAb to the *C. albicans* C3d-binding protein (lane 5); 1, Molecular mass standards (95, 55, 36, and 29 kDa, from top to bottom), indicated by arrows.

with wells containing the other samples to construct standard curves. These curves always demonstrated a linear relationship between the number of organisms and the OD. The effects of each fraction on endothelial cell adherence were tested at least three times for both blastospores and germ tubes, and each experiment was performed in replicates of five to eight. The results of similar experiments performed on different days were compared by analysis of variance with the Bonferroni correction for multiple comparisons. P values of <0.05 were considered significant.

RESULTS

Effects of cell wall fractions on adherence. A whole-cell hyphal extract was initially fractionated with ConA-Sepharose. Soluble, nonbinding proteins (fraction I) and α-methylmannoside-eluted proteins (fraction II) were assayed for their effects on the adherence of germ tubes or blastospores to human endothelial cells. Fraction I at a concentration of 200 μg/ml inhibited the adherence of germ tubes by 50% ± 23% ($P < 0.001$), compared with the adherence of germ tubes in controls. In contrast, fraction II, which contained mannan and mannosylated proteins, was not inhibitory (10% ± 21% inhibition; $P > 0.5$ [not significant]). These same fractions at 200 μg/ml were also tested for their effects on the adherence of blastospores to human endothelial cells. Fraction I inhibited the adherence of blastospores by 41% ± 26% ($P < 0.001$). Fraction II was also inhibitory (27% ± 16% inhibition; $P < 0.001$). Although there were significant day-to-day differences in the magnitude of the blocking ability of each fraction, fraction I always blocked both blastospore and germ tube adherence to endothelial cells, and fraction II always blocked blastospore but not germ tube adherence.

Western blot analysis. Fraction I was analyzed by SDS-PAGE and Western blotting (Fig. 1). A number of bands were observed in Aurodye-stained gels and nitrocellulose transfers from gels. The major bands ranged in molecular mass from 95 to 25 kDa (Fig. 1, lane 2). When a serum sample from a patient with mucocutaneous candidiasis was used, major bands were observed at 85, 55, and 50 kDa (Fig. 1, lane 3). Fraction I was also tested with MAbs to mammalian CR3 (MAb OKM-1) (Fig. 1, lane 4) and to the *C. albicans* C3d-binding protein (MAb CA-A) (Fig. 1, lane 5). Both OKM-1 and CA-A MAbs reacted with proteins of 55 to 60 and 45 kDa. Thus, fraction I contained proteins with reactivity to antibodies against CR2 and CR3. Proteins with similar molecular masses were not found on immunoblots of fraction II with OKM-1.

DISCUSSION

In 1985, Heidenreich and Dierich (13) found that sheep erythrocytes coated with complement component iC3b or C3d bound to *C. albicans* and *C. stellatoidea* hyphae, suggesting the presence of CR2- and CR3-like molecules on the surface of the organisms. This finding was confirmed by Edwards et al. (6), and the recognition of the hyphal or germ tube antigen by an MAb (Mo-1) directed against human CR3 was established. CR3 is known to be important in the adherence of leukocytes to endothelium (1, 23), and the possibility that the CR3-like molecules on the surface of germinated *Candida* cells may be operative in the adherence of the germinated form to endothelial cells remains open to further investigation.

More recently, the similarity between *Candida* and human complement receptors has been further determined. Gilmore et al. (11) found on *C. albicans* blastospores surface receptors for iC3b that shared homology with the alpha chain (CD11b) of human CR3 but not the beta chain (CD18). Calderone et al. (2) found receptors for both iC3b and C3d on *C. albicans* hyphae but not blastospores, and they extracted fractions that inhibited the rosetting of C3d-coated erythrocytes. Eigentler et al. (7) concluded that human CR3 and *C. albicans* complement receptors were antigenically related but not identical on the basis of anti-CR3 MAb binding. In their studies, an anti-CR3 MAb (OKM-1) was blocked from binding to *C. albicans* germ tubes when the cells were first reacted with an anti-*Candida* antibody that presumably bound to CR3-like molecules on the hyphae. However, an $F(ab')_2$ fragment of this anti-*Candida* immunoglobulin G was antigenically too different to bind directly to the true CR3 site found on a macrophage cell line (U-937).

On the basis of the experimental data that we are presenting now, we have identified a protein fraction extracted from *C. albicans* that blocks the adherence of the germinated form of the organism to human vascular endothelial cells. While this fraction contains constituents that react with an anti-CR3 MAb, OKM-1, blocking studies with purified *C. albicans* CR3-like molecules need to be pursued. There is additional evidence for a role of CR3-like molecules as adhesins for *C. albicans*. Frey et al. (9) and Gustafson et al. (12) showed that the iC3b ligand blocked the adherence of *C. albicans* to cultured endothelial cells, and Klotz and Smith (17) demonstrated that the adherence of *C. albicans* to subendothelium was inhibited by RGD peptides, which are common to ligands such as iC3b. Additionally, Gustafson et al. (12) have recently reported that some MAbs directed against CR3 block the adherence of *C. albicans* to cultured endothelial cells. Thus, the data obtained in our present study and work by others continue to point to an interaction of *Candida* CR3-like molecules with host cells.

Interestingly, an MAb (CA-A) to the *C. albicans* C3d-binding protein reacted in a Western blot with the same

proteins as OKM-1, the MAb to mammalian CR3. Unresolved is the question of whether the *C. albicans* iC3b and C3d MAb-binding activities are associated with a single protein. While not definitive, the data from our Western blots indicate that both binding activities may be associated with the same protein. However, the C3d-binding protein of *C. albicans* is glycosylated. Reactions with MAb CA-A in Western blots of fraction I were very weak, perhaps suggesting incomplete binding of CR2-like molecules to ConA (20). In this study, blocking (of germ tube adherence) was associated with the nonmannosylated fraction of whole-cell extracts, perhaps indicating that these activities are actually associated with different proteins. Ollert et al. have shown that a mutant of *C. albicans* deficient in adherence has normal CR2-like molecule activity but substantially reduced CR3-like molecule activity (22).

We conclude also that the surface of the blastospore form has at least one additional determinant not present on the hyphal form, since a component(s) from both fraction I and fraction II blocked the adherence of blastospores, while a compent from only fraction I blocked the adherence of germinated organisms. Recently, Critchley and Douglas (5) described an adhesin of *C. albicans* blastospores for epithelial cells that appeared to be a lectinlike protein that recognized fucose residues of host cells. This adhesin appears to be different from the CR3-like component of *C. albicans*, which recognizes peptide sequences. Thus, blastospores may contain both types of adhesins.

In conclusion, we found that fraction II blocked only blastospore adherence to endothelial cells, while fraction I blocked both blastospore and germ tube adherence to endothelium. Since the two fractions had dissimilar effects on the adherence of blastospores and germ tubes, the adherence mechanisms of the two forms of *C. albicans* are not identical. Fraction II contains mannosylated proteins, which may block constituents not present on germ tubes by a mechanism different from that of the proteins of fraction I. Fraction I contains proteins that have a low carbohydrate content, that also react with OKM-1 and CA-A, and that therefore may be CR2- or CR3-like proteins. The fact that both the soluble proteins (fraction I) and the mannosylated proteins (fraction II) blocked adherence, coupled with the lack of blocking by pure mannan (24), points to both proteins and the protein moiety of mannosylated proteins as being adhesins. Further studies are necessary to characterize the similarities and differences between these CR2- or CR3-like proteins on *C. albicans* and the human proteins.

ACKNOWLEDGMENTS

This research was supported by U.S. Public Health Service grants HLB-21370 and AI-25738 to R.A.C., AI-19990 to J.E.E., and AI-08258 to S.G.F.

The IMT-2 Olympus phase-contrast microscope used in this study was graciously donated by Toyota U.S.A.

REFERENCES

1. **Anderson, D. C., F. C. Schmalsteig, M. A. Kohl, M. F. Tosi, N. Dana, G. J. Buffone, B. J. Hughes, B. R. Brinkley, W. D. Dickey, J. S. Abramson, T. Springer, L. A. Boxer, J. M. Hollers, and C. W. Smith.** 1984. Abnormalities of polymorphonuclear leukocyte function associated with a heritable deficiency of a high molecular weight surface glycoprotein (GP138): common relationship to diminished cell adherence. J. Clin. Invest. **74**:536–551.
2. **Calderone, R. A., L. Linehan, E. Wadsworth, and A. L. Sandberg.** 1988. Identification of C3d receptors on *Candida albicans*. Infect. Immun. **56**:252–258.
3. **Calderone, R. A., and E. Wadsworth.** 1987. Characterization with crossed immunoelectrophoresis of some antigens differentiating a virulent from its derived avirulent strain. Proc. Soc. Exp. Biol. Med. **185**:325–334.
4. **Chattaway, F. W., M. R. Holmes, and A. J. E. Barlow.** 1968. Cell wall composition of the mycelial and blastospore forms of *Candida albicans*. J. Gen. Microbiol. **51**:367–376.
5. **Critchley, I. A., and L. J. Douglas.** 1987. Role of glycosides as epithelial cell receptors for *Candida albicans*. J. Gen. Microbiol. **133**:637–643.
6. **Edwards, J. E., Jr., T. A. Gaither, J. J. O'Shea, D. Rotrosen, T. J. Lawley, S. A. Wright, M. M. Frank, and I. Green.** 1986. Expression of specific binding sites on *Candida* with functional and antigenic characteristics of human complement receptors. J. Immunol. **137**:3577–3583.
7. **Eigentler, A., T. F. Schulz, C. Larcher, E. Breitwieser, B. L. Moynes, A. L. Petzer, and M. P. Dierich.** 1989. C3bi-binding protein on *Candida albicans*: temperature-dependent expression and relationship to human complement receptor type 3. Infect. Immun. **57**:616–622.
8. **Filler, S. G., L. C. Der, C. L. Mayer, P. D. Christenson, and J. E. Edwards, Jr.** 1987. An enzyme-linked immunosorbent assay for quantifying adherence of *Candida* to human vascular endothelium. J. Infect. Dis. **156**:561–566.
9. **Frey, C. L., J. M. Barone, and D. Drutz.** 1990. The role of the iC3b receptor in fungal adherence to endothelial cells, abstr. F-101, p. 112. Abstr. 90th Annu. Meet. Am. Soc. Microbiol. 1990.
10. **Gilmore, B. J., and M. K. Hostetter.** 1986. Inducible binding site for iC3b on virulent *Candida albicans*. Clin. Res. **34**:518A.
11. **Gilmore, B. J., E. M. Retsinas, J. S. Lorenz, and M. K. Hostetter.** 1988. An iC3b receptor on *Candida albicans*: structure, function and correlates for pathogenicity. J. Infect. Dis. **157**:38–46.
12. **Gustafson, K. S., G. M. Vercellotti, C. M. Bendel, and M. K. Hostetter.** 1991. Molecular mimicry in *Candida albicans*. Role of an integrin analogue in adhesion of the yeast to human endothelium. J. Clin. Invest. **87**:1896–1902.
13. **Heidenreich, F., and M. P. Dierich.** 1985. *Candida albicans* and *Candida stellatoidea*, in contrast to other *Candida* species, bind iC3b and C3d but not C3b. Infect. Immun. **50**:598–600.
14. **Jaffe, E. A.** 1984. Biology of endothelial cells, 1st ed. Martinus Nijhoff, Boston.
15. **Kessler, G., and W. J. Nickerson.** 1959. Glucomannan-protein complexes from cell walls of yeasts. J. Biol. Chem. **234**:2281–2285.
16. **Kimura, L. H., and N. N. Pearsall.** 1978. Adherence of *Candida albicans* to human buccal epithelial cells. Infect. Immun. **21**:64–68.
17. **Klotz, S., and R. L. Smith.** 1990. *Candida albicans* adherence to subendothelial extracellular matrix is inhibited by arginine-glycine-aspartic acid peptides. Clin. Res. **38**:13A.
18. **Kuo, S. C., and J. O. Lampen.** 1974. Tunicamycin—an inhibitor of yeast glycoprotein synthesis. Biochem. Biophys. Res. Commun. **58**:287–295.
19. **Lee, J. C., and R. D. King.** 1983. Characterization of *Candida albicans* adherence to human vaginal epithelial cells in vitro. Infect. Immun. **41**:1024–1030.
20. **Linehan, L., E. Wadsworth, and R. A. Calderone.** 1988. *Candida albicans* C3d receptor, isolated by using a monoclonal antibody. Infect. Immun. **56**:1981–1986.
21. **McCourtie, J., and L. J. Douglas.** 1985. Extracellular polymer of *Candida albicans*: isolation, analysis and role in adhesion. J. Gen. Microbiol. **131**:495–503.
22. **Ollert, M. W., E. Wadsworth, and R. A. Calderone.** 1990. Reduced expression of the functionally active complement receptor for iC3b but not for C3d on an avirulent mutant of *Candida albicans*. Infect. Immun. **58**:909–913.
23. **Pohlman, T. H., K. A. Stanness, P. G. Beatty, H. D. Ochs, and J. M. Harlan.** 1986. An endothelial cell surface factor(s) induced in vitro by lipopolysaccharide, interleukin 1, and tumor necrosis

factor-α increases neutrophil adherence by a CD218-dependent mechanism. J. Immunol. **136**:4548–4553.

24. **Rotrosen, D., J. E. Edwards, Jr., T. R. Gibson, J. C. Moore, and A. H. Cohen.** 1985. Adherence of *Candida* to cultured vascular endothelial cells: mechanisms of attachment and endothelial cell penetration. J. Infect. Dis. **152**:1264–1274.
25. **Sandin, R. L.** 1987. The attachment to human buccal epithelial cells by *Candida albicans*: an in vitro kinetic study using concanavalin A. Mycopathologia **98**:179–184.
26. **Sandin, R. L.** 1987. Studies on cell adhesion and concanavalin A-induced agglutination of *Candida albicans* after mannan extraction. J. Med. Microbiol. **24**:145–150.
27. **Sandin, R. L., and A. L. Rogers.** 1982. Inhibition of adherence of *Candida albicans* to human epithelial cells. Mycopathologia **77**:23–26.
28. **Sandin, R. L., A. L. Rogers, R. J. Patterson, and E. S. Beneke.** 1982. Evidence for mannose-mediated adherence of *Candida albicans* to human buccal cells in vitro. Infect. Immun. **35**:79–85.
29. **Segal, E., and D. C. Savage.** 1986. Adhesion of *Candida albicans* to mouse intestinal mucosa in vitro: development of the assay and test of inhibitors. J. Med. Vet. Mycol. **24**:477–479.
30. **Sobel, J. D., P. G. Myers, D. Kaye, and M. E. Levison.** 1981. Adherence of *Candida albicans* to human vaginal and buccal epithelial cells. J. Infect. Dis. **143**:76–82.