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Antigenic Differences in the Surface Mannoproteins of *Candida albicans* as Revealed by Monoclonal Antibodies

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Monoclonal antibodies to *Candida albicans* were prepared with blastoconidia bearing germ tubes used as the immunogen. Four antibodies reacted by immunofluorescence with surfaces of *C. albicans* as well as *Candida stellatoidea*, *Candida tropicalis*, and several strains of *C. albicans*, but not with *Torulopsis glabrata*. One antibody reacted with *Saccharomyces cerevisiae*. In addition, the monoclonal antibodies precipitated material of approximately 200 kilodaltons when tested against metabolically labeled blastoconidia digests. The monoclonal antibodies exhibited heterogeneous staining of *C. albicans* surfaces, as shown by immunofluorescence. None of the monoclonal antibodies were specific to germ tubes. More importantly, however, two of the monoclonal antibodies reacted with the mannoprotein precipitin arc of *C. albicans* that was produced by reference rabbit polyclonal antisera by crossed immunoelectrophoresis, thus linking the heterogeneity seen by immunofluorescence to the heterogeneity in mannoproteins. Finally, three of the monoclonal antibodies reacted with a glycan fraction of cell digests, indicating their reactivity with the carbohydrate portion of the mannoprotein.

In previous studies on the dimorphic yeast *Candida albicans*, we showed that the primary surface epitopes of this organism reside on mannoprotein components of 155 kilodaltons (kDa) or greater which are released by enzymatic digestion of cell walls of whole organisms (18–20). In addition, these components, when prepared from digests of germ tubes, contain germ tube-specific epitopes. In this study, we sought to obtain specific monoclonal antibody probes for surface antigens of these two forms of growth. With such probes, it might be possible to isolate surface components and determine the chemical structures that are responsible for the immunological differences that have been observed. Furthermore, the production of monoclonal antibodies could facilitate the development of serological assays specific for filamentous or spherical growth. In studies by others in which monoclonal antibodies directed against the surface of *C. albicans* were used, it has been shown that some surface antigens are variably expressed, as shown by agglutination assays and electron microscopy (4, 6, 9). Results of this study confirmed the variability of expression of surface antigens by immunofluorescence assays. In addition, results of this study indicated that the mannoprotein surface material is itself antigenically heterogeneous, suggesting that surface variability is, at least in part, the result of heterogeneity in surface carbohydrates.

MATERIALS AND METHODS

Organisms and culture. *C. albicans* 441B and B311 were the same strains as those used in previous studies (18). Organisms were grown either as blastoconidia in glucose-salts-biotin media or as blastoconidia bearing germ tubes in medium 199, as described previously (18). *C. albicans* 28367 (serotype A) and 38696 (serotype B), *Candida stellatoidea* 36232, *Candida tropicalis* 44508, and *Torulopsis glabrata* 32312 were purchased from the American Type Culture Collection, Rockville, Md. *Saccharomyces cerevisiae*, diploid and without markers, was obtained from Breck Byers, University of Washington, Seattle, Wash. *S. cerevisiae* and *T. glabrata* were grown in Sabouraud broth instead of glucose-salts-biotin media. All organisms were cloned before use.

For immunization, live *C. albicans* bearing germ tubes were washed three times and suspended in TES [*N*-tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid; pH7.4]–saline (TES-saline). For the enzyme-linked immunosorbent assay (ELISA), washed oganisms (10^8/ml) in 0.15 M NaCl containing 10 mM TES–saline were stored at −20°C until use. For the immunofluorescence assay, washed organisms were fixed in Formalin as described previously (18).

Immunization protocol. Two female BALB/c mice were immunized with *C. albicans* B311 for the first fusion, and one BALB/c mouse was immunized with strain 441B for the second fusion. The first immunization consisted of 0.1 ml containing 10^5 organisms administered subcutaneously. Subsequent immunizations (seven injections of 5×10^5 to 1×10^7 organisms at 2-week intervals) were given intraperitoneally; except for the last injection, which consisted of 1×10^5 organisms in 0.5 ml given intravenously 3 days before the animals were sacrificed.

Hybridization procedure. Cells were hybridized as described previously (16), except that immune lymphocytes were combined at a ratio of 5:1 and 4:1 in the first and second fusions, respectively. Cells were isolated and cloned as described elsewhere (12).

ELISA. ELISA was used to detect monoclonal antibodies in cell culture supernatants and ascites fluids and was carried out as described previously (18), with a few modifications. In the first fusion, supernatants were initially assayed with three antigenic preparations: blastoconidia, germ tubes, and autoclaved blastoconidia (to detect antibodies to heat-stable antigens) from *C. albicans* B311. For the second fusion,

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similar antigens were prepared from strain 441B. *S. cerevisiae* cells were also used for both fusions. Frozen antigens in 0.01 M phosphate buffer (pH 7.4) were plated on microtiter plates (Immulon II) at a concentration of 10^6/ml. Preparations containing germ tubes were sonicated briefly to disperse clumps before the microtiter plates were coated, but organisms remained as whole cells. Affinity-purified, horseradish peroxidase-conjugated, heavy chain-specific goat anti-mouse immunoglobulin G (IgG) and IgM (Zymed, San Francisco, Calif.), at a 1:3,000 dilution, were used to detect monoclonal antibodies. Immune mouse serum which was positive by immunofluorescence was used as a positive control at a 1:100 dilution. Normal mouse serum served as a negative control.

Immunofluorescence assay. The immunofluorescence assay was carried out as described previously (18), except that fluorescein isothiocyanate-conjugated, goat anti-mouse IgM, μ-chain-specific antibodies (1:100 dilution; Cappel Laboratories, West Center, Pa.) were used to detect monoclonal antibodies in cell culture supernatants or ascites fluid which were diluted 1:500 (monoclonal antibody 4G8) to 1:1,000 (all monoclonal antibodies). Immune mouse serum was used at a 1:10 dilution as a positive control, and normal mouse serum was used as a negative control.

Crossed immunoelectrophoresis. A soluble antigen from germ tubes was prepared by homogenization (Braun homogenizer) of a mixture of washed organisms (4 g [wet weight])–4 ml of 40 mM TES-saline–21 g of glass beads (diameter, 0.5 mm; VWR, Seattle, Wash.) for 20 min (two 10-min periods) with continuous cooling with CO_2. The homogenate was filtered through glass wool to remove beads and cell walls, dialyzed against 10 volumes of TES-saline (10 mM) and 5 volumes of Veronal (Winthrop Laboratories, Div. Sterling Drug Co., New York, N.Y.) buffer with an ionic strength of 0.02, lyophilized, and suspended to a protein concentration of 3.5 mg/ml, as determined by the method described by Lowry et al. (13). The antigen was fractionated and stored at −70°C until use.

Two-dimensional (crossed) electrophoresis was done as described previously (10, 21). For first-dimension electrophoresis, an agarose (SeaKem agarose; FMC Corp., Marine Colloids Div., Rockland, Maine) (electroendosmosis, $[-M_r]$ = 0.16 to 0.19) bed (0.7% in barbital buffer [pH 8.6]; ionic strength, 0.2) layer (depth, 2 mm) was poured on to agarose-precoated glass slides (43 mm^2). Antigen (7 μl or 24 μg) was placed in a well (depth, 4 mm); and when appropriate, bovine serum albumin (5 μl of a 1-mg/ml solution) stained with 1 mg of bromophenol blue per 5 ml (Sigma Chemical Co., St. Louis, Mo.) was used as a tracer. The sample was electrophoresed at 8 V/cm for 35 to 40 min. The second-dimension gel contained rabbit polyvalent antiserum (7.5%) or monoclonal antibody (0.5 to 1%) ascites fluid and was run at 1.5 V/cm overnight.

For intermediate gel electrophoresis (2), a 0.7-cm-wide gel containing 10% ascites fluid or 9.5% concanavalin A (wt/vol; Sigma) was interposed between the first and second gels, which contained 7.5% rabbit polyclonal antibody. Electrophoresis was carried out as described above.

After electrophoresis, slides were washed exhaustively in saline and then were washed with water, dried, and stained with Coomassie blue R-250 (Bio-Rad Laboratories, Richmond, Calif.).

Radiolabeling of germ tubes with [2-^{3}H] mannose. A homogeneous population of blastoconidia in the stationary phase was prepared by inoculating a loopful of organisms from a Sabouraud agar slant into 100 ml of medium modified from that of Lee et al. (11), which did not contain amino acids but which contained sucrose instead of glucose; the medium also contained the following, in grams per liter: $(NH_4)_2SO_4$, 5 g; $MgSO_4 \cdot 7H_2O$, 0.2 g; K_2HPO_4, 2.5 g; NaCl, 5 g; sucrose, 10 g; biotin, 0.04 g. The pH was adjusted to 6.8 with HCl. This mixture was incubated on a gyratory shaker (New Brunswick Scientific Co., Inc., Edison, N.J.) at 200 rpm for 48 h at 25°C. Organisms were centrifuged and washed twice in TES-saline and held at room temperature overnight. For radiolabeling of germ tubes, washed blastoconidia (8×10^6/ml) were suspended in the medium described above containing 1% bovine serum albumin (Sigma), methionine (0.01 g/liter), and 11 μCi of D-[2-^{3}H]mannose (22 Ci/mM; ICN Radiochemicals, Irvine, Calif.) per ml (1 μCi = 37 kBq). Organisms in the labeling medium were incubated at 37°C for 4 h until germ tubes formed that were 2 to 3 times the blastoconidia diameter. Labeled blastoconidia were prepared in the same medium but were inoculated at 10^7/ml and incubated at 25°C with shaking for 7 h. Under these conditions, blastoconidia incorporated approximately 90% of the radioisotope and germ tubes incorporated 80%.

Antibody binding of the glycan fractions and cell digests. To obtain immunoreactive carbohydrate free from proteins, a Zymolyase 60000 (Kirin Brewery, Tokyo, Japan) digest of [2-^{3}H]mannose-labeled germ tubes (*C. albicans* 441B) was exhaustively degraded with pronase (Calbiochem-Behring, La Jolla, Calif.) exactly as described previously (1, 20). Radioimmunoprecipitations with the glycan fraction and cell digests were done as described previously (20), except that an additional 1 h of incubation with affinity-purified rabbit anti-mouse IgM (20 μl; μ-chain specific; Zymed) was done after the initial 1 h of incubation and before the addition of staphylococci bearing protein A. The amount of radiolabeled glycan precipitated by the monoclonal antibodies was quantitated by scintillation counting. Antigen precipitated from the Zymolyase digest was visualized by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and autoradiography as described previously (19, 20). An IgM monoclonal antibody, LE-4, directed against the C complex of *Chlamydia trachomatis*, which had an IgM titer of 11,600, as measured by immunofluorescence, served as a negative control.

RESULTS

Isolation of hybridomas producing anti-*Candida albicans* antibodies. Screening by ELISA was designed to detect clones that produced antibodies which reacted with organisms bearing germ tubes but which did not react with blastoconidia. No stable clones with this reaction pattern were isolated. In the first fusion after 12 days of culture, 52 of 576 wells contained culture fluids which reacted with at least one of the antigens tested in the ELISA, whereas only 13 of 576 wells from the second fusion were positive. Of the 65 ELISA-positive clones, 5 were positive by immunofluorescence. Four remained stable after further propagation and were cloned twice by limiting dilution, frozen, and used in ascites production. All monoclonal antibodies (4E6, 5B7, 5B2, and 4G8) reacted with the three *C. albicans* antigens tested: blastoconidia, blastoconidia bearing germ tubes, and autoclaved blastoconidia. Only one antibody (4G8) reacted with *S. cerevisiae*. All were IgM, as determined by immunofluorescence and ELISA.

Reaction patterns of monoclonal antibodies with *Candida* species by immunofluorescence. The titer of monoclonal antibodies 4E6, 5B7, and 4G8 was 1,000 and that of 5E2 was

TABLE 1. Immunofluorescent reactions of different yeast species with polyvalent and monoclonal antibodies

Organism	Reaction of the following antibodies		
	Unadsorbed polyvalent	Blastoconidium-adsorbed polyvalent (stained only germ tubes)	Monoclonal[a]
C. albicans (includes five strains)[b]	+	+	+
C. stellatoidea[b]	+	+	+
C. tropicalis[c]	+	−	+
T. glabrata[c]	−	−	−
S. cerevisiae[c]	+	−	−

[a] Monoclonal antibodies 4E6, 5B7, 4G8, and 5E2. A + sign indicates any immunofluorescent reaction.
[b] Blastoconidia bearing germ tubes.
[c] Blastoconidia only.

5,600, when tested against *C. albicans* 441B by immunofluorescence. All monoclonal antibodies reacted to some degree with the *Candida* species tested but not with *T. glabrata* (Table 1). Monoclonal antibody 4G8, which reacted with *S. cerevisiae* in the ELISA, did not react with *S. cerevisiae* by immunofluorescence. Neither boiling of the organisms in saline for 10 min nor treatment with pronase altered the reactivity patterns that were observed. While all the monoclonal antibodies reacted with the *C. albicans* strains tested, the reaction patterns varied from strain to strain. For example, monoclonal antibody 4E6 stained both blastoconidia and germ tubes of strain B311 evenly (Fig. 1A), but reacted unevenly with strain 441B (Fig. 1B and C) and stained predominantly blastoconidia in strains 28367 and 38696 (Fig. 1D, E, and F). In addition, the different monoclonal antibodies reacted differently to the same strain. For example, monoclonal antibody 4E6 reacted weakly with the germ tubes of strain 28367, but the other three monoclonal antibodies failed to react with germ tubes of this strain (data not shown). In the case of monoclonal antibody 4E6, the presence or absence of the antigen was related to the age of the organisms. While 100% of log-phase blastoconidia of strain 441B reacted with monoclonal antibody 4E6, approximately 50% of blastoconidia scraped from a 2-month-old refrigerated slant stained, although organisms were 95% viable, as judged by exclusion of methylene blue dye and germ tube formation (data not shown).

Identification of the yeast surface antigen with monoclonal antibodies. When the monoclonal antibodies were tested by Ouchterlony double diffusion or rocket immunoelectrophoresis, precipitin bands were detected with ascites fluids containing monoclonal antibodies 4E6 and 5B7 (data not shown). To compare the reactivities of monoclonal antibodies with that of the rabbit polyclonal antiserum, intermediate gel electrophoresis was performed. Monoclonal antibody 4E6 (Fig. 2C, arrow) precipitated a component directly under the large, diffuse band precipitated by the polyclonal rabbit antibody (Fig. 2A, arrow), suggesting that the diffuse band was heterogeneous and that the monoclonal antibody reacted with a part of this peak. The large, diffuse peak precipitated by the rabbit polyclonal antibody was mannoprotein, as shown by its ability to be precipitated by concanavalin A (Fig. 2B). In addition, both the peak recognized by the monoclonal antibody and the one recognized by polyclonal antibody contained antigens that were present on the surface of the blastoconidia. When the polyclonal antiserum or ascites fluid was adsorbed exhaustively with live blastospores, antibodies to this component were removed (Fig. 3B and D). Similar results were seen with monoclonal antibody 5B7. Thus, monoclonal antibodies 4E6 and 5B7 precipitate a mannoprotein, surface component of yeast cells.

Carbohydrate nature of the determinants recognized by monoclonal antibodies. Three of the four anti-*C. albicans* monoclonal antibodies (4E6, 4G8, and 5E2) reacted strongly with a glycan fraction (20) as compared with controls in which only the rabbit anti-mouse IgM second antibody (no monoclonal antibody) or an irrelevant monoclonal antibody (anti-*Chlamydia trachomatis* IgM) and a second antibody (Table 2) were used. Anti-*C. albicans* monoclonal antibody 5B7 reacted only weakly or not at all with the glycan fraction. None of the monoclonal antibodies reacted with peak II glycan (data not shown). A component of approximately 200 kDa from Zymolyase-digested [2-^{3}H]mannose-labeled blastoconidia of *C. albicans* 441B was precipitated by three of the four monoclonal antibodies (4E6, 5B7, and 4G8). Controls in which only rabbit anti-mouse IgM second antibody (no monoclonal antibody) or an irrelevant monoclonal antibody (anti-*Chlamydia trachomatis* IgM) was used were negative (Fig. 4). Anti-*C. albicans* monoclonal antibody 5E2 did not precipitate components from this cell digest.

DISCUSSION

The immunofluorescent reactions of the monoclonal antibodies differed from those observed with rabbit polyclonal serum in that individual cells showed variable expression of surface epitopes. Variable surface antigen expression, as detected by immunofluorescence and electron microscopy

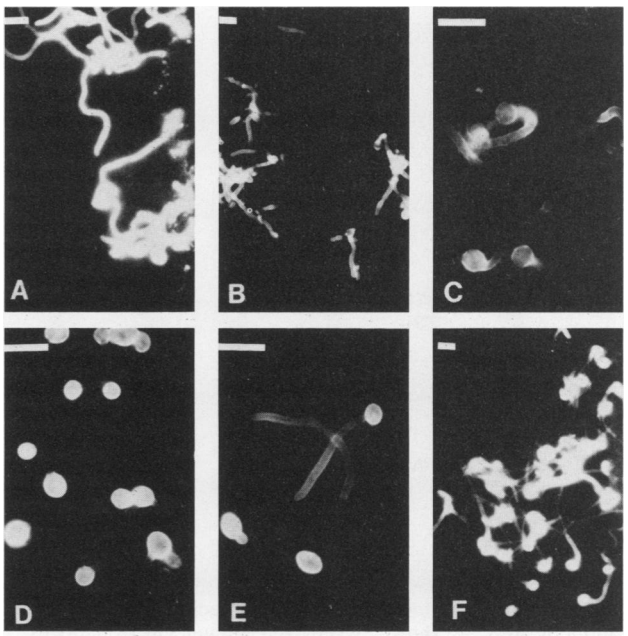

FIG. 1. Variable immunofluorescent staining patterns of different strains of *C. albicans* stained with ascites fluid containing monoclonal antibody 4E6. All assays were done for 3 h on cells which formed germ tubes. Ascites fluid containing monoclonal antibody 4E6 was used at a 1:1,000 dilution. Even staining was seen with *C. albicans* B311 (A), while other strains showed heterogeneous staining: 441B (B and C), ATCC 28367 (D and E), ATCC 38696 (F). Bars, 10 μm.

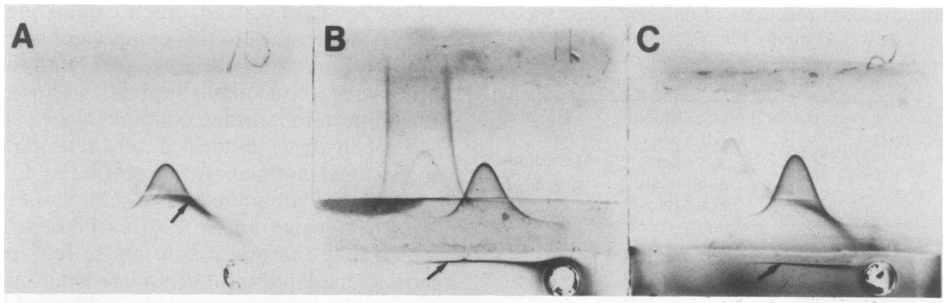

FIG. 2. Recognition of a mannosaccharide-containing component of *C. albicans*, as shown by intermediate gel electrophoresis. The wells contained a homogenate (Braun homogenizer) of *C. albicans* 441B (24 μg), and upper gels contained polyvalent rabbit antiserum (7.5%). Intermediate gels contained fetal calf serum (A) and concanavalin A (B). The tall peak on the left in panels A and B is a bovine serum albumin marker. (C) Monoclonal antibody 4E6. The diffuse peak was not altered by the fetal calf serum control (arrow in panel A), but was completely precipitated by concanavalin A (arrow in panel B) and partially precipitated by monoclonal antibody 4E6 (arrow in panel C).

with colloidal gold-labeled monoclonal antibodies, also has been seen by Brawner and Cutler (4) and has been observed with polyclonal antiserum as well. As we found with monoclonal antibody 4E6, the antibodies of Brawner and Cutler (6) reacted with a lower percentage of blastoconidia in the stationary phase than in the log phase. While one of the monoclonal antibodies of Brawner and Cutler (6) also detected variable expression on germ tube formation of one strain of *C. albicans*, we noticed differences among strains in the production of a given determinant on germ tubes, as well as differences in staining of individual germ tubes within the same culture. The mechanism for this expression is unclear; but it could represent shedding, as cytological evidence suggests (4, 9); degradation of surface components; or synthesis of new surface components that which mask the old growth.

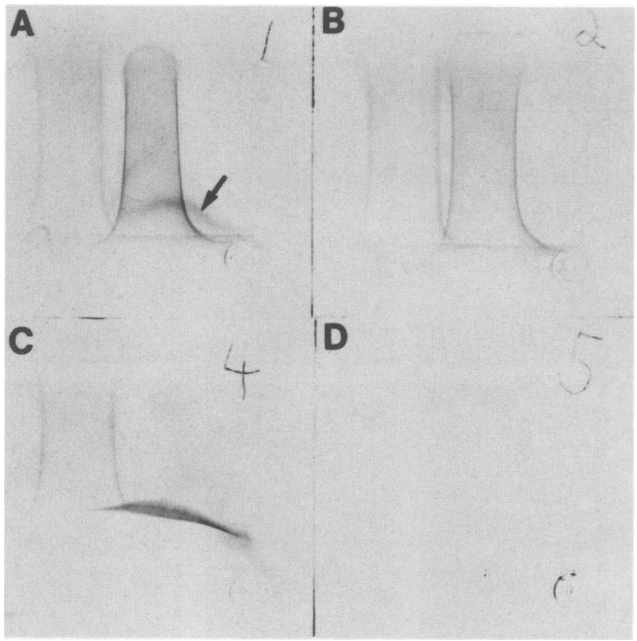

FIG. 3. Surface location of antigen precipitated by monoclonal antibody 4E6, as shown by crossed immunoelectrophoresis. Adsorption of rabbit polyclonal antiserum or ascites fluid containing monoclonal antibody 4E6 with blastoconidia of *C. albicans* 441B removed antibodies to the diffuse peak. The wells contained a soluble extract of *C. albicans* 441B (24 μg); and the upper gels contained unadsorbed rabbit polyclonal antibody (7.5%) (A), blastoconidium-adsorbed rabbit polyclonal antibody (7.5%) (B), unadsorbed monoclonal antibody 4E6 (1%) (C), and blastoconidium-adsorbed monoclonal antibody (1%) (D).

Several studies involving monoclonal antibody production to cell wall extracts or whole cells of *C. albicans* or *C. tropicalis* have produced antibodies with characteristics similar to the ones that we have described (4–6, 8, 14, 15; T. M. Kerkering and A. Espinel-Ingroff, Abstr. Annu. Meet. Am. Soc. Microbiol. 1983, F41, p. 389; E. Reiss, L. de Repentigny, R. R. Kuykendall, A. W. Carter, and C. H. Aloisio, Abstr. Annu. Meet. Am. Soc. Microbiol. 1984, F41, p. 299; W. L. Chaffin, J. Szkudlarek, and K. J. Morrow, Abstr. Annu. Meet. Am. Soc. Microbiol. 1985, F11, p. 366). As a class, these monoclonal antibodies react with epitopes which are heat resistant, trypsin resistant, periodate-sensitive, or all three. They also react with mannoprotein-containing extracts from *Candida* cells or cell walls; are specific to yeast cell surfaces, as shown by agglutination or immunofluorescence; and are of the IgM class, with the exception of one IgG-producing clone (Reiss et al., Abstr. Annu. Meet. Am. Soc. Microbiol. 1984). These characteristics suggest that carbohydrates are the principal antigenic determinants on *Candida* cell surfaces that are recognized by monoclonal antibodies. This conclusion is supported by our results, which indicated that three of four monoclonal antibodies react with a glycan fraction which is essentially free of amino acids and that monoclonal antibodies react with antigen which was exhaustively digested with protease (4). It should be noted, however, that hybridomas selected for propagation have been those which were positive by immunofluorescence or agglutination, most likely the ones directed against mannoprotein. Antibodies to less-abundant surface epitopes might not be detected.

The precipitation of components from [2-^{3}H]mannose-labeled blastoconidia digests, which migrated close to the

TABLE 2. Radioimmunoprecipitation of the *C. albicans* glycan fraction by monoclonal antibodies

Antigen[a]	cpm of the following monoclonal antibodies[b]:					
	Control[c]	4E6	5B7	4G8	5E2	None
10	ND[d]	41,760	505	3,637	1,886	ND
20	180	57,885	313	4,314	2,679	115

[a] Micrograms (dry weight) of [2-^{3}H]mannose-labeled *C. albicans* glycan.
[b] Ascites fluid (4 μl) containing anti-*Candida* monoclonal antibodies 4E6, 5B, 4G8, and 5E2.
[c] Ascites fluid (4 μl) containing anti-*Chlamydia* monoclonal antibody LE4.
[d] ND, Not done.

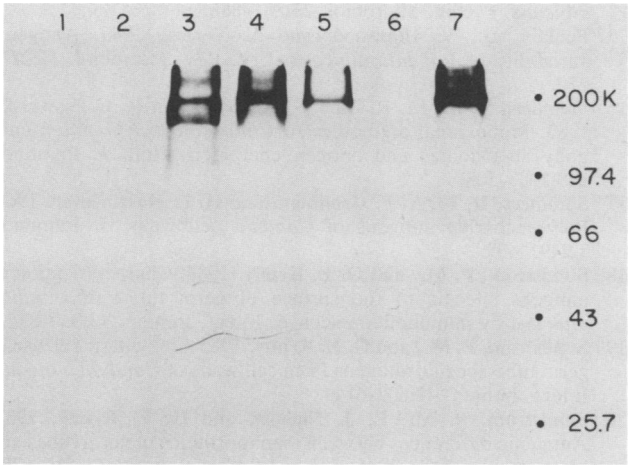

FIG. 4. Monoclonal antibody binding of Zymolyase blastospore cell wall digests. Sodium dodecyl sulfate-polyacrylamide gel electrophoretograms of D-[2-^{3}H]mannose-labeled blastoconidia cell wall digests (5×10^6 cpm) from *C. albicans* 441B without monoclonal antibody (lane 1) or with 3 μl of ascites fluid containing anti-*Chlamydia trachomatis* IgM LE4 (lane 2); anti-*C. albicans* monoclonal antibodies 4E6, 5B7, 4G8, and 5E2 (lanes 3 to 6, respectively); or rabbit polyclonal antiserum to *C. albicans* (lane 7). Bands were detected by autoradiography. K, Molecular weight in thousands.

200,000-molecular-weight marker, confirmed results of studies with polyclonal antiserum (18), in which it was shown that these are primary surface components. In addition, the reactivity of two of the monoclonal antibodies with both the glycan fraction and the 200-kDa component proves that the 200-kDa component carries carbohydrate structures. The reasons for the failure of monoclonal antibody 5E2 to react with the 200-kDa component are unknown, especially since this monoclonal antibody precipitated a component with a similar molecular weight from [^{125}I]- or [^{35}S]methionine-labeled blastospore digests of strain 441B (data not shown). One possibility is that the antigen and antibody concentrations were not optimal, resulting in a small amount of precipitated material which would be detectable with the stronger isotopes ^{125}I and ^{35}S, but not with ^{3}H.

The different patterns of reactivity of the monoclonal antibodies with the same digest (Fig. 4) reflect the unique specificity of each monoclonal antibody. In addition, the various reaction patterns suggest that multiple high-molecular-weight components are released in Zymolyase digests, and that these vary in their carbohydrate structures, further emphasizing the variability in surface material. The fact that monoclonal antibody 4E6 precipitated the most major bands from Zymolyase digests could explain, in part, its increased precipitation of the glycan fraction as compared with precipitation of the glycan fraction by the other monoclonal antibodies (Table 2).

A fortuitous outcome of studies with monoclonal antibodies has been further evidence for the chemical heterogeneity of mannoprotein within a strain (4, 8). This extends findings of studies with polyclonal antiserum in which it has been shown that differences in mannoprotein are responsible for the serological differences between A and B strains of *C. albicans* (17) and possibly for other strain differences (15). Two of the monoclonal antibodies that we detected (4E6 and 5B7) reacted with a portion of the diffuse concanavalin A-reactive peak precipitated by polyclonal serum, suggesting that this diffuse peak (likely mannoprotein) is composed of several different components. In addition, the precipitin lines formed by the same two monoclonal antibodies showed nonidentity when tested by Ouchterlony immunodiffusion (data not shown), an observation made by Brawner and Cutler (4) with their monoclonal antibodies. Interestingly, monoclonal antibody 5B7 reacted poorly or not at all with the glycan fraction, in spite of the fact that it had a titer (1,000) that was equivalent to those of the other monoclonal antibodies in the immunofluorescence assay against whole organisms. This suggests that monoclonal antibody 5B7 reacts with a peptide portion of the mannoprotein.

Several factors may have contributed to our inability to elicit hybridomas that secrete monoclonal antibodies specific for germ tubes. First, the immunization schedule may not have been appropriate. We immunized the mice with small numbers of live organisms, so as not to destroy any antigens, for a period of several months. The rabbit polyclonal serum was produced after comparatively large doses of Formalin-fixed antigens were administered over a shorter period. Even in the rabbit polyclonal serum, most of the antibodies produced were against common antigens; thus, it is possible that a large number of organisms is required to attain immunizing levels of the germ tube-specific determinant. Second, mice may be unable to make germ tube-specific antibodies. Third, inbred mouse strains vary greatly in their immune responses to various antigens, including *C. albicans* (3, 7). Finally, it is possible that the germ tube-specific immunofluorescent reaction seen with rabbit antiserum was the result of the fact that several different epitopes reacted with their homologous antibodies and did not consist of one repeating epitope that was present at a high density on the surface, which may be required for immunofluorescence to be seen with monoclonal antibodies. Our results indicate that, even when whole organisms bearing germ tubes are the immunogen, the monoclonal antibodies produced are similar to those produced by other investigators who have used blastoconidia, blastoconidia cell walls, or cell wall extracts devoid of filamentous components.

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