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RESEARCH ARTICLES

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The cell wall structure: developments in diagnosis and treatment of candidiasis

Summary Candidiasis are among the fungal infections the most difficult to diagnose and treat. Research focused on specific fungal components which are absent in the host, such as the cell wall has lead to a better understanding of *Candida albicans* pathogenicity and clinical impact. The cell wall is responsible for antigenic expression and primary interaction with the host. It is composed mainly of β-glucans, chitin and mannoproteins, which account for the rigidity of the wall and for the fungal morphology. Of these components, mannoproteins might carry a "morphogenetic code" which might modulate the molecular architecture of the cell wall. The features of specific cell wall proteins as part of building blocks to form this structure is revised, and the usefulness of monoclonal antibodies obtained against cell wall components to study those processes, together with their clinical applicability, is discussed.

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Introduction

Importance of candidiasis In the last few decades the incidence of fungal infections by *Candida albicans* and other related human opportunistic yeast species has increased dramatically due to the rise in the number of immunocompromised patients and their life expectatives [44]. Approximately 50% of fungal nosocomial infections are caused by *C. albicans*, as has been indicated by the US National Nosocomial Infections Surveillance System (NNISS). Several factors have contributed to this increase, such as abuse of antibiotics which has favored the emergence of fungal resistances, immunosuppresion originated by cancer therapy and transplantations, and the progression of HIV infection to AIDS. The use of catheters and protheses, together with surgical techniques and the augmentation of intensive-care units, contribute also to the expansion of these infections.

All types of candidiasis, particularly invasive candidiasis, are infections difficult to diagnose and to treat, and are often fatal. Although the diagnostic methods for candidiasis on the surface are relatively effective, no satisfactory methods have been available when the fungus invades human tissues and blood.

Success in diagnosis involves efficient techniques for the isolation of the pathogen, which allows to obtain sufficient samples for the identification assays. Progress in diagnosis requires the development of new serological and molecular assays.

C. albicans being a diploid eukaryotic organism, it possesses enough genomic information to grow, multiply and colonize different environments, from simple culture media to the complex human body. Devoid of sexual reproduction, this fungus can face multiple environmental conditions by undertaking major phenotype and karyotype variations [93]. To understand *Candida* pathogenicity and clinical impact, it is necessary to analyze the host-fungus interaction. This interaction includes the expression of fungal determinants of pathogenicity as opposed to natural and adaptative host immune response. To switch from saprophytic to pathogenic behavior, *C. albicans* has to develop some phenotypic characteristics which allow penetration into the host organism. Two main steps are required: adhesion to host constituents, and production of lytic enzymes to facilitate the fungal progression. These two processes are associated with morphological variations [137]. By operating a dimorphic transition from the blastospore to the filamentous stage, *C. albicans* increases in adhesive properties and proteinase secretion [28]. *C. albicans* cells, particularly the hyphal form, can bind to epithelial cells via lectin-like surface

components to mono- or disaccharides [133]. Fixation to the endothelial layers involves protein-protein interactions, including adhesion to either basement membrane or extracellular matrix components such as fibronectin, laminin, collagen, entactin, and even fibrinogen, suggesting multiple mechanisms for settlement and colonization [133].

The cell wall as a differentiating structure Cell wall is the fungal structure responsible for the primary interaction with the host. It is also responsible for antigenic expression, adhesion, and cell-cell interactions [123, 124]. Besides, the cell wall plays a crucial role by shielding the fungal cell against osmotic, chemical, biological harm, and because of its rigidity, it is essential for the integrity and shape of the cell [64, 124]. The cell wall maintaing the shape of the fungal cell, it should be involved not only in morphogenetic changes, but also in morphological responses. B- and T-cell mediated immunity against *C. albicans* focuses upon surface constituents which are also very relevant in the adherence of the fungus to the target host cell, antigen variation, in avoidance of phagocytosis and the suppression of host immune response itself [28].

The cell wall is a structure unique to fungal cells. It is not present in mammalian cells, thus is ideal search for new specific antifungal drugs. These antifungals should interfere with the synthesis and/or assembling of the biopolymers which compose the cell wall (chitin, glucan and mannoproteins). Many of the enzymes involved in the synthesis and crosslinking of these components are essential targets for antifungal treatment. Thus, some antibiotics have been isolated, such as polyoxins and nikkomycins, which inhibit chitin synthases, and echinocandins and pneumocandins, which inhibit β-glucan synthases.

Knowledge of the cell wall is thus essential for the design of new antifungals in the treatment of candidiasis. Furthermore, basic studies on cell wall composition and architecture during dimorphic transition are generating important information which might help to produce useful tools for the diagnosis of candidiasis. Various monoclonal antibodies against *C. albicans* cell wall mannoproteins have been developed and successfully used in the diagnosis of candidiasis by different means, including detection of the pathogen in tissues, and the rapid, specific detection in cultures from clinical isolates by using diagnosis kits [32, 45, 76, 84, 102, 105].

C. albicans continues to be a good model to study basic cell biology problems such as cell multiplication and morphological transition. Methods for selective gene disruption and expression are now available [43, 92] that allow a genetic approach to study such processes. Particularly interesting is the genetic control of cell wall biosynthesis and assembly of macromolecules, especially those covalently and non-covalently linked proteins which have been recently found as integral components of cell wall structure [2, 28, 40, 41, 69, 131]. Thus, dimorphic transition from yeast to hyphal cell remains a major problem for addressing key issues of *C. albicans* biomedicine.

Cell wall composition

The cell wall of *C. albicans*, as in other yeasts such as *Saccharomyces cerevisiae*, makes up approximately 30% of the dry weight of the cell [134, 141].

Approximately 90% of the cell wall is composed of polysaccharides, and 5–10% of protein. Some studies have shown that the main components of fungal cell walls are β-1,3-D-glucans, β-1,6-D-glucans, chitin and mannoproteins [110, 126], whereas lipids and inorganic salts are minor components [110, 127]. In most fungi, glucans and chitin polymers account for the rigidity of the cell wall and also for its morphology [124]. These cell wall components form a layered structure, where the mannoproteins are mainly on the outside and the glucan layer on the inside [42, 64].

Glucans Glucans may be divided into two groups, α- and β-glucans, according to the type of binding. β-Glucans are the main structural polysaccharides present in *C. albicans*, *S. cerevisiae* and other fungi [39, 134, 145]. β-Glucans can be chemically fractionated into six different fractions considering whether they are linear or branched, and the type and size of branches [110]. However, practically they could be divided in three different types: alkali-soluble (1,3)-β-glucans with (1,6)-β-linkages; alkali-insoluble (1,3)-β-glucans without branches and highly branched (1,6)-β-glucans. The two β-glucan fractions, alkali-soluble and insoluble are currently considered to be one only fraction [51]. Glucans may play different roles in the physiology of fungi, but the most important one is their structural function. Thus, treatment of fungal cells with substances that inhibit β-glucan synthesis leads to morphological alterations [4, 7, 82, 97], and cell fragility [4, 7].

Several genes involved in the biosynthesis of β-1,6-D-glucans have been identified through mutations in *S. cerevisiae* that confer resistance to the K1 killer toxin [6, 12, 13, 80, 107, 108]. These mutans carry some defect in the process of biosynthesis and incorporation of β-1,6-D-glucan and have been named *kre* (for **k**iller **re**sistant). Analysis of the *kre* mutants have shown that β-glucans synthesis follows the secretory pathway; *Kre5*p and *Cwh41*p are found in the endoplasmic reticulum, *Kre5*p in the Golgi-apparatus [80], and *Kre11*p is a cytoplasmic protein involved in the control of *Kre6*p [64]. In the cell surface, *Kre1*p acts in the maturation of the β-1,6-D-glucan polymer [5, 107], and *Kre9*p may be involved either in the final assembly of β-1,6-D-glucan or in its cross-linking to other cell wall components [12]. A *KRE1* functional homologous gene has been isolated from *C. albicans* [6].

Unlike β-1,6-D-glucan synthesis, little is known about genes involved in β-1,3-D-glucan synthesis. β-1,3-D-glucan synthase is a membrane protein [17], whose activity is reduced by 50% in *kre6* mutants of *S. cerevisiae* [106]. This enzyme is made up of at least two components, a catalytic subunit and a regulatory subunit [83]. Following different approaches, two genes termed *FKS1* and *FKS2* [23, 30, 79, 103] or *GSC1* and

GSC2 [54, 79], have been cloned that codify for β-(1,3)-glucan synthase subunits. *Gsc/Fks* proteins have a regulatory subunit with GTPase activity known as *Rho1*p [31, 101]. In *S. cerevisiae,* β-1,3-glucan synthase is bound to chitin [66], this linkage being important to retain the rigidity of the cell wall [125]. Recently a *GSC/FKS* gene has been cloned from *C. albicans* and other fungi [63, 81], and the high homology between them suggests a similar mechanism of β-1,3-glucan synthesis for these organisms.

Chitin Chitin is a linear polysaccharide of β-(1,4)-linked N-acetylglucosamine. The structure found in fungal cells is α-chitin, and the sugar chains run antiparallel to each other. Chitin chains have the tendency to form hydrogen bonds resulting in a ribbon-like structure [110]. Chitin is found in small amounts in yeast but play a major role in wall structure. Mutants with low levels of chitin are osmotic sensitive [14, 95], and exhibit an abnormal morphology, among other phenotypic effects. Chitin biosynthesis is carried out by chitin synthase, which has been partially purified from different organisms [25, 58, 71, 85]. It is suggested that chitin synthase could be a protein complex of approximately 500 kDa. It is accepted that chitin synthase in fungi is localized in two compartments, chitosomes [111] and in the plasma membrane [112]. Several genes have been reported the protein products of which are implicated in chitin biosynthesis. *CHS1*, the gene that codifies for chitin synthase 1 (*Chs1*p), was cloned by complementation of a mutant lacking in chitin synthase activity in vitro [16]; *Chs1*p activity has been found on the plasma membrane in a zymogenic form [33, 34] and in the chitosomes [70]. *Chs1*p is involved in a reparing function at the end of cytokinesis [18]. A *CHS1 C. albicans* homologous gene has been cloned by complementation of *S. cerevisiae chs1* mutant [1]. *CHS2* is the structural gene for chitin synthase 2 (*Csh2*p), which is involved in primary septum formation [91, 95, 118, 130]. *CHS3* (*CSD2, DIT101, KTI2*) codes for chitin synthase 3 (*Chs3*p) [14, 15, 95, 117, 139]. It was cloned by complementing Calcofluor white resistance mutant phenotype, and its disruption leads to a 10-fold reduction in chitin level. Chitin synthase activity is affected by protein products of *CHS4* (*CSD4*, *CAL2*), *CHS5* (*CAL3*) and *CHS6* (*CSD3*) [15, 26, 117]. *CHS6* may be involved in localization and *CHS4* in activation, respectively, of *Chs3*p.

Mannoproteins Cell wall mannoproteins can be divided into two groups according to the methods used for their extraction. One is formed by mannoproteins loosely associated with other components of the cell wall, which can be solubilized by detergents or chaotropic agents [22, 38, 96, 140]. The second group can be released only following enzymatic digestion of the cell wall with β-glucanases [38, 95, 144], or chitinase [77]. Glycosylation in mannoproteins may be N-linked to asparagine, O-linked to serine/threonine, by attachment of a GPI (glycosyl-phosphatidyl-inositol) membrane anchor, or by β-1,6-glucan containing carbohydrate chains [64]. Most of the mannoproteins identified to date released by β-glucanases are very rich in serine/threonine in their C-terminal end [88, 104, 143], and are likely to be highly O-glycosylated. The heavily O-glycosylated proteins may adopt a rod-like structure that enable proteins to expose their active domains to the extracellular medium [55]. Most of the glucanase extractable mannoproteins are N-glycosylated, some of which are GPI modified. The presence of a GPI anchor has been demonstrated in α-agglutinin and various other cell wall proteins of *S. cerevisiae* [75], but not in other β-glucanase-released cell wall mannoproteins [89, 104]. By using antibodies against β-1,6-glucan it has been demonstrated that, in the cell wall of *C. albicans* and *S. cerevisiae* [86], there are β-glucanase-released proteins which possess a β-1,6-glucan moiety. This moiety is likely to be responsible for anchoring mannoproteins to the cell wall [62, 114, 115].

The exact mechanism by which mannoproteins are retained in the cell wall is not yet known, but there is certain evidence indicating that mannoproteins are, probably, involved in determining the morphology of the cells. One such evidence is the presence of specific mannoproteins that might carry a "morphogenetic code" responsible for modulating the molecular architecture of the cell wall [122].

Molecular organization of ***Candida albicans*** cell wall

We now focus on the morphogenetic pathways that govern the positioning of specific components in the cell wall to produce its correct molecular organization. Cellular morphogenesis requires integration of multiple cellular functions, from signal reception to bud site selection to synthesis, secretion and assembly of cell wall proteins.

Processes involved in cell wall morphogenesis The main steps in cell wall construction are summarized in Table 1. We concentrate mainly on point 6. The interaction and assembly of cell wall components lead to the formation of covalent bonds, and to the final organization of the cell wall. In organisms such as *C. albicans*, with more than one morphology, this process involves different interactions between the original components and/or the synthesis of new ones.

Table 1 Critical steps involved in cell wall formation

1. Signal reception and transduction
2. Differential expression of genes
3. Selection site for budding or germ-tube formation (relationship with cell cycle)
4. Cytoskeleton organization at the selected bud or germ-tube site
   4.1 Activation of glucan and chitin synthetases
   4.2 Polarization of secretion
5. Synthesis and secretion of wall proteins
   5.1 "Morphogenetic" proteins
   5.2 "Functional" proteins
6. Interaction and assembly of macromolecules to give rise to the final molecular architecture of the cell wall

We propose that variations in the expression of certain genes (which may be involved in the selection for site budding or germ-tube formation, in the cytoskeletal organization and/or in the interaction between macromolecules in the wall itself) govern the positioning of components in the wall structure. As a consequence, alternative morphologies result. In addition, certain enzymes for the biosythesis of the cell wall are required only in specific steps in the cell cycle, and certain proteins such as Phr1 [116], Phr2 [90] and Pra1 [120], are required for proper morphogenesis. The function of these proteins, whose synthesis is pH regulated, is unknown, but, in their absence, there is an alteration in the location of certain cell wall components, which results in cells with an abnormal morphology. Indirect evidence suggests that the gene products may be involved in the correct organization of the cytoskeleton and therefore regulate polarized secretion of proteins and extrusion of chitin and β-glucans.

Cell wall proteins and their common features As mentioned above, some proteins are solubilized by ionic detergents (i.e., SDS) or by chaotropic agents (urea), illustrating that they are retained by non-covalent bonds; others are solubilized only following enzymatic (β-glucanases and chitinase) degradation of the glucan and chitin structural networks, which suggests that they are retained by covalent bonds to these polymers. In fact, the released proteins from *C. albicans* cell walls carry β-1,6-glucose residues [115]. These proteins, which could be called "intrinsic proteins", have been found in all the fungal species studied.

The presence of specific proteins [2, 48, 130], or specific antigens in mycelial cell walls [21, 94, 99], indicates that new gene products are used in the construction of alternative morphologies. It is therefore possible that these proteins are needed to guide, by some not yet known manner, the positioning of other wall components in a different way than that in the case of the yeast morphology. Ultrastructral studies suggest that rearrangements and/or losses of wall components may occur during the yeast-mycelial transition.

Molecular genetic studies have identified *C. albicans* genes encoding hyphal-specific cell surface proteins [131], and *S. cerevisiae* pseudohyphal surface proteins [53]. In addition, other genes coding for cell wall proteins of *S. cerevisiae* [75, 88, 89, 141], *Yarrowia lipolytica* [104] and *Thrichoderma harzianum* [73] have been cloned. The common characteristics of these proteins, which are rich in proline and threonine, highly O-glycosylated with potential N-glycosylation sites, and which contain distinct repetitive structural domains, do not inform us about how they are really organized in the cell walls.

Cell wall building blocks Knowledge about the chemical bonds that interconnect the wall components (β-glucans, chitin, and mannoproteins) that form the basic building blocks may help us to uncover morphogenetic pathways leading to their formation. The treatment of isolated walls with hot alkali leaves insoluble blocks made of β-glucan-chitin and small amounts of proteins. N-acetyl glucosamine residues of chitin chains are directly bound to glucan through glycosidic linkages [135], and mannoproteins are linked to β-1,6-glucan [115]. Glucans and chitin are synthesized at the level of the plasma membrane and are extruded to the periplasmic space. Besides, wall proteins follow the secretory pathway. So, linkages between glucans, chitin and proteins must be formed externally to the plasma membrane. This conclusion has been confirmed by two complementary observations: (i) the alkali-soluble glucan present in the walls decreases during cell growth with a concomitant increase in the alkali-insoluble glucan [37], and (ii) glucan remains alkali-soluble when the synthesis of chitin is inhibited with nikkomycin [37]. Similar results have been reported in *S. cerevisae* [50]. Other kinds of linkages connect the cell wall polymers of *C. albicans*, *S. cerevisiae* and other yeast species (Table 2).

Table 2 Linkages connecting cell wall polymers

Wall polymers involved	Type of connection	References
β-glucans-chitin	basic amino acids	129
β(1,3)glucan-chitin	β(1,4)linkage	50, 66, 67
β(1,6)glucan-chitin	glycosidic linkage	135
β(1,6)glucan-protein	—	59, 115
β(1,3)glucan-protein	—	37, 106
β(1,3)glucan-protein	phosphodiester linkage	61
β(1,6)glucan-protein	phosphodiester linkage	61
β(1,6)glucan-protein	GPI remnant	67
β(1,3)glucan-β(1,6)glucan	glycosidic linkage	60
chitin-protein	amino acids	77
	mainly lysine	108
protein-protein	—	36, 78, 87
protein-protein	S—S	20, 77, 99
glucan-mannan	glycosidic linkage	87, 144

Glycosyl-phosphatidyl-inositol (GPI) anchors have been already mentioned. In *S. cerevisiae,* this type of anchoring has been proposed to localize certain proteins initially to the plasma membrane, and their final wall anchorage involves releasing from the GPI anchor to produce a periplasmic intermediate, followed by their linkage to the cell wall [75, 88, 142, 143]. Other *S. cerevisiae* and *Y. lipolytica* cell wall proteins lack this GPI signal, implying that other types of attachments are implicated in their binding to the cell wall structure [89, 104].

Enzymatic activities that catalyse the formation of wall covalent linkages The results mentioned above imply that the linkages between different polymers are formed externally to the plasma membrane, probably in the domains of the cell wall itself, and raise the questions of how and when these linkages are formed.

Very little is known about the catalytic activities responsible for the formation of the covalent linkages present in the wall. A secreted β-glucan branching enzyme with glucosyl transferase activity has been detected in *C. albicans* [49], and a glutaminyl-peptide-γ-glutamylyl-transferase activity (tranglutaminase, EC 2.3.2.13) has been described in the cell walls of *C. albicans*, *S. cerevisiae* and *Y. lipolytica* [113]. This latter enzyme catalyzes the formation of pseudo-peptidic crosslinks. The reaction

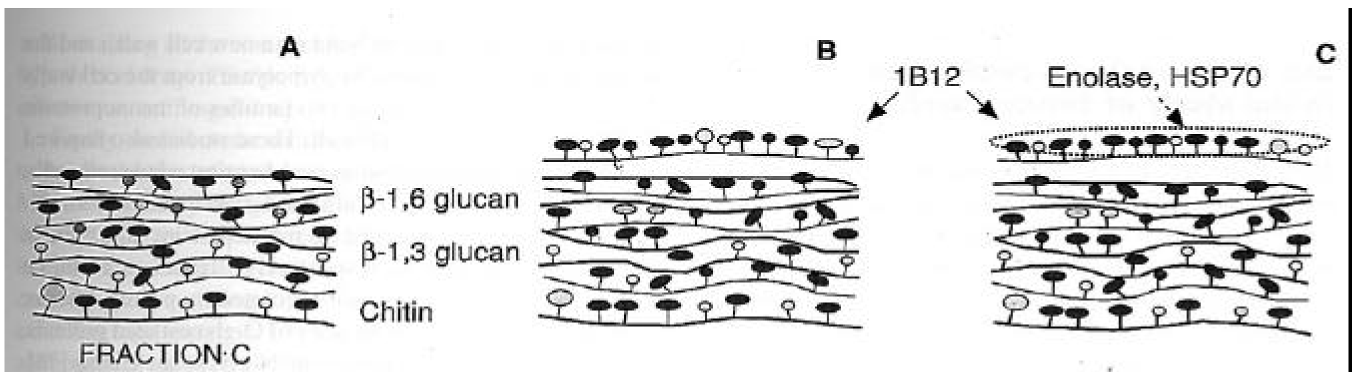

Fig. 1 Organization of *Candida albicans* cell wall. Mannoproteins (indicated as small umbrellas) are connected covalently to the skeleton of β-glucans and chitin (fraction C, panel A). Other proteins externally located in the wall (i.e. proteins bearing the epitope recognized by MAb1B12, reference [78]) are linked covalently to glucan (panel B). Adventitous proteins such as enolase or Hsp70 (reference [41]) cover the outermost external surface of the cell wall (panel C)

involves the carboxamide group of a glutamine residue in one protein, and the γ-amino group of a lysine residue of another peptide [74].

It seems, therefore, that transglutaminase is one of the enzymes that catalyzes one of the final steps in the formation of *C. albicans*, *S. cerevisiae* and *Y. lipolytica* fungal cell walls. But little is known about other potential enzymes that must participate in later steps of cell wall formation.

Topology of the cell wall polymers We know the polymers and some of the building blocks, but: what do we know about their location in the cell wall? This question has been answered by two complementary experiments: (i) by the use of monoclonal antibodies to mark cell wall epitopes (discussed below), and (ii) by the controlled degradation of the cell walls with ethylenediamine. By the first approach, β-1,6- and β-1,3- glucan have been found deep in the cell wall [68], they being accessible only after elimination of the outer mannoprotein layer [52, 65, 115]. This layer seems to be formed by a set of specific proteins [114].

By the second approach it has been shown that the cell walls are mainly layered β-1,3-glucan structures that lose their organization following digestion with chitinase. This latter result evidences that chitin, though a minor component, is a crucial element in maintaining the fungal cell wall organization.

In addition to the external specific proteinaceous layers, several cytoplasmic proteins (enolase, helicase, HSP70, etc.) have been found in the outermost external surface of *C. albicans* cell walls of both yeast and mycelial cells [40, 69, 121]. These proteins seem to be released by dead cells and then to be absorbed by the walls of living cells due to their sticky nature [41].

Organization of *C. albicans* cell wall We propose a scheme where the cell wall of *C. albicans* is an extracellular matrice constructed upon an initial skeleton made of β-glucans and protein blocks, which are insolubilized by small amounts of chitin (Fig. 1). The protein(s) present in this skeleton might modulate the incorporation of the other wall components. If that were the case, these proteins would form part of a "morphogenetic code", as mentioned before, and would be responsible of the fungal morphology. For instance, in *C. albicans* and other dimorphic species, they would be responsible for alternative morphologies (Fig. 1A). These proteins would co-ordinate the interaction between glucans, chitin and the so-called structural proteins. The result would be the production of cell walls that would be species and morphology specific. External to this inner core, other proteins are found connected to glucan by covalent bonds (Fig. 1B). These proteins might be responsible for social activities of cells (adhesion, etc.). Finally, enolase and other adventitious cytoplasmic proteins would cover the outermost external surface of the walls (Fig. 1C). They would be responsible for the high title of anti-*C. albicans* antibodies exhibited by patients with deep candidiasis infections. This high title of antibodies has been suggested to be a useful marker in the diagnosis of candidiasis. Another function for these proteins would be to form a "smoke-screen" to the host immunological system, representing as a consequence, an additional protective shield for the fungal parasite.

We may conclude that *C. albicans* cell walls are dynamic extracellular structures formed by an intricate network of proteins and polysaccharides that are secreted and assembled externally in the domains of the structure itself and probably in close association with the plasma membrane.

The formation of cell wall appears to be a highly controlled process. Both the rate of synthesis of different components and the timing with respect to the cell cycle must be adjusted to produce the corresponding building blocks. Furthermore, transition to mycelial morphology as the result of the external stimuli needs changes in the synthesis and/or in the assembly of wall components.

Knowledge about cell wall assembly and regulation may be of help for the search for new antifungal drugs, for the understanding of how morphogenesis operates in apparent simple organisms, and to unravel how morphogenesis takes place in higher cells and organisms.

Use of monoclonal antibodies in the study of the cell wall

The low inherent antigenicity of β-glucans and chitin [93, 100] require studies of the immunogenic expression in *C. albicans* cell wall to focus on mannoproteins. Several of these antigens also carry remains of glucan and chitin (glucose and glucosamine residues) because of the strong interconnections between those components in the fungal cell wall [36, 98, 119, 128]. In such complexes, glucan can act as an adjuvant [27]. These reasons explain why most of the polyclonal and monoclonal antibodies (MAbs) obtained against the *C. albicans* cell wall detect portions of mannoproteins, particularly their glucidic moiety [56, 93]. The antigenic differences between the mannans of distinct *Candida* species are due to the variation in the location of mannose residues in the polydisperse chains present in mannoproteins. Thus mannans necessarily share a high degree of similarity, which explains the cross-reactivity observed between distinct species of the genus *Candida* [47], and even between species of other genera [109, 136, 138].

To reduce the incoveniences of isolating highly polydisperse molecules, several research groups have focused their efforts on the production of specific monoclonal antibodies. Since the first description of a monoclonal antibody raised against *C. albicans* cell wall molecules in 1984 [132] several monoclonal antibodies have been described, most of which belong to the IgM isotype, recognize glucidic epitopes, and show cross-reactivity with different species of *Candida* [3, 19, 46, 72].

MAbs are frequently used in studies which include: (i) rapid identification of *C. albicans* and other related species; (ii) analysis of the corresponding epitopes in the process of cell wall assembly (as mentioned before); and (iii) the detection of circulating antigens in patients with disseminated candidiasis. These studies have focused either on the analysis of antigenic expression during the cellular age [8–11, 24] or on serospecificity [57]. Moreover, these MAbs have been used to show differential expression of their epitopes among species, in particular clinical isolates, or depending of growth rating, or even among individual cells [9, 24]. Some of these MAbs have been shown to react in vivo with antigens present in tissue of infected animals [11].

Our group has generated ten MAbs by using, as immunogens, distinct materials solubilized from isolated cell walls of *C. albicans* (Table 3). Only one of them proved to be an IgM, the rest belonging to the IgG isotype. With the exception ot the MAb JRR1, which recognizes epitopes of β-1,6-glucan, the others detect specifically proteinaceous material [21, 76, 78, 115]. Three of the MAbs raised against the material released by zymolyase (essentially a β-1,3-glucanase) from yeast and mycelial cell walls (1B12, JRR1, and 4C12, respectively) have been used to study how specific molecules are secreted and incorporated into growing cell walls. The use of MAbs 1B12 and 4C12 in the analysis of the mannoproteins present in membranes, secreted by regenerating protoplasts (which have to build up a new cell wall), and the mannoproteins solubilized by zymolyase from the cell walls has revealed the existence of two families of mannoproteins covalently linked to the cell wall. These studies also required the use of polyclonal antibodies raised against whole cell walls, and drugs which either inhibited the formation of N-glycosilation or deglycosilated the mannoproteins (tunycamicin and endoglycosidase H, respectively). Hence, it could be established that the precursors secreted by protoplasts are constitued at least by two families of O-glycosilated proteins, one of which is able to incorporate N-glycosidic chains [78]. The findings that MAbs detected high molecular weight materials solubilized by degradation of the glucan network of the wall with zymolyase, and that those materials also carried mannan moieties, suggest that both families of mannoproteins, although secreted independently, could be released from the wall as part of supramolecular structures [36]. Therefore, the incorporation of defined proteins into the wall architecture involves significant modifications [35]. Supporting this notion, recent studies have shown that MAb JRR1, which recognizes β-1,6-glucan, reacts with mannoproteins released from the cell wall by zymolyase, which confirms the existence of direct links between the mannan moiety of the mannoproteins and glucan in *C. albicans* [115]. MAb JRR1, however, failed to react with the mannoproteins secreted by regenerating protoplasts, which suggests that the modification suffered before their secretion by those mannoproteins is not a β-1,6 glycosilation [114, 115].

Table 3 Monoclonal antibodies produced against *Candida albicans* cell wall materials

Antibody	Subclass	Immunogen[1]	Specificity[2]	Reference
4C12	IgG	Zym M	Mp M	21
1B12	IgM	Zym Y	Mp Y M	78
3H8	IgG	Zym Y	Mp Y M	76
3G10	IgG	Zym Y	Mp Y M	76
B5	IgG	Zym M	ND	76
G11	IgG	Zym M	Mp Y M	76
H11	IgG	Zym M	Mp Y M	76
R2	IgG	2-ME[1] M	Mp Y M	76
R3	IgG	2-ME M	Mp Y M	76
JRR1	IgG	Zym Y	β-1,6 glucan	115

[1] Zym, material released by zymolase20T after washing with SDS from isolated yeast (Y) or mycelial (M) cell walls; 2-ME, material released by 2-mercaptoethanol after washing with SDS.
[2] Mp, mannoproteins (mainly O-glycoproteins), ND, not determined.

Previous studies have reported the existence of linkages between β-1,3-glucan and mannoproteins in *S. cerevisiae* [86, 144], whose nature has been later revealed. They are established between the C-terminal glycan (derived from a glycosyl-phosphatidyl-inositol anchor, GPI) from a mannoprotein and β-1,6-glucan [67], as it had been previously suggested for *S. cerevisiae* and *C. albicans* [29, 115]. In this context, β-1,6-glucan seems to play a key role in the organization of the cell

wall, retaining all the components. These findings suggest that hypothetical drugs affecting β-1,6-glucan synthesis could prove effective as antifungal drugs.

Finally, studies on the nature of the cell wall have given raise to the production of useful reagents in diagnosis. Two of the MAbs obtained by our group, 1B12 and 3H8, have been used successfully in the identification of *C. albicans* by immunohistochemical techniques in tissues from patients with candidiasis, they being highly specific for *C. albicans* and exhibiting high sensitivity [76, 84]. Furthermore, the monoclonal antibody 3H8 has been used to produce a diagnostic kit for rapid identification of *C. albicans* in culture (Bichro-latex albicans®, Fomouze Laboratories, France). This test is easy to perform and has proven highly specific and sensitive for *C. albicans* [32, 45, 102, 105]. In developing the test, it was observed that the addition of zymolyase to the kit improved the detection of the epitope by the antibody [105], which confirmed previous observations related to the location of the epitope in the inner part of the cell wall in different clinical isolates of *C. albicans* [76].

The results mentioned afford good examples of the use of monoclonal antibodies in both basic and applied research. The understanding of the function of individual cell wall mannoproteins opens the possibilities to obtain excellent tools for both rapid diagnosis and efficient treatment of candidiasis.

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