CHAPTER 3

REVIEW LITERATURE

Biology

The heterogeneous genus *Candida* belongs to the Family Cryptococcaceae within the Phylum Deuteromycetes and Class Blastomycetes (30).

Family : Cryptococcaceae

Genus 1: *Cryptococcus*; unicellular budding cells only; reproduce by blastoconidia pinched off the mother cell. Most are urease – positive. Cell surrounded by a heteropolysaccharide capsule and produces starchlike compounds; carotenoid pigments are usually lacking.
Example : *Cryptococcus neoformans* (caused cryptococcal menigitis)

Genus 2 : *Torulopsis;* some as above, but do not have capsules or produce iodine – positive, starchlike substance; urease negative, and there is no assimilation of inositol.
Example : *Torulopsis glabrata* (caused torulopsosis)

Genus 3 : *Malassezia;* Mostly unicellular budding cells which reproduce by blastoconidia that develop from a reduced phialide. Cells may adhere, forming short hyphal strands. Growth stimulated by lipids. There is no fermentative ability.
Example : *Malassezia furfur* (caused pityriasis versicolor)

Genus 4 : *Rhodotorula;* Unicellular budding forms that rarely produce pseudomycelium, are generally encapsulated, but do not produce starchlike substance. They do not

assimilate inositol or ferment sugars. Carotenoid pigments are produced.

Example : *Rhodotorula rubra* (caused rare pulmonary and systemic infections)

Genus 5 : *Candida*; Reproduction is by pinched blastoconidia. They may form pseudomycelium or true mycelium; urease is generally negative; capsules are not formed; strach or carotenoid pigments are not produced; inositol is not assimilated.

Example : *Candida albicans* (caused Candidiasis)

Genus 6 : *Trichosporon*; Reproduction is by blastoconidia and arthroconidia. Mycelium and pseudomycelium are formed.

Example : *Trichosporon beigelii* (caused white piedra and systemic infections)

Genus 7 : *Geotrichum*; Reproduction is by arthroconidia only. A true mycelium is formed

Example : *Geotrichum candidum* (caused rare pulmonary geotrichosis)

Candida can find on many plants and as normal flora of mucosal commensals. It can be isolated so frequently from the vagina, stool, normal skin and throat of normal individuals. *Candida* can be present in clinical specimens as a result of contamination, colonization, or actual disease processes *Candida* that are normal flora can invade tissue and produce life – threatening disease.

C. albicans, C. parapsilosis, C. tropicalis and *C. guilliermondii* play a major role in the etiology of urinary tract infections, meningitis, pyelonephritis and fungemia (31). *C. lusitaniae* has been recognized as an important pathogen because of its resistance to amphotericin B (32). Other species of *Candida* are emerging as an opportunistic pathogens.

There are five conditions in which the normal equilibrium between *Candida* and host may be sufficiently upset to lead to a pathologic state (30, 33).

1. Extreme youth : the restricting factors for *Candida* may be absent, and a clinical condition is produced.
2. Physiologic change : Pregnancy, administration of steroids and diabetics.
3. Prolonged administration of antibiotics.
4. General debility and the constitutionally inadequate patient.
5. Iatrogenic and barrier - break.

The most infections have an endogenous source same as it is in *Staphylococcus aureus* infections. Occasionally the infections is contagious, and true epidemics have occurred under unusual circumstances (34). Oropharyngeal candidiasis is an expected finding sometime during the course of human immunodeficiency virus infection and constitutes one of the opportunistic infections in the case of AIDS.

Morphology

Candida appears as a gram positive oval budding yeast and/or pseudohyphae, measuring 2 – 3 x 4 – 6 μm, (Figure 1). On Sabouraud's agar incubated at room temperature within 2 or 3 days, soft, cream – colored colonies with a yeasty odor develop. The surface growth consists of oval budding cells. The submerged growth consists of pseudomycelium, composed of blastoconidia at the nodes and sometimes chlamydoconidia (Figure 2). Blastoconidia of *Candida* spp. vary in shape, from round to oval to elongate. Asexual reproduction is by multilateral budding, and mycelia may be present. If sexual reproduction occurs, the yeasts are classified by their teleomorphic state. Growth on fungal media can be detected as early as 24 hours : however, colonies usually are visible in 48 – 72 hours as white cream colored or tan. They are creamy and may become more membranous and convoluted with age. Most

Candida grow well aerobically at 25 – 30 °C and may with grow at 37 ° C or above. Carbon assimilation and occasionally fermentation studies are needed to differentiate the species (35). Production of pseudohyphae is one of the major differentiating factors separating *Candida* from *Torulopsis* spp. Observation of germ tubes (only the budding cells of 24 hours old cultures of *C. albicans* (and *C. stellatoidea*) and not of other species will form germ tubes (Figure 3) in 2 – 3 hours when placed in serum at 37 °C and chlamydoconidia is helpful in identifying *C. albicans*.

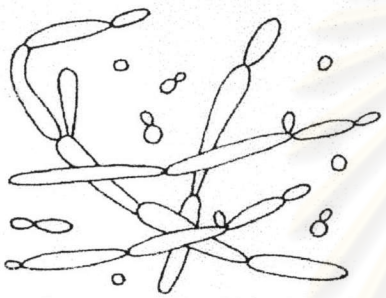

Figure 1. Blastoconidia and pseudohyphae in exudate (29).

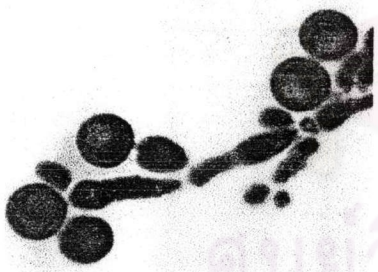

Figure 2. Chlamydoconidia

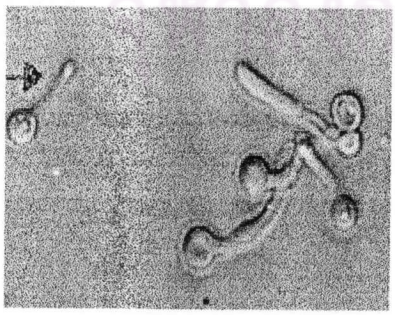

Figure 3. Young culture forms germ tubes when placed in serum for 3 hours at 37 °C

Pathogenesis of Candidiasis

C. albicans is a human commensal so that the infectious sources are primarily endogenous and secondary exogenous.

1. endogenous : The gastrointestinal tract is considered a major reservior for *C. albicans*, e.g. in vaginitis or diaper rash. Moreover, the fungus can invade the bloodstream from gastrointestinal tract after damage to the gastrointestinal mucosa, such as that induced by anti – cancer treatment (irradiation or chemotherapy) or major surgery; it can then spread heamatogenously into various organs, causing deep – seated / disseminated infections.

2. exogenous : *C. albicans* can, however, be introduced from exogenous sources as well. These may include introduction through various catheters and lines, or other dwelling / prosthetic medical devices (36). This route is of particular importance in the development of deep - seated and systemic candidiasis because most of these therapeutic modalities are used primarily in compromised hosts whose defence systems are unable to combat the introduced pathogen. Person – to – person transmission is not a predominant mechanism of pathogenesis in candidiasis. It is seen primarily in the oral thrush of those newborns whose mothers have vaginal infections, acquiring them during birth, or is noted in the sexual transmission from patients with vaginitis to their sexual partners.

Clinical disease (30):

1. Infectious Diseases.

1.1 Mucocutaneous involvement

(1) Oral candidiasis : thrush, glossitis, stomatitis, cheilitis, perleche.

(2) Alimentary candidiasis : esophagitis, enteric and perianal disease.

(3) Vaginitis and balanitis

(4) Chronic mucocutaneous candidiasis

1.2 Cutaneous involvement

(1) Intertrigenous and generalized candidiasis

(2) Paronychia and onychomycosis

(3) Diaper disease (napkin candidiasis)

(4) Candidal granuloma

1.3 Systemic involvement

(1) Bronchial and pulmonary candidiasis

(2) Urinary tract infection

(3) Endocarditis

(4) Meningitis

(5) Septicemia

(6) Iatrogenic candidemia

(7) Disseminated candidiasis

2. Allergic Diseases.

2.1 Candidids

2.2 Eczema

2.3 Asthma

2.4 Gastritis

The normal innate mammalian defend system against *Candida* infection include intact dermal surface, intact mucosa, unspecific humoral factors and the immune system – humoral immune response (HMIR) and cell - mediated immune response (CMIR). The first line of defence, which protects primarily against the mucocutaneous forms of candidiasis, is unbreached skin and mucosa. Current concepts attribute an inflammatory - immunological activity to the skin, in addition to its function as a barrier. The former may involve specific skin cell – epidermal Langerhans' cells and keratinocytes, which may function as antigen - presenting cells engage in phagocytosis and/or produce various cytokines. As indicated above, breaching the intact nature of the skin (e.g. through intravenous catheters) or the gastrointestinal mucosa can also lead to introduction of the organisms into the bloodstream, either from exogenous or endogenous (gastrointestinal tract) sources,

respectively. Thus damage to the integuments may be considered as a risk factor for the development of systemic candidiasis. Additional non-specific, non-immune factors, to which a possible role in defence has been attributed, include such iron-binding proteins as transferrin or lactoferrin (37, 38).

The second line of defence, which comes into action following fungal penetration, includes the phagocytic and candidacidal activity of polymorphonuclear (PMN) (primary neutrophils) and mononuclear (primary monocytes) cells. These process involve myeloperoxidase and superoxide, or cationic proteins. PMN activity is of crucial significance in protection from the deep-seated forms of candidiasis, as can be judged by epidemiological data which indicate that an increased rate of these infections is associated with neutropenia or inactive PMNs (39, 40).

Despite numerous experimental studies, clinical studies and clinical observations, there is no clear - cut concept of the activity of the various elements of the system and of the mechanisms underlying that activity. The T lymphocytes involved in cell - mediated immune response seem to be of significance, particularly in defence against the mucocutaneous forms of candidiasis (40). Patients with T - cell deficiencies, such as AIDS patients, are especially prone to these infections. The role of these cells in resistance to systemic candidiasis is more controversial. Opsonizing antibodies (IgG) seem to contribute to phagocytosis, complement fixation and thereby to defence. An additional humoral element of the immune system involved in defence is apparently the complement cascade (41).

Virulence factors

Virulence in *Candida albicans* includes host recognition. Binding of the organism to host cells, host cell proteins or microbial competitors (co – aggregation) more than likely prevents or at least reduces the extent of clearance by the host. *C. albicans*, expresses several virulence factors that contribute to pathogenesis. These factors include host recognition biomolecules (adhesins), morphogenesis (the reversible transition between unicellular yeast cells and filamentous, growth forms), secreted aspartyl proteases and phospholipases (42).

Adhesins : The interactions of host cells and fungi during infection represent a complex interplay. *C. albicans* requires adherence, particularly to endothelial cells, which in turn are stimulated to express various cell – markers and pro – inflammatory cytokines as part of a proactive resistance to invasion (43, 44). Adherence is achieved by a combination of specific (ligand – receptor interactions) and non – specific (electrostatic charge, van der Waals forces) mechanisms which allow the yeast to attach to a wide range of tissue types and inanimate surfaces (45). In oral cavity, *C. albicans* selectively adheres to salivary proteins, which are absorbed to many oral surfaces. This mechanism enables the cells to colonize surfaces of the oral cavity (46). In addition, saliva molecules, including basic proline – rich proteins, adsorbed to many oral surfaces promote *C. albicans* adherence (47). The identification of genes that encode a host – recognition protein are described as followed (Table 4)

- Als family : has features typical of secreted proteins and a hydrophobic carboxyl terminus that suggests a glycosylphosphatidylinositol (GPI) anchor. Both Als 1p and Als 5p (Ala 1p) appear to provide an adhesin function (48, 49, 50).
- *HWP1* : encodes an outer surface mannoprotein that is believed to be oriented with its amino – terminal domain surface – exposed and the carboxyl terminus most probably covalently integrated with cell wall β - glucan and was found to resemble transglutaminase (TGase) substrates. Although, *hwp 1* – knockout strain of *C. albicans* was constructed and found to have reduced activity as a substrate for TGase and lower levels of stabilized, covalent adherence to HBEC, and importantly, the null mutant was less virulent than parental or single – gene – deleted strains in a hematogenously disseminated murine model (51).
- *Int 1p* : plays important roles in adherence and filamentation of *C. albicans*. And a cytoskeleton protein, might interact with Int

Table 4. Adhesins of *Candida albicans* and *Candida glabrata* (54).

Adhesin	Ligand	Mutant phenotype[b]		other
		Adherence *in vitro*	Virulence[c]	
Als 1p	HBEC, endo[a]	Reduced	Reduced	Agglutinin – like sequence proteins
Ala 1p (Als5p)	FN	ND	ND	Agglutinin – like sequence proteins
Hwp 1p	HBEC	Reduced	Reduced	Stabilized adherence, TGase substrate
Int 1p	Epithelial	Reduced	Reduced	The 'integrin' – like protein
Mnt 1P	HBEC	Reduced	Reduced	Type I mannosyl transferase

a = Abbreviations:endo, human umbilical vein endothelial cells; FN, fibronectin; HBEC,human buccal epithelial cells;ND,not determined; TGase, transglutaminase.
b = Compared with parental cells, heterozygote or reconstituted strains; *ALS*1 and *ALS*5 (*ALA*1), heterologous expression in *Saccharomyces cerevisiae.*
c = Hematogenously disseminated murine model.

Morphogenesis : *C. albicans* is a dimorphic fungus has two distinct morphological stages : a unicellular yeast stage known as the blastoconidia and a filamentous, or hyphal stage. It is believed that the developmental switch from

the blastoconidia to the filamentous stage confers pathogenic properties to *C. albicans* (31). Several candidate genes involved in switching have been identified, including genes encoding proteins involved in signal transduction and gene repression. The situation is further complicated by the observation that there are form – specific genes, whose expression is controlled by the same regulatory molecules controlling morphological development. Thus, it is impossible to attribute attenuation to the morphological defect versus the altered expression of form – specific genes that might be crucial for virulence. Some observations related to the genetics of morphogenesis and virulence are described below (Table 5).

- Cph 1p : whose phosphorylation is regulated by the proteins of the mitogen – activated protein kinase pathway. A first morphogenesis pathway in *C. albicans* is identified by the transcription factor. Deletions of this gene in *C. albicans* result in morphogenesis defects on certain agar media (55).
- Egf 1p : which is a member of the family of basic helix – loop – helix transcription factors. A second morphogenesis pathway in *C. albicans* is identified by the transcription factor (55, 56).
- Tup 1p and Rbf 1p : that are suppressors of morphogenesis.
    - Strained *C. albicans* deleted in *TUP1* constitutively formed hyphae (57).
    - Rbf 1p is a DNA – binding protein and, like Tup 1p mutants of *C. albicans* deleted in *RBF1* are stimulated to form filamentous growth (58).
- Czf 1p : a third transcription factor has been identified from *C. albicans* regulates morphogenesis when cells are embedded in agar (59).

Table 5. Transcriptional proteins of *Candida albicans* and morphogenesis[a] (54).

Protein	*S.cerevisiae* homologue	Mutant phenotype[b]	
		Morphogenesis	Virulence
Cph 1p	Ste 12p	Reduced on spider agar	10^6 = to wt 10^4 = to avir.
Efg 1p	Phd 1p	Abnormal filaments	10^7 ~ to wt 10^5 = less vir.
Tup 1p	Tup 1p	Constitutive filaments	ND
Rbf 1p	?	Constitutive filaments	ND
Czf 1p	None	Reduced hyphal growth	ND
Rim 101p	Rim 101p	Reduced hyphal growth	Reduced
Tec 1p	Tec 1p	Filamentation	Reduced

a = Abbreviation: avir, avirulent; ND, not determined; vir, virulent; wt, wild type
b = Compared with matched strains.

Enzymes that contribute to invasiveness : The secreted asparatyl proteinases (*SAP*) and phospholipases (*PL*) are two rather large families of *C. albicans* enzymes, some of which have been associated with virulence.

- *PLs (PLA, PLB, PLC* and *PLD*) : of the four PLs identified thus far, only *PLB1* has been required for virulence in an animal

- model of candidiasis. (*In vitro* was less virulent 40% killing versus 100% by wild – type). Plb1 activity has been detected at hyphal tips during tissue invasion, it is an 84 kDa glycoprotein that both hydrolase and a lysophospholipase – transacylase activity, and is probably secreted (54).
- *SAP* (at least 9 proteins comprise in *SAP* family) : in guinea pig and murine models, deletions in *SAP 1 – 6* attenuated virulence (60, 61). It appear that *Saps* 1 – 6 are required for invasive disease. The order of expression was *SAP1*, 2, followed sequentially by *SAP8*, *6* and *3*. Expression was correlated with invasion of the tissue, that is, early invasion (*SAP1,2*), extensive penetration (*SAP8*) and extensive hyphal growth (*SAP6*). In vaginitis models, although all *Saps* have not been evaluated, *SAP2* is required for disease development.

Cell Wall Structure

The initial step in the pathogenesis of candidiasis is adherence of the fungus to host tissue (62, 63). Many adhesins of *C. albicans* that play a role in attachment of the fungus to various host tissues or to inanimate surfaces have been characterized (62, 64, 65, 66), and the cell wall phosphomannoprotein complex (PMPC) antigen has received the most attention (66, 67, 68, 69). The cell wall of *C. albicans* is composed primarily of the polysaccharides mannan, glucan, and chitin. The components of the walls of *C. albicans* are chitin, β – glucans and mannoprotein. Glucan and chitin form a skeleton for the mannoproteins, which are localized mainly at the outer surface (70, 71). Wall mannoproteins can be divided into two major groups depending upon their degree of interaction with the structural network : one type interacts by non – covalent bonds while the second group seems covalently bound to other wall components (intrinsic or structural mannoproteins). The budding of the wall occurs in two steps: during the first one the skeleton of chitin is formed retaining protein molecules by non – covalent bonds. The second steps take

place by deposition of glucan molecules that allows incorporation of mannoproteins probably by covalent linkages (72).

Composition: The major components of the cell wall of *C. albicans* are

1. Carbohydrates (95%) :
    1) mannan or polymers of mannose covalently associated with proteins to form glycoproteins, also referred to as mannoproteins.
    2) β - glucans that are branched polymers of glucose containing β - 1,3 and β - 1,6 linkages.
    3) chitin, which is an unbranched homopolymer of *N* – acetyl – D – glucosamine (GlcNAc) containing β - 1,4 bonds.
2. Proteins (6 – 25%).
3. lipids (1 – 7%).

Proteins and lipids are present as minor wall constituents (62, 73, 74). The general features of cell wall mannoproteins in *C. albicans* are basically identical to those found for *Saccharomyces cerevisiae,* one of the most throughly investigated yeasts in this regard. Several studies have resulted in a detailed knowledge of the structure of this cell wall constituent in *C. albicans* (75, 76, 77, 78). Thus, mannose polymers are linked to the protein moiety through asparagine (by *N* – glycosidic bonds through two GlcNAc [di – *N* – acetylchitobiose] residues) and threonine or serine (by *O* – glycosidic, alkali – labile linkages) residues. The *N* – glycosidically linked carbohydrate is composed of backbone chains of α – 1,6 – linked mannopyranosyl residues to which oligosaccharide side chains are attached. The side chain mannopyranosyl residues contain α – 1,2, α – 1,3, β - 1,2, β - 1,6 and phosphodiester linkages as well as branches (α – 1,6) that are over synthesized under acidic growth conditions (79, 80, 81, 82). The *O* – glycosidically – linked sugar component consists of single mannose residue and short, unbranched mannose oligosaccharides (83).

Structure: The outer fibrillar layer of the cell wall of both yeast and filamentous cells is composed of mannan or mannoprotein, although this material is probably deposited at several sites within the cell wall also. The outer layer has also been described as a mucous coat or capsule and is sloughed off during infection. The amount of mannoprotein produced by cells depends on the growth medium and cell age. For example, incorporation of galactose or sucrose instead of glucose in the culture medium results in a greater production of the fibrillar outer layer. Sucrose – or galactose – grown cells tend to be more adhesive. Surarit R, *et al.* (84) reported the presence of glycosidic linkages between glucan and chitin in the nascent wall of *C. albicans*. Recent evidence indicates that mannoproteins may also establish covalent associations with β – glucans (85, 86, 87). It is suggested that β - 1,3 – and β - 1,6 – glucans are linked to proteins by phosphodiester linkages, a process that may involve the participation of a GPI (glycosyl phosphatidylinositol) anchor (88). Protein and mannoprotein species that are released only after digestion of the glucan cell wall network with β - glucanases may play a key role in configuring the final cell wall structure characteristic of growth form (yeast and mycelium) of *C. albicans* (85, 87). Cell wall architecture has been studied most extensively in *S. cerevisiae* and is likely to be a model for *C. albicans* since there are some similar observations, in particular sensitivity to enzymatic digestion, glucan – mannoprotein linkages, and candidate proteins, that fit the same model (89, 90, 91, 92). In a very recent study, Kolla´r R, *et al.* (93) detected the presence of material containing all four major cell wall components, β - 1,3 – glucan, β - 1,6 – glucan, chitin, and mannoprotein. Their analysis indicated that β - 1,6 – glucan has some β - 1,3 – glucan branches that may be linked to the reducing end of chitin. The β - 1,6 – glucan and mannoprotein are attached through a remnant of the mannoprotein GPI anchor. Reducing ends of β - 1,6 – glucan may also be attached to the nonreducing end of β - 1,3 – glucan. The proportion of cell wall polysaccharide involved in this type of structure is not clear. The following cell wall building block, where the linkages are indicated by the long dashed, is proposed (92, 93): mannoprotein – GPI remnant - β - 1,6 – glucan - β - 1,3 – glucan – chitin. Chitin

and β - 1,3 – glucan are synthesized at the plasma membrane and extruded into the periplasm, mannoprotein is synthesized in the cytoplasm and transported through the secretory pathway, and β - 1,6 – glucan synthesis may occur partially in the endoplasmic reticulum or Golgi complex (94) (Figure 4).

Mannoprotein structure : Yeast mannoproteins comprise a heterogeneous mixture of large molecules in which carbohydrate (mannan), accounting for as much as 95% of the overall weight, is covalently linked to protein. The basic structure of *C. albicans* mannorptein appears to be similar to that of *S. cerevisiae* (Figure 4). Carbohydrate are joined to protein via either *O* - or *N* - glycosidic linkages. The *O* - linked chains consist of 1 to 4 mannose residues attached to serine or threonine, from which they can be released by treatment with dilute alkali. The *N* - linked chains are highly branced polysaccharides joined to asparagine residues in the protein via an *N* - acetylglucosamine dimer bridge. They are composed of an α - 1,2 – and α - 1,3 – linkages. The first segment of the polysaccharide, designated the "inner core", can be distinguished biosynthetically from the "outer chain" region which also contains diesterified phosphate groups (Figure 6).

It appears that the mannoprotein of *C. albicans* is more highly phosphorylated than that *S. cerevisiae,* has longer side chains, and contains some β - linked mannose residues. The side chains contain the epitopes that determine serospecificity (serotype A or B) (80).

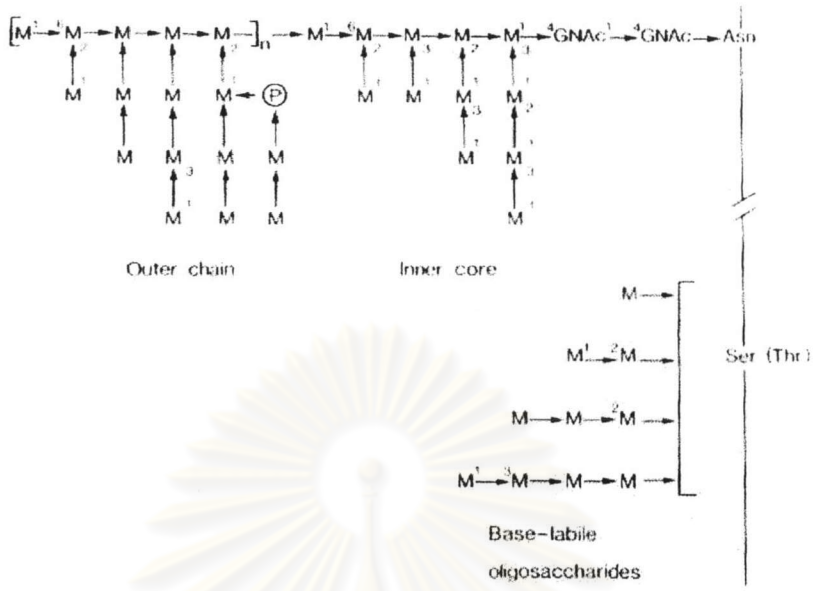

Figure 4. Generalized structure for yeast mannoprotein as first proposed by Ballou (1976) (94) for *S.cerevisiae*. M, Mannose; P, phosphate; GlcNAc, *N* – acetyl – D- glucosamine; Asn, asparagine; Ser, serine; Thr, threonine.

Evidence for mannoprotein adhesins : Although other wall components such as chitin and lipids (95, 96) have been proposed as candidate adhesins, most experimental evidence indicates a role for mannoprotein in mediating yeast attachment to buccal and vaginal cells. Adhesin can be inhibited by pretreating the epithelial cells with a crude mannoprotein preparation obtained from culture supernatants of yeasts grown in medium containing a high concentration of galactose (68). This extracellular material is thought to originate, at least in part, from the surface fibrillar layer whose synthesis is promoted by growth under such conditions; its ability to inhibit adhesion indicates that it contains an adhesin capable of binding to, and thus blocking, epithelial receptors. The interaction is quite specific (97), since mannoprotein isolated from one *C. albicans* strain (GDH 2023) fails to inhibit adhesion of a second strain (GDH 2346).

Further evidence for the mannorptein nature of the adhesin has come from experiments with tunicamycin. In yeasts, this antibiotic suppresses synthesis of mannoprotein but not that of the other major wall components, glucan and chitin (98). Addition of tunicamycin to cultures of *C. albicans* in

high galactose medium at the onset of stationary phase inhibited formation of the fibrillar layer, resulting in a decrease in adhesion to buccal cells of over 60% as compared with untreated yeasts (99).

Layering : Since polysaccharides are poorly reactive to the ordinary fixatives and stains used for transmission electron microscopy (Figure 5A, 5B). However, transmission electron microscopy studies performed with more special techniques or with cytochemical stains and contrasting agents show several layers in the cell wall of *C. albicans* (Figure 5C, 5D and 5E). The appearance of these layers is variable and seems to be related to the strain examined, growth conditions, morphology, and preparation of the specimens (62, 100). Thus, there is no consensus about the number of layers present in the cell wall. Different authors have reported the presence of three to eight different layers (54, 101, 102). The outer cell wall layer appears as a dense network with a fibrillar or flocculent aspect (73, 86), whereas the inner wall layer appears contiguous with the plasmalemma with extensive membrane invaginations involved in anchoring of the cell wall to the membrane (103, 104). The microfibrillar polysaccharides glucan and chitin, the components that supply rigidity to the overall wall structure, appear to be more concentrated in the inner cell wall layer, adjacent to the plasma membrane, in contrast, proteins and mannoproteins appear to be dominant in the outermost cell wall layer, although they are also present through the entire wall and at the inner regions of the cell wall. Some of the latter proteins may be covalently associated with glucans. Evidence from several cytochemical and cytological studies indicate that the cell wall layering may be due to the distribution of mannoproteins at various levels within the wall structure (73). In any case , it seems clear that layering may be the result of quantitative differences in the proportions of the individual wall components (β - glucans, chitin, and mannoproteins) in each layer rather than of qualitative differences (105).

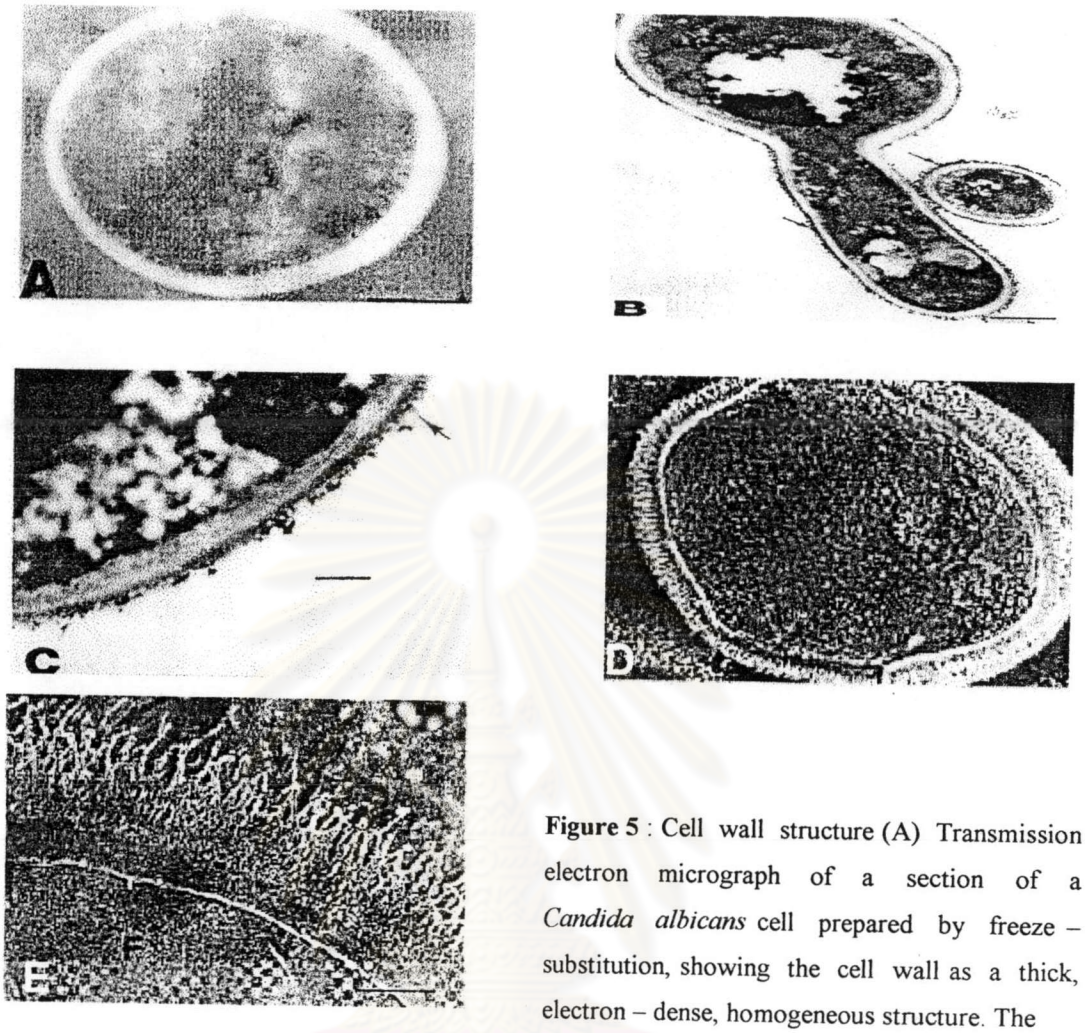

Figure 5 : Cell wall structure (A) Transmission electron micrograph of a section of a *Candida albicans* cell prepared by freeze – substitution, showing the cell wall as a thick, electron – dense, homogeneous structure. The presence of distinct layers was not evident in this preparation. Reprinted from reference 142 with permission of the publisher. (B) Thin sections of cells treated with gold – conjugated concanavalin A, showing an intense labeling with gold particles of the external wall surface. The surface exhibits a fibrillar appearance (arrows), suggesting that concanavalin A – reactive cell wall components, e.g., mannoproteins, are particularly abundant at the most external wall layers. The remaining wall structure also appeared as a homogenous structure in this transmission electron micrograph. Bar, 0.5 μm. Reprinted from reference 170 with permission of the American Society for Microbiology. (C) Other procedures for transmission electron microscopy examination of thin sections of *C. albicans* cells revealed more clearly the presence of an outer floccular layer (arrow) and showed that the remaining cell wall structure is not homogeneous and that some layering exists. Bar, 200 nm. Reprinted from reference 91 with permission of the publisher (D and E). Complexity of the wall ultrastructure and presence of distinct layers in the cell wall of *C. albicans* as revealed by different scanning electron microscopy – based procedures such as cryo – scanning electron microscopy.

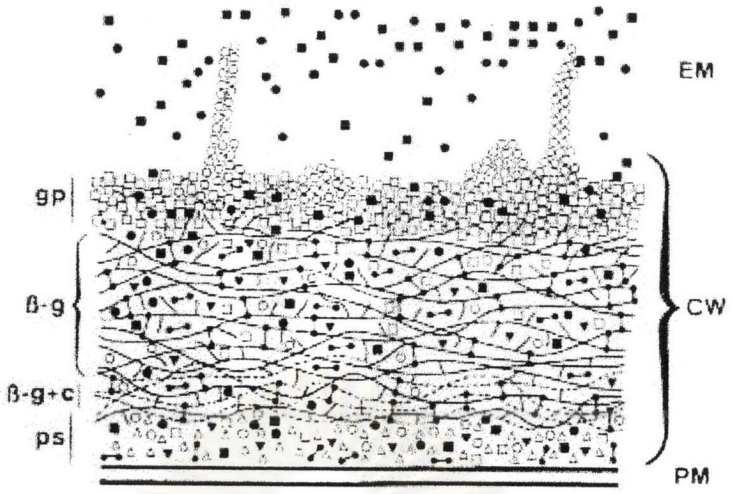

Figure 6: Schematic diagram of the cell wall (CW) structure of *C.albicans*, showing the presence of different layers enriched in particular components. The microfibrillar polymers of β - glucans (β - g) and chitin (c) appear to be more heavily concentrated in the inner cell wall domains; β - glucan – chitin complexes that appear to be formed by glycosidic linkages between both polymers will be located adjacent to the plasma membrane (PM) and the periplasmic space (ps). Proteins and glyco (manno) proteins (gp) appear to be dominant in the outermost cell wall layer, although they are also distributed throught the entire wall structure. Once secreted through the plasma membrane, some protein and glycoproteins species will remain at the periplasmic space, possibly playing enzymatic roles (Δ), some other will establish functional (i.e., β - glucanases [∇]) or structural covalent associations with β - glucans and possilbly also with chitin (●–●) adjacent to the plasma membrane; and, finally, other moieties will constitute the most external layer, where the different molecular entities may be homogeneously (□) or heterogeneously (fimbriae, cluster of receptor – like molecules, etc. [○]) distributed or specifically released (i.e., extracellular enzymes) to the extracellular medium (EM) (□ , ○). Proteins and glycoprotein species in the outermost wall layer (● , ■) may establish different types of covalent (disulfide linkages) and noncovalent (hydrophobic and hydrogen ionic bonds) interactions. During their passage through the wall from the plasma membrane and periplasmic space to the outermost cell wall layers (■ , ●) and possibly the extracellular environment, proteins and glycoproteins are most likely to be in quilibrium with other proteinaceous constituents, thus contributing, at least from a functional point of view, to the cell wall layering. In any case, protein and glycoprotein species other than proteinaceous constituents, thus contributing, at least from a functional point of view, to the cell wall layering. In any case, protein and glycoprotein species other than those specifically secreted to the exocellular medium may also be released to such locations by dying (lysed) cells or as a consequence of unbalanced processes of synthesis and degradation of the cell wall structure, required for wall expansion during cell growth. To simplify the scheme, some aspects such as possible interactions of cell wall components with the plasma membrane and proteins retained in the cell wall, apparently by either covalent or noncovalent linkages, are not depicted.

Immune response and Immunodiagnosis

The normal human has a high innate immunity to infection by *Candida* (115). The organism lives as a normal flora on body surfaces, and disease is very uncommon unless there is alteration of host defenses or predisposing environmental conditions. Exposure to *Candida* stimulates both humoral (HMI) and cell-mediated immune (CMI) responses and there is a renewed interest in the study of the host antibody response to *C. albicans* (17, 28, 116). Anti-candida antibodies can be detected in experimental animal infections (117), in humans with naturally acquired infections, as well as in healthy individuals harboring the fungus in a carrier state. The antibodies represent the different immunoglobulin types: IgG, IgM, IgA or IgE, not all immunoglobulin types can be detected in all the clinical entities, under all conditions and in different body fluids

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

In table 6 (28), 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 6. Antibody response to *C. albicans* cell wall proteins and mannoproteins

Component	Immuno globulin isotype[b]	Epitope	Antibodies detected in : Humans (Healthy)	Humans (Patients)	Animals (Models)	Comments
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
		O-linked	+	+		
		β-1,2 linked	+	+		
		Various, acid stable			+	
HMW – MP[a]	IgG 3	Unknown	+	+		
14-18 kDa moiety	IgG3?	β-1,2 linked	+	+		Antibodies cross – react with β-1,2 linked mannooligosaccharides of mannan
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
Proteins						
SAP	IgG, IgA	Unknown	+	+	+	Elevated antibody levels in patients may be due to contaminating mannan
Hsp90	IgG, IgM	Unknown, LKVIRK	-	+		Antibodies associated with recovery from infection

Hsp70	IgG	Unknown	+	+	+	Antibodies may recognize epitopes among microbes and self
Heat shock mannoproteins	IgA	Polysaccharide		+		Apparently induce secretory immune response during mucosal candidiasis
Enolase	IgG, IgE	Unknown	ND[c]	+	+	Antigen-based serodiagnostic test; species differences in epitopes recognized
PGK	IgG, IgE	Unknown				Not a universal immunogen/ allergen
GAPDH	IgM,IgG?	Unknown	ND	+		
mp58	IgG,IgM?	Unknown	ND	+	+	Positive in patients with systemic but not superficial candidiasis

[a]HMW – MP, high – molecular – mass mannoproteins.

[b]Not all Ig isotypes may have been tested.

[c]ND, Not detected.

IgG and IgM are generally found in sera of patients with deep-seated candidiasis, except in highly immunosuppressed patients who are unable to mount an immune response. The presence of IgM anti - *candida* antibodies, as an indicator of a recent infection has, contrary to other microbial infections, no proven validity. Thus, differentiation between the two types of immunoglobulins has limited diagnostic value in candidiasis, with the consequence that it is generally not carried out for this purpose. 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. Systemic candidiasis can be a life – threatening opportunistic infection, the treatment may be delayed because diagnosis is often difficult. *Candida* are associated with the normal flora, the positive cultures often require supportive diagnostic evidence. So there is an urgent need to develop serologic procedures to diagnose systemic candidiasis

Numerous serological procedures have been developed to assess levels of *C. albicans* – specific antibody in patient sera. These have been disturbed by false – positive results because human are exposed to its antigen and develop antibodies early in life (118). The detection of specific *C. albicans* antibody by counterimmunoelectrophoresis or immunodiffusion has been of some clinical value, as high levels of precipitins are diagnostically significant (119). The antigen was shown to be present in the cytoplasm, in the periplasmic space, and at the cell surface of *C. albicans* by indirect immunofluorescence (IF) (120). A latex agglutination test for antigen, it was available as a commercial kit (Ramco) (121, 122). Serological tests for specific serum antibody are frequently false – negative because many patients with candidiasis are immunocompromised and produce little, if any antibody (123, 124). As the results of antibody tests in immunocompromised patients are generally unreliable, antigen testing has been tried. An ELISA test for antigen (125) is useful for some investigators but its results have been disappointing.

Immunodiagnosis of candidiasis is based mainly on the detection of the humoral immune response as expressed in antibody production, and detection of fungal antigens (126, 127). The serodiagnosis of candidal disease has been the subject of controversy in the literature. An enzyme – linked immunosorbent assay (ELISA) can differentiate IgA and IgG antibodies (128) and is positive in patients. It has diagnostic accuracy for some types of *Candida* infections. The studies of immune responses have been extensively carried out. As many evidences showed that *C. albicans* antibody immunity may contribute to host defense by direct candidacidal activity, preventing attachment, providing opsonins for more efficient phagocytosis, binding to immunomodulating polysaccharides, neutralizing extracellular protease, and inhibiting the yeast-to-mycelium transition, which is associated with increased adherence and invasion (128, 129). Costantino *et al.* (130) reported that the levels of IgG anti – *C. albicans* antibodies detected in ELISA was