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Adherence and Receptor Relationships of *Candida albicans*

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INTRODUCTION

As a commensal organism of the mucous membranes, *Candida albicans* becomes invasive when some aspect of host immunity becomes impaired (1, 106). This statement is not meant to imply that the organism plays a very benign role in pathogenesis. Instead, several virulence factors, each of which promotes the successful colonization or invasion of tissues, have been described. The cell wall of the organism is essential to its success as a pathogen, since it is required for growth (104), provides rigidity and protection against osmotic insult, and is the site of contact between the organism and its environment. Cell surface ligands and receptors promote colonization of host cells and tissues. A proteolytic enzyme (acid carboxyl proteinase) associated with the cell surface and external environment is probably responsible for tissue invasion, which occurs as the organism undergoes morphological conversion from a yeast (blastoconidial) cell to its filamentous form. This conversion is thought to be important to the infectious process (104, 144).

The complexity of the organism is well-known to its investigators. At least three growth forms are observed in vitro. Blastoconidia normally colonize skin folds and the mucous membranes of the oral, vaginal, and gastrointestinal tracts. As stated above, conversion to a filamentous growth form occurs following germination of blastoconidia. Blastoconidia may also grow by budding or may undergo elongation, forming pseudohyphae. The influence of environmental factors on these various conversions has been studied in great detail and is not the subject of this review. For such information, the reader is directed to several other sources (104, 143, 149). In addition to this level of complexity, colony phenotypic variation has been reported (2, 71). Associated with this switching between colony phenotypes are changes in antigen expression (2) and adherence to epithelial cells (71). Ligand-host cell receptor interactions appear to be complex and strain specific (33). Finally, the host response to *C. albicans* is also complex. The type of *Candida* infection depends on the specific host defect. In general, systemic disease is usually an outcome of neutrophil depletion or defects, while cutaneous or mucocutaneous disease is a result of defects in cell-mediated immunity (104). These few examples illustrate the diversity of the organism and point to the highly adapted nature of this pathogen. The purpose of this review is to focus on the cell wall of *C. albicans*, especially the outer cell surface macromolecules that confer ligand- and receptorlike activity on the organism.

THE CELL WALL: AN OVERVIEW

The cell wall of *C. albicans* is composed primarily of the polysaccharides mannan, glucan, and chitin. Although the synthesis of the cell wall components is dynamically influenced by growth conditions and metabolic states, the literature contains fairly consistent data regarding the chemical composition of the cell wall. Mannans represent about 15.2 to 22.9% of the yeast cell wall (dry weight), or about 40% of the total cell wall polysaccharide (120). β-1,3-D-Glucans and β-1,6-D-glucans account for 47 to 60% by weight of the cell wall (30, 149). Proteins (3, 4, 6, 100, 115, 117, 129) have been reported to account for 6 to 25%, lipids for 1 to 7%, and chitin for 0.6 to 9% by weight of the cell wall (46, 72, 124, 168). The percent compositions of cell walls from yeast cells and germ tubes are relatively similar, although the amounts of alkali-soluble and -insoluble glucans and chitin from *C. albicans* vary according to the growth form (30). The spatial relationships of these polymers to each other are presented in Fig. 1.

Ultrastructural studies of the *C. albicans* cell wall have indicated a complex microarchitecture. The wall is of variable thickness and is composed of several layers that are revealed by differences in electron density. The number of layers and their morphology are variable and may be related

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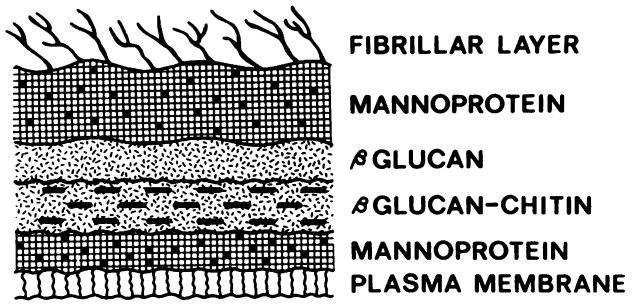

FIG. 1. Schematic diagram of the cell wall of *C. albicans*. The layers are enriched in the indicated components. However, mannoprotein is found throughout the wall (139).

to the stage of growth, growth form (yeast form or germ tube), strain selected for study, medium used to grow the cells, or the fixation procedure (51). Most investigators have described five layers within the cell wall (26, 35, 65, 136); however, Poulain et al. (112, 113) have observed as many as eight or nine cell wall layers in *C. albicans*, although these results covered a variety of wall types from cells grown on different media for various amounts of time. Cassone et al. (24) have shown that after prolonged starvation of *C. albicans*, the cell wall layers composed of mannan gradually disappear. Subsequently, the cell wall increases in thickness as the other cell wall layers also disappear. This process was reversed when favorable environmental conditions were restored. Although the appearance of the cell wall suggests a discrete packaging of the various macromolecules, the layering is more likely a result of differences in the proportions of the components in each layer rather than absolute differences in chemical composition (104).

The major chemical components in the wall layers of *C. albicans* have been determined by experiments using various extractants, chemical stains, conjugated antibodies, and conjugated lectins. The literature is in general agreement that the mannan polysaccharides are located throughout the cell wall and that they are predominant in areas of high electron density (25, 47, 50, 112, 113, 155, 160, 162). The inner cell wall layers are composed mostly of chitin and glucan (139). These components provide rigidity and appear essential for cell division (59, 103, 140, 161). There are three types of β-glucan (139): a highly branched β-1,6-glucan, a highly branched β-1,3-glucan, and a mixed β-1,3–β-1,6-glucan complexed with chitin. Extraction of cell walls with either acid or alkali yields glucans with various proportions of either β-1,3- or β-1,6-linked glucose moieties. As stated above, the proportions of certain kinds of glucans are quite different for yeast forms and germ tubes. During early stages of germ tube formation, there is almost an exclusive synthesis of β-1,3-glucans (139). Chitin is found in yeast forms, germ tubes, and hyphae, although the proportion is higher in hyphae (30).

MANNOPROTEINS OF *C. ALBICANS*

The outer fibrillar layer of the cell wall of both yeast and filamentous cells is composed of mannan or mannoprotein, although this material is probably deposited at several sites within the cell wall also. The outer layer has also been described as a mucous coat or capsule (40, 116) and is sloughed off during infection (121, 122). The amount of mannoprotein produced by cells depends on the growth medium and cell age (36–39, 77, 92–94, 130). For example, incorporation of galactose or sucrose instead of glucose in the culture medium results in a greater production of the fibrillar outer layer (92, 94). Sucrose- or galactose-grown cells tend to be more adhesive (see below). The outer layer is easily removed and appears to extend into strands that link the fungal cells together or allow cells to adhere to other surfaces (77, 157, 162). The fact that intact *C. albicans* cells can be agglutinated with concanavalin A (ConA) or with monoclonal antibodies (MAb) specific for mannan epitopes confirms that mannan is present at the outer surface of the cell wall (25, 66, 139).

Mannan was first identified as the major cell surface antigen of *C. albicans* before the chemical structure was understood (150). On the basis of adsorption and agglutination studies, *C. albicans* was initially grouped into two major serotypes, designated A and B, by Hasenclever and Mitchell (54). Since then, many attempts have been made to determine the structure of the major epitopes of each serotype. The current structural model of the mannoprotein holds that the oligosaccharide is covalently linked to protein either as short oligosaccharides (O linkages via serine or threonine residues) or as a larger, complex oligosaccharide linked via N linkages involving two units of *N*-acetyl-D-glucosamine and the asparagine residues of the polypeptide. In this regard, the general structure of the *Candida* mannan is quite similar to that of the *Saccharomyces cerevisiae* mannan (49). The O-linked and N-linked oligosaccharides account for about 10 and 90%, respectively, of the total mannose residues. The N-linked mannan consists of mannose residues linked by α-1,6 linkages to form a backbone that is highly substituted with short oligomannoside chains containing major internal α-1,2 linkages and a small proportion of α-1,3 linkages (49, 139, 154) (Fig. 2). There is some disagreement over the precise structures that function as specific immunodeterminants; however, there is general agreement that the mannan side chains contain the epitopes that confer serospecificity. Data from three types of analysis—acetolysis, methylation-fragmentation, and hapten inhibition—have led to the conclusion that the types of mannose linkages are responsible for serospecificity (120, 123). For example, the major epitope of serotype A contains predominantly a straight chain of α-1,2-linked mannose residues with a terminal α-1,3 mannose residue. Determinants from serotype B are shorter but more complex, lacking a terminal α-1,3 linkage but having a single C-1–C-2–C-3 branch point and an additional internal α-1,6 mannosyl residue, which probably links the chain to the mannan backbone but does not play a direct role in conferring serospecificity. A model of the type-specific epitope for serotype A is shown in Fig. 3.

Tsuchiya and his colleagues studied many *Candida* species in cross-agglutination tests and classified 22 species on the basis of their complement of heat-stable and heat-labile antigens (164). Several serofactors were delineated, resulting in the scheme shown in Table 1. Thus, *C. albicans* serotype A possesses factor 6 (Fig. 3) and occasionally factor 13b, while serotype B possesses factor 13b and occasionally factor 7 (164). Cross-reactivity among *Candida* species is observed; *C. albicans* serotype A is more similar antigenically to *Candida tropicalis* than it is to serotype B *C. albicans*. *Candida stellatoidea* is similar to *C. albicans* serotype B.

Serological and DNA typing of *C. albicans* strains from a variety of human populations has been done (5, 16, 114, 147).

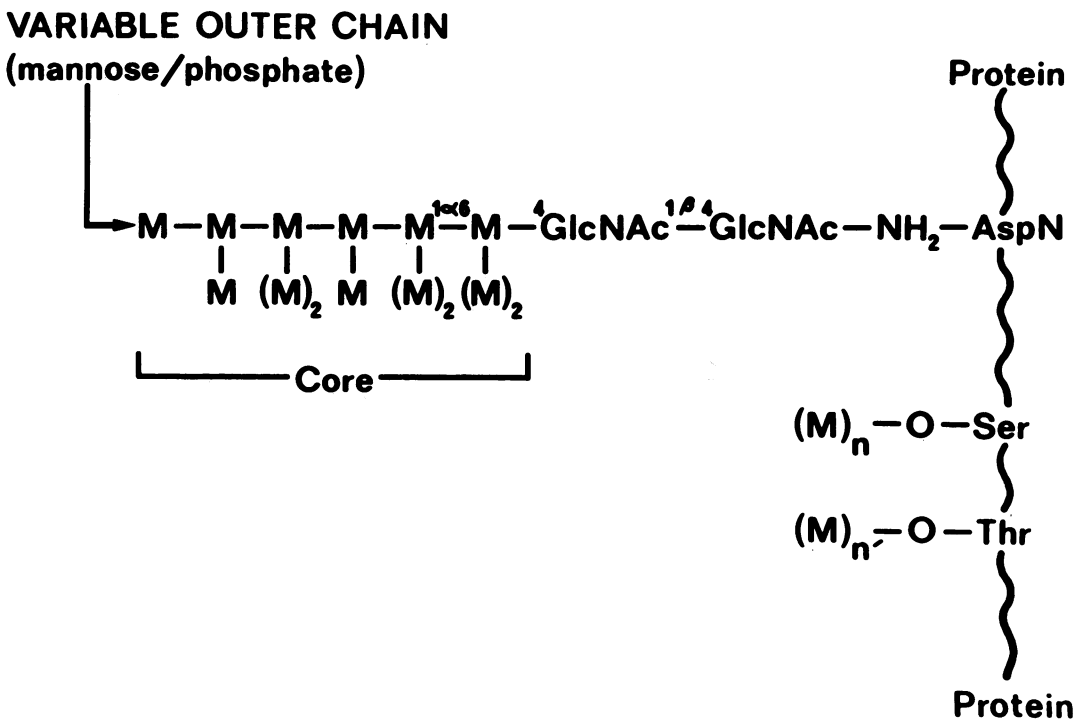

FIG. 2. Representation of the cell wall mannoprotein of *C. albicans* (139). M, Mannose.

The data of Auger et al. (5) indicate that approximately 74% of the clinical isolates are serotype A. More recently, Brawner and Cutler examined the serotypes of isolates from normal carriers, immunocompetent hospitalized patients, and immunocompromised patients with or without AIDS (16). Immunocompetent individuals colonized by oral *C. albicans* were just as likely to carry serotype A or serotype B organisms. However, immunocompromised individuals were at least twice as likely to be infected with serotype B instead of serotype A oral strains. In the former study, immune status of the patients was not considered. Therefore, the differences in data between the two studies may be related to immune status, although geographical or hospital biases may also explain the differences in serotypes. These

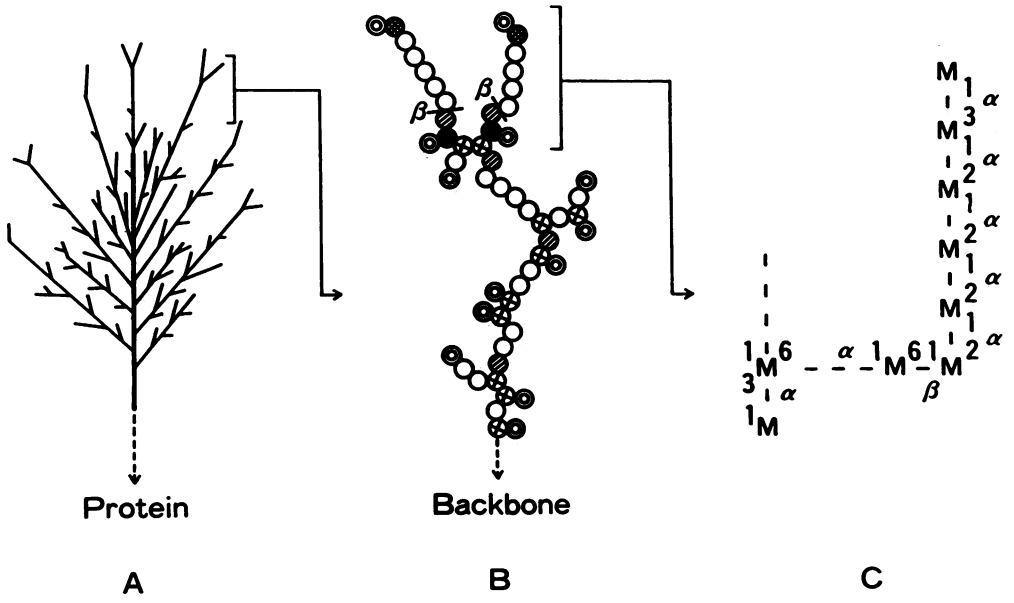

FIG. 3. Model of serospecific factor 6 of serotype A *C. albicans* (154). Detail of linkages is provided in the bracketed regions of Fig. 3B and C. M, Mannose.

TABLE 1. Factor-specific epitopes of *Candida* spp.

Species	Mannan-specific epitopes
C. albicans serotype A	1, 4, 5, 6
C. albicans serotype B	1, 4, 5, 13b
C. tropicalis	1, 4, 5, 6
C. krusei	1, 5,[a], 11
C. parapsilosis	1, 5b, 13[a]
C. pseudotropicalis	1, 8

[a] Variable in reactivity.

data do point out, however, the ambiguity in the relationship between serotype and virulence.

Immunochemical analysis of the *Candida* cell surface has been hastened by the development of highly specific MAb. To date, 19 MAb that recognize cell surface epitopes of *C. albicans* have been reported (12–15, 20, 27, 29, 61, 69, 84, 98, 107, 110, 121, 153, 156). As can be seen in Table 2, most are immunoglobulin M (IgM) and react with carbohydrate epitopes. Also, most of the MAb recognize determinants shared by two or more *Candida* species.

In general, the data listed in Table 2 are taken from studies of antigen expression as a function of cell age (12–15, 29), studies that identify germ tube- and yeast-specific antigens (12–15; see below), or studies of the dissection of some of the serospecific factors from *C. albicans* (69). For example, Brawner and Cutler (12–15, 17) as well as other investigators (29) have produced MAb specific for various surface determinants and have used them to show that expression of these epitopes varies among *Candida* species, between individual isolates of the same species, between stages of growth in culture, and between individual cells. The structural differences of these epitopes may be associated with the protein component or mannan side chains. Therefore, the cell wall of *C. albicans* should be viewed as a dynamic structure that responds to a variety of changes in the growth environment. However, other epitopes are expressed at all stages of the growth cycle (28). The MAb reported by Brawner and Cutler not only react with *C. albicans* antigens in vitro but can also be detected in tissues of infected animals associated with growing *C. albicans* (15).

In regard to the dissection of *Candida* serospecific factors, Miyakawa et al. (98) have recently isolated two agglutinating MAb (CA4-2 and CA5-4), which react with a mannan preparation of *C. albicans* serotype A. The cross-reactivity patterns of the MAb are closely related to those of polyclonal antibodies against factors 5 and 6. Kagaya et al. have also reported the isolation of two other MAb, CA1-2 (4b) and CB13-3 (4c), against *C. albicans* serotypes A and B (69). Competitive binding studies using their MAb indicated that polyclonal antibody against factor 4 reacts with a complex containing a number of epitopes that bind different MAb. From the results of experiments with their MAb to factor 4, a hypothetical model of the determinants of antigenic factor 4 was constructed (Fig. 4).

YEAST FORM- AND HYPHAL (GERM TUBE)-SPECIFIC ANTIGENS

The expression of cell surface components on *C. albicans* is, in part, growth phase specific. For example, hyphal forms of the organism bind fibrinogen, laminin, and the complement (C3) conversion product C3d, while yeast cells (blastoconidia) do not (9–11, 20, 163). Quantitative differences probably exist in regard to the level of iC3b (C3 conversion product) binding to yeast and hyphal forms, since standard assays for binding (by rosetting with conjugated sheep erythrocytes bearing iC3b) are positive only for hyphal forms of the organism (41, 52). That hyphal- or germ

TABLE 2. Characteristics of MAb to cell surface components of *C. albicans*

MAb	Ig class	Epitope[a]	Growth form[b]	Mass (10^3 kDa) by Western blot	Growth stage[c]	Specific for *C. albicans*	Reference(s)
CA-A	G	MP	Y, GT	60, 68	ND[d]	Yes	20, 84
1.183	G	MP	GT	55, 60	ND	ND	107
24	M	C	Y	ND	STA[e]	ND	29
5B2	M	C	Y, GT	ND	ND	No	61
UCSC-1	M	C	Y	ND	ND	Yes	110
4E6	M	MP	Y, GT[f]	200	EXP	No	153
5B7	M	MP	Y	200	ND	No	153
4G8	M	C	Y	200	ND	No	153
5E2	M	C	Y	NR[h]	ND	No	153
H9	M	C	Y, GT	ND	EXP	No	13, 14
C6	M	C	Y, GT	ND	EXP	No	13, 14
CA4-2	M	C (6)[g]	Y	ND	ND	No	99
CA5-4	M	C (5b)[g]	Y	ND	ND	No	98
18d7	G	C	Y	ND	ND	No	156
8H4	M	C	Y	ND	ND	ND	156
4Cl2	G	MP	H	260	ND	ND	23
CA1-2	M	C (4b)[g]	Y	ND	ND	No	69
CB13-3	M	C (4c)[g]	Y	ND	ND	Yes	69
AF1	M	GMP	Y	ND	ND	No	27

[a] MP, Mannoprotein; C, carbohydrate; GMP, glucomannoprotein.
[b] Y, Yeast form; GT, germ tube.
[c] STA, Stationary-phase cells; EXP, exponential-phase cells.
[d] ND, Not determined.
[e] At 37°C, exponential growth.
[f] Weakly reactive with germ tubes.
[g] Epitopes for these MAb represent a component of factors 6, 5, and 4.
[h] NR, No reactivity in a Western blot.

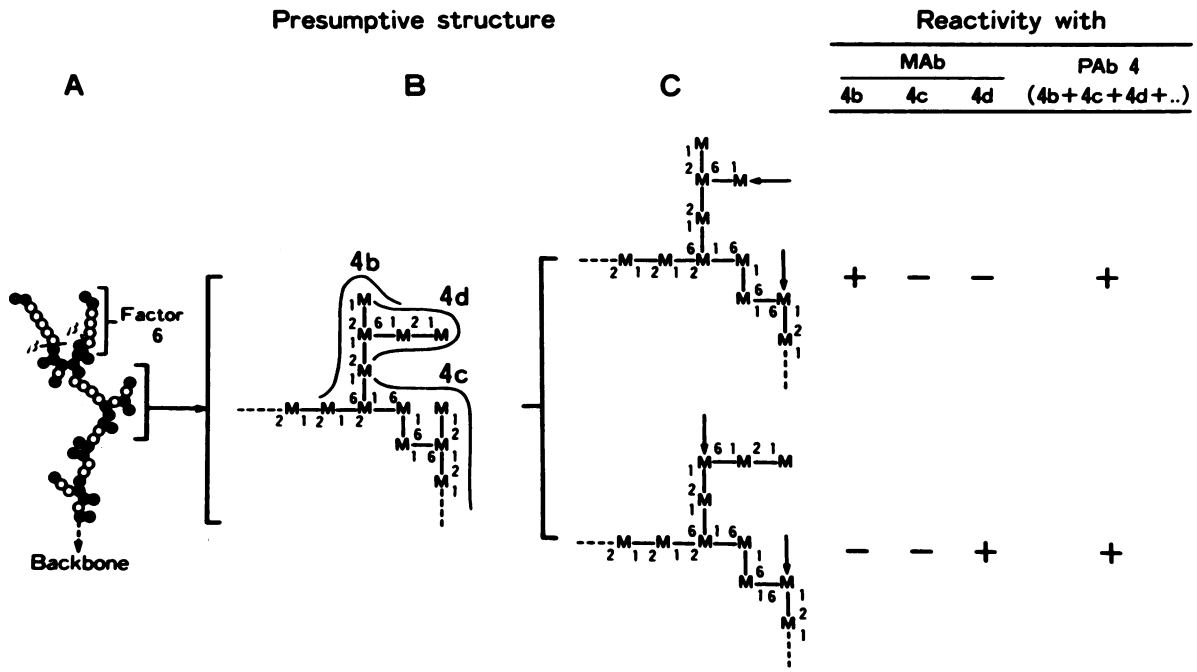

FIG. 4. Proposed model of the factor 4 and factor 6 epitopes of *C. albicans*. The factor 4 epitope is further dissected into epitopes detected by specific MAb that react within the factor 4 epitope. The reactivity of the polyclonal monospecific antibody (PAb) to each of the epitopes within factor 4 is also indicated (69). M, Mannan.

tube-specific antigens exist has been demonstrated by a number of investigators (17, 20, 23, 43, 107, 111, 112, 149, 151–153). With most of these observations, it has not been possible to determine if these phase-specific antigens represent de novo synthesis of new proteins or a topological rearrangement of yeast antigens from a subsurface to a surface site in the cell wall. Regarding the latter possibility, Ollert and Calderone (107) have shown that a MAb that reacted by immunofluorescence (IF) with hyphal forms of the organism but not yeast cells did, however, react with yeast protoplasts by IF and in Western blotting (immunoblotting) of a yeast protoplast extract. As pointed out by Brawner and Cutler (17), comparisons between growth forms should be carried out with several isolates, with cells of different ages, and with different media. Most studies fail to include these variables. Nevertheless, growth form-specific mannoproteins may be critical to the process of morphogenesis. Additionally, the hyphal form of the organism is most often associated with its invasiveness. Therefore, the identification of hypha-specific cell surface components may result in the identification of proteins associated with morphogenesis or invasiveness.

A variety of techniques have been used to study the cell surface macromolecules of both yeast and hyphal (or germ tube) forms of *C. albicans*. Most of the studies have focused either on extraction of cells with sodium dodecyl sulfate (SDS) or dithiothreitol (DTT) or on digestion of the cell wall with Zymolyase. Subsequently, such extracts are analyzed by either crossed immunoelectrophoresis or SDS-polyacrylamide gel electrophoresis (PAGE) with Western blotting (21–23, 43–46, 68, 84, 101, 108, 111, 149, 151–153). DTT extraction tends to remove components associated with the outermost layers of the cell wall (111), while Zymolyase digestion releases residual wall mannoproteins that are probably covalently linked to structural cell wall glucans (44). Elorza et al. (43–46), for example, used SDS to extract cells of *C. albicans* and detected about 40 bands corresponding to molecular masses of 15 to 80 kDa. Further treatment with Zymolyase yielded mannoproteins of 160 and 250 kDa, two of higher molecular mass from yeast cells, and only two corresponding to the lower-molecular-mass components from hyphal cells. Molloy et al. (101) have questioned the use of SDS in the extraction of cell wall components and have shown contamination in such preparations with cell membrane proteins. For this reason, DTT extraction is preferred to SDS extraction, although the former method can still remove proteins from the cell interior in addition to the outer wall mannoproteins. Zymolyase treatment is believed to remove mannoproteins covalently linked to glucan (44). These components probably represent structural entities of the cell wall. Thus far, studies of Zymolyase-extracted mannoproteins from both yeast and hyphal forms have been pursued by Casanova et al. (23) and Sundstrom et al. (151–153). Mannoproteins of 180 and 260 kDa appear to be hyphal specific, while mannoproteins of 200, 340, 500, and 650 kDa were obtained from yeast-phase cells (Table 3). Each of these components appeared to be synthesized de novo and did not represent rearrangement of existing polymers. A MAb to the hyphal-specific 260-kDa mannoprotein (MAb 4C12) was obtained. By an IF assay, MAb 4C12 reacted specifically with the surfaces of mature hyphae and not of yeast cells. Reactivity of the MAb was lost if hyphae were treated with pronase prior to digestion with Zymolyase; this may indicate that the epitope recognized by the MAb is a protein.

Mannoproteins of 155 and 200 kDa from germ tubes, extracted by Zymolyase, have been reported by Sundstrom et al. (151–153). These components were also released from germ tubes by treatment of hyphae with proteolytic enzymes, as described by Casanova et al. (23). Thus, the

TABLE 3. Phase-specific mannoproteins extracted from *C. albicans* with Zymolyase or DTT and analyzed by SDS-PAGE

Extracting agent and reference(s)	Mass (kDa)	
	Germ tubes/ hyphae	Yeast form
Zymolyase		
45	34, 160, 205	>205
23	180, 260	500, 650 340, 200
151, 152	200,[a] 155	200
DTT		
111	19, 245	ND[b]

[a] Endoglycosidase H sensitive for hyphal form but not for yeast form.
[b] ND, Not Determined.

change in surface architecture as cells convert from the yeast to the hyphal phase in part reflects differences in cell surface proteins. More recently, Sundstrom et al. (152) described a 200-kDa mannnoprotein from germ tubes of *C. albicans* that was extracted with Zymolyase. This component reacted with germ tube-specific antiserum obtained by exhaustive adsorption with yeast-phase cells. When adsorbed with germ tubes, this antiserum failed to react with the 200-kDa mannoprotein, indicating the surface nature of the mannoprotein. The antigen was labeled by both ^{125}I-mannose and [^{3}H] mannose, indicating both the surface nature of the component and its glycosylated nature. A 200-kDa component was also extracted from yeast-phase cells. However, it did not react with germ tube-specific antiserum. Also, the 200-kDa component from germ tubes was susceptible to endoglycosidase H, whereas the yeast antigen was not (Table 3). Finally, the germ tube-specific antiserum did not react with glycan fractions obtained from protease-degraded cell wall digests. The glycan fraction was essentially free of amino acids. This study and that of Casanova et al. (23) demonstrate that germ tube-specific determinants are probably protein and not carbohydrate.

Germ tube-specific mannoproteins have also been observed in DTT-solubilized extracts (68, 111, 142). DTT extracts from mycelial-phase cells blocked binding of an antiserum to hyphae according to IF. A total of 12 antigens was observed in DTT extracts from mycelial-phase cells, while seven antigens were observed in yeast-phase cells with unadsorbed serum. Using tandem crossed immunoelectrophoresis, mycelial, yeast, and cytoplasmic extracts were compared with an antimycelium antiserum. A dense, cross-reactive arc from all three extracts was observed. When this same antiserum was adsorbed with yeast cells and compared with DTT mycelial extracts, several arcs remained, indicating the presence of phase-specific antigens. Adsorbed antiserum still reacted with mycelial cells when examined by IF. Experiments were performed with tissues from patients with systemic *Candida* infections. When adsorbed serum was used, staining was limited to the germ tube segments of the organism within tissues, indicating that these phase-specific antigens are expressed in vivo. Patient sera adsorbed with yeast cells stained germ tubes when tested in vitro (14 of 17 patients).

In subsequent studies, Ponton and Jones (111) compared several methods of extraction of yeast and mycelial cells by SDS-PAGE. DTT and iodoacetamide extraction yielded the most satisfactory array of components for study. Western blots of a yeast extract appeared densely stained until the antiserum prepared against mycelium was adsorbed with yeast cells prior to blotting. Adsorbed serum, however, revealed well-defined bands that ranged in molecular mass from 19 to approximately 80 kDa. Also, the adsorbed serum still reacted with yeast-phase antigen preparations, even though the serum reacted only with mycelium and not yeast cells by IF. Thus, these cross-reacting antigens could represent components within the yeast cell wall or on the cell surface, but at extremely low density. Previous work by Smail and Jones (142) indicated that if adsorption of the antimycelial serum was carried out at a 10-times-higher concentration of yeast cells, reactivity of the serum with mycelial cells was significantly reduced. These data again indicate that the identification of germ tube- or mycelial-specific antigens may be tenuous in a number of instances.

The composition of specific mannoproteins extracted by either Zymolyase or DTT has been studied (44, 101). Zymolyase-extracted mannoprotein contains high quantities of carbohydrate and relatively little protein. For example, Elorza et al. (44) determined that Zymolyase-solubilized material contains 92% carbohydrate (86% mannose and 6% glucose) and 7% protein. Over 85% of the carbohydrate is N glycosidically linked to the protein, while the rest is O glycosidically linked. Molloy et al. (101) found that the hexose/protein molar ratio for yeast-phase *C. albicans* is 38.3:1 for Zymolyase-extracted material and 1.7:1 for SDS-extracted material. Zymolyase-solubilized material is devoid of discretely migrating cellular proteins. A 260-kDa species in ^{125}I-labeled cells accounts for 95% of the wall-bound ^{125}I. In contrast, DTT-extracted material has been found to contain less carbohydrate. Ponton and Jones (111) showed that most proteins extracted by DTT contain little if any mannose. However, the C3d-binding protein of *C. albicans* (extracted with DTT) is composed of approximately 36% protein and 64% carbohydrate, the latter being composed of mannose and glucose in equimolar amounts (84). The extracellular form of the C3d-binding protein is composed of approximately 30% carbohydrate as determined by the phenol-sulfuric acid method (133). The predominant hexose is mannose. On the basis of digestion patterns with various endoglycosidases, the oligosaccharide was thought to be linked to protein via N-glycosidic bonds.

The vectorial synthesis of a specific mannoprotein has been studied by Elorza et al. (44). As stated previously (see above), one of the major mannoproteins released by Zymolyase treatment of mycelial cells is a 260-kDa species. A MAb to this mannoprotein also recognizes an epitope on a mannoprotein of 180 kDa if the extraction with Zymolyase is carried out for a longer time and at a higher concentration of Zymolyase. If cells are treated with endoglycosidase H, the 260-kDa species is not observed in Western blot analysis. Instead, only the 180-kDa species is recognized. Furthermore, protoplasts of yeast-phase cells during early stages of wall regeneration synthesize the 180-kDa but not the 260-kDa species. Interestingly, the 180-kDa species is susceptible to degradation by mild alkali treatment, which releases *O*-glycoside-linked sugars. It is probable, therefore, that the 180-kDa mannoprotein is initially composed of *O*-glycoside-linked carbohydrate that is modified by the addition of *N*-glycoside-linked carbohydrate. This may account for the increase in molecular mass to 260 kDa. Thus, the reactions responsible for the increase in the molecular mass of this protein take place during the process of its incorporation into the cell wall (44). Unfortunately, there are few studies of this type, so that speculation about the assembly and biosynthesis of other cell surface components is not possible. In Table

TABLE 4. Characteristics of mannoproteins extracted by different methods

Mannoprotein characteristics	
Zymolyase extraction	DTT extraction
High C/P[a]	Low C/P
Unknown function; structural?	Receptor and enzymatic activities
Covalently linked to glucan	Primarily disulfide linked
20–25% of wall protein	50–59% of wall protein

[a] C/P, Carbohydrate/protein ratio.

4 the functions and characteristics of mannoprotein extracted by Zymolyase or DTT are described.

RECEPTORS-LIGANDS OF *C. ALBICANS*

C3d-Binding Proteins

C. albicans hyphae and pseudohyphae rosette with antibody-sensitized sheep erythrocytes conjugated with the complement C3 conversion product C3d. This observation was first made by Heidenreich and Dierich (58) and has been reported also by several other investigators (20, 41, 52). Yeast forms of the organism seem to have lower levels of expression when binding is measured by a rosetting assay using EAC3d (20). The *Candida* complement receptor for C3d (CR2-like protein) from mycelial and yeast cultures has been studied, but comparisons of the proteins from the two growth forms have not been pursued.

Heidenreich and Dierich (58) also initiated studies on the biochemical nature of the C3d-binding protein of *C. albicans*. These investigators attempted to block binding of EAC3d to *C. albicans* by using solubilized mannan (from bakers' yeast) or various monosaccharides such as L-mannose, *N*-acetylglucosamine, and D-galactose. While these substances were without effect, D-glucose and D-mannose did block binding of the EAC3d ligand to hyphae, although the amount of inhibition was small (approximately 30%).

Further purification and characterization of the *C. albicans* C3d-binding protein(s) has recently been accomplished (20, 84, 133). As reported by Heidenreich and Dierich, the protein was found only on strains of *C. albicans* and *C. stellatoidea* (58). Yeast forms of the organism did not possess binding activity. Additionally, when hyphae were heat killed or treated with proteases such as trypsin or pronase, binding activity was significantly inhibited, indicating that the receptor may be a protein.

Purification of the C3d-binding protein(s) was accomplished by a combination of techniques (Fig. 5), including fractionation of hyphal extracts on DEAE-Trisacryl with an NaCl step gradient followed by affinity chromatography with C3d-Thiol-Sepharose of the active material collected from the DEAE column (20). Two proteins with molecular masses of 60 to 62 and 70 kDa were observed by silver stain in SDS-PAGE profiles of eluates from the C3d affinity column.

Proteins of similar molecular mass were also isolated by affinity chromatography by using a MAb (CA-A) that previously had been shown to block the rosetting of EAC3d with hyphae of *C. albicans* (Fig. 5) (84). Of five such MAb screened, two (CA-B and CA-E) did not inhibit binding of EAC3d to hyphae, although both reacted with the cell surface of *C. albicans* hyphae as determined by IF (20). Additionally, the antigen which was purified by using CA-E coupled to Sepharose did not block rosetting of EAC3d to hyphae, while eluates from a CA-A–Sepharose column did block rosetting with EAC3d.

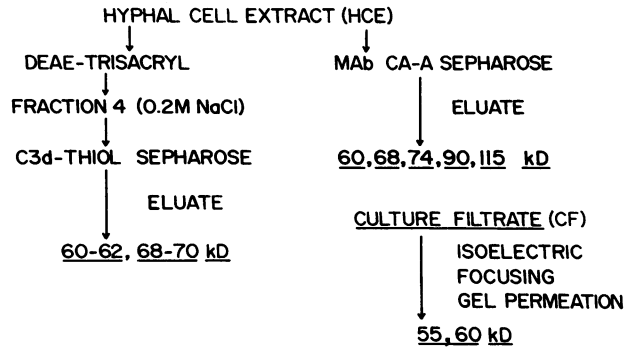

FIG. 5. Fractionation and purification of C3d-binding proteins of *C. albicans*. The proteins were purified by either ligand (C3d) or MAb (CA-A) affinity chromatography.

Eluates from the MAb CA-A affinity column contained proteins of 60, 68, 74, 90, and 115 kDa. This eluate was further fractionated by high-pressure liquid chromatography (HPLC) using a Bio-Sil 400-size exclusion column. The protein of 60 kDa was isolated in a purified form and found to block rosetting when EAC3d was reacted with hyphal forms of the organism (84). It is not clear what relationship the 68- to 70-kDa form has to C3d binding activity. The protein was isolated from a C3d affinity column and was purified by using MAb CA-A, which, as stated previously, blocked rosetting of the organism by EAC3d. However, fractions from HPLC that contained the protein were noninhibitory. Therefore, either the protein nonspecifically binds the ligand or it is specific for EAC3d but isolated in concentrations too low to effectively block rosetting. Similarly, the relationships of the 74-, 90-, and 115-kDa proteins to C3d binding need to be determined. There is some indication that MAb CA-A reacts with an oligosaccharide epitope (unpublished data). Therefore, in an MAb affinity chromatography system using CA-A, any protein with a similar oligosaccharide epitope would be isolated, regardless of its specificity for C3d. Further work is needed to verify the relationships of these proteins and their C3d-binding activities.

A C3d-binding protein was also isolated and purified from medium supernatants (culture filtrates) of hyphae grown in phytone peptone broth (133). The protein was obtained by centrifugation of cultures and concentrated by tangential ultrafiltration with a membrane exclusion size of 10 kDa. Culture filtrate was fractionated by isoelectric focusing. Fractions containing the active material were pooled and further purified by gel permeation chromatography on a Protein Pak 300 column. SDS-PAGE analysis of silver-stained gels (as well as Western blotting) revealed a doublet of 55 and 60 kDa, similar in molecular mass to proteins purified by ligand and MAb affinity chromatography (see discussion above). Overall, a 30-fold purification and a yield of approximately 25% were obtained by these purification techniques. As described above, purification of the C3d-binding protein was aided by the fact that solubilized preparations retained their biological properties.

Additional studies (133) on the C3d-binding protein revealed that (i) the native protein purified by isoelectric focusing and gel permeation had a molecular mass of approximately 240 kDa when partitioned by sucrose density gradi-

TABLE 5. Antibodies to mammalian complement receptors and their reactivity with *C. albicans*

Antibody	Specificity[a]	Reactivity[b]		Mass (kDa) of reactive *Candida* protein(s)[c]	Blocking[d] of:		Reference
		Yeast form	Hyphae		Binding	Adhesion	
OKM-1	CR3, α chain	±	+	130, 100, 50	+	ND	42, 52
M522	CR3, α chain	−	+	ND	ND	ND	42
MO-1-94	CR3, α chain	+	+	165	ND	+	53
MO-1	CR3, α chain	±	+	ND	(−)	ND	41, 52
M1/70	CR3, α chain	+	+	ND	(−)	ND	52
BU-15	p150.95, α chain	+	+	185	ND	ND	62
Integrin	β Chain	ND	ND	95	ND	ND	89
Anti-CR2	CR2	−	−	ND	+	ND	41
Anti-GP140	CR2	−	−	ND	+	ND	41
Anti-CR1	CR1 (3D9)	−	−	ND	−	ND	41

[a] Of MAb or polyclonal antibody for the mammalian complement receptor.
[b] As determined by IF. +, Fluorescence.
[c] As determined by Western blot or immunoprecipitation and SDS-PAGE; reducing gels except for BU-15.
[d] +, Antibody blocks ligand (iC3b or C3d) binding to *C. albicans* or adherence of *C. albicans* to endothelial cells; −, no blocking; ND, not determined.

ent centrifugation; (ii) the isoelectric point of the purified protein was approximately pH 3.9 to 4.1, which differs significantly from that of the mammalian CR2 (approximately pH 8.0) (133); and (iii) the total amino acid composition of the *Candida* C3d-binding protein was substantially different from that of the mammalian CR2. For both, the two predominant amino acids were glycine and glutamic acid-glutamine. However, the protein from *C. albicans* contained almost three times the amount of glutamic acid and almost five times less tyrosine than the mammalian complement receptor. Since cysteine determinations have not been made with the protein from *C. albicans*, it is not possible to predict the extent of intrachain (or interchain) bonding. In fact, there are no data to indicate whether the protein is composed of a single polypeptide or of subunits or whether the protein also possesses iC3b-binding activity. With mammalian cells, CR2 is a single polypeptide with minor iC3b-binding activity (125).

On the basis of the previous discussion, it would seem as if the *Candida* and mammalian CR2 have a low degree of homology. Other investigators have also observed the lack of reactivity of *Candida* cells with both MAb and polyclonal antibodies directed against mammalian CR2 (41, 52). In the study by Edwards et al. (41), both a MAb (HB-5) and a polyclonal antibody (anti-GP140) failed to react with *C. albicans* when tested by IF. Those authors did note that in the presence of high concentrations of each antibody (480 μg of protein per ml) and at low concentrations of C3d (5 U), *C. albicans* did not rosette with EAC3d when an enhancing antibody was also included. The specificity of this interaction remains to be tested. Furthermore, Eigentler et al. (42) reported that other MAb (2G7, 6F7, and 1C8) specific for the mammalian CR2 also failed to react with *C. albicans* when examined by indirect IF antibody assays. In comparison, those investigators did show reactivity of MAb with mammalian CR3 (iC3b receptor) and *C. albicans*. These studies will be discussed below.

Both indirect and direct observations indicate that the C3d-binding protein of *C. albicans* is glycosylated (20, 84). These observations include the following. (i) *C. albicans* eluates from a ConA-Sepharose column (eluted with α-methylmannoside) block rosetting of hyphae with EAC3d, while nonmannosylated fractions, which do not bind to ConA, possess minimal blocking activity. (ii) Semipurified fractions that possess C3d-binding activity lose activity when incubated with Sepharose beads conjugated with ConA but not when incubated with the lectin from *Bandieraeae simplicifolia* (*N*-acetylgalactosamine). Correspondingly, eluates from the ConA-Sepharose beads possess binding activity for C3d. (iii) Proteins of 60 and 68 kDa obtained by CA-A–affinity chromatography stain with ConA when analyzed by SDS-PAGE. (iv) Direct HPLC analysis for sugar composition of the purified protein has been performed; both mannose and glucose have been detected in approximately equimolar concentrations and account for 36% (by weight) of the binding protein (133). In contrast, the form of the protein in the culture filtrate contained about 30% carbohydrate and also differed in that *N*-acetylglucosamine was found instead of glucose. (v) The binding protein in the culture filtrate consists of a doublet in the 55- and 60-kDa range, as stated above. However, incubation of the purified protein with endoglycosidase F but not with *O*-glycanase, endoglucosidase H, or *N*-glycanase resulted in the complete conversion of the doublet to a 45-kDa species. This observation indicates that perhaps the doublet observed prior to glycosidase treatment represents one protein with different degrees of glycosylation.

Evidence against the glycosylated nature of the C3d-binding protein is that *C. albicans* hyphae saturated with ConA still bind EAC3d (20). The interpretation of this observation, seemingly the opposite of the data presented above, is that the oligosaccharide component of the protein is not associated with ligand binding. In fact, the mammalian CR2, while glycosylated, has a protein domain associated with ligand binding (166). The oligosaccharide may function in stabilizing the protein as suggested by these investigators.

iC3b-Binding Proteins

Using a rosetting assay similar to that described for C3d, Heidenreich and Dierich described the binding of antibody-coated sheep erythrocytes conjugated with iC3b (EAiC3b) to hyphae and yeast forms of *C. albicans* (58). Binding was specific for *C. albicans* and *C. stellatoidea* and could be inhibited by sugars such as D-mannose and D-glucose. These studies have been extended by other investigators (20, 41, 52). Edwards et al. (41) have shown that a MAb to an epitope of the α chain of CR3 of mammal cells (MO-1) reacted with hyphae of *C. albicans*. Reactivity was determined by an IF assay. Interestingly, while anti-MO-1 reacted with the organism, it did not block rosetting of EAiC3b and *C. albicans* hyphae (Table 5). In fact, blocking of binding to EAiC3b was

not inhibited by any anti-CR3 that was tested. In addition, Gilmore et al. (52) demonstrated the reactivity for *C. albicans* of two other MAb (anti-OKM1 and M1/70) with specificities for the α chain of the mammalian CR3 (Table 5). In light of these studies as well as those of Eigentler et al. (42), the evidence is strong for a higher degree of homology between the *Candida* iC3b-binding protein(s) and mammalian CR3. As stated above, the opposite appears to be true for the C3d-binding protein of *C. albicans* and mammalian CR2.

While the C3d-binding protein of *C. albicans* has been characterized to a great extent, the biochemistry of the iC3b-binding protein is not well understood. Expression of this protein is observed on hyphae but not yeast forms assayed by rosetting with EAiC3b (84). However, binding of anti-mammalian CR3 antibodies to both yeast and hyphal forms of the organism occurred (52, 53, 63). The differences in reactivity of the growth forms as reported by Hostetter et al. (52) probably reflect a higher degree of sensitivity in their assay. For example, the anti-CR3 MAb OKM1, M1/70, and MO-1 reacted with both yeast and hyphal forms of the organism when measured by fluorescence-activated cell sorting analysis (52).

Identification of the iC3b-binding protein from *C. albicans* was pursued by Eigentler et al. (42). Expression of the iC3b-binding protein on pseudohyphae of *C. albicans* was temperature dependent. The attachment indices (determined by counting the number of adherent erythrocytes per pseudohyphae) were 3.45, 1.45, and 0.17 for pseudohyphae grown at 30, 37, and 38.5°C. Adherence of EAiC3b was never observed on yeast forms. Pseudohyphae obtained from cultures grown at 30°C were shifted to temperatures ranging from 30 to 70°C for an additional 10 min. After exposure to a temperature of 56°C or higher (for 10 min), an abrogation of EAiC3b binding was observed, demonstrating that the binding molecule for iC3b was heat labile. The binding protein was also shown to be trypsin sensitive (50% inhibition at approximately 0.8 μg of trypsin) but was not dependent on the presence of divalent cations.

IgG from a hyperimmune rabbit serum obtained by immunization with whole cells inhibited the binding of EAiC3b to pseudohyphae, whereas IgG from nonimmunized rabbits did not (42). Preadsorption of the rabbit IgG against *C. albicans* pseudohyphae but not yeast cells abrogated the inhibitory effect of the IgG, showing the specificity of IgG for a surface component on hyphal cells. Preincubation of the organism with rabbit IgG directed against *C. albicans* resulted in a loss of binding of an OKM-1 MAb to *C. albicans* when cells were examined by IF. Conversely, an $F(ab')_2$ of the IgG anti-*Candida* antibody did not react with a macrophage cell line (U937) expressing CR3, and the IgG did not inhibit EAiC3b binding to U937 cells. The amount of OKM-1 needed to inhibit binding of EAiC3b to *C. albicans* (300 μg/ml) was about 25 times higher than the amount of OKM-1 needed to inhibit binding of EAiC3b to U937 cells. Also, while the anti-*Candida* antibody inhibited binding of OKM-1 to *C. albicans*, the opposite reactivity was not observed. These differences might be explained by a stronger avidity of the OKM-1 antibody than of the anti-*Candida* antibody or some other inherent property of the antibodies. Nevertheless, while some degree of homology exists between mammalian CR3 and the *Candida* iC3b-binding protein (based on the reactivity of OKM-1 with *Candida*), there are obvious differences between the CR3 of both cell types. First, immunoprecipitation of *Candida* extracts surface labeled with iodine revealed proteins of 130, 100, and 50 kDa in molecular mass in contrast to the CR3 of mammalian cells, which consists of a dimer of 165 and 95 kDa (125). Second, functional differences exist, for, as stated previously, in contrast to iC3b binding to human CR3, the binding of iC3b to *C. albicans* did not require the presence of divalent cations and anti-*Candida* IgG did not inhibit binding to U937 cells.

Suggested Functions of the *Candida* Complement-Binding Proteins

The complement receptors of mammalian cells may be involved in cell proliferation, like the CR2 of B cells (48, 97); in adhesion and homing mechanisms (148), like the CR3 from neutrophils, monocytes, and lymphocytes; or in phagocytosis, in which CR1, CR3, and CR4 participate (125). The tendency among investigators of the *Candida* complement-binding proteins is to point toward some role for these components in virulence. In fact, there is some evidence, although only correlative, that the CR3-like protein(s) of *C. albicans* may be associated with virulent strains only (108). In somewhat related studies, the up regulation of these proteins was correlated with a corresponding decrease in the ability of neutrophils to phagocytize the organism (52, 53, 63). Several investigators have noted that only *C. albicans* (and *C. stellatoidea*) exhibit CR-like proteins, while relatively nonpathogenic *Candida* spp. do not (20, 41, 58). However, among the nonproducing strains, certainly *C. tropicalis* deserves classification as a pathogenic *Candida* species. Thus, the relationship between the distribution of these proteins among the *Candida* species and virulence is not absolute.

Recent work by Ollert et al. (108) demonstrated the presence of an iC3b-binding protein on a virulent strain of *C. albicans* but not on a derived, avirulent, nonadhering strain (strain m-10). In comparison, C3d-binding activity was similar for both the virulent and avirulent strains. *C. albicans* m-10 is a spontaneous, cerulenin-resistant strain impaired in its ability to attach to buccal epithelial cells (BEC) (60), vaginal epithelial cells (81), fibrin platelets (18), and endothelial cells (40a). It was also relatively avirulent in animal models of both vaginitis (81) and endocarditis (18). When examined by crossed immunoelectrophoresis, strain m-10 yeast cells, unlike parental cells, lacked a cell wall mannoprotein of slow electromobility (21). The cell surface antigens from hyphal-phase cells of both parental and m-10 strains have also been studied (22, 108). Proteins of 68 to 71, 55, and 50 kDa were observed in Western blots of parental cells when serum from a patient with chronic mucocutaneous candidiasis was used, but these proteins from m-10 cells were nonreactive or only weakly reactive. The fractions analyzed by Western blot from both strains either were from whole-cell extracts or were DEAE-purified fractions known to be rich in the complement-binding proteins of this organism (108). Since the antiserum used in this study inhibited the attachment of EAiC3b to *C. albicans*, these proteins may represent the iC3b-binding proteins. Recently, Hostetter and Kendrick (62) used MAb BU-15, which recognizes the CD11c (α subunit) of the p150.95 receptor (a member of a family of mammalian receptors which includes CR3), to identify similar molecules from *C. albicans*. Interestingly, those investigators (62) reported that three proteins of molecular masses 70, 67, and 55 kDa reacted with BU-15 under reducing conditions similar to those of Ollert et al. (108). A 185-kDa protein reacted with MAb BU-15 under nonreducing conditions. There is some overlap in regard to the

Candida proteins that react with MAb OKM-1 as reported by Eigentler et al. (42). The protein common to each of these studies is 50 kDa; however, differences in reactivity as reported by each group may reflect differences in antigen preparation and/or growth conditions. Variation in the amount of each growth form (pseudohyphae, hyphae, and yeast forms) among strains of *C. albicans* has been reported (104), and this variation may account for the variation in molecular mass of the putative iC3b-binding proteins. Additional strains of *C. albicans*, derived from parental cells by mutagenesis, have reduced adherence to EAiC3b and EAC3d and have altered Western blot profiles within the molecular mass range mentioned above (167). These strains have not been compared thoroughly in an animal model, although one of the three strains with reduced EAC3d adherence was as virulent as the parental strain in a rabbit model of gastrointestinal colonization. It should be mentioned, however, that rabbits were treated with antibacterial antibiotics, and their cell-mediated immunity was suppressed to allow the organism to gain a foothold in its host. Thus, the ability of deficient and wild-type parental strains to persist in a untreated host was not evaluated.

Hostetter et al. (63) and Gilmore et al. (52) have reported that iC3b receptor expression in *C. albicans* is augmented by high concentrations of glucose in the growth medium. Binding of [^{3}H]iC3b increased by 44% when cells were cultured in 50 mM instead of 5 mM D-glucose. The increase in receptor expression was also confirmed by using fluorescence-activated cell sorting analysis following incubation of cells with MAb MO-1. Correlated with the increase in iC3b binding when cells were grown in high glucose was a reduction in the phagocytosis of yeast cells by human neutrophils. However, both observations (increased iC3b binding and a reduction in phagocytosis) were strain dependent, as a second strain used in this study did not respond in this manner. More recently, these studies have been extended by Hostetter et al. (63) to include clinical isolates of *C. albicans*. Thirteen of 22 patients examined had plasma glucose levels of >200 mg/dl within 3 days of a *C. albicans* culture, and the frequency of hyperglycemia was greater among patients with blood isolates of *C. albicans* (7 of 8 patients). Wide variation in iC3b receptor expression was observed among urinary, mucosal, and blood isolates, so that no correlation could be made between receptor expression and isolates that colonized mucosal surfaces versus those that invaded tissues.

Gustafson et al. (53) measured the adherence of ^{35}S-methionine-labeled *Candida* cells to human umbilical vein endothelium in the presence of 20 mM glucose (high receptor expression) or 20 mM glutamine (low receptor expression). Cells grown in the presence of glucose adhered to a greater extent (36%) than those grown in glutamate (20%; $P < 0.01$). More importantly, an anti-MO-1 MAb (MO-1-17) inhibited glucose-enhanced adhesion by 72%, while anti-MO-1 MAb 94, which is known to inhibit iC3b binding but not adhesion-dependent functions of neutrophils, did not block binding of yeast cells to the endothelium. More recently, Frey et al. (48a) have shown that iC3b (10 μg/ml) inhibits adherence of *C. albicans* by 71%, again implying a role for the *Candida* CR3 in adherence.

Other investigators have postulated additional ways in which the CR-like molecules of *C. albicans* promote the organisms' virulence (41, 58). (i) *Candida* complement receptors may participate as cofactors in the enzymatic degradation of bound complement, much as the mammalian complement receptor has been shown to function, and thus remove opsonins necessary for the phagocytosis of the organism. (ii) The complement receptor molecules may promote clumping of the organism through complement receptor–fixed-complement interactions. (iii) *Candida* complement receptors may compete with mammalian (phagocyte) receptor for ligand, thus reducing the efficiency of phagocytosis. Such a competition could be promoted by cell-bound or released C3-binding proteins. (iv) *Candida* cells may escape eradication because of nonrecognition (molecular mimicry).

In addition to proteins that mimic mammalian complement receptors, fibrinogen (9, 10, 158, 163), laminin (11)-, and fibronectin-binding-proteins (75, 76, 141) have been reported for *C. albicans*. Should these binding proteins turn out to be homologous to the integrins of mammalian cells, *C. albicans* may be able to escape recognition by phagocytes. This last mechanism seems unlikely, however, since we have observed that patient serum contains antibody(ies) that recognizes proteins identified as having C3-binding activity (unpublished data).

Nevertheless, it is tempting to speculate that the *Candida* complement receptor-like molecules may contribute to the invasiveness of the organism. Certainly, the evidence indicates a role for these proteins in virulence, since they are nonreactive in avirulent strains. Even more important, MAb to complement receptor of mammalian cells blocks adherence of the yeast form to host cells (both lines of evidence are discussed above). Additional experimentation is needed to substantiate this claim.

Fibrinogen-, Laminin-, and Fibronectin-Binding Proteins of *C. albicans*

The binding of fibrinogen to *C. albicans* and *Aspergillus* species has been described elsewhere (9, 10). With *Candida* spp., all isolates were positive for binding when assayed by indirect IF with an anti-fibrinogen antibody subsequent to incubation in purified human fibrinogen (9, 10). The IF pattern was similar for all isolates; i.e., the cell walls of germ tubes and hyphae (24 h) were positive, while nonbudding yeast cells were negative. In some cases fluorescence was observed in the buds of yeast cells. The fluorescence observed with purified fibrinogen was similar to that observed with whole fresh plasma, although Page and Odds reported that binding of whole plasma was different from that of the purified fibrinogen (109). Tronchin et al. observed the binding of fibrinogen by using scanning electron microscopy of cells incubated with fibrinogen-coated latex microspheres or gold particles (163). Cells examined by transmission electron microscopy showed dense gold particles associated with the flocculent surface layer of germ tubes but also within the cell wall. Gold labeling was also observed within the cytoplasm of germ tubes, suggesting that fibrinogen receptors may be synthesized and transported to the cell surface during germination of the organism.

By using ^{125}I-fibrinogen, binding was quantitated for both yeast and hyphal forms (10). Binding was 12-fold higher for mycelium and 7.7-fold higher for germ tubes than for yeast forms. In arriving at these figures, corrections were made for differences in surface area among the three growth forms. Binding of ^{125}I-fibrinogen was inhibited by 50% with approximately 10 μg of a crude culture filtrate from hyphae. However, mannan prepared from yeast cells and chitin (from horseshoe crab) were noninhibitory compared with the filtrate. Other agents inhibiting binding of fibrinogen to the mycelium included 2-mercaptoethanol, trypsin, and α-mannosidase, while mycelia treated with pronase E and chitinase could bind fibrinogen as readily as untreated cells. The cell

surface moieties that bind fibrinogen were not identified in these studies.

More recently Bouchara et al. (11) have reported the presence of laminin receptors on *C. albicans* germ tubes. As with the studies described above in regard to fibrinogen, nongerminating blastoconidia did not interact with soluble laminin. Binding in this study was measured by incubating cells with laminin and subsequently with rabbit anti-laminin antibody and a fluorescein-labeled goat anti-rabbit immunoglobulin. Older mycelia were also able to bind laminin, but septa of the mycelia were not labeled. This binding pattern was confirmed by transmission electron microscopy. ^{125}I-laminin was used to quantitate binding to germ tubes. These studies indicated that binding was time dependent, saturable, and highly specific. A single dissociation constant (K_d) was calculated (1.3×10^{-9} M), suggesting that germ tubes possessed only one class of receptor (10). Binding was inhibited when germ tubes were heated (80°C, 10 min) or treated with trypsin, and, of several proteins tested for their abilities to block laminin binding (including fibronectin, laminin, fibrinogen, and bovine serum albumin) only laminin (100% inhibition) and fibrinogen (57% inhibition) inhibited binding of ^{125}I-laminin. The extraction of the laminin receptors from germ tubes was accomplished by using DTT and iodoacetamide. Following SDS-PAGE and transfer to nitrocellulose paper, binding proteins were identified by reaction with laminin in a Western blot assay using anti-laminin and alkaline phosphatase-labeled antibody. Two proteins of 68 and 60 to 62 kDa were identified in this manner.

Tronchin et al. (158) recently used a similar method (ligand affinity) to compare the fibrinogen-, laminin-, and C3d-binding proteins from *C. albicans*. Interestingly, by Western blot analysis, proteins of similar molecular masses, i.e., 68 and 60 to 62 kDa, were observed for each of the above mentioned ligands. The results of this group thus confirm the work of Calderone et al. (20) and Linehan et al. (84) in regard to the C3d-binding proteins (mentioned previously). Also significant was the apparent temporal reorganization of cell surface mannoproteins from the yeast cell to the germ tube wall as germination occurred. This observation may profoundly influence our concept of the assembly of cell surface mannoproteins in this organism. An additional point of interest in regard to the 68- and 60- to 62-kDa proteins is the finding of Tronchin et al. (159) that adherence of *C. albicans* to plastic is probably promoted by proteins similar in molecular mass. Our studies with the native C3d-binding protein indicated a molecular mass of 240 kDa. It is tempting to speculate that *C. albicans* has a cell surface mannoprotein with a number of binding activities. The relationships between ligand-binding proteins as proposed by several groups of investigators are presented in Table 6. Further work is needed to confirm any relationships.

TABLE 6. Cell surface proteins of *C. albicans* and ligand binding

Mass (kDa) of:				
Cell surface protein				Plastic[b]
C3bi[c]	C3d[a,d]	Laminin[a]	Fibrinogen[a]	
130				200, >200
100				
68–70	68–70	68–70	68–70	68
55	60–72	60–72	60–72	60

[a] See reference 158.
[b] See reference 159.
[c] See references 42 and 62.
[d] See references 20 and 84. The native protein is approximately 240 kDa.

ADHERENCE OF *C. ALBICANS* TO HOST CELLS

Adherence of *C. albicans* to human BEC and vaginal epithelial cells was first studied by King et al. (74) and Lee and King (79). Since that time, a number of investigators have pursued the characterization of the *C. albicans* ligand for epithelial cells (19, 31, 33, 36–40, 57, 65, 71, 73, 80–83, 92–95, 126, 130–132, 138, 145, 146), fibrin platelets (87, 88), endothelial cells (76, 96, 137), human epidermal corneocytes (118, 119, 135), and plastic substrates (159) (for reviews, see references 36–38 and 126). Several generalizations can be made as a consequence of these studies. (i) A hierarchy exists among the *Candida* spp. such that the more commonly presenting pathogens such as *C. albicans* and *C. tropicalis* adhere to host cells in vitro to a greater extent than the relatively nonpathogenic species such as *Candida krusei* and *Candida guillermondi*. (ii) The growth medium profoundly affects the extent of adherence of *C. albicans* in vitro to both epithelial cells and plastics. (iii) Strains with reduced abilities to adhere in vitro also have impaired abilities to cause infection in animal models. (iv) Increased adherence in vitro is correlated with an increase in the production of a surface fibrillar-floccular layer that in some ways is comparable to the adhesins associated with bacterial fimbriae (7). (v) There is both indirect and direct proof that the *C. albicans* ligand (adhesin) is a mannoprotein. (vi) Filamentous forms of the organism adhere to a greater extent to a variety of substrates than do yeast forms (126). Emphasis in this review will focus on medium-induced effects on adherence and the identification of the *Candida* adhesin.

Influence of Growth Media on Adherence

Samaranayake and MacFarlane (130) and McCourtie and Douglas (92) first showed that yeast cells of *C. albicans* grown in medium supplemented with a high concentration of galactose, sucrose, glucose, or maltose adhered to acrylics to a greater extent than cells grown in a medium containing a lower concentration of glucose. This observation may be highly relevant when one considers that a carbohydrate-rich diet can predispose individuals to oral *Candida* infections (106). Increased adherence of *C. albicans* may, in part, explain this clinical observation. The increased adherence with galactose-grown cells was not strain dependent (92). McCourtie and Douglas (92) noted that several phenotypic properties of galactose-grown cells were identifiable. First, such cells were more resistant to spheroplast formation when treated with Zymolyase. Second, electron microscopy of thin sections of yeast cells stained with ruthenium red revealed an outer, fibrillar-floccular layer which was absent from or reduced in glucose-grown cells. This observation suggests the presence of an acid polysaccharide on cells that were more adherent. In a related study, these investigators examined the adherence to BEC and acrylic of several strains of *C. albicans* isolated from active infections and of two strains obtained from asymptomatic carriers (93). Each of the seven strains from patients adhered to the same extent when grown in 50 mM glucose but adhered significantly more when grown in a high concentration of galactose or sucrose. Although only two strains from asymptomatic carriers were used, neither of these strains exhibited galactose-induced enhancement of adherence. Interestingly, the strains of *C. albicans* isolated from patients with a candidal

infection exhibited a higher virulence for mice when grown in galactose than when grown in glucose (relative virulence was 5 to 24 times greater than that of cells grown in low-glucose medium). The two strains of *C. albicans* isolated from carriers exhibited similar 50% infectious doses regardless of the type of sugar used to grow the cells. Thus, these data indicate that adherence in vitro and virulence are greatly influenced by the types of sugar used to propagate cells. Concomitantly, a fibrillar-floccular layer appears on the surface of cells incubated in a medium containing a high level of galactose. The fibrillar-floccular layer of *C. albicans* is not peculiar to in vitro-grown cells. A number of investigators have described a prominent fibrillar-floccular layer on *C. albicans* in scrapings taken from the tongue and buccal mucosa of patients with oral candidiasis (65, 90, 102). This material seemed to mediate adhesion to host cells. Attachment of yeast cells to BEC appeared to be dependent on fibrillar structures composed of ConA-rich polysaccharide (158, 162). This external layer may also influence interactions of the organism with neutrophils (64).

More recent investigations (33, 94) have detailed the isolation, analysis, and partial characterization of the fibrillar-floccular cell surface material. Yeast cells grown in a liquid medium containing galactose, sucrose, or glucose produce an extracellular polymeric material (EP) that is easily recovered in culture supernatants. Galactose-grown yeasts produce more EP than sucrose or glucose-grown cells. An analysis of this EP revealed approximately 65 to 82% carbohydrate (which was mostly mannose), 7% protein, 0.5% phosphorus, and 1.5% glucosamine. Regardless of which sugar was used to obtain the EP, each preparation was immunologically identical; however, galactose-grown cells had a higher number of antigenic determinants. For example, antiserum raised against cells grown in 500 mM galactose exhibited significantly higher titers of antibody by agglutination to cells than did antiserum raised against cells grown in glucose. Also, antiserum raised against galactose-grown cells reacted more strongly with galactose-grown cells used in the agglutination assay than with those grown in glucose. However, antiserum raised against cells grown in galactose and adsorbed with cells grown in glucose lost all ability to agglutinate the homologous organism. Hence, the EP from organisms grown in each sugar was immunologically identical.

EP from culture supernatants was coated on acrylic, and adherence by yeast forms to the acrylic was measured (94). EP from galactose-grown cultures enhanced the ability of yeast forms to adhere to the acrylic. In fact, cells grown in glucose adhered to a greater extent on acrylic coated with EP from galactose-grown cultures than on acrylic coated with EP from glucose-grown cultures. Thus, EP derived from the most adherent yeast type increased the adherence of yeast cells, which were the least adherent on acrylic. Additionally, adherence to BEC treated with EP from galactose-grown cells was reduced when yeast cells were grown in either galactose or sucrose but was unaffected when cells were grown in glucose. EP from strain to strain varied. Thus, an antiserum prepared against EP from *C. albicans* 2346 grown on galactose, sucrose, or glucose did not agglutinate *C. albicans* 2023 to any appreciable extent. In addition, adherence of *C. albicans* 2346 to BEC pretreated with EP from *C. albicans* 2023 was not affected. The results from both the agglutination and adherence studies indicate that there may be strain-specific differences in the EP.

Increased adherence of cells grown in 500 mM galactose is apparently limited to *C. albicans* and perhaps *C. tropicalis* (31). Other species, including *C. stellatoidea*, *Candida parapsilosis*, *Candida pseudotropicalis*, *C. guilliermondi*, and *S. cerevisiae* showed little or no increased adhesion to acrylic or to BEC (31).

Purification of the component from EP that inhibited the adhesion of *C. albicans* yeast cells to BEC has been pursued (33). Yeast cells were grown at 37°C in yeast nitrogen base containing 500 mM galactose for 24 h (stationary phase). The culture was centrifuged, and supernatants were collected and freeze-dried following extensive dialysis against distilled water. Crude EP was fractionated by ConA-Sepharose chromatography. Nonbinding material was resolved into two protein fractions, and the material eluted with α-D-mannose also yielded two fractions. Each fraction was then examined for ligand activity by a blocking assay in which BEC were first treated with each fraction and then incubated with *C. albicans* yeast cells. Fraction 4, eluted from the ConA column with α-D-methylmannoside, inhibited attachment by approximately 50%, while the other three fractions were relatively noninhibitory. Thus, the active component was presumed to be a mannoprotein. Additional fractionation was accomplished by using DEAE-cellulose chromatography of the mannoprotein fraction eluted from the ConA-Sepharose column. This material could be further resolved into a single fraction (fraction 5) that possessed blocking activity. Following DEAE-cellulose chromatography, a 30-fold purification of the active material was done. However, the investigators did not reveal the purity of the preparation following DEAE-cellulose chromatography. The carbohydrate/protein ratio of the material from the active fraction was 6:7 (wt/wt). Blocking studies were also performed with crude EP subjected to treatment with heat, chemicals, and enzymes. Heat (60°C, 15 min), DTT, mild acid, or the proteases chymotrypsin, bromelin, and pronase significantly destroyed the inhibitory property of EP, while agents such as periodate and α-mannosidase had little if any effect on the inhibitory activity of EP. These studies demonstrate the importance of the protein portion of EP in mediating adhesion.

A different approach, taken more recently, focuses on identification of the epithelial cell receptor for *C. albicans* (32). Knowledge of its characteristics would be useful, in turn, in the isolation and identification of the *Candida* ligand. When BEC were treated with various lectins of known sugar specificity, the Winged-pen lectin inhibited adhesion of *C. albicans* GDH 2346 but not strain GDH 2023. This suggested that glycosides containing L-fucosyl residues might serve as a receptor for this strain. Other lectins such as wheat germ agglutinin (specific for *N*-acetyl-D-glucosaminyl residues) or peanut lectin (D-galactosyl residues) had very little effect on the adherence of *C. albicans* GDH 2346. Recall that attachment of *C. albicans* 2023 to BEC was unaffected by EP from *C. albicans* GDH 2346. When sugars were included in the assay medium, the results closely paralleled those obtained with the lectins, in that L-fucose inhibited adhesion of *C. albicans* GDH 2346 but not *C. albicans* GDH 2023. On the other hand, *N*-acetyl-D-glucosamine (and D-glucosamine) inhibited attachment of *C. albicans* GDH 2023 but only slightly inhibited adherence of *C. albicans* GDH 2346. These observations on lectin and sugar inhibition were not restricted to BEC, as similar results were obtained with vaginal epithelial cells (32).

The observation that *N*-acetylglucosamine and glucosamine inhibited attachment of *C. albicans* to host cells may explain the results of other investigators, who observed similar effects but postulated that chitin is the *Candida*

adhesin (80, 82, 137). It is possible that the *N*-acetylglucosamine and glucosamine effects are due to the binding of these sugars to the *Candida* protein ligand, since some strains would attach to receptors on epithelial cells that have these terminal sugar residues.

To determine whether there were lectinlike components in EP that might mediate attachment of the yeast cell to fucose-containing receptors on epithelial cells, EP preparations from a variety of *C. albicans* strains were applied to affinity columns containing various sugars immobilized on Sepharose (38). Binding of EP from various *C. albicans* to immobilized sugars varied according to the strain tested. However, EP preparations contained a significant amount of lectin protein with binding specificities for L-fucose, D-mannose, or *N*-acetyl-D-glucosamine. As might be expected from the lectin inhibition studies described above, EP from *C. albicans* GDH 2346 recognized L-fucose predominantly, while *C. albicans* GDH 2023 recognized D-mannose and *N*-acetyl-D-glucosamine predominantly and L-fucose least. These results indicate that glycosides containing L-fucose, *N*-acetyl-D-glucosamine, and perhaps D-mannose can each function as epithelial cell receptors for *C. albicans* and that the specific interaction of yeast and host cells may be strain specific. The involvement of L-fucose in the adherence of *C. albicans* is not unique for this organism. In fact, L-fucose, an important constituent monosaccharide of epithelial cell membranes, appears to function as a receptor for the bacteria *Vibrio cholerae* and *Campylobacter* sp. (7, 67). It should be stated that other cell receptors, including fibronection, have been associated with adherence of *C. albicans* to epithelial cells (70, 75, 141).

The findings obtained from the studies on lectin binding by EP have been used to purify the yeast adhesin that binds to fucose-containing receptors (38). EP was first treated with *N*-glycanase, papain, and dilute alkali to cleave both the carbohydrate and protein portions of the mannoprotein. The *Candida* fucose-containing lectin was then isolated by using an affinity system containing the H blood group antigen (containing a terminal L-fucose residue). The material eluted from this column inhibited adherence to BEC more efficiently than the crude EP (220-fold-greater activation when EP and purified material were standardized to protein). The major features of the EP are as follows: it contributes to the fibrillar-floccular surface appearance, reacts with ConA and ruthenium red, is produced when cells are cultivated in high-hexose medium, can be isolated from culture supernatants, promotes stickiness of cells binding to acrylic, blocks adhesion of yeasts to BEC and vaginal epithelial cells, is strain specific, has lectin-binding activity that is also strain specific, and its active protein (ligand) can be purified by using a fucose-affinity column.

It is not possible to determine from the work presented above if the ligand of *C. albicans* that promotes attachment to epithelial cells is identical to the *Candida* complement receptor-like proteins or the laminin-fibrinogen-binding proteins. The only feature in common is the mannoprotein nature of each moiety.

The data presented thus far indicate that the *Candida* ligand (adhesin) is a mannoprotein; i.e., mannoprotein-rich fractions inhibit the binding of *C. albicans* to epithelial cells. Other more indirect proof supports this contention. For example, *C. albicans* pretreated with ConA does not attach to epithelial cells (132). Additionally, anti-*Candida* antibody with a specificity for mannan blocks binding of the organism to host cells (126). Lee and King (79) were able to purify cell walls from yeast forms of *C. albicans*. When incubated with vaginal mucosal epithelial cells, the cell wall fragments adhered to the epithelial cells. However, pretreatment with trypsin, papain, or α-mannosidase reduced adherence by 77, 93, or 99%, respectively, again pointing to a mannoprotein type of ligand. The data obtained with cell wall fragments are very similar to those obtained on the adherence of whole yeast cells to vaginal mucosal epithelial cells. Adherence of whole cells was significantly reduced when cells were pretreated with trypsin and other proteolytic enzymes or β-mercaptoethanol (79). Lipase, high-molar salts, and detergents had little if any effect on the adherence of whole cells to vaginal mucosal epithelial cells. Other investigators have demonstrated a critical role for cell surface protein(s) (mannoproteins) in the adherence of the organism to fibrin platelets (87, 88). Treatment of yeast cells with tunicamycin, an antibiotic that inhibits the synthesis of mannoprotein, blocked the adherence of galactose-grown but not glucose-grown *C. albicans* to BEC when the drug was used at a concentration of 5 μg/ml (40). An alkali-soluble fraction obtained from cell walls of *C. albicans* coupled to sheep erythrocytes promoted the adherence of the erythrocytes to fibrin platelets (87). Adherence was almost totally abolished when conjugated sheep erythrocytes were pretreated with α-mannosidase or by periodate oxidation. This same alkali preparation adsorbed with an anti-*Candida* antiserum prepared against whole cells reversed the inhibitory effect of the antiserum on the adherence of whole yeast cells to fibrin platelets. Partial characterization of the alkali-soluble fraction has been accomplished by Reiss et al. (124). The material is highly antigenic, eliciting a delayed-type hypersensitivity as measured by skin testing and specific inhibition of macrophage migration. This fraction was designated a peptidoglucomannan since it contained glucose and mannose (ration of 2:3) as well as amino acids (7.3%). Partition on BioGel A5M yielded two species with molecular weights exceeding 5×10^4. Resolution of the active mannoproteins from this fraction has not been pursued.

As previously stated, strains of *C. albicans* with reduced adherence to various types of mammalian cells have been reported (21, 60). These strains appear to have cell surface defects involving mannoproteins that can be correlated with their reduced adherence (22). The mannans of these strains have been studied and compared with those of parental cells (134). Mannan fractions were subjected to either mild acid hydrolysis, alkali hydrolysis, or acetylation followed by acetolysis. The apparent molecular weights of mannan from each strain were in excess of 600,000. Thin-layer chromatography as well as HPLC analysis of acid hydrolysates revealed mannose as the major (99%) and glucose as a minor sugar component. Of mannan obtained from parental yeast cells, 15% was acid labile, while mannans from nonadhering strains m-2 and m-10 were found to be 56 and 36% acid labile, respectively. The increased susceptibility of the mannan from the mutant strains to acid hydrolysis may indicate changes in the numbers or sizes or both of mannose side chains attached to the mannan backbone via phosphate bonds. The mannan from the mutant strains was also more susceptible to alkali hydrolysis. Hexasaccharide release following acetolysis was also elevated with mutant mannan. Taken together, these data indicate that structural alterations in cell surface mannan have occurred. Since mannans (and mannoproteins) are thought to function as cell surface ligands, alterations in their structures may be responsible for the reduced level of adherence seen with these strains.

Other cell surface mutants of *C. albicans* have been described; these appear to have defects in their mannan

structures and, correspondingly, to adhere less readily to host cells. Whelan et al. used agglutination of mutagenized *C. albicans* with IgG from a hyperimmune rabbit serum prepared against whole cells to isolate agglutination-negative clones (167). Nuclear magnetic resonance spectra of nonagglutinating clones revealed a lack of Man $\alpha 1 \rightarrow PO_4$ and Man $\alpha 1 \rightarrow 6$ resonances, although a variety of changes specific for each agglutination-negative mutant was observed. Studies correlating specific alterations in mannan structure and reduced functional activity have been initiated with these strains. At least four of nine such mutants lack specific binding activity for iC3b or C3d or both, and one strain, A9V2, appeared to adhere less readily to human BEC (unpublished observation).

Miyakawa et al. (99) recently used MAb CA4-2 to isolate agglutination-negative strains of *C. albicans*. Cultures were mutagenized and subsequently reacted with CA4-2 in a manner similar to the method of Whelan et al. (167). Wild-type cells were agglutinated and discarded, while nonagglutinated cells were cloned and tested further for reactivity with CA 4-2. ^1H nuclear magnetic resonance spectral analysis of the purified mannans for the parent mutants revealed a loss associated with the α linkage of the oligosaccharide side chain in the mutants. Interestingly, *C. albicans* mutant MYU 4-24 adhered less readily to a cell line of a human mouth squamous-cell carcinoma, indicating that the factor 6 determinant may play an important role in the colonization of host tissues (Table 2).

Finally, Kennedy et al. (71) described differences in the adherence of isolates of *C. albicans* that switch at a high frequency between two phenotypes (white and opaque). Each phenotype is readily distinguishable by the size, shape, and color of colonies when cells are grown at 25°C. Kennedy et al. compared the ability of these two phenotypes to attach to BEC and plastic. White cells of three different strains were significantly more adhesive to BEC than their opaque counterparts (88 to 96% of BEC with white cells versus 31 to 50% of BEC with opaque cells). Adherence to plastic by cells of each phenotype was similar. The ultrastructure and antigenicity of the *Candida* opaque phenotype have been studied further (2). Opaque budding cells are larger than white cells, are bean shaped, and possess cell wall pimples, some of which appear by transmission electron microscopy to have channels. Additionally, a large vacuole predominates in the opaque-cell cytoplasm, which is composed of membranous material. It was thought that the pimples might serve as origins of blebs that appear on the cell surface. The investigators used a Western blot assay with hyperimmune antiserum against opaque cells to study proteins unique to opaque cells. Proteins of 31, 21, and 14.5-kDa were unique to opaque cells, but of the three proteins, only the 14.5-kDa protein appeared to be associated with the cell surface. The relationship of these ultrastructural and protein differences to adherence was not postulated.

Adherence of yeast cells has also been examined in an ex vivo assay (34) patterned after studies that measured lymphocyte receptors associated with homing mechanisms (148). In this assay, tissues such as spleen, kidney, lymph nodes, or liver are obtained from mice and quick frozen. The tissues are sectioned, yeast-phase cells are added and incubated, and adherence of *Candida* spp. is subsequently measured. In experiments performed by Cutler et al. (34), adherence of yeast cells was associated with specific regions of each tissue. For example, adherence in lymph node tissue was confirmed to the subcapsular spaces, trabecular sinuses, and marginal zones of splenic tissue. *Candida* spp. appeared to be associated with tissue macrophages in both spleen and lymph nodes. During experimental infection, the same pattern of adherence for each tissue was observed, although in the kidney, binding to the renal arterioles was not seen in the animal infections. A significant variation in the extent of binding was observed among strains of *C. albicans*. This assay should prove useful in characterizing the adhesin(s) of this organism.

Hydrophobicity of *Candida* Cell Surface and Adherence

Hydrophobicity of the cell surface of *C. albicans* may be associated with properties of the organism that cause it to adhere to epithelial cell surfaces and to plastic medical devices (57).

The temporal shift in the hydrophobicity of the organism has been studied by Hazen and Hazen (55). Stationary-phase yeast cells remained hydrophobic after subculture in fresh medium regardless of incubation temperature. After 1 h of incubation, cells cultured at 37°C gradually became hydrophilic, while those cultured at room temperature (RT) remained hydrophobic. As these results were obtained with complex media, experiments were also carried out in defined media. Stationary-phase yeast cells subcultured at RT or 37°C either remained hydrophobic or gained cell surface hydrophobicity (CSH). The occurrence of CSH during germ tube formation was also studied. CSH increased prior to morphological evidence of germination. These results indicate that CSH expression is a dynamic process that is involved in growth of either the yeast or the mycelial form of the organism.

More recently, the expression of CSH was examined in 19 isolates of *C. albicans* (56). Yeast-phase cells of 13 isolates were more hydrophobic at RT than at 37°C, while three isolates were hydrophobic and two were hydrophilic regardless of growth temperature. One isolate was more hydrophobic at 37°C. The isolates that expressed greater CSH at RT were also more adherent to HeLa cells (11 of 13 isolates). The adherence of isolates with equal amounts of CSH at RT and 37°C was similar. Correlation between adherence and expression of CSH was not significant if the isolates were compared as a group; however, when compared as single isolates, better correlation between adherence and expression of CSH was obtained. Since some isolates did not display a correlation between CSH production and adherence, the results suggest the existence of other primary mechanisms that promote adherence. Variants of *C. albicans* that are hydrophilic at RT and hydrophobic at 37°C in complex media have been isolated (56). Functional studies of these variants have not been undertaken. The identification of specific cell surface macromolecules that confer hydrophobic properties on the cell has not been reported.

Aspartyl Proteinase

Extracellular proteinase activity of *C. albicans* may be associated with virulence. The enzyme has been purified (129), its gene has been sequenced (85), and its association with virulence of *C. albicans* has been verified (8, 78, 86, 105, 127). In regard to its role in virulence, the enzyme may function in two ways. First, its proteolytic activity may be associated with tissue invasion. Second, enzyme production seems to be associated with the ability of the organism to colonize host tissues (8).

The proteinase was purified from culture filtrates of *C. albicans* by ion-exchange chromatography using DEAE-

cellulose and affinity chromatography by pepstatin-linked Sepharose (129). SDS-PAGE revealed a major peak of 45 kDa, although with disc gel electrophoresis in the absence of detergent, the protein appeared to be slightly larger (approximately 68 kDa). The isoelectric point of the protein was found to be pH 4.45, although some variation does exist in regard to this characteristic among strains of *C. albicans*. The optimum pH for enzyme activity is also variable, ranging from 2.2 to 4.3. Thermal stability of the enzyme was influenced by pH, and the enzyme, like other carboxyl proteinases, was found to be denatured irreversibly at alkaline pH. The proteinase cleaved human transferrin, α-1 antitrypsin, α-2 macroglobulin, and both IgA1 and IgA2 myeloma proteins. The glycoprotein nature of the enzyme was determined by its staining with sugar-specific dyes.

Candida proteinase may be directly involved in fungal invasion and tissue destruction. The role of the proteinase in the invasion of human oral epithelium was studied by Borg and Ruchel (8). Blastoconidia of the organism adhered to excised human epithelium, and by 4 h, germ tubes could be observed penetrating the epithelial surface. Scanning electron microscopy with specific antiproteinase antibody conjugated with horseradish peroxidase that then was reacted with a diaminobenzidine-hydrogen peroxide (DAB) substrate was used to monitor proteinase activity. DAB colloids were visible on the surfaces of blastoconidia attached to epithelial cells and on germ tubes of *C. albicans* that had penetrated the epithelial surface. Interestingly, only serotype A *C. albicans* presented this pattern of proteinase production. A *C. albicans* serotype B strain exhibited DAB colloids only on its blastoconidia and not on germ tubes. As a correlate, this serotype showed little tendency to invade the epithelium. In this regard, it has been observed that *C. albicans* serotype A is much more common among isolates from lesions of patients with denture stomatitis (91). Those investigators also showed that adherence of *C. albicans* blastoconidia to oral epithelia was inhibited by the proteinase inhibitor pepstatin, implying a role for the proteinase in adherence.

In experiments similar to the ones described above, Ray and Payne (119) investigated the role of the acid proteinase in the adherence of *C. albicans* to human epidermal corneocytes. Pepstatin did not inhibit the attachment of the organism but prevented the formation of cavitations, which usually form as a result of the invasion of the corneocytes. The cavitations were observed around blastoconidia and probably represent sites of tissue destruction caused by the *Candida* proteinase. More recently, Frey et al. (48b) have shown that all protease inhibitors tested blocked adherence of yeast-phase cells by 19 to 53%. Growth of the organism was also affected by the inhibitors, but the effect was strain specific. The most effective inhibitors, which are nonhydrolyzable, synthetic peptide substrate analogs, generally appear to be highly hydrophobic. Other investigators have shown that proteinase inhibitors may also inhibit the growth of *C. albicans* (165). These data suggest a role for aspartyl proteinase in the pathogenesis of candidiasis.

A proteinase-deficient mutant of *C. albicans* has been described by Kwon-Chung et al. (78). The mutant was obtained by nitrous acid mutagenesis. In vitro assays for proteinase production and growth as well as virulence studies using a murine model were conducted with the parental strain (C9), the proteinase-negative mutant (C9M1), and a spontaneous revertant with levels of proteinase about 50% of parental strain (C9M1M) levels. The revertant strain induced infection in 90% of the mice, while the proteinase-negative strain was avirulent. Even though the growth rate of the revertant was slower than that of the parental strain and similar to that of the proteinase-negative strain, virulence of the revertant was essentially equal to that of the parental type.

The enzyme has been found in the vaginal fluid of women affected with *Candida* vaginitis at significantly higher levels than in carriers or in control, healthy individuals (34a).

Other *Candida* species, including *C. tropicalis* and *C. parapsilosis*, secrete a proteinase (128). The proteinase from *C. tropicalis* is similar to that from *C. albicans* in that it is glycosylated. The proteinase of *C. parapsilosis* has a slightly smaller molecular mass and is not produced during the infection of phagocytes as the proteinases of the other two species are. Thus, in the case of *C. parapsilosis*, lack of production may be associated with the reduced virulence of this species.

The mechanism by which the *Candida* proteinase promotes adherence is not known. It is unlikely that the proteinase is also the iC3b-binding protein (a probable ligand), although this possibility has not been ruled out. Nevertheless, it is also reasonable to assume that multiple types of adhesins exist for this organism. A more likely mechanism might involve the proteolytic cleavage of some component that masks a host cell receptor. This model would require that the protease (or some other component) function as a ligand, resulting in a yeast cell ligand-host receptor interaction. A similar mechanism has been described for a bacterial system (7).

HOST CELL RECEPTORS

The nature of the epithelial cell receptor that recognizes the *Candida* ligand has been discussed above (see Adherence of *C. albicans* to Host Cells). In brief, fucose or *N*-acetylglucosamine containing surface glycoproteins of epithelial cells recognizes a lectin(s) on the *Candida* surface (32). However, blood-borne *Candida* cells interact with endothelial cells or the subendothelial extracellular matrix (ECM). From these initial points of contact, the organism may gain entry into tissues as conversion to its filamentous form occurs and extracellular hydrolyses are produced. Adherence of the organism to both endothelial cells and ECM has been reported in both in vitro and in vivo studies (75, 76, 96). In some instances, the organism adheres to the ECM to a greater extent than to endothelial cells (75). In recent studies by Klotz (75), the adherence of *C. albicans* to substrates in the vascular basement membrane and stromal ECM has been measured. The organism adhered to type IV collagen, laminin, and fibronectin but not to substrates associated with the surface of normal endothelium. Adherence to these substrates was enhanced in the presence of divalent cations, while solubilized laminin or fibronectin inhibited the attachment of the organism to surface-coated laminin or fibronectin, respectively. In a more recent study, Klotz has demonstrated that trypsin digests of gelatin inhibit the adherence of *C. albicans* to type 1 and type 1V collagen, fibronectin, and laminin (74a). Low-molecular-weight gelatin fragments were obtained by Sephadex purification and found to inhibit adherence. Since each of these substrates contains the arginine-glycine-aspartic acid (RGD) amino acid sequence, this peptide and others, specifically peptite, a synthetic RGD-containing peptide, were assayed for their abilities to inhibit adherence of yeast cells to collagen or ECM (76a). The degree of inhibition varied depending on the

peptide used, with peptite exhibiting the greatest inhibition (>90%).

These data establish the role of components of the ECM in the adherence of *C. albicans* to host tissues and indicate that, much like the mammalian integrin family of receptors, the RGD sequence of ligands is important in host-yeast interactions.

Previously, Skerl et al. (141) had demonstrated the binding of *C. albicans* fibronectin coated in petri dishes. Adherence was reduced by approximately 50% if the yeast cells were heat killed and treated with proteases or if calcium was omitted from the medium. *C. albicans* and *C. tropicalis* adhered to a significantly higher degree than *C. krusei* or *S. cerevisiae*. Solubilized fibronectin inhibited attachment to BEC or vaginal epithelial cells by 50 and 40%, respectively. More recently, Kalo et al. (70) demonstrated that yeast cells of *C. albicans* adhered to a greater extent to those human vaginal epithelial cells which were also positive for fibronectin than to cells which did not possess fibronectin.

CONCLUSIONS

On the basis of the preceding discussion, it appears that the *Candida*-host cell recognition systems are extremely complex. It is, however, possible to categorize these systems by considering the components of each ligand-receptor. Thus, category 1 includes interactions between *Candida* cells and host cells occurring as a result of protein-protein interactions. An example of this is interactions concerning the CR3-like protein of *C. albicans*, which recognizes a protein ligand of host cells. There is sufficient evidence to indicate that the *Candida* CR3-like protein recognizes the RGD sequences that are common to many mammalian cell proteins, such as iC3b, fibrinogen, fibronectin, and laminin. Each of these proteins has been shown to bind to *C. albicans*. Category 2 includes those interactions in which a *Candida* protein with lectin activity recognizes sugar residues associated with the host cell plasma membrane. Thus far, two such proteins have been described, i.e., those that recognize fucose and *N*-acetylglucosamine (glucosamine) residues of epithelial cells. Category 2 appears to include strain-specific interactions, since in the example given above, the type of lectin is specific for certain strains of *C. albicans*. Category 3 is based on the observation that a MAb with a specificity for *C. albicans* mannan, specifically a factor 6 epitope, blocked adherence of the organism to host cells (99). This observation implies a carbohydrate ligand on the *Candida* cell surface that is recognized by an unknown host receptor. Whether or not this simplified explanation of a complex problem is valid will be revealed in future investigations. Adding to the complexity is the finding that the *Candida* aspartyl proteinase is associated with adherence of *C. albicans* to epithelial cells.

The prevailing data strongly indicate that the ligand-receptors of *C. albicans* are mannoproteins. These components are located at the cell surface, where they are associated with the outer, fibrillar-floccular layers. Among the questions that remain unanswered is that of the relatedness of each of these proteins. It is clear that the CR3-like protein of *C. albicans* promotes adherence of the organism to host cells. It is unclear whether this protein also has a lectin activity (as described above) or can bind other ligands such as C3d, fibrinogen, fibronectin, and laminin, as reported for the C3d-binding protein. This information is close at hand as binding studies and sequence analysis are completed.

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