Serologic Response to Cell Wall Mannoproteins and Proteins of *Candida albicans*

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INTRODUCTION

The dimorphic fungus *Candida albicans* is both a commensal and opportunistic pathogen of humans. Predisposing factors for candidiasis include immunosuppressive, antibiotic, and cytotoxic therapies; the presence of intravenous catheters and indwelling devices; very low birth weight; AIDS; diabetes; and drug abuse. Depending on the underlying host defect, this microorganism is able to cause a variety of infections that range from mucosal candidiasis to life-threatening disseminated candidiasis (15, 61, 214).

C. albicans pathogenicity also depends on a complex array of microorganism-related putative virulence factors. These include the yeast-to-mycelium transition, antigenic variability, phenotypic switching, adhesion to host cells and tissues, cell surface hydrophobicity, molecular mimicry, and production of extracellular enzymes (24–26, 50, 64, 113, 233, 246, 277). Most of the biological functions related to pathogenicity and virulence reside in the fungal cell wall, whose main features are presented later in greater detail.

As the outermost cellular structure, the cell wall plays an essential role in the interactions with the host, including the triggering and modulation of the anti-*Candida* host immune responses, which appear to rely on a complex interplay between natural and adaptive immunity (89). Fungal antigens may stimulate specific cell-mediated and humoral immune responses. The importance of cellular defense mechanisms for protection against fungal infections is supported by the clinical observation that most invasive fungal infections occur in patients with defective cellular immunity (81, 155). The role of antibodies in resistance to candidiasis is poorly understood, and reports exist in the literature providing evidence both in favor of and against the importance of antibody immunity as a main host defense mechanism against fungi. However, several authors have suggested the need to reexamine the contribution of antibodies in fungal infections (28, 60, 185, 189), and there is renewed interest in the study of the host antibody response to *Candida* and the possibility of immunointervention as a feasible prophylactic or therapeutic approach for the management of candidiasis.

Another controversial aspect is the laboratory diagnosis of *Candida* infections. The diagnosis of invasive candidiasis is usually difficult to establish by clinical criteria; therefore, culture techniques and serodiagnostic tests for antigen and antibody detection have been used as laboratory aids to diagnosis. However, blood cultures for *Candida* species generally exhibit low sensitivity (129, 214, 240), and the tests for determination of marker antigens need further fine-tuning to improve their sensitivity and specificity so that they will be valuable in guiding

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TABLE 1. Antibody response to *C. albicans* cell wall proteins and mannoproteins

Component[a]	Immunoglobulin isotype[b]	Epitope	Humans		Animal models	Comments	Reference(s)
			Healthy	Patients			
Carbohydrates							
Mannan	IgA, IgE, IgG, IgM	Multiple	+	+	+	Antigen-based serodiagnostic test; strain serotyping is defined by specificity of antibodies to fine structural antigenic motifs of mannan	6, 12, 19, 37, 62, 104, 111, 118, 128, 129, 151, 154, 211, 220, 238, 240, 253, 303
		O-linked	+	+			112
		β-1,2 linked	+	+			119, 231
		Various, acid stable			+		106
HMW-MP	IgG3	Unknown	+	+			119, 231
14–18-kDa moiety	IgG3?	β-1,2 linked	+	+		Antibodies cross-react with β-1,2 linked mannooligosaccharides of mannan	119, 231
Blood group I-related antigens	IgM	Carbohydrate other than mannose	+	+		Antibodies present as part of response to blood group antigens and are presumed to be independent of the fungus	202
Proteins							
SAP	IgG, IgA	Unknown	+	+	+	Elevated antibody levels in patients may be due to contaminating mannan	37, 123, 172, 205, 236, 245
Hsp90	IgG, IgM	Unknown, LKVIRK	−	+		Antibodies associated with recovery from infection	186, 190–193
Hsp70	IgG	Unknown	+	+	+	Antibodies may recognize epitopes among microbes and self	46, 47, 152, 161
Heat shock mannoproteins	IgA	Polysaccharide		+		Apparently induce secretory immune response during mucosal candidiasis	226
Enolase	IgG, IgE	Unknown	ND[c]	+	+	Antigen-based serodiagnostic test; species differences in epitopes recognized	84, 124–126, 183, 199, 252, 253, 281, 286, 305, 306, 309, 310
PGK	IgG, IgE	Unknown				Not a universal immunogen/allergen	124
GAPDH	IgM, IgG?	Unknown	ND	+			98
mp58	IgG, IgM?	Unknown	ND	+	+	Positive in patients with systemic but not superficial candidiasis	169, 262

[a] HMW-MP, high-molecular-mass mannoproteins.
[b] Not all Ig isotypes may have been tested.
[c] ND, not detected.

clinical treatment decisions (203, 238, 240). Likewise, standard immunological tests to detect anti-*Candida* antibodies usually have low specificity and/or sensitivity (i.e., in most cases they failed to discriminate between disseminated and superficial candidiasis), since they mainly recognize antibodies against *Candida* cell wall mannan, which are ubiquitous in human sera (129, 306, 318). Furthermore, the crude preparations of candidal antigens cannot be standardized enough to allow good test reproducibility among laboratories (129). A simple, reliable, and easy-to-perform serological assay for the diagnosis of systemic *Candida* infections is still not available but is urgently needed. In this context, the detection of defined fungal antigens and/or antibodies to such antigens may provide a suitable procedure for diagnosis of invasive candidiasis.

In this review, we will focus specifically on the antibody response to defined protein and glycoprotein *C. albicans* cell wall components in humans and in animal models of experimentally induced candidiasis (Table 1), with special emphasis on the implications for developing novel diagnostic, prophylactic, and therapeutic techniques for candidiasis. Since these proteins and glycoproteins are integral candidal components, we will first consider their main biological features in the context of their natural environment within the fungal cell. Readers who wish to know more about basic aspects of cell wall

biology are referred to a number of excellent recent reviews discussing the composition and structure, physiological role, and antigenic composition of the cell wall of *C. albicans* (26, 36, 41, 62, 88, 210, 233, 259, 260, 265, 266).

C. ALBICANS CELL WALL

The cell wall may be envisaged as a dynamic and plastic, multilayered structure located external to the plasma membrane. The presence of the wall is essential to several aspects of the biology and, as noted above, the pathogenicity of *C. albicans*. Thus, the cell wall is the structure responsible for maintaining the shape that characterizes each growth form (yeast and hyphae) of the fungus, and it acts as a permeability barrier that protects the protoplast against physical and osmotic injuries. In addition, the cell wall plays nutritional roles and is the structure that mediates the initial interaction between the microorganism and the environment.

Composition, Structure, and Functions

The major components (80 to 90%) of the cell wall of *C. albicans* are carbohydrates: (i) mannan or polymers of mannose covalently associated with proteins to form glycoproteins,

also referred to as mannoproteins; (ii) β-glucans that are branched polymers of glucose containing β-1,3 and β-1,6 linkages; and (iii) chitin, which is an unbranched homopolymer of *N*-acetyl-D-glucosamine (GlcNAc) containing β-1,4 bonds. Proteins (6 to 25%) and lipids (1 to 7%) are present as minor wall constituents (26, 36, 265, 266). Yeast cells and germ tubes are similar in their cell wall composition, although the relative amounts of β-glucans, chitin, and mannan may vary with the morphology (26, 259).

β-Glucans and chitin are the structural components of the wall. Quantitatively, β-glucans contribute to 47 to 60% by weight of the cell wall. Chitin is a minor (0.6 to 9%) but structurally important component, particularly associated with cell-cell connections in the ring between parent and daughter cells, in the bud scar, and in the septa of dividing independent cells.

Mannose polymers do not exist as such but are found in covalent association with proteins (mannoproteins) (26, 36, 259, 265, 266). They are the main material of the amorphous cell wall matrix in which the structural polymers (β-glucans and chitin) are embedded, and they represent 40% of the total cell wall carbohydrate. The mannoproteins of *C. albicans* share several general features with the mannoproteins of *Saccharomyces cerevisiae*, a model organism that is one of the most thoroughly investigated yeasts in this regard. The structure of the carbohydrate component of *C. albicans* mannoproteins is described in detail below. Although mannose is the main monosaccharide component of *C. albicans* cell wall glycoproteins, there is evidence that other sugar residues are also present in some wall glycoprotein species (see below).

Electron microscopy studies have shown the existence of several layers in the cell wall of *C. albicans*. The structural appearance is variable and seems to be related to the strain examined, growth conditions, morphology, and the experimental conditions used to prepare the specimens (26, 36, 233). Several cytochemical and cytological studies indicate that the cell wall layering is essentially due to the distribution of mannoproteins at various levels within the wall structure (36). The microfibrillar polysaccharides, glucan and chitin, appear to be more concentrated in the inner cell wall layer, adjacent to the plasma membrane. Proteins and glyco(manno)proteins fill the network of structural polymers and appear to be dominant in the outermost cell wall layer, which has a fibrillar or flocculent aspect (36, 259).

The different cell wall components interact with each other to give rise to the overall architecture of the cell wall. Besides hydrogen and hydrophobic bonds, there is also experimental evidence for the presence of covalent linkages between different components (249, 260). In this context, Surarit et al. (288) reported the presence of glycosidic linkages between glucan and chitin in the nascent wall of *C. albicans*. On the other hand, recent evidence indicates that mannoproteins may establish covalent associations with β-1,3- and β-1,6-glucans through phosphodiester linkages (139, 140, 251), suggesting that such associations may play a key role in configuring the final cell wall structure characteristic for each growth form (yeast and mycelium) of *C. albicans* (249, 257–260). Finally, interactions between mannoproteins and chitin also appear to exist in the wall of *C. albicans* cells (176). Hence, it is possible that the so-called layering, as concluded mainly from electron microscopy observations, is the result of quantitative differences in the proportions of the individual wall components (β-glucans, chitin, and mannoproteins) in each layer rather than absolute qualitative differences (212). Nevertheless, since proteins and mannoproteins appear to be involved in a variety of essential processes for *C. albicans* such as morphogenesis and several

pathogenicity-related aspects, and since they may be responsible for the hydrophobic or hydrophilic status of the cell surface (24–26, 36, 65, 66, 114–116, 121, 162, 249, 257–260), an asymmetric distribution of wall protein and glycoprotein constituents as a consequence of the physiologic role played by each moiety should not be ruled out (41).

A complex array of protein-containing components has been solubilized from isolated cell wall preparations and from intact cells of both candidal growth forms by different treatments (29–31, 33, 39, 71, 162, 176, 178, 227–229, 285). Analysis of these components has revealed quantitative and qualitative differences in the protein composition of yeast and mycelial cell walls. Some components have been characterized as high-molecular-mass (>120-kDa) mannoproteins that appear to be covalently linked to the structural polysaccharides. These species, which contain large amounts of carbohydrate and consequently could be major elicitors of anti-candidal host immunity, seem to play also an important and active role in modulating the organization of the different cell wall constituents to obtain the final supramolecular structure of the wall that is specific for each morphologic form of *C. albicans* (31, 34, 70, 95, 96, 164, 167, 179, 180, 228, 259, 285). Several studies have identified 20 to 40 polypeptide species in the medium-to-low-molecular-mass (from 80 to 15 kDa) range (33, 39, 71). Most molecules that appear to be present in the outermost wall layers and that exhibit receptor-like activities and adhesin properties are medium- and low-molecular-mass species (41). The high-molecular-mass species seem to be homogeneously distributed on the cell surface (31, 34, 95), whereas protein and mannoprotein moieties that may represent receptors for different host ligands exhibit clustering or asymmetric cell surface distribution (32, 163, 181). This further supports the contention, noted above, that differences in the distribution or topological location of wall mannoproteins appear to be related to the distinct functional or structural role played by individual species.

Variability and Dynamics of Cell Wall Antigens

The expression, distribution, chemical characteristics, and behavior observed in vitro and the biological properties of *C. albicans* cell wall-bound proteins and glycoproteins appear to be dependent on multiple environmental and organism-related factors. These include growth conditions, growth state, morphology of the cells, strain and serotype, phenotypic switching, cell surface hydrophobic or hydrophilic status, and the nature of the biological specimens (intact cells or isolated wall preparations) that are subjected to analysis (3, 5, 14, 21, 29, 35, 59, 96, 114, 115, 120, 122, 164, 167, 179, 229, 233, 278). Particularly, differential expression of antigens at the cell surface level of both *C. albicans* growth forms has received considerable attention from numerous research groups. A number of different possibilities have been suggested to explain the changes in the cell wall composition observed during the morphologic transition. Possible explanations are as follows: (i) form-specific components reflect de novo synthesis of proteins; (ii) topological rearrangement of preexisting cell wall constituents occurs; (iii) differences are due to major quantitative variations in the composition of surface components on both fungal morphologies; (iv) changes in the glycosylation pattern of the same molecule occur depending on the cell morphology considered; and (v) a combination of all the above could occur (21, 31, 33, 42, 215, 287, 301, 308). Since the ability to grow in the filamentous form appears to be one of the important virulence traits in *C. albicans*, and since the presence of hyphal elements in deep tissues is related to infection (214), special emphasis

has been directed towards the characterization of hypha-specific moieties and their possible use as antigen markers in the serodiagnosis of systemic candidiasis (208, 209, 230, 235, 307). In this context, the ideal marker antigen should be expressed by all *C. albicans* strains under all different environmental conditions. However, since the cell wall appears to be a highly dynamic "organelle" that exhibits both the capacity of differentially expressing variable constituents useful for switching between the commensal and pathogenic lifestyles and the capacity for modulating and/or evading the immune host defense barriers, this variability must be taken into consideration when trying to identify potential marker antigens of infection. Therefore, this variability accounts, at least partly, for the poor specificity and sensitivity displayed by some of the methods currently available for immunodiagnosis of systemic candidiasis.

Structural peculiarities in the cell wall mannan (see below) are responsible for the antigenic specificity of *C. albicans* serotype A and B strains (111). Antigenic variability in the epitopes that accounts for this specificity has been detected also. In fact, there is evidence suggesting that both serotypes are determined phenotypically rather than genotypically. Poulain et al. (234) showed that serotype B yeast cells and germ tubes express antigen 6 (the factor characteristic for serotype A strains) in infected tissues and that germ tubes of serotype B strains, but not the mother yeast cells from which the germ tubes emanate, express the antigen in vitro. Kobayashi et al. (144) have shown that serotype A strains behave as serotype B strains at pH 2 since they do not express both factors 5 and 6, the latter being specific for serotype A *C. albicans* cells. Barturen et al. (14) reported that the expression of antigens responsible for serotype A specificity is modulated by the pH of the culture medium. These observations may be related to the activity of the enzyme 1,2-β-mannosyltransferase, which appears to be suppressed or lowered at low pH (148).

This antigenic variability detected for epitopes conferring serotype specificity is also extended to other polysaccharide epitopes. The surface antigen makeup of *C. albicans* is in general variable both in vitro and in vivo, as already indicated. Recently, De Bernardis et al. (56) studied the expression of a polysaccharide epitope in yeast and mycelial cells in a model of candidal vaginitis in rats. This epitope is shared by a family of strongly antigenic cell wall mannoproteins including a 65-kDa component, which is recognized as a main target of cell-mediated anti-*Candida* immunity in humans (99, 294, 296). They showed that this epitope is efficiently incorporated in all layers of the cell wall and abundantly expressed on the yeast cell surface soon (1 h) after the vaginal challenge but is no longer expressed on the surface of most of the cells harvested after 24 h of intravaginal growth. This represents the first clear indication of cell surface antigenic variation of *C. albicans* during active infection. Since mannans are strongly immunogenic and immunomodulatory constituents of *C. albicans*, changes associated with the composition of these wall polymers and epitope expression on the cell surface may theoretically represent a mechanism that modulates the host immune response.

CARBOHYDRATE COMPONENT

As stated above, β-glucans, chitin, and mannan are the three major polysaccharide components of the cell wall of *C. albicans*. Although β-glucans are present in greater abundance than mannan in the wall of this fungal species (44), they are immunologically less active (233).

Most of the mannan is found as large N-linked polymers containing several hundred mannose residues associated with high-molecular-mass species, yet smaller N-linked moieties and/or O-linked mannooligosaccharides are associated with smaller glycoproteins. Antibodies to all these carbohydrate immunodeterminants are readily detectable in serum samples. Although mannose appears to be the major monosaccharide constituent, the potential for other complex carbohydrates that may be also present in candidal cell wall glycoproteins to elicit an antibody response has been reported.

Mannan

Initial studies focusing on the characterization of antigen patterns for the serologic classification or identification of medically important *Candida* species demonstrated that cell wall mannan is the main candidal antigen responsible for the specificity of the different serologic reactions (91, 111, 282, 302, 303). Consequently, considerable effort has been expended to determine the specific chemical structure of mannan and to define the epitopes present in this wall component that may account for serospecificity (for a review, see reference 210).

As mentioned above, mannan does not exist as such in the wall structure but exists in covalent association with proteins (mannoproteins) (36, 62, 265, 266). However, the term "mannan" has also been used to refer to the main soluble immunodominant component present in the outer cell wall layer of *C. albicans*. This component, also called phosphomannoprotein or phosphopeptidemannan complex (Fig. 1), contains homopolymers of D-mannose (as the main component), 3 to 5% protein, and 1 to 2% phosphate (239). A detailed structure of this cell wall constituent has emerged from several studies (10, 144–149, 267–274). The mannose polymers are linked to proteins by N-glycosidic bonds (through two GlcNAc [di-*N*-acetylchitobiose] units) to asparagine residues and by O-glycosidic, alkali-labile linkages to threonine or serine residues. The N-glycosidically linked carbohydrate has a backbone chain of α-1,6-linked mannopyranosyl residues with oligosaccharide branches containing mannopyranosyl residues with α-1,2, α-1,3, β-1,2, β-1,6, and single α-1,6-linked mannose units and phosphodiester bonds (87, 90, 145, 150, 271). Single mannose residues and short, unbranched mannose oligosaccharides constitute the O-glycosidically linked sugar component (222).

The antigenic specificity of serotypes A and B of *C. albicans*, described by Hasenclever and Mitchell (111), appears to be determined by structural peculiarities of the carbohydrate moiety of mannan present in the cell wall of strains belonging to a particular serotype. In this context, Kobayashi et al. (147) showed that oligomannosyl side chains containing both β-1,2 and α-1,2 linkages were serotype A-specific epitopes (factor 6) whereas β-1,2-linked oligomannosyl side chains, which are attached to phosphate, serve as major common epitopes (factor 5) for both *C. albicans* serotypes (267). Thus, individual oligomannosides obtained by depolymerization of the phosphopeptidemannan complex, represent epitopes that have been described as the basis of rabbit polyclonal antibody specificities (267). Further characterization of epitopes recognized by antisera to other *C. albicans* antigenic factors revealed that antiserum to factor 1 is directed to the O-linked mannose chains of the cell wall mannoproteins whereas the epitope recognized by antisera to factor 9 is the α-1,6-linked mannose backbone of the outer chain of the N-linked oligosaccharide moiety (10). Single mannose units linked by α-1,6 bonds to the oligosaccharide branches containing α-1,2- and α-1,3-linked mannopyranosyl residues make the epitope corresponding to antigenic factor 4 (271). The structures defining the different antigenic factors mentioned above are depicted in Fig. 1.

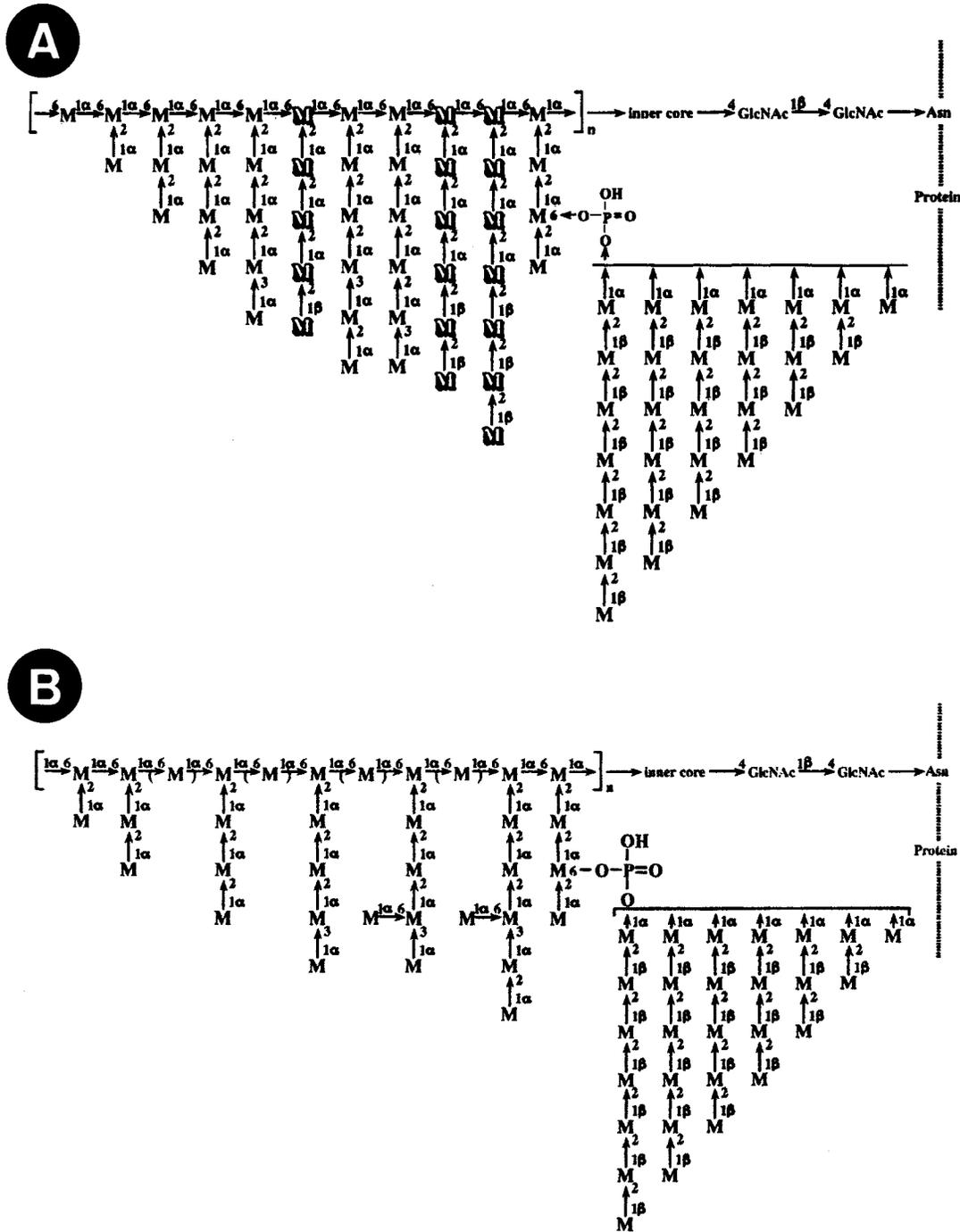The mannan component appears to be involved in the ad-

FIG. 1. Structures of *C. albicans* mannan (M, D-mannopyranose units; GlcNAc, *N*-acetylglucosamine units; Asn, asparagine residue). (A) Representative structure of serotype A strain mannan. Side chains shown by the outlined letter M indicate putative serotype A-specific structures containing β-1,2-linked mannopyranosyl residues at the nonreducing terminal (i.e., antigenic factor 6); other side chains depicted are common in mannans from both serotypes. Reprinted from reference 267 with permission of the publisher. (B) Representative structure of serotype B strain mannan. Single mannose units linked by α-1,6 bonds to the oligosaccharide branches containing α-1,2- and α-1,3-linked mannopyranosyl residues make a serotype B-specific epitope (i.e., antigenic factor 4); α-1,6-linked mannose residues in brackets indicate the partial absence of these units in mannan from *C. albicans* B cells. Reprinted from reference 271 with permission. Structures corresponding to other antigenic factors present in mannans from both *C. albicans* serotypes are described in the text.

herence of *C. albicans* cells to macrophages located in the splenic marginal zone and in the subcapsular and medullary sinuses of peripheral lymph node tissue of mice (40, 51, 109, 135–137, 157). Kanbe et al. (136) showed that the mannan portion of the phosphomannoprotein isolated from yeast-form

C. albicans cells by β-mercaptoethanol extraction and concanavalin A-agarose affinity chromatography contained the adhesins responsible for attachment to macrophages in both types of tissues. Li and Cutler (157) identified one adhesin site on the acid-labile portion of the mannoprotein as a β-1,2-

linked tetramannosyl residue (Ag10G). However, the results indicated that additional adhesins in the phosphomannoprotein complex were involved in the adherence to macrophages. In fact, Kanbe and Cutler (135) later reported evidence of strong adhesin activity in the acid-stable moiety of the phosphomannoprotein complex. The adhesin site implicated is a carbohydrate but is devoid of epitopes for factors 5 and 6 or for Ag10G, which contains the β-1,2-linked oligomannosyl adhesin site. The diagnostic implications of using these carbohydrate epitopes or monoclonal antibodies against them to detect specific antibody or antigen, respectively, in sera from patients with disseminated candidiasis need to be investigated. In any case, immunization of mice with the mannan adhesin fraction implicated in adhesion to macrophages elicits protective antibody responses against experimental *Candida* infection (106) (see below).

Anti-mannan antibodies have been shown to be ubiquitous in human sera, presumably because the immune system can be stimulated as a result of colonization by *C. albicans* in the absence of disease (45, 62, 129). In any defined population, levels of anti-mannan antibodies are usually distributed about a mean; sera with the highest levels give positive precipitin tests when tested against cell wall mannan (128). When anti-mannan antibodies were measured in serially drawn sera from neutropenic patients, a frequency distribution plot showed that the levels of antibodies from patients with invasive candidiasis were elevated and tended to skew the normal distribution curve to the right. However, a clear bimodal distribution of these antibody levels was not observed; therefore, after establishing a cutoff value for anti-cell wall mannan antibodies, the best sensitivity value was about 65% (102).

The applications for the diagnosis of disseminated candidiasis by detection of (i) mannan that circulates during infection or (ii) anti-mannan antibodies have been discussed elsewhere (129, 238, 240). Although most of the recent serological tests for invasive candidiasis are directed to the detection of specific circulating cell wall-bound or cytoplasmic candidal antigens other than mannan, special emphasis has been put on the detection of mannan, as a major cell wall antigen, by different procedures including counterimmunoelectrophoresis, radioimmunoassay, enzyme-linked immunosorbent assay (ELISA), and latex agglutination (11, 12, 19, 23, 57, 74, 82, 85, 92, 94, 131, 143, 154, 156, 198, 211, 220, 256, 311–313). Sensitivities of 23 to 100% and specificities of 92 to 100% have been reported for ELISAs for mannan detection (57). Although early studies showed good sensitivity and specificity of latex-agglutination tests (92), recent studies have been less favorable (19, 23, 154), particularly for sera from patients with malignancies (74). A commercial test for mannan detection, the Cand-Tec system (Ramco Laboratories Inc., Houston, Tex.) has been shown to be relatively insensitive (30 to 50%) compared to other methods (6, 154, 220). Another commercial system to detect *Candida* mannan in serum, the Pastorex Candida test (Sanofi Diagnostics Pasteur, Marnes-la-Coquette, France [118]), appeared promising, but Gutiérrez et al. (104), who conducted a prospective clinical trial, found a 0% sensitivity for this test. The sensitivity of the Pastorex system was improved when serial assays with multiple consecutive serum samples were used (103). In a recent study, Savolainen et al. (253) examined the reactivity of immunoglobulin E (IgE), IgG, and IgM antibodies to mannan by a radioallergosorbent test (RAST) and ELISA and to proteins (some of which are in the cell wall) by immunoblotting and concluded that (i) the IgE titer rose early and was more useful for detection than were IgG and IgM titers, (ii) RAST and immunoblotting analyses were both required for maximum sensitivity and specificity, and (iii) invasive disease could be distinguished from mucosal colonization by this procedure.

Most serologic studies used whole phosphopeptidemannan complexes as antigenic preparations and did not identify individual epitopes recognized by different antibodies. To determine the molecular basis of recognition of oligomannoside antigenic motifs by human and animal (in experimentally induced candidiasis models) immune systems, Faille et al. (76) developed a method of rendering oligomannosides antigenic by coupling them to a carrier molecule that was able to bind to conventional substrates for immunoanalysis. The oligomannosides were coupled to a lipid, 4-hexadecylaniline by a method leading to a mole-to-mole binding whose efficiency can be visualized and assessed by thin-layer chromatography. The hybrid molecules (neoglycolipids [NGL]) exhibited both immunogenicity and antigenicity.

Trinel et al. (298) constructed NGL from three families of oligomannosides released by sequential depolymerization of *C. albicans* phosphopeptidemannan by acid hydrolysis (NGLH), β-elimination (NGLO), and acetolysis (NGLA) and studied their reactivity by ELISA with six monoclonal antibodies reacting with polysaccharide moieties of *C. albicans* mannoproteins. The six monoclonal antibodies were also tested by immunoblotting against germ tube antigens obtained by alkali extraction under reducing conditions. By this extraction method, the authors obtained highly standardized profiles of *C. albicans* germ tube cell wall and cytoplasmic proteins and mannoproteins ranging from 300 to 10 kDa (119). Comparing the epitope mapping of antibodies by ELISA with NGL and by immunoblotting with the extract, they provided evidence that the reactivity of monoclonal antibodies with NGLH (corresponding to homopolymers of β-1,2-linked mannopyranosyl units [77, 268]) was correlated with reactivity with a 14- to 18-kDa *C. albicans* antigen. Conversely, monoclonal antibodies reacting with NGLA also reacted with polydisperse *C. albicans* high-molecular-mass mannoprotein species (298). Oligomannosides released after mild acetolysis have been described as being composed of from 1 to more than 11 mannosyl residues (75) linked mainly through α-1,2 and α-1,3 bonds (146, 150, 268, 273) and to a lesser extent through β-1,2 bonds (145, 268).

By using the method of NGL construction, an analysis of the human antibody response to *C. albicans*-derived oligomannosides was undertaken to define the molecular basis of the recognition of the mannan molecule by human Igs (75, 112, 231, 299). Construction of NGL from *C. albicans* O-linked oligomannosides provided evidence that human antibodies reacted with these residues (112). O-linked oligomannosides released by mild-alkali degradation contained one to seven mannose residues, among which the quantitatively major components, mannobiose and mannotriose, were shown to contain exclusively α-1,2 linkages (112). The pool of oligomannosides was converted to NGL and tested by ELISA with human sera. The sera were from patients who had seroconverted during the course of a mycological and serological survey for candidiasis as detected by indirect immunofluorescence and co-counterimmunoelectrophoresis assays (232). The results of ELISA-NGLO tests appeared to correlate with the results of ELISA on the original phosphopeptidemannan molecule and with the results of routine serologic tests (112). Subsequently, Poulain et al. (231) selected sera with different IgG3 levels against NGLH and NGLA from patients with candidiasis to check the previously correlated reactivity between NGLH and the 14- to 18-kDa antigen and between NGLA and high-molecular-mass mannoprotein species (298). Patient sera recognized saccharide epitopes distributed over the 14- to 18-kDa antigen. These epitopes, which were identified by IgG3, are thought to consist

of β-1,2-linked oligomannosides because of the observed reactivity of the same sera with NGL constructed from these residues (231). Subsequent analysis has led to the identification of the 14- to 18-kDa antigen as a phospholipomannan because of its resistance to proteolysis, its ability to be extracted by chloroform-methanol-water, and its content of phosphate, mannose, and palmitic acid as revealed by metabolic labelling with radioactive probes (299). Poulain et al. (231) also concluded that the oligomannosides distributed over high-molecular-mass mannoprotein moieties, which are susceptible to release from mannan by acetolysis, may act as epitopes for IgG3. Hernando et al. (119) tested sera from patients with candidiasis and from healthy individuals by immunoblotting for reactivity with high-molecular-mass mannoprotein species and the 14- to 18-kDa antigen described above that is present in the extracts of yeast cells and germ tubes. All sera tested recognized high-molecular-mass moieties, and recognition by control sera was invariably associated with reactivity with the 14- to 18-kDa antigen. However, despite a high level of antibodies against high-molecular-mass mannoproteins, some serum samples failed to react with the 14- to 18-kDa antigen or lost this reactivity during the course of the disease. Hernando et al. (119) also found specific recognition of bands in the molecular mass range of 20 to 54 kDa. This is in agreement with the generally accepted contention that antibodies directed to medium- to low-molecular-mass (60- to 29-kDa) antigens may be of value for serodiagnosis (93, 281, 318).

Nonmannan Carbohydrates

Undoubtedly, mannose is the main monosaccharide present in *C. albicans* cell wall glycoproteins. Nevertheless, some experimental evidence indicates that wall glycoproteins may contain sugar residues other than mannose (e.g., sialic acid, galactose, and fucose) that may define additional functional and/or antigenic motifs (2, 29, 130, 202, 308) and raises the possibility that antibodies to other carbohydrate antigens are present in sera from colonized or infected individuals. Antibodies to the human blood group I antigen reacted with *C. albicans* cells in vitro and in infected tissue sections and cross-reacted with protein moieties present in β-mercaptoethanol extracts from yeast and hyphal cells, suggesting that complex carbohydrates related to the blood group I antigen are present at the surface of *Candida* cells (202). Blood group-related antigens such as the I and i antigenic determinants, the precursors of the ABH system, are developmentally regulated (105). The I antigen is the precursor of the H antigen, to which it is converted by fucosylation. Blood group-related antigens are present not only on human erythrocytes but also on epithelial and endothelial cells and in soluble form in plasma and other body fluids (48). Hence, the spectrum of individuals who have antibodies to the I antigen that will react with the analogous antigenic determinants on the surface of fungal cells will be the same as the distribution of anti-I antibodies in the population. The extent to which the presence of cross-reactive antigens on a commensal/opportunistic pathogen such as *C. albicans* may contribute to the development of host antibodies is unclear. In any case, anti-I antibodies are cold-reactive Igs, and the extent to which they may interact with the fungus in vivo is yet unknown.

PROTEIN COMPONENT

Numerous *C. albicans* proteins and glycoproteins play their structural and physiological role outside the plasma membrane barrier (secreted species). They can be divided into two broad categories: moieties whose primary destination is considered to be the cell wall, and molecules whose final destination is the exocellular environment (41). Members of both categories may elicit a host humoral response, and antibodies to several secreted candidal proteins have been detected in sera from both human patients and animals with experimentally induced candidiasis (Table 1).

Secreted Proteins

A complex assortment of hydrolytic enzymes such as proteinases (secreted aspartyl proteinase), phospholipases, acid phosphatase, chitinases, esterase, and glucoamylase can be found in culture filtrates of *C. albicans* cells (41). Although some of the proteins present in culture filtrates may represent products specifically secreted, some others may be components shed from the cell wall or, alternatively, released from the cytoplasm as a consequence of spontaneous cell lysis (see below). Several of the enzymes mentioned above are putative virulence factors of *C. albicans* (26, 41, 50); consequently, neutralizing antibodies to them could represent an effective host defense barrier. Unfortunately, except for aspartyl proteinase, the humoral response of the host to these candidal moieties is essentially unexamined.

Antigenic components released by *C. albicans* cells may be of potential utility as specific marker antigens for the serodiagnosis of systemic candidiasis. During the 1960s, yeast phase cultures incubated for up to 4 weeks were widely used to obtain soluble antigens (170, 174, 182, 276). These antigens cross-reacted with antigen preparations from the cell wall and the cytoplasm and may therefore be considered autolytic products rather than secreted components (182, 291). More recently, Torosantucci et al. (295) studied the material released after 24 h by yeast- and mycelial-phase *C. albicans* cells growing in a chemically defined simple medium. These authors characterized concanavalin A-reactive mannoprotein constituents and demonstrated that cells of both growth forms released comparable amounts of such mannoproteins. No qualitative differences in individual molecular species between the two extracts were found (295).

Later, Aguiar et al. (1) characterized the exocellular antigens obtained from concentrated filtrates of mycelial- and yeast-phase cultures grown on synthetic medium. The only difference between the extracts was the presence in mycelial culture supernatants of a high-molecular-mass, polysaccharide-rich component, migrating in sodium dodecyl sulfate-polyacrylamide gel electrophoresis gels at a position that would correspond to proteins with molecular masses of 245 to 265 kDa. The authors suggested that this molecular entity may correspond to the previously described 260-kDa mycelium-specific high-molecular-mass mannoprotein species (31). Sera from immunized rabbits clearly recognized medium- to low-molecular-mass antigens (21 to 44 kDa) in the culture filtrate supernatants (1).

Recently, we have characterized a complex array of protein and mannoprotein components released into a chemically defined simple medium by cells of both *C. albicans* morphologies (165). A short incubation time (6 to 8 h) was used to prevent potential contamination with cytosolic components released by autolysis. In addition to high-molecular-mass (i.e., >180 kDa) species, which may include the 260-kDa high-molecular-mass mannoprotein moiety (1, 31), several mannoprotein species within a molecular mass range of 58 to 180 kDa were also detected. Qualitative and quantitative differences in the electrophoretic patterns of the materials released by yeast and mycelial cells along the entire range of molecular mass were

resolved by the 5 to 15% slab gradient gels used in this study (165). The soluble material cross-reacted with polyclonal antisera to known cell wall components, including the 58-kDa fibrinogen-binding mannoprotein (mp58) that we described (32, 33, 168).

Other candidal cell surface-related moieties that have been purified and characterized from culture supernatants are the receptor for the complement C3d fragment (255), a fucoside-binding adhesin with lectin-like properties (49, 63, 297), an exo-β-glucanase activity (43, 171), and enolase (284). Except for enolase and preliminary observations in the case of the mp58 receptor (see below), the literature contains no reports of studies on the serologic response in humans and/or animal models to other candidal moieties present in supernatant filtrates of *C. albicans* cultures. The presence of cell wall-bound components in the extracellular medium could be a consequence of an imbalance in degradation and synthesis occurring during cell wall expansion (101, 259, 260) and/or of fibril shedding. This imbalance could occur during changes in hydrophobicity related to environmental and nutritional influences (114, 115), which may result in the liberation of minor but significant amounts of protein and mannoprotein wall components (165). If this situation also occurs in vivo, the release of wall-associated antigenic moieties may trigger a complex humoral response in the host.

Secreted aspartyl proteinase. An extracellular proteinase activity from *C. albicans*, reported by Staib (279), Remold et al. (241), and Rüchel (243), has been characterized as a carboxyl proteinase. Among putative virulence factors in candidiasis, this secreted aspartyl proteinase (SAP) ranks with those which are most widely investigated (246), since several observations suggest a major role for SAP in the pathogenesis of candidiasis (16, 17, 52, 54, 55, 68, 69, 132–134, 173, 216, 237, 244, 247, 248).

MacDonald and Odds (172) compared the titers of antibodies to candidal SAP and to cytoplasmic antigens in the sera of healthy individuals, patients with candidiasis, and other hospitalized patients without candidiasis. The levels of anti-SAP antibodies in human sera were higher in patients with candidiasis than in healthy individuals, while the antibody titer against candidal cytoplasmic antigens was almost equal in the two groups. The authors concluded that SAP could be a specific antigen for the serological diagnosis of candidiasis (172). Detection of anti-SAP antibodies for serodiagnosis has been attempted (213). Elevated levels of IgG antibodies to SAP were also observed in patients with invasive or disseminated candidiasis (236, 245).

Ishiguro et al. (123) studied the serological characteristics of patients with *C. albicans* vaginal infection. The frequency of detection of antibodies and their binding patterns against cellular antigens did not differ greatly between healthy females and patients with vaginitis. In contrast, IgG antibodies against SAP purified from culture medium were found at a higher frequency among patients who carried *C. albicans* in their vaginal discharge than among healthy individuals. Vaginal infection by *C. albicans* might contribute to a rise in the level of anti-proteinase antibodies. However, antibody production may be stimulated as a result of gastrointestinal colonization or other types of infection, and this may explain why 18 and 13% of healthy females and patients in whom the absence of *Candida* was assessed by microbiological culture and supported by clinical signs and symptoms, respectively, had anti-proteinase antibodies.

The purified enzyme used in the studies by Ishiguro et al. (123) was contaminated with mannan. Later, Morrison et al. (204) purified SAP and removed mannan by column chromatography with sequential anion-exchange, gel permeation, and

linear gradient anion-exchange steps. The polyclonal antibodies prepared against this purified SAP were used in a competitive binding-enzyme immunoassay in an immunosuppressed-rabbit model of disseminated candidiasis (205). This assay was able to detect SAP antigenuria within 24 h of intravenous challenge and could discriminate between gastrointestinal *C. albicans* colonization and disseminated candidiasis.

In a study by Cassone et al. (37) demonstrating that antibodies mediate protection in rat vaginal candidiasis (see below), anti-proteinase IgA antibodies were found to be present in the vaginal fluid from most of the infected animals. The presence of anti-proteinase antibodies in the vaginal fluid may be expected, taking into account that this enzyme is actively produced in vivo by *C. albicans* cells during infection (52). Preadsorption of the vaginal fluid from infected rats with *C. albicans* cells previously grown in vitro under conditions in which synthesis of SAP was not induced significantly reduced the ability of the vaginal fluid to transfer protection to healthy animals. Hence, it seems likely that a major protective role in this model is played by anti-mannan antibodies (37). However, the possibility of a protective effect exerted by specific anti-SAP antibodies cannot be completely ruled out (53).

Immunosuppressive and B-cell mitogenic protein. Tavares et al. (293) have purified from the supernatants of *C. albicans* cultures an immunosuppressive B-cell mitogenic (ISM) protein that plays an important role in the survival of the microorganism in the host. They showed that the immunosuppressive and B-cell mitogenic properties of this protein, designated p43, were quantitatively associated with the host susceptibility to *C. albicans* infection. Moreover, treatment of C57BL/6 mice with p43 decreases the resistance of the host and subsequently facilitates fungal growth. The loss of the capacity to produce p43 by *C. albicans* correlates with the loss of the fungal virulence. Immunosuppressive B-cell mitogenic proteins produced by bacteria and viruses have been described previously (8, 9, 80). These ISM proteins are virulence factors because the immunosuppression, a consequence of B-cell overstimulation induced by such proteins, is crucial for the survival of the producing microorganisms in the host (158). These observations suggest that a strategy of vaccine development may involve the neutralization of microbial metabolites or substances that are virulence factors like the ISM protein. Tavares et al. (292) investigated whether neutralization of p43 by previous selective immunization of the host with this protein would protect against *C. albicans* infection. They found that immunization of BALB/c mice with p43 fully protected the mice against the fungal infection and that passive administration of specific anti-p43 antibodies significantly protected the animals against challenge with living microorganisms. However, antibodies to p43 have been not detected in naturally infected human individuals.

Heat Shock Proteins

All living organisms have the ability to respond to sudden changes of temperature by increasing their production of a set of proteins, the so-called heat shock proteins (hsps). *C. albicans* is no exception to this rule (317). hsps are also immunodominant antigens and major targets of host immune response during different types of infections (141, 142, 177, 316). An interesting feature of some hsps of *C. albicans* is that they are not confined to the intracellular compartment but are also present at the cell (wall) surface. Members of the hsp70 family of proteins of this fungus, along with the 47-kDa fragment of hsp90, have been reported as being present in the cell wall (161, 197). As components of fungal cell walls, hsps may play

roles similar to those described for hsps in the cytoplasm (160). This surface location implies that the antigenic determinants are naturally exposed and may be responsible for the immunodominant nature of *C. albicans* hsps. Moreover, the presence of hsps at the candidal cell surface may facilitate potential protective roles of host antibodies.

In any case, the ubiquitous nature of hsps, along with the high degree of homology among them, poses interesting challenges to the immune system of the host. First, the presence of epitopes shared by a number of infectious agents may provide the immune system with a universal signal for infection, and antibodies to these conserved regions could provide some natural resistance to infection, bridging the gap between innate and acquired immunity. Second, epitopes shared by the parasite and the host may trigger deleterious autoimmune responses (141). This could also be true for the immune responses against highly conserved immunodominant glycolytic enzymes (see below).

hsp90. Immunoblotting experiments with sera from patients with systemic candidiasis showed the presence of a 47-kDa immunodominant antigen in whole-cell extracts of the fungus (186, 190, 191). This 47-kDa antigen was further identified as a heat-stable breakdown product of hsp90 (187). Monovalent antibodies against the 47-kDa fragment were used in immunoelectron microscopy to demonstrate its cell wall location (197). Antibody to the 47-kDa antigen is present in serum samples from a high proportion of patients with chronic mucocutaneous candidiasis and with AIDS (20, 186, 193). Patients who recover from systemic candidiasis produce a major antibody response to the 47-kDa component, whereas patients with fatal cases have little antibody or falling titers (186, 190, 191, 193). In a mouse model of systemic candidiasis, prior administration of sera from two infected patients containing antibodies to hsp90 resulted in decreased mortality rates (194). Epitope mapping of *C. albicans* hsp90 with patient sera revealed that patients recovering from systemic candidiasis produce antibodies against both fungus-specific and conserved epitopes of hsp90 (192). In particular, a highly conserved oligopeptide epitope (LKVIRK) was recognized by sera from all patients with antibody to the 47-kDa antigen (192). Moreover, when given prophylactically, a murine monoclonal antibody raised against this epitope reduced mortality in a mouse model of invasive candidiasis (194). The LKVIRK epitope is central to the proposed protein-binding site of human hsp90, suggesting a possible mechanism whereby the monoclonal antibody was protective. If extraneous circulating candidal hsp90 binds to serum proteins, causing them to malfunction, an antibody preventing this would be beneficial (185). Thus, it was concluded that autoantibodies against hsp90 can protect against infection. "Human recombinant antibodies" are entirely human in origin, thus avoiding problems inherent to the human anti-mouse antibody response; therefore they are the antibodies of choice for an Ig-based therapy for candidiasis. In this context, the protective potential of a human recombinant antibody to the LKVIRK motif was assessed in two models of murine invasive candidiasis. A statistically significant improvement in survival (acute model) or renal clearance of infection (chronic model) was apparent in both experiments (195, 196).

C. albicans hsp90 circulates in body fluids of patients with disseminated candidiasis, and the 47-kDa antigen was isolated from patient sera by affinity chromatography (191). An enzyme-linked immunodot assay with affinity-purified antibody to the 47-kDa moiety was capable of detecting circulating antigen in the patient sera (186). By using this assay, systemic candidiasis was detected in 77% of neutropenic patients and in 87% of nonneutropenic patients. The sensitivity and specificity of

detection were improved over those of other commercially available products.

In another study, Zoller et al. (318) used purified somatic antigens of *C. albicans*, including a 47-kDa component, in enzyme immunoassays for antibody detection in sera from patients with confirmed disseminated candidiasis. The assay had a sensitivity of 81.5% and a specificity of 97%. However, the identity of this 47-kDa antigen remains to be resolved, since it could actually be enolase (see below).

hsp70. Two members of the hsp70 family of proteins (Ssa1p and Ssa2p) have been found in *C. albicans* (72, 152, 161). A cell wall location of at least one member of this family of proteins has been shown for both *S. cerevisiae* and *C. albicans* (160, 161). The presence of hsp70s in the cell wall and surface implies that the antigen is present in intact cells, without the necessity for cellular disruption, and is readily exposed to the host immune defenses. La Valle et al. (152) reported that sera from three healthy individuals contained antibodies to *C. albicans* Ssa1p and suggested that the presence of such antibodies could contribute to protection against infection. It was also pointed out that the variability in this generally conserved protein was predominantly in the C-terminal region, which is also the immunodominant region (142). In the case of the product of *C. albicans SSA2* gene, it was reported that serum samples from both healthy people and patients with candidiasis contained antibodies to the C-terminal portion of Ssa2p (161). Constantino et al. (47) have analyzed the humoral response of CBA/H mice to systemic infection with *C. albicans*. The major antibody response identified was directed against a protein antigen of 96 kDa (which was not induced by heat shock) and against a 75-kDa candidal hsp, which, according to these authors, appears to be a member of the hsp70 family. Due to the high homology among the different members of the hsp70 family, the antibody responses reported by the different groups could result from a combination of antibodies to the different hsp70s present in *C. albicans*, as well as to hsp70s from other organisms. These reactivity patterns are also likely to be the result of combination of antibodies to conserved epitopes and epitopes unique to individual *C. albicans* hsp70s.

Other heat shock proteins. Swoboda et al. (290) probed hsps (with molecular masses of 38 to 42, 66 to 68, 70 to 72, and 74 to 76 kDa) of *C. albicans* isolated by ATP affinity chromatography in Western immunoblots and ELISA experiments with sera from patients with oral and/or esophageal *C. albicans* infections. These hsps were recognized by the different serum samples assayed; in addition, the levels of IgA correlated with the severity of candidal infection in each case. Heat shock mannoproteins with approximate molecular masses of 180 to 200, 130 to 150, 90 to 110, and 60 to 70 kDa have been identified as being involved in the secretory immune response during mucosal candidiasis (226). These molecules were overexpressed after a temperature shift from 25 to 37°C. The antigenic determinants recognized by secretory IgA antibodies in saliva and vaginal washings were polysaccharide in nature. The 180- to 200-kDa component also enhanced tumor necrosis factor secretion by a murine macrophage cell line (221).

Glycolytic Enzymes

Glycolytic enzymes are abundant immunodominant antigens during *C. albicans* infections, and *C. albicans* enolase (2-phospho-D-glycerate hydrolase; EC 4.2.1.11) phosphoglycerate kinase (PGK; EC 2.7.2.3), alcohol dehydrogenase (ADH; EC 1.1.1.1), pyruvate kinase (PYK; EC 2.7.1.40), aldolase (EC 4.1.2.13), and glyceraldehyde-3-phosphate dehydrogenase (GAPDH; EC 1.2.1.12) have been described as major

allergens or immunogens during candidiasis (97, 98, 104, 123, 124, 199, 263, 281, 286, 289, 304–306, 310). All of these enzymes appear to be highly conserved.

The presence of glycolytic enzymes in the cell wall of *C. albicans* has been reported recently. Thus, enolase was found to be associated with glucan in the inner layers of the cell wall as well as in culture supernatants (7). PGK was found in the cell wall and at the outermost cell surface (4). The presence of ADH in the wall of *C. albicans* has been suggested also (219). Finally, our group has recently found evidence indicating the presence in *C. albicans* of a wall-associated, enzymatically active form of GAPDH, which appears to be located at the most external cell surface layer (97, 98). The cell wall location of other glycolytic enzymes such as PYK or aldolase has not been examined.

It has recently been suggested that the cytoplasm may be the origin of several protein species (including enolase and hsp70) found in the cell wall of *C. albicans* and that these moieties may mask other essential cell surface antigens, thus subverting specific host immune responses to such antigens (73). However, although the localization of presumably "intracellular" glycolytic enzymes in the wall of *C. albicans* cells is intriguing, the presence of glycolytic enzymes on microbial surfaces is not unprecedented. Thus, GAPDH has been identified as a constitutive protein component of the cell wall of *Streptococcus pyogenes*, where it not only is enzymatically active but also serves as a binding protein for fibronectin, lysozyme, myosin, and actin (218, 314), and in the cell wall of another yeast species, *Kluyveromyces marxianus*, where its concentration increases substantially upon induction of flocculation (78, 79). In addition, Goudot-Crozet et al. (100) presented evidence for an active form of GAPDH on the surface of *Schistosoma mansoni* and showed that sera from subjects with low susceptibility to infection by this parasite reacted with this protein while sera of susceptible individuals had little or no reactivity. ADH is also a surface protein of *Entamoeba histolytica* (315). Finally, PGK and triose-phosphate isomerase have been found on the surface of *S. mansoni* (153, 275) and have been suggested as potential candidates for vaccine development against this parasite.

Glycolytic enzymes of *C. albicans* are immunogens during candidiasis. Of these, enolase seems to be the most immunodominant antigen in humans. Enolase stimulates both humoral and cellular immune responses in mice, and these responses are indicative of *C. albicans* proliferation in the host. Antibodies to other glycolytic enzymes (PYK, ADH, PGK, and GAPDH) have also been found in the sera of patients with different forms of candidiasis. Enolase, PGK, aldolase, and ADH of *C. albicans* are also allergens for allergic patients.

Enolase. Despite the considerable heterogeneity of the humoral responses to antigens of *C. albicans* in humans (175, 190, 281), several immunodominant antigens have been identified. These include enolase, the glycolytic enzyme that catalyzes the reversible dehydration of 2-phospho-D-glycerate to high-energy phosphoenolpyruvate. Strockbine et al. (281) characterized the antigenic components in cytoplasmic extracts of *C. albicans* that were recognized by sera from patients with disseminated candidiasis. They found that these patients had circulating antibodies directed against a 48-kDa protein antigen purified by anion-exchange chromatography from the candidal extract. Individuals colonized with *C. albicans* or without evidence of candidiasis did not have detectable levels of antibodies to this antigen, which was subsequently identified as enolase (84, 183). Circulating anti-enolase antibodies may have potential value for the diagnosis of candidiasis (199, 305, 306). Thus, an ELISA with purified *C. albicans* enolase as the target was

devised to detect antibodies in sera from patients with proven candidiasis. Statistical analysis of the results obtained indicated that the assay was able to discriminate between invasive infection and simple colonization (305). However, the test suffered from low sensitivity. Using purified candidal enolase as the antigen in immunoblotting experiments, another group detected anti-enolase IgG antibodies in serial samples drawn from 92.5% of the patients with systemic candidiasis examined, with a specificity of 95% (199).

Antigenemia with the 48-kDa antigen as detected by ELISA was observed in a murine model of disseminated candidiasis in the absence of fungemia and correlated with deep tissue infection (309). To investigate the expression of this candidal cytoplasmic antigen in the serum of patients with cancer, who are at high risk for deep invasive candidiasis, Walsh et al. (310) conducted a prospective clinical trial with patients from four medical oncology centers over a 2-year period. They concluded that *C. albicans* enolase antigenemia is a marker for deep tissue invasion even in the absence of fungemia. The serum enolase immunoassay complemented rather than replaced blood cultures for the diagnosis of such infections (240, 310). The assay was very specific (96%), but the sensitivity was low (only 54% in patients with proven deep tissue invasion). Testing of multiple samples improved the sensitivity for antigen detection to 85% for patients with proven deep tissue infection and to 64% for patients with proven candidemia (310). A commercial assay (Directigen; Becton Dickinson) for the detection of candidal enolase has been evaluated, in comparison with other serodiagnostic reagents for invasive *Candida* infection, by Gutiérrez et al. (104). They showed that the most useful markers in patients with first-time *C. albicans* sepsis are IgM antibodies to blastoconidial antigens. However, for adequate detection of invasive candidiasis, they proposed that levels of both IgG and IgM antibodies and of circulating 48-kDa antigen should be determined.

Some major protein allergens of *C. albicans* have been identified by immunoblotting with anti-human IgE antibodies. In one initial study, 77% of the serum samples from asthmatic patients reacted with fractions containing a 46-kDa protein (252). Ishiguro et al. (124) also focused on the identification and characterization of IgE binding of *Candida* antigens. Several moieties with molecular masses of 175, 125, 46, 43, and 37 kDa were detected. The 46-, 43-, and 37-kDa antigens were purified, and their polypeptide sequences were found to have significant levels of homology to the *S. cerevisiae* glycolytic enzymes enolase, PGK, and aldolase, respectively. Although enolase from *S. cerevisiae*, in addition to the *C. albicans* enzyme, is a major allergen (125, 151), *S. cerevisiae* enolase did not react with IgE of patient sera, suggesting that IgE antibodies whose levels are elevated in allergic patients recognized a limited set of epitopes. In this context, characterization of candidal enolase antigenic motifs eliciting IgE responses revealed that the C-terminal portion of the protein was the more immunogenic (126). The molecular mass and cytoplasmic localization point to the possibility that the 46-kDa allergen detected by Savolainen et al. (252) was also enolase.

Recently, the reactivity toward mannan of antibodies (IgE, IgG, and IgM) present in postoperative patients with invasive candidiasis has been examined by different methods including immunoblotting (253). Several protein antigens were detected by this technique, none by all patient sera, although a 46-kDa band, believed to be enolase was the most conspicuous immunogen detected (253). Enolase thus appears to be an immunodominant protein both in allergic reactions to fungal antigens and in fungal infections. In this context, Breitenbach et al. (22) have reported that serum from a patient allergic to

the molds *Alternaria alternata* and *Cladosporium herbarum* reacted equally well with the enolases from *Alternaria*, *Saccharomyces*, and *Candida*, concluding that enolases are highly conserved major fungal allergens.

Since a Th2 cell response that predominates over a Th1 response has been suggested to underlie the IgE response in atopic individuals (58), and since disseminated candidiasis in murine models is associated with the Th2 response (242), IgEs may be a better indicator of infection than other Ig classes. In one study (253), IgE titers rose early during infection and the greatest sensitivity (93%) and specificity (73%) were seen with the combined use of two immunoassays (RAST and immunoblotting) for IgE detection, while IgG and IgM detection resulted in lower sensitivities and specificities. Whether IgE responses are similar and potentially useful for diagnosis in immunocompromised patients has not been examined.

The humoral and cellular immune responses to enolase have been studied in mice (286). Both lymphocyte activation and antibody production to enolase were evident in germ-free mice colonized with *C. albicans*. Immune responses arising as a result of gastrointestinal tract colonization can be studied in this model because the alimentary tracts of germ-free adult mice are easily colonized by oral administration of *C. albicans* and the microorganism does not persist in the bloodstream or invade internal organs (13). Moreover, lymphocytes from intravenously challenged mice responded to enolase, and in these animals enolase was the immunodominant humoral antigen. This study demonstrated that enolase stimulates cellular and humoral responses and that specific immune responses to enolase are sensitive indicators of the presence of proliferating *C. albicans* in mice (286).

Sundstrom and Aliaga (283) isolated and sequenced a clone coding for *C. albicans* enolase from a cDNA library. Comparison of the predicted *C. albicans* enolase sequence with that of crystallized *S. cerevisiae* enolase (280) showed extensive homology in regions of secondary structure, thus illustrating the similarity in the architecture of the two enzymes. The protein product of the cloned cDNA has been purified as a recombinant protein fused to glutathione *S*-transferase and has been shown to have enolase enzymatic activity. Consistent with the presumed cytoplasmic location of this enzyme, no reactivity of whole cells with antiserum to the fusion protein was observed by immunofluorescence and no enolase activity was detected when intact cells were used in the enzymatic assays (284). These results indicated that enolase was not present on the surface of *C. albicans*, however, enolase was detected by radioimmunoprecipitation in cell wall extracts obtained after digestion of the wall β-glucan network with β-glucanases (Zymolyase). The enzyme was also found in culture supernatants. The presumed cytoplasmic location of enolase suggests that enolase released from fungal cells spontaneously or as a consequence of damage inflicted by host cells and factors may account for the circulating levels of the enzyme in the bloodstream of patients with disseminated candidiasis. Moreover, the release of enolase may be important in defining the selective stimulation of the host antifungal responses during infection. In this context, it has been recently reported that anti-enolase antibodies may have an immunoprotective effect in mice (304).

Although the cytoplasmic location of enolase appeared to be established, Angiolella et al. (7) have recently shown that this enzyme is also present in the cell wall of *C. albicans*. These authors studied the effect of cilofungin, a lipopeptide antibiotic affecting β-1,3-D-glucan synthesis, and showed that subinhibitory, nonlytic doses of this antibiotic inhibited the incorporation of a 46- to 48-kDa glucan-associated protein (46K pro-

tein), which seems to be a cell wall-associated form of enolase, into the growing cell wall. Several lines of evidence supported this contention: (i) the 46K protein exhibited cross-reactivity toward anti-enolase antibodies; (ii) two internal peptide fragments of the purified 46K protein were identical in amino acid sequence to the peptide sequence of the recombinant enolase protein; and (iii) by immunoelectron microscopy with anti-enolase antibodies, the 46K protein was clearly detected in the inner layers of the fungal cell wall (7, 83, 184, 283). The presence of the protein in the inner layers but not in the outermost regions of the cell wall is consistent with previous results of Sundstrom and Aliaga (284). Results reported by Angiolella et al. (7) strongly suggested that enolase is a bona fide cell wall protein, in contrast to previous results obtained by other authors (73, 261). In any case, it remains to be demonstrated whether the cell wall-associated form of enolase is enzymatically active, what its role in the cell wall could be, if the postulated cell-wall location of the enzyme contributes to its antigenic properties, and/or if the wall-associated form may account for the enolase found in the external milieu.

Other glycolytic enzymes. A number of recent reports indicate that in addition to enolase, several other candidal glycolytic enzymes act as elicitors of the host immune response. IgE antibodies to candidal antigens present in 41% of serum samples from allergic patients reacted with PGK (124). The IgE response seemed to be directed against specific epitopes of the *C. albicans* PGK, since the antibodies did not cross-react with the homologous enzyme from *S. cerevisiae* (124). Similar to enolase (7), evidence indicating that PGK is also a bona fide component of the cell wall of *C. albicans* has recently been reported (4).

A dual location (cytoplasm and cell wall) has been suggested for the candidal ADH (219). Although the presence of this enzyme in the wall of *C. albicans* cells requires further confirmation, ADH has been shown to be a major allergen and to elicit an immune response during candidiasis. Thus, IgE antibodies from allergic patients reacted with ADH (263), and antibodies to candidal ADH have been reported to be present in the sera of patients with superficial candidiasis (289). An immunoblot analysis of *C. albicans* components cross-reacting with human IgE antibodies revealed the existence of an immunodominant candidal allergen that was recognized by 77% of serum samples obtained from asthmatic patients (264) and that was identified as ADH (263).

Recently, a cDNA clone coding for a protein exhibiting 76% homology to the GAPDH from *S. cerevisiae* has been isolated by immunoscreening of a cDNA library with a pooled antiserum preparation obtained by mixing sera from two patients with systemic candidiasis confirmed by blood culture and five neutropenic patients with a high level of anti-*C. albicans* IgM antibodies (97, 98). The most reactive isolated clone contained a 0.9-kb cDNA encoding a polypeptide immunoreactive only with sera from patients with confirmed systemic candidiasis. The deduced amino acid sequence from the cDNA clone corresponded to the GAPDH of *C. albicans*, and the enzyme was found to be present and enzymatically active both at the outer surface of the wall and in the cytoplasm of *C. albicans* cells (97, 98).

Finally, IgE antibodies present in sera from allergic patients have also been found to react with aldolase (124), and serum from individuals affected by superficial candidiasis contains antibodies to candidal PYK (289). However, as noted above, these proteins are not known to be in the wall of *C. albicans* cells.

Receptors and Binding Proteins for Host Ligands

C. albicans displays a large repertoire of cell wall-bound receptor-like molecules that mediate the interaction between the fungus and the host cells and tissues. Although there is detailed information about the biochemical characteristics and functional features of most of these candidal adhesins and receptors (24–26, 41, 65, 66, 121, 219), information on the cell-mediated and/or humoral host responses to these moieties is scant. Among the molecules displaying receptor-like characteristics, perhaps two of the best characterized are the C3d receptor mannoprotein (CR2) and the 58-kDa fibrinogen binding mannoprotein (mp58). Although several similarities appear to exist between these two candidal cell wall mannoproteins (27, 32, 159, 255), the receptors appear to be biochemically and functionally independent entities (166).

The ability of *C. albicans* cells to bind serum proteins such as fibrinogen was initially described by different authors (18, 217, 300). Later, our group identified, in β-mercaptoethanol extracts from intact *C. albicans* yeast and hyphae, mp58 which specifically binds fibrinogen (32) and which appears to be heterogeneously distributed on the surface of cells in vivo (181). This molecule has N- and O-glycosidically linked mannose; O-linked sugar residues seem to be involved in the interaction of the molecule with fibrinogen (32). By using a polyclonal monospecific antiserum raised against the mp58 species (32) as a probe, it was found that the mp58 species is expressed by *C. albicans* cells both in culture and in infected human tissues (32, 166, 168, 181). In addition, a large number of *C. albicans* clinical isolates examined contained cell wall-bound functional (i.e., as detected by their ability to bind fibrinogen) mp58-like moieties (169, 262). The presence of antibodies to the mp58 species in sera from patients with different types of candidiasis has also been investigated by immunoblotting. All sera from patients with confirmed systemic candidiasis reacted with this antigen, whereas none of the sera from control individuals and patients with superficial candidiasis contained detectable levels of antibodies to the mp58 (169, 262). These results suggest the potential usefulness of mp58 as a marker antigen for the serodiagnosis of systemic candidiasis.

The ability of *C. albicans* to rosette antibody-sensitized erythrocytes coated with complement fragments C3d and iC3b was initially reported by Heidenreich and Dierich (117) and later reported by other investigators (for a review of this topic, see references 26 and 121). With respect to candidal cell surface moieties that may represent receptors for C3d, a 60-kDa component that exhibits the ability to bind C3d has been detected in both growth forms of the fungus (27, 138, 159, 255, 308). The candidal receptor for C3d is expressed in vivo by fungal cells in kidney tissue sections and peritoneal lavage fluid from infected mice (138) and also appears to be immunogenic in vivo, eliciting a cell-mediated response (86). However, a serologic host response to this *C. albicans* cell wall component, which may play a role in virulence and pathogenesis, has not yet been evaluated.

ANTIBODIES TO CELL WALL ANTIGENS: PROTECTIVE AND DIAGNOSTIC VALUE

Clinical observations indicate that antibodies appear to play an important role in host defense against disseminated candidiasis, because individuals with defects in cell-mediated immunity mechanisms are particularly prone to superficial but not disseminated candidiasis (for reviews, see references 185 and 189). The role of the host humoral response to *C. albicans* in providing protection against disease, however, remains contentious. In this context, the literature is full of conflicting data that either support or refute the importance of anti-*Candida* antibodies as effective mechanisms for fighting infection. In a recent review, Casadevall (28) pointed out that the antibody response to *Candida* is complex, with the presence in immune sera of protective, nonprotective, and deleterious (infection-enhancing) antibodies that may be isotype and epitope specific. As a result, immunoprotection experiments with polyclonal antibodies that may differ in their isotype and in their recognition of epitopes are difficult to interpret and usually lead to inconclusive observations. Also, a number of variables must be considered when searching for and assessing protective antibody effects. Adding further complexity to this problem is the fact that antibodies may exert protective effects by an assortment of mechanisms (which may be difficult to evaluate) and that the fungus may use a variety of "tricks" to evade the host immune defenses (mainly antigenic variability and immunomodulation). However, in recent years, there has been increasing evidence that some *Candida*-specific antibodies can be immunoprotective during infection, thus suggesting the viability of an immunotherapy and/or vaccine approach for the treatment and management of candidiasis (28, 60, 185, 189).

All fungal antigens that appear to be potential elicitors of these immunoprotective antibody responses are secreted proteins or *C. albicans* cell wall-bound components (Table 2). These include several adhesins, most of which are wall mannoproteins, which play a key role in attachment of the fungus to host tissues and cells or to inert surfaces or materials (reviewed in references 24–26, 41, 50, 65, 66, and 121).

Several authors have found that the carbohydrate portion of many candidal cell surface adhesins and receptors is important in fungal adherence to host epithelial cells and proteins (32, 40, 51, 65, 66, 109, 135–137, 157, 200, 201, 250). Han and Cutler (106) immunized mice with a mannan fraction (previously encapsulated into liposomes) implicated in the adhesion of *C. albicans* cells to macrophages (see the section on carbohydrate component, above) (135) to induce protective antibody responses (106). Circulating agglutinins specific for this fraction correlated with an increased resistance to disseminated candidiasis. Specific circulating antibodies that recognized the acid-labile mannan portion of the phosphomannoprotein adhesin complex were at least partly responsible for the protection. Encapsulation of the antigen in lipid was necessary to produce the protective response, thus suggesting that some undetermined coadjuvant factors may participate in triggering the host humoral response.

In the same study, these authors tested two monoclonal antibodies specific for different mannan epitopes in the adhesin fraction. Both antibodies agglutinated *Candida* cells, but only one of them protected mice against disseminated candidiasis. The protective antibody recognized the acid-labile adhesin, while the nonprotective antibody recognized a different epitope in the fraction. Although further experimentation is necessary to determine the mechanism by which anti-adhesin antibodies protect the host against disseminated candidiasis, the authors suggest that such antibodies may alter the adherence of yeast cells in vivo or may enhance phagocytosis (106). Taking into account that patients with disseminated candidiasis vary not only in whether they express a good humoral response to candidal antigens but also in the antigen specificity and class of antibodies produced and the epitopes they recognize on particular antigens (189), these observations underscore the importance of defining precisely which fungal epitopes can induce protective responses and indicate that only antibodies with certain specificities are protective. In this context, it has been reported recently that a mannan vaccine (li-

TABLE 2. Putative protective antibodies to *Candida* cell wall components: models and strategies

Component	Infection or vaccination	Antibodies	Model	Comments	Reference(s)
Mannan	Infection	Polyclonal	Rat vaginitis	Passive transfer with antibodies to mannan and SAP	37, 53
β-1,2 linked oligo-mannoside	Vaccination with liposomal-packed component	Polyclonal	Mouse and mouse vaginitis	Correlation between agglutinating antibodies and resistance	106, 108
	Passive transfer	Monoclonal (IgG)	Mouse and mouse vaginitis	Apparent epitope and antibody specificity; agglutination not sufficient for protection	106, 108
	Vaccination with mannan-protein conjugate	Polyclonal	Mouse vaginitis	Correlation between agglutinating antibodies and resistance	110
Yeast killer toxin receptor (β-1,6 glucan)	Infection; vaccination with anti-idiotypic antibodies	Polyclonal (IgA)	Rat vaginitis	Direct candidacidal activity	38, 224, 225
SAP	Infection	Polyclonal (IgA)	Rat vaginitis	Polyclonal antibodies contained both anti-mannan and anti-SAP immunoglobulins; anti-SAP antibodies may contribute to resistance	37, 53
hsp90	Human infection	Polyclonal	Human and mouse	Presence of antibody correlates with protection; passive transfer reduces mortality rate	188, 193, 194
LKVIRK epitope		Monoclonal (IgG)	Mouse	Prophylaxis reduced mortality	194
	Human infection	Human recombinant antibody	Mouse	Reduced mortality in acute infection and reduced organ burden in chronic model	196
hsp70	Human infection	Polyclonal	Human	Proposed as a general intermediate mechanism between innate and specific immunity	141
hsp75	Infection	Polyclonal (IgG)	Mouse	High levels of antibodies correlated with resolution of infection	46, 47
Non-hsp 96-kDa moiety	Infection	Polyclonal (IgG)	Mouse	High levels of antibodies correlated with resolution of infection	47
Enolase	Vaccination	Polyclonal	Mouse	Passive transfer in a lethal but not acute model	304
p43 immunosuppressive factor	Vaccination	Polyclonal	Mouse	Neutralization of immunosuppressive properties; no lethal model	292

posome-encapsulated cell surface mannans) and the protective monoclonal antibody previously described by Han and Cutler (106, 107) protect mice against *Candida* vaginitis (108) but that a mannan-protein conjugate vaccine can also induce a protective immune response against the same infection in mice (110). Hence, the diagnostic implications of using these carbohydrate epitopes or monoclonal antibodies against them to detect specific antibody or antigen, respectively, in sera from patients with disseminated candidiasis needs to be investigated.

Polonelli et al. (224) demonstrated that secretory anti-idiotype IgA elicited by intravaginal vaccination with a monoclonal antibody with specificity for a yeast killer toxin from *Pichia anomala* (which kills *Candida* cells) protected rats from infectious challenge with *C. albicans* by molecular mimicking of yeast killer toxin activity as its internal image. In this case, the protective antibodies were not anti-*Candida* antibodies. Consistent with this observation was a previous report from this group showing that yeast killer toxin-like anti-idiotypic antibodies specifically react with the *C. albicans* killer toxin receptor (223). The killer toxin receptor is β-1,6 glucan and not a wall-associated protein or glycoprotein (254). Later, Polonelli et al. (225) showed that exposure to *C. albicans* bearing the

killer toxin receptor stimulated the production of antibodies that were functionally equivalent to anti-idiotypic antibodies because they were also able to confer a significant protection against *Candida* cells in a model of rat vaginitis. Antibodies that mimicked killer toxin were found in unvaccinated mice that were exposed to the fungus. Such antibodies were recovered from the vaginal fluid of women with candidiasis, and, once purified, they were able to afford passive protection in the animal model. The authors speculated that the host immune response may exploit the killer toxin receptor of microbial pathogens to produce microbicidal antibodies (38). In any case, additional clinical studies (with a large number of subjects) are required to demonstrate a correlation between infection or simple colonization by *C. albicans* and levels of antibodies to anti-killer toxin.

In another study, antibodies to the cell wall carbohydrate component were also implicated in mediating protection in rat vaginal candidiasis. Cassone et al. (37) showed that the spontaneous clearing of the initial infection rendered the animals highly resistant to a second vaginal challenge with the fungus. The vaginal fluid of these *Candida*-resistant rats contained antibodies to mannan constituents and SAP and was capable of

transferring a degree of protection against *Candida* cells to naive rats. This protection was mediated by the immunoglobulins present in the vaginal fluid. These results suggest that acquired anti-candidal protection in this vaginitis model is mediated at least in part by antibodies, among which those directed against the mannan antigen(s) might play a key role.

Recently, a protective role for anti-enolase antibodies has been suggested (304). Thus, passive protection against a subacute but lethal challenge with *C. albicans* cells in a murine system was provided by repeated administration of immune serum. The protective effect appeared not to cause the initial clearance of fungi but to afford protection at some later stage in infection.

As noted in a previous section of this review, antibody to the p43 immunosuppressive factor provided protection against a fungal challenge following vaccination with the factor or passive transfer of anti-factor serum (292).

As discussed above, a protective role for antibodies to hsp90 has been suggested on the basis of both correlates of antibody titers in patients recovering from candidiasis and passive-protection experiments in murine models (186, 188, 190–193, 196). A human recombinant monoclonal antibody to an epitope recognized by all positive sera has been found to provide passive protection in mice (196). hsp70s are immunodominant antigens that elicit an antibody response in different types of microbial infection (141, 142, 177). Such responses to conserved epitopes may confer a level of cross-species protection intermediate between innate and acquired immunity (141).

The potential of additional candidal cell wall proteins to provide targets for protective antibodies has been pointed out by Casadevall (28) and Matthews and colleagues (185, 189). We have previously shown that Fab fragments of a monoclonal antibody to a putative structural protein of the wall of *C. albicans* germ tubes (31) inhibited morphogenesis in vitro (34). Casadevall (28) also emphasized that protective antibodies are epitope and isotype specific. On the one hand, this raises questions about whether complex antigen sources such as intact cells or cell extracts will be successful in producing a general protective antibody response; on the other hand, it suggests that specific antigens and epitopes may be successful in this regard. In addition, antibodies may be able to enhance the efficacy of antifungal drugs. Certain monoclonal antibodies to the glucuronoxylomannan of *Cryptococcus neoformans* not only provide passive protection against infection but also enhance the efficacy of antifungal drugs (67, 206, 207). Preliminary experiments with *C. albicans* suggest that a monoclonal antibody to the acid-labile mannan adhesin can prolong survival in infected mice receiving nontherapeutic doses of amphotericin B and fluconazole (107).

Another controversial issue is the diagnosis of candidiasis, mostly in its invasive form. Systemic candidiasis is difficult to diagnose antemortem because of the lack of a typical clinical picture; in addition, although advances in blood culture methods have improved the detection of fungemia, routine blood cultures are relatively insensitive and make take several days to establish the diagnosis of candidemia (129, 240). The need for a rapid noninvasive test for the diagnosis of invasive *Candida* infections has focused the interest of many research groups for more than a quarter of a century (reviewed in references 57, 129, 209, and 240). In this context, the potential of several candidal antigens and antibodies to them, including several cell wall-associated antigenic species, has been examined. The principal cell wall components that have been used in these tests are mannan and enolase in both antigen and antibody detection schemes. Other antigens have also been detected in immunoblot analyses. The diagnostic applications of these spe-

cies were described more extensively in previous sections. As discussed elsewhere in this review, some of these tests in the hands of their developers provided both sensitivity and specificity. Thus, the answer to the general question whether serologic tests for candidiasis can be diagnostic is yes. However, a distinct question is whether these tests are clinically useful and feasible to perform. In this area, the answer is more ambiguous. The predictive value of serologic tests generally increases when serial serum samples are obtained, tests for more than one antigen or antibody are used, or serological tests are used in conjunction with blood culture procedures. A successful commercially available test, which simply does not exist to date, will require both high specificity and high sensitivity to discriminate between colonization and infection. New tests must improve on previous methods in these areas and be more timely, allowing a more rapid diagnosis of the invasive forms of the disease. This is clearly an aspect in which our increasing understanding of the antibody response to *Candida* could have direct implications. Thus, detection of specific immunodominant antigens, rather than undefined antigenic preparations, and/or detection of antibodies to such antigens may prove suitable for diagnosis of invasive candidiasis. The ideal "antigenic marker" for deep infection needs to meet certain criteria in order to be useful in the diagnosis of candidiasis. First, it has to be expressed in all strains of *C. albicans* and under different growing conditions. Second, it has to be expressed and trigger an immune response during infection, preferably at the early stages. Finally, it should not elicit a major immune response during the interactions of *C. albicans* with the host as a component of the normal human flora. As mentioned earlier in this review in the discussion of individual cell wall molecules, a number of cell wall antigens with potential utility as antigenic markers have been identified, characterized, cloned, and sequenced. With their amino acid sequences readily available and the application of new epitope-mapping techniques, it is now easier to identify specific epitopes within these antigens and to adapt this knowledge to the development of improved serologic tests for the diagnosis of candidiasis.

SUMMARY AND OUTLOOK

Morbidity and mortality rates associated with systemic infections by *C. albicans* remain unacceptably high, the main reasons being the difficulties in the diagnosis and treatment of this type of infection. Many research laboratories have examined the utility of detecting antigenemia with candidal moieties or host antibodies produced to fungal antigens for diagnosis of disseminated candidiasis, and studies towards this objective continue in several laboratories (127, 253, 305). However, although urgently needed, an early, accurate, and reliable serology-based procedure for diagnosis is not commercially available and in general use, nor are alternative approaches for the therapy and prophylaxis of candidiasis available. Clearly, the development of these innovative tools can directly benefit from a better understanding of the mechanisms of pathogenicity displayed by *C. albicans*, especially the role of the antibody response during disseminated candidiasis. As stated above, cell wall proteins and mannoproteins of *C. albicans* are major elicitors of the host immune response, and our increasing knowledge of the identity and expression of these components may assist in the development of innovative tools leading to a better management of candidiasis.

The demonstration of protective antibody effects has benefited from major advances in different fields such as (i) our increasing knowledge of the pathogenicity mechanisms associated with *Candida* infections, which can help identify oppor-

tunities for immunointervention; (ii) the identification and characterization of individual cell wall moieties displaying antigenic properties, which may represent potential candidates for vaccine development; and (iii) the advent of the monoclonal antibody and antibody engineering technologies, which have renewed interest in serum therapy as a safe, inexpensive alternative to treat infections. As stated by Matthews and Burnie (189), a number of questions need to be answered when assessing the therapeutic potential of antibodies to candidal antigens (e.g., which types of candidal infections and groups of patients would respond to antibodies, which *Candida* species are potentially treatable with which antibodies, and whether there is synergism between antibodies to different antigens and between antibodies and antifungal agents). However, it is expected that the currently expanding knowledge in these areas could ultimately result in the design of innovative prophylactic and therapeutic strategies for these infections.

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