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Variability in Expression of a Cell Surface Determinant on *Candida albicans* as Evidenced by an Agglutinating Monoclonal Antibody

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The expression of a surface immunodeterminant of *Candida albicans* was investigated with an agglutinating immunoglobulin M monoclonal antibody. The 96 strains of *C. albicans* tested, of which 76% were recent clinical isolates, were capable of expressing the antigen. The antigen was also produced by strains of *Candida tropicalis* and *Torulopsis glabrata*, but not by other yeast species. Expression of the surface immunodeterminant in *C. albicans* varied as a function of growth as indicated by agglutinin reactions and indirect immunofluorescence tests. When yeast cells were tested with the agglutinin, three patterns of reactivity were observed. In general, cells in the early logarithmic phase were less reactive than cells in the mid-logarithmic phase. Antigen expression, as determined by agglutinin reactivity, was also influenced by the nutritional composition of the growth medium, and in general, cells from broth cultures were usually more reactive than cells grown on solid media. The antigen was solubilized from the cell surface of *C. albicans* by hot 1 M NaCl. These water-soluble extracts were capable of binding antibody, and a single precipitin band formed when soluble antigen was reacted with the monoclonal antibody in an Ouchterlony double-diffusion test. Whole cell preparations and hot NaCl extracts from yeast strains which did not agglutinate when mixed with the antibody also did not absorb the agglutinin from solution. These data indicate that the expression of surface antigens on *C. albicans* is a dynamic process which may be influenced by a number of environmental factors. The use of monoclonal antibodies may allow characterization of surface antigens presented to the host during candidiasis.

The immunocompetent host, probably through a multitude of mechanisms, is capable of resisting candidiasis. Although many investigators have attempted to define the various innate and acquired host mechanisms involved, they have had limited success. Principle foci in current studies have been the cell-mediated arm of acquired immunity (2, 4, 15–17, 27, 33, 42) and innate defenses, including the role of neutrophils (5, 18, 19), macrophages (5, 29), and complement (13, 22).

Except for early, poorly defined studies, few investigators have examined the role of antibody in host defense against candidiasis. The general dogma is that *Candida albicans*-specific antibody is not protective because most candidiasis patients are not only B-cell competent, but often develop high precipitin and agglutinin titers against the fungus (8, 16, 18, 26). However, several investigators (23, 24, 28) have presented evidence that humoral immunity may contribute to protection.

The antigenic mosaic surface of *C. albicans* remains undefined which may account for the confusion regarding the roll of antibody. Antigens may not be equally expressed in all strains, and recent studies suggest that the fungus may undergo antigenic modulation in vivo (31).

Confusion surrounding the role of antibody may be due to a lack of understanding of the antigenic mosaic surface of *C. albicans*. By using polyclonal antisera to serologically classify yeasts, investigators have shown that antigen may not be uniformly expressed in all strains of *C. albicans* (11, 12, 21, 31, 35, 38, 41). Additionally, although other investigations have shown that individual yeast strains may express antigen variability as a function of their metabolic or morphological states (1, 14, 20), none of these studies specifically addresses or identifies the physical location of the occurrence of the antigenic changes. From the standpoint of host-parasite encounters, the definition of surface changes which occur seems imperative. Actually, results of recent studies indicate that a single strain of *C. albicans* may undergo antigenic variation during pathogenesis of candidiasis (30).

In the present study, an agglutinating monoclonal antibody which reacts with a cell surface immunodeterminant of *C. albicans* was used to investigate expression of the antigen during growth of the fungus. Our data show antigen expression to be a dynamic process.

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MATERIALS AND METHODS

Organisms and culture conditions. *C. albicans* 9938 (Mycology Unit, Tulane Medical School, New Orleans, La.) was used to immunize mice. Other organisms used in either agglutination or growth-curve studies were *C. albicans* 394 (R. P. Morrison, University of Oklahoma, Norman, Okla.); 22 additional strains of *C. albicans*; a single strain of *C. albicans* var. *stellatoidea*; and 7 strains of *Candida tropicalis*; and strains of *Candida parapsilosis*, *Candida krusei*, *Torulopsis glabrata*, *Cryptococcus terreus*, *Cryptococcus neoformans*, *Saccharomyces cerevisiae*, *Rhodotorula* sp., *Trichosporon beigelii*, *Phaeococcomyces exophialae*, and *Blastoschizomyces pseudotrichosporon* were from the Montana State University mycological collection. Seventy-one recent clinical isolates, transferred no more than twice onto synthetic media, were obtained from the University of Washington Clinical Microbiology Laboratories, Seattle, Wash. Autoagglutination, particularly prevalent in strains of *Candida tropicalis*, which masked agglutinin reactivity, prevented the use of 39 additional strains of yeast. The identification of each organism was reconfirmed with API 20C Yeast Identification Strips (Analytab Products, Plainview, N.Y.) or by conventional nitrate assimilation and carbohydrate fermentation tests. Germ tube tests were positive on all strains of *C. albicans*. The fungi were grown at 22 to 24°C on potato flakes agar slants (32) and, subsequently, stored at 4°C on the same medium.

Organisms were grown in either glucose (2%; J. T. Baker Chemical Co., Phillipsburg, N.J.)–yeast extract (0.3%; BBL Microbiology Systems, Cockeysville, Md.)–peptone (1.0%; Difco Laboratories, Detroit, Mich.) (GYEP) broth or brain heart infusion (BHI) broth (Difco). Additional media utilized in comparison studies included potato dextrose broth and agar, Sabouraud dextrose broth and agar, BHI agar (Difco), synthetic amino acid medium broth and agarose (GIBCO Diagnostics, Madison, Wis.), and a chemically defined medium, synthetic amino acid medium for fungi broth and agarose (GIBCO).

Immunization of mice. BALB/cByJ mice, obtained from Jackson Laboratories (Bar Harbor, Maine), were immunized by using a protocol which promotes agglutinin responses (43). This entailed six weekly, intraperitoneal, 0.4-ml injections of a saline suspension of heat-killed *C. albicans* 9938 blastoconidia (10^8/ml). Three days after the last booster injection, mouse sera were checked for *C. albicans*-specific agglutinins, spleens were removed from agglutinin-positive animals, and splenocytes were prepared for cell fusion.

Hybridoma production. The hybridoma techniques utilized were those of Chesebro et al. (3), with minor modifications. Maintenance and selective media were supplemented with amphotericin B (1 μg/ml), penicillin (100 U/ml), and streptomycin (100 μg/ml, Irvine Scientific, Santa Ana, Calif.). Cell fusions were effected with a 35% (wt/vol) solution of polyethylene glycol 1540 (Baker) in RPMI 1640 medium.

Agglutinin reactivity was detected directly in combining hybridoma culture supernatant material with heat-killed *C. albicans* 9938 blastoconidia. Hybridoma antibody production was determined indirectly by use of an enzyme-linked immunosorbent assay (BRL Hybridoma Screening Kit; Bethesda Research Laboratories, Gaithersburg, Md.). One of the hybrids obtained produced antibody that agglutinated *C. albicans* 9938 and was twice cloned by limiting dilution. The remainder of this report is devoted to studies involving this monoclonal candidal agglutinin.

Heavy-chain-specific anti-mouse immunoglobulins (Meloy Laboratories, Springfield, Va.) were used in agar double-diffusion (Ouchterlony) and immunoelectrophoretic analyses (7) to identify the antibody class of the agglutinin.

Growth-curve experiments. Growth-curve analyses were carried out in duplicate on seven yeast strains to determine whether agglutinin reactivity varied during the growth cycle. Each strain was first grown in GYEP broth or BHI broth for 48 h. The inoculum was adjusted at time zero to give 7×10^6 to 5×10^7 blastoconidia in 200 ml of fresh medium (either GYEP broth or BHI broth) in 500-ml Erlenmeyer flasks. Cultures were incubated at 37°C with rotation (160 rpm). At hourly intervals, portions of broth sufficient to provide 5×10^8 per ml of cell suspension were aseptically removed and counted (hemocytometer), and reactivity with the agglutinating monoclonal antibody was determined by the slide test.

Media comparison. *C. albicans* 9938, *C. albicans* 394, *C. albicans* 17, and *C. albicans* 34 were chosen to investigate the effects of growth environment on agglutinin reactivity. Each strain was grown in 100 ml of GYEP broth and in 100 ml of BHI broth in 250-ml Erlenmeyer flasks for 24 h at 37°C while rotating at 160 rpm. The yeast cells were washed in saline three times and enumerated (hemocytometer). Cells grown in GYEP broth were used to inoculate 10 ml (10^5 yeast cells per ml) of Sabouraud dextrose broth in 25-ml Erlenmeyer flasks and in agar plates, 10 ml of GYEP broth in flasks and in agar plates, and 10 ml of potato dextrose broth in flasks and in agar plates. Cells grown in BHI broth were used to inoculate 10 ml of BHI broth (10^5 cells per ml) and BHI agar plates. Broth flasks were incubated at 37°C and rotated at 160 rpm for 24 h. Agar plates were streaked for the isolation of *C. albicans* and incubated for 24 h at 37°C. At 24 h, growth was gently swabbed from the surface of the agar plates. After washing three times in saline, suspensions (5×10^8 cells per ml of saline) were made of the agar and broth-grown yeast cells for slide agglutination tests.

Agglutination tests. Standardization of qualitative and semi-quantitative slide agglutination tests was achieved with ring slides (2-cm diameter rings; Scientific Products, Inc., Detroit, Mich.). Twenty-five microliters of the agglutinin, which was undiluted, serial twofold diluted, or ammonium sulfate precipitated (50% at room temperature prepared by methods [7] similarly described by others) was added to the ring slide and mixed with one drop of antigen suspension (heat-killed *C. albicans* 9938 blastoconidia at 5×10^8 cells per ml of saline). The degree of reactivity (1 to 4+) and endpoint titers were determined macroscopically and microscopically. Semiquantitative tube agglutinin titers were carried out in serial twofold dilutions in a total volume of 0.3 ml with 5×10^5 *C. albicans* 9938 per tube and were incubated for 2 h at 37°C, refrigerated for 22 h, and allowed to sit at 22 to 24°C at least 1 h before endpoint titers were determined.

Soluble antigen extraction. The agglutinin-specific antigen was solubilized from whole cells by the following procedure. Yeast cells were grown in 500 ml of GYEP broth at 37°C with rotation at 160 rpm for 24 h, washed three times in saline, suspended in 100 ml of 1 M NaCl, heated to 68°C for 40 min, and centrifuged at 1,800 × *g* at 22 to 24°C. The supernatant fluid was exhaustively dialyzed (as determined by conductivity measurements) against distilled water at 4°C and lyophilized. Antigen preparations (5 mg/ml), resolubilized in 0.15 M NaCl, produced a single precipitin band when reacted with the monoclonal antibody in an Ouchterlony double-diffusion test.

Absorption experiments. Soluble antigen extracts were tested to determine their capacity to absorb out agglutinin reactivity by combining 0.1 ml of monoclonal antibody solution (agglutinin titer, 128) with 0.1 ml of antigen extract (5 ml per mg of saline). These were allowed to react at 22 to 24°C for 15 min. Tubes were then centrifuged at 300 × *g* for 3 min, and the supernatant material was tested for agglutinin reactivity by the slide test.

Several yeast strains which were used in growth-curve analyses were also tested for their ability to absorb the agglutinin from solution. Whole cell suspensions were prepared from 24-h cultures (37°C, 160 rpm) in GYEP and BHI broths. The monoclonal antibody (0.5 ml) was added to the first of three tubes, each of which contained a pellet of 5×10^7 washed yeast cells. The cells in the first tube were suspended, incubated for 5 min at 22 to 24°C, and centrifuged at 300 × *g* for 3 min, and the supernatant fluid was removed and added to the second tube. This procedure was followed through the third tube, and the agglutinin titers of both the resulting supernatant fluid and the original agglutinin preparation were determined against *C. albicans* 9938 to test for anti-μ reactivity with goat anti-mouse immunoglobulin M (IgM) (Meloy Laboratories) in Ouchterlony double-diffusion plates.

Indirect fluorescent antibody test to detect binding of the monoclonal antibody to *C. albicans*. *C. albicans* 9938 was grown in GYEP broth at 37°C with rotation at 160 rpm. At 5, 24, 48, and 96 h of incubation, portions of the broth culture were removed, and the cells were washed in 0.01 M phosphate-buffered saline (pH 7.2). A solution of 20 μl containing 2×10^6 *C. albicans* cells was mixed with 50 μl of monoclonal

antibody (agglutinin titer, 64) for 30 min at 22 to 24°C. The cells were washed four times in 0.01 M phosphate-buffered saline and 20 μl of fluorescein isothiocyanate-labeled goat anti-mouse immunoglobulin (Cappel Laboratories, Cochranville, Pa.), diluted 1:10 in 0.01 M phosphate-buffered saline, was added to the cell pellet. After incubating for 30 min at 22 to 24°C, the cells were washed four times in 0.01 M phosphate-buffered saline and examined with a fluorescent microscope (Leitz Ortholux II).

RESULTS

Characterization of the agglutinin. Enzyme-linked immunosorbent assays were positive for antibody production 3 weeks after the fusions. Agglutinin reactivity of *C. albicans* 9938 yeast cells was simultaneously observable. A precipitin band developed between anti-mouse IgM and the agglutinin (agglutinin titer, 128). This band did not develop after absorption of the antibody preparation with *C. albicans* 9938. The concentrated agglutinin was nonreactive with antibodies specific for other immunoglobulin heavy chains. The observations described above were substantiated by the appearance of a single precipitin band which developed in immunoelectrophoretic analysis between the electrophoresed agglutinin and anti-whole mouse serum. This band was coincident with IgM migration.

By heating the agglutinin to 60°C for 5 min, its ability to agglutinate *C. albicans* 9938 cells was destroyed.

Growth-curve analyses. To examine cell surface-determinant expression as a function of growth, growth-curve analyses were carried out on nine yeast strains. Six of these strains were chosen on the basis of their reactivity with the unconcentrated agglutinin: *C. albicans* 9938 and *Torulopsis glabrata* agglutinated strongly, whereas *C. albicans* 394, *C. albicans* 17, *C. albicans* 19, and *C. albicans* 34 were weakly reactive; *S. cerevisiae* was nonreactive; and *C. albicans* 4 and *C. tropicalis* 4 autoagglutinated in saline. The agglutinin reacted similarly during the growth of *T. glabrata* and *C. albicans* 9938, and the results of the reaction of these two organisms are represented by the results of the reaction of *C. albicans* 9938 (Fig. 1c). *C. albicans* 17 represents growth-curve results obtained for *C. albicans* 4, *C. albicans* 19, and *C. albicans* 34 (Fig. 1a). With the exception of *S. cerevisiae*, all organisms, regardless of growth medium, exhibited transient autoagglutination (i.e., agglutination in saline and in uninoculated culture medium) in the first 2 to 6 h of growth. After 6 h, autoagglutination occurred only in *C. tropicalis* 4. Autoagglutination of *C. albicans* 394 appeared to be transient and media dependent, occasionally masking the observation of agglutinin reactivity in the early logarithmic and late stationary phases (Fig. 1b). At no time during growth-curve analysis was agglutinin reactivity observed in *S. cerevisiae*.

Nutritional environment (GYEP versus BHI) had an effect on agglutinin reactivity during growth (Fig. 1). In general, when agglutination reactions were positive at any point for an organism during growth, BHI broth-grown cells agglutinated more strongly than GYEP broth-grown cells.

Effect of environment on agglutinin reactivity. Because agglutinin reactivity of cells grown in GYEP differed from cells grown in BHI, additional studies were carried out to determine the effect of the nutritional environment on immunodeterminant expression. Yeast cells were generally more reactive with the agglutinin when the fungus was grown in broth media rather than on agar plates (Table 1). Enhanced agglutinin reactivity by BHI appeared to be dependent on actively growing cells because enhancement did not occur after incubation of cells in BHI at 4°C for 1 h.

TABLE 1. Effect of *C. albicans* nutritional environment on subsequent monoclonal agglutinin reactivity[a]

Growth medium[b]	Agglutinin reactivity of the following *C. albicans* strains[c]			
	9938	17	34	394
Broth				
SAB	4+	−	1+	2+
GYEP	4+	1+	1+	2+
BHI	4+	3+	2+	3+(1+)
PD	2+	1+(1+)	−	−
SAAM	4+	1+	1+	−
SAAMF	4+	3+	2+	−
Agar				
SAB	4+	−	−	−
GYEP	4+	−	1+	−
BHI	4+	1+	1+	−
PD	2+	−	−	−
SAAM	4+	2+	1+	−
SAAMF	4+	1+	2+	−

[a] Four strains of *C. albicans* were grown in various broth or solid media at 37°C for 24 h, harvested, counted, washed in saline, and tested by slide agglutination for reactivity with the agglutinin (agglutinin titer, 128).

[b] Abbreviations: SAB, Sabouraud dextrose; PD, potato dextrose; SAAM, synthetic amino acid medium; SAAMF, synthetic amino acid medium for fungi. Each medium, including GYEP and BHI, was either prepared as broth or was solidified by appropriate addition of agar (or agarose for synthetic amino acid medium and synthetic amino acid medium for fungi).

[c] Slide agglutination reactions were scored from no evidence of agglutination (−) to maximum agglutination (4+). Autoagglutination in saline or uninoculated culture medium occurred in only two instances which are indicated within parentheses.

Agglutination tests. The slide agglutination titer of pooled hybridoma culture fluid was 8 with *C. albicans* 9938. Concentration of the agglutinin by 50% $(NH_4)_2SO_4$ fractionation increased the titer to 32 and produced a tube agglutination titer of 256.

Of the 96 strains of *C. albicans* tested for agglutinin reactivity, the 19 strains of *C. albicans* which were nonreactive with unconcentrated agglutinin did agglutinate when tested with the $(NH_4)_2SO_4$-concentrated agglutinin (Table 2). All strains of *C. tropicalis* and three strains of *T. glabrata* were reactive, but none of the other yeast strains agglutinated with unconcentrated or concentrated agglutinin.

Absorption and extraction procedures. Whole cell preparations were used to test the ability of individual yeast strains to absorb the agglutinin from hybridoma culture supernatant fluid. These results are compared with absorption tests which were carried out with soluble antigen extracts prepared from several of the same strains (Table 3). In general, strains which reacted strongly with the agglutinin completely absorbed out agglutinin reactivity, whereas the capacity to absorb out the agglutinin could not be detected from either whole cells or soluble antigen extracts prepared from strains of yeast which were nonreactive. Whole cell extracts and soluble antigen extracts prepared from weakly agglutinin-positive strains (*C. albicans* 394 and *C. albicans* 19) minimally reduced agglutinin titers in absorption experiments. These results indicate that cells which do not agglutinate when mixed with the monoclonal antibody are, in fact, not expressing the antigen on their surface.

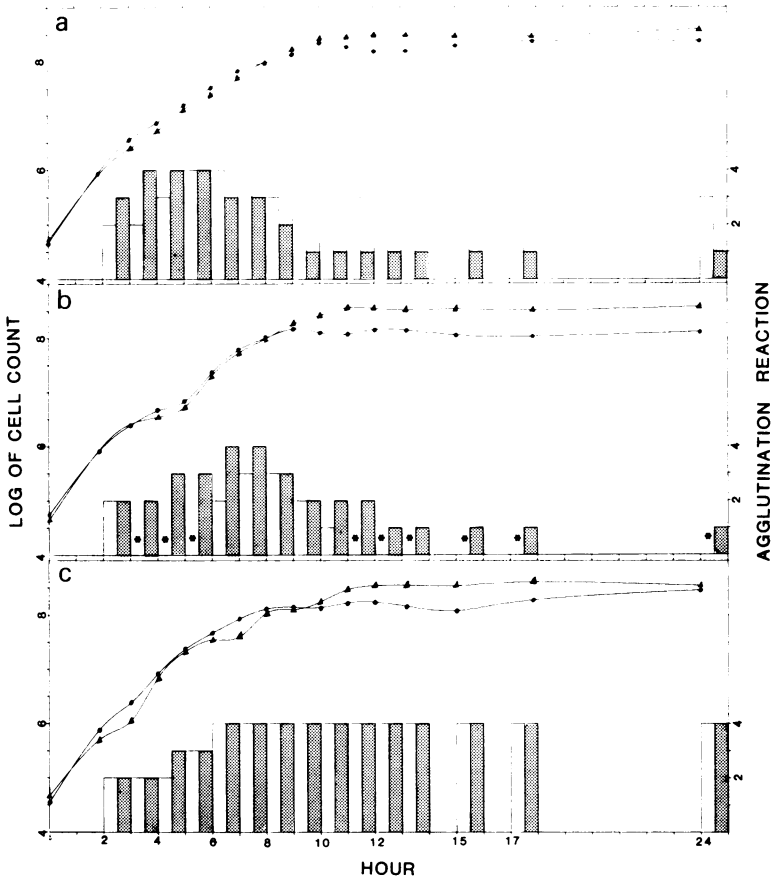

FIG. 1. Growth-curve studies carried out on several yeast strains. Yeast cells were grown for 24 h at 37°C with rotation at 160 rpm in either GYEP broth (▲) or BHI broth (●). At hourly intervals, portions were removed from each broth, and the cells were counted (hemocytometer), washed, and tested for monoclonal agglutinin reactivity (agglutinin reactivity of GYEP broth-grown cells and reactivity of BHI broth-grown cells are indicated by stipled and unstipled bars, respectively). (a) Growth curves and agglutinin reactivity of *C. albicans* 17. (b) Growth curves and agglutinin reactivity of *C. albicans* 394. (c) Growth curves and agglutinin reactivity of *C. albicans* 9938. Asterisks indicate autoagglutination of cells in both saline and concentrated growth medium. All other spaces without asterisks were untested intervals.

T. glabrata 74 agglutinated when mixed with the monoclonal antibody, and the cells absorbed the antibody from solution (Table 3). However, the antigen did not appear to be solubilized by hot NaCl since these extracts did not reduce the titer of a solution of the monoclonal antibody (Table 3).

Fluorescein isothiocyanate-labeled monoclonal antibody studies. To examine expression of the antigen on the surface of individual cells of *C. albicans* 9938, we harvested the cells from GYEP broth after various incubation periods, and the cells were assessed for reactivity with the monoclonal antibody by an indirect fluorescent antibody test.

At 5 h of incubation, ca. 50% of the blastoconidia and 50% of the pseudohyphae fluoresced strongly (4+). Only about 30% of the cells examined at 24, 48, and 96 h of incubation were reactive with the antibody, and the fluorescence was weaker (2 to 3+). In addition to these observations, *C. albicans* 394, which produces weak agglutination in the presence of the monoclonal antibody, produced weak fluorescent reactions (1 to 2+) by this test. These data further support the concept that the monoclonal antibody does not bind to cells which do not agglutinate.

DISCUSSION

Monoclonal antibodies directed against candidal cell surface determinants represent a potentially powerful tool for gaining a better understanding of fungal-host interactions. Antigens expressed on the surface of the fungal cells, which should be critical in host recognition of the parasite, may be defined during pathogenesis of candidiasis.

In the present study, a monoclonal antibody which agglutinates *C. albicans* was isolated from culture filtrates of cloned splenocyte-myeloma hybrid cells. Although several hybridomas which produced antibody against cell wall determinants of *C. albicans* (as evidenced by the enzyme-linked immunosorbent assay test) were detected, only 1 of 250 hybridomas (unpublished data) yielded an antibody which also agglutinated the yeast cells.

The agglutinin was characterized as an IgM antibody retaining activity after storage at 6 to 8°C or at −20°C, but inactivated at 60°C. The characteristics of this agglutinin are similar to those of an agglutinin which appears in mouse sera after immunization with heat-killed *C. albicans* cells (38).

Although our intent was to address the dynamic nature of antigen expression in *C. albicans*, we noted that the immunodeterminant appeared to vary in concentration on different strains of *C. albicans* of undetermined serogroups and was detected on *C. tropicalis* and *T. glabrata* (Table 2). The antibody did not agglutinate other *Candida* sp. The association of the antigen with the cell wall of *T. glabrata* appeared to differ from its association with the cell wall of *C. albicans* in that 1 M NaCl treatment did not remove the antigen from

TABLE 2. Monoclonal agglutinin reactivity with various yeasts

Organism[a]	No. of strains	Agglutination reaction[b]
C. albicans	42[c]	3–4+
	15[d]	2+
	19[e]	1+
	19[f]	–[g]
C. albicans var. stellatoidea	1	+
C. tropicalis	7	2–4+
C. parapsilosis	1	–
C. krusei	1	–
T. glabrata	2	4+
	1	2+
Cryptococcus neoformans	1	–
Cryptococcus terreus	1	–
Rhodotorula sp.	1	–
S. cerevisiae	1	–
Trichosporon beigelii	1	–
Phaeococcomyces exophiliae	1	–
Blastoschizomyces pseudotrichosporon	1	–

[a] Yeasts were grown in GYEP broth at 37°C for 24 h (48 h for *Trichosporon beigelii*, *Phaeococcomyces exophiliae*, and *Blastoschizomyces pseudotrichosporon*).

[b] Scoring of reactions from no evidence of agglutination (–) to maximum agglutination (4+).

[c] Includes *C. albicans* 9938, *C. albicans* 9; 81% were recent clinical isolates.

[d] 67% were recent clinical isolates.

[e] Includes *C. albicans* 17, *C. albicans* 34, and *C. albicans* 394; 79% were recent clinical isolates.

[f] Includes *C. albicans* 19; 68% were recent clinical isolates.

[g] Positive agglutination occurred with antibody concentrated by $(NH_4)_2SO_4$ fractionation.

cells of *T. glabrata*. The absence of agglutinin reactivity with some strains of yeast is apparently due to the lack of primary binding of antibody rather than inhibition of agglutination because (i) *S. cerevisiae* neither agglutinated with antibody nor absorbed the agglutinin from solution, (ii) weakly reactive strains of *C. albicans* effected only small reductions of agglutinin titer after absorptions, (iii) a weakly reactive strain which was mixed with the agglutinin and then washed with saline was weakly reactive with fluoresceinated anti-mouse immunoglobulin.

Our results indicate that the expression of at least one cell wall determinant is dependent upon species, strain, nutrition, and growth phase. Growth-curve analyses revealed three patterns of reactivity. (i) Agglutinin reactivity in several organisms steadily increased from 2 to 4+ during the early logarithmic phase, reaching 4+ reactivity by 6 h and remained 4+ throughout the remainder of the experiment (Fig. 1c). (ii) Agglutinin reactivity in several organisms reached its maximum (4+) by 5 h, and reactivity after 8 h was media dependent (Fig. 1a). (iii) Agglutinin reactivity peaked (4+) only briefly at 6 to 8 h before and after which time both agglutination and autoagglutination were transient and media dependent (Fig. 1b). Furthermore, in media comparison studies, cells grown in either complex or chemically defined broth media tended to react with the agglutinin more strongly than cells grown on the surface of solidified media (Table 1).

Fluorescent antibody studies of surface antigen expression on *C. albicans* 9938 correlated well with agglutinin reactivity observed at 5 h of incubation. The 24-h fluorescence of *C. albicans* 9938 cells appeared weaker than the 5-h fluorescence, whereas slide agglutinin reactivity remained strong. This may reflect the increased sensitivity of fluorescent antibody tests over agglutination tests. During the most reactive growth phase (5 h), only 50% of the cells appeared to react with the monoclonal antibody. This may indicate either that some cells are suppressed from antigen expression or that cells alternate from expression to nonexpression and are shedding the antigen. To support the latter hypothesis, antigen is readily solubilized by relatively mild (1 M NaCl) extraction procedures, and soluble antigen is detectable in culture filtrates (unpublished data). These ideas may be most appropriately tested by studying cells in synchrony, a feat we were unable to maintain in *C. albicans* for more than one generation.

Many previous investigators who used absorbed polyclonal antisera (6, 9–12, 21, 34–40) have examined cell surface determinants and cytoplasmic antigens mainly for taxonomic purposes. Results of these studies disagree on points as basic as whether *C. albicans* exhibits antigenic homogeneity or heterogeneity (6, 11, 40). These studies may presuppose a static antigen display on the surface of *C. albicans*. In accordance with some of these studies (11, 12), we found the antigen to be expressed by all strains of *C. albicans* tested and by strains of *C. tropicalis* and *T. glabrata*. Furthermore, although the quantity of antigen expressed on the cell surface is dependent on growth phase and nutritional factors, yeast species which do not react with the antibody seem incapable of antigen production under any condition.

Our observations suggest that classifications of *C. albicans* into two or three serological groups (6, 11, 25, 40) may be oversimplified in that antigenic expression appears to be a dynamic process influenced by strain, growth phase, and environment. For example, using only two types of media, we observed three patterns of agglutinin reactivity during the growth of various strains of *C. albicans* (Fig. 1). Disagreements regarding serological classification of yeasts (6, 11, 25, 40) may reflect problems which stem from the varied methods of antisera production, the varied nature of polyclonal antibody responses, and the different immunological tech-

TABLE 3. Agglutinin absorptive ability of various yeast

Organism used for absorption[a]	Agglutination titer[b]	
	After whole cell absorption	After solubilized antigen extract absorption[c]
C. albicans 9938	2	4
C. albicans 394	32	128
C. albicans 4	2	4
C. albicans 19	16	ND[d]
T. glabrata 74	2	128
S. cerevisiae	32	128
C. tropicalis 4	2	ND
Saline control	32[e]	128[f]

[a] Organisms used for absorption studies were grown in both BHI and GYEP broths for 24 h at 37°C. Absorption results were identical for organisms grown in either medium.

[b] Semiquantitative tube agglutination titers were determined with *C. albicans* 9938 as the antigen. The numbers represent the reciprocal of the highest dilution of agglutinin which retained reactivity.

[c] Water-soluble extract obtained from whole cells after treatment with 1 M NaCl at 68°C.

[d] ND, Not done.

[e] Monoclonal antibody (pooled culture supernatant fluid from cloned hybridoma).

[f] Monoclonal antibody concentrated by $(NH_4)_2SO_4$ fractionation.

niques employed to produce them, as well as the different growth environments of the test organisms.

Although our observations have been carried out only on cells grown in vitro, to find profound surface changes occurring during pathogenesis of candidiasis would not be surprising. In fact, this has been recently suggested by others who have used conventional antisera in their studies (30, 31). Thus, surface antigenic changes may provide an effective method by which *C. albicans* eludes host defenses.

Antibodies specific to antigens transiently expressed by the fungus would be expected to have minimal impact during pathogenesis of candidiasis. Alternatively, antibody directed against surface antigens expressed constitutively may have important effects on the outcome of this disease. Further studies are in progress to test some of these ideas.

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