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Adherence of *Candida* Species to Host Tissues and Plastic Surfaces

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Adherence of *Candida* species to host tissues and nonbiologic materials has been studied in vivo and in vitro. Attachment of *Candida albicans* to mucosal cells, fibrin-platelet matrices, vascular endothelial cells, and plastic materials has been examined to elucidate early events in the pathogenesis of mucosal colonization and infection, candidal endocarditis, tissue invasion from the intravascular space, and infection of prosthetic devices. Adherence of *C. albicans* and *Candida tropicalis* exceeds that of less virulent *Candida* species, and germinated *C. albicans* cells adhere to host tissues more readily than do yeast-phase organisms. The adhesin of *Candida* that mediates attachment has yet to be characterized at the molecular level; however, on the basis of competitive inhibition by crude and purified cell wall products, blocking by antibody and lectin, and controlled degradation of the cell surface of *Candida*, it appears that mannans and mannoproteins are important constituents of the adhesin. The methods currently used to assay adherence of *Candida* all have limitations, and an approach to resolving these limitations is one of several areas that warrant further investigation.

During the past decade extensive investigations have resulted in the elucidation of mechanisms that mediate attachment of bacteria to host cells. Bacterial adhesins (surface constituents that mediate specific attachment) and host cell receptors have been characterized at the molecular level, as have factors governing their expression [1–3]. Bacterial adherence to host tissues has been generally accepted as an important step in the pathogenesis of infection, and knowledge of bacterial attachment mechanisms has been translated into approaches for the modification or prevention of infection in humans and experimental animals. Future developments seem likely in the areas of vaccination and passive immunization against bacterial adhesins, pharmacologic modulation of adhesin or receptor expression, and competitive inhibition by administration of adhesin and receptor analogues.

Knowledge of fungal adherence mechanisms is in a comparatively early state; nevertheless, a rapidly expanding literature attests to the potential importance of adherence in the pathogenesis of candidal infections. Attachment of *Candida* to epithelial cells has been studied to define factors relevant to the pathogensis of oral, gastrointestinal, vaginal, and urinary candidiasis. In addition, the attachment of the organism to fibrin-platelet matrices and to vascular endothelial cells has been examined to elucidate early events in the induction of candidal endocarditis and hematogenously disseminated infection. Finally, the attachment of *Candida* to "plastic" surfaces has been demonstrated and may be important in infections involving dental prostheses, intravascular and urinary catheters, and prosthetic cardiac valves. This discussion (*1*) reviews the present understanding of attachment factors of *Candida* to receptors on host cells; (*2*) considers the laboratory methods used to quantitiate adherence of *Candida* and to elucidate mechanisms of attachment; and (*3*) identifies major issues warranting further exploration.

A detailed discussion of the physicochemical factors involved in cell-cell and cell-surface interactions is beyond the scope of this review. Nonetheless, fundamental concepts underlying bacterium–host cell interactions may provide a useful framework in

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which to view attachment of *Candida*. Mammalian cells are endowed with a net negative surface potential resulting largely from ionization of sialic acid residues of the glycocalyx; other surface groups contributing to surface potential include acidic and basic amino acid side chains and glycolipid and phospholipid amines [4, 5]. The cell surface of *Candida* is likewise negatively charged, and ultrastructural delineation of the superficial mannoprotein coat has been possible with the use of ruthenium red, a marker for acidic polysaccharides [6]. The fixed-charge groups on eukaryotic cell surfaces attract ions of opposite charge from the surrounding milieu, creating an electrical double layer, which for practical purposes is part of the cell surface. Electrostatic repulsion plays an important role in cell-cell interactions and electrostatic attraction in the attachment of cells to positively charged surfaces. The paradoxical adhesion of cells of like charge has been explained in part by the lyophobic colloid theories of Derjaguin and Landau [7] and Verwey and Overbeek [8]. These theories state that the energy of interaction of two charged spherical particles of like sign and magnitude is the sum of the electrostatic energy of repulsion and the energy of attraction provided by van der Waal's forces. The net forces between approaching surfaces vary with their separation such that at certain distances (the primary and secondary minima) the surfaces attract one another, while at other distances they repel one another. The attractive forces are considerably stronger at the primary than at the secondary minimum, and the energies and distances of separation at primary minima correspond to those of molecular interactions. Interactions of a long-range nature (occurring at secondary minima) have been viewed as a preliminary and likely necessary step that precedes the essentially irreversible adhesin-receptor interactions [4, 9]. These theories inadequately describe the latter interactions, in which steric constraints as well as hydrophobic and polymeric interactions may play a major role [9].

It follows from these theories that the close approach of two surfaces would be favored by surface appendages or protrusions of low radii of curvature [4]. Pseudopodia and microvilli of animals cells may promote adhesion in such a manner, as may bacterial or fungal fimbriae. It is of note that fungal fimbriae have only rarely been demonstrated, and their role (if any) in adherence and their distinction from the fibrillar glycoprotein coat common to many fungi remain areas of controversy.

The hydrophobicity of microbial surfaces can be measured indirectly by partition of a microbial suspension in a biphasic aqueous system [10], by relative adsorption to hydrophobic gels [11], and by measurement of the contact angles between an aqueous phase and the surface in question [12]. With the use of these techniques, the potential importance of hydrophobic forces in certain bacterium–host cell interactions has been convincingly shown; however, only limited information has accrued regarding the role of hydrophobicity in adherence of *Candida* [13].

Mechanisms of Attachment of Candida and Host Cell Invasion

Adherence to mucosal epithelial cells. *Candida* species adhere readily to a variety of mucosal cells, including exfoliated buccal and vaginal epithelial cells [14–24] and uroepithelial cells [25], and to epithelium-derived cultured cell monolayers [21, 26–28]. The species with the highest rate of adherence to these cells is *Candida albicans* [14]. Which host cell type has the highest affinity for *Candida* is unclear; attachment of *Candida* to vaginal cells exceeded that to buccal cells in the study of King et al. [14], but the opposite result was noted by Sobel and co-workers [20]. Viability of either the organism or the host cell is not essential for attachment. However, nonviable *Candida* cells do not adhere as readily as do viable organisms, and the degree to which adherence is reduced depends on the method of killing [15, 20, 22, 27]. It is of interest that the extent of adherence of *Candida* is greater with formalin-killed than with viable HeLa cell monolayers [27] and that bacterial species preferentially attach to desquamating cultured vaginal epithelial cells [21]. These observations may lend validity to the routine use of a largely nonviable population of exfoliated mucosal cells in adherence assays [29].

To unravel mechanisms of attachment, investigators have subjected the adherence of organisms to a variety of growth conditions and have studied in vitro the adherence of purified cell wall components or partially degraded cell walls. Blastospores grown at 25–28°C adhered to exfoliated vaginal epithelial cells to a greater extent than did organisms grown at 37°C [15, 17]. King et al. [14] showed greater adherence of organisms harvested in the stationary

phase of growth than of organism harvested in the logarithmic phase, a result which suggests that a cell surface constituent that mediates attachment accumulates with increasing time in culture. In contrast, Segal et al. [17] showed greater vaginal epithelial adherence of logarithmic- than of stationary-phase *Candida*. Methodologic as well as strain-related differences between the two studies may account for the differences in the observed results. Preincubation of viable *Candida* with sucrose promoted adherence to buccal cells and acrylic surfaces and resulted in the appearance of a ruthenium red–staining fibrillar material (most likely mannoprotein) on the yeast cell surface [6, 27, 30, 31]. The adherence-promoting effects of saccharides were limited to cells exposed during the stationary phase of growth. In the presence of tunicamycin (an inhibitor of mannoprotein synthesis but not of chitin or glucan synthesis in *Saccharomyces*), enhanced adherence was not observed; this result suggests a role for surface mannoprotein of *Candida* in attachment [32]. Transmission electron microscopic studies suggest that a fibrillar cell surface polysaccharide likewise mediates attachment of *Candida* to vaginal epithelial cells [16].

Extracted cell wall fragments of *Candida* that are rich in mannan adhere to vaginal epithelial cells, and their adherence is diminished following selective degradation with α-mannosidase [15]. Additional evidence for mannan-mediated adherence to buccal cells is derived from studies showing diminished attachment of formalin-killed germ tubes in the presence of α-D-methylmannopyranoside (α-D-mM) or following treatment of the organisms with concanavalin A, a mannose-binding lectin [18, 19]. However, inhibition in the presence of α-D-mM was only partial (53%) and occurred only at relatively high saccharide concentrations (>200 mg/ml). An isolated report describes partial inhibition of vaginal epithelial cell adherence in the presence of D- and L-fucose [20].

Of interest are the observations that proteolysis of cell wall fragments also diminishes their adherence to mucosal cells [15] and that epithelial adherence of intact *Candida* is likewise decreased following exposure to proteolytic enzymes [15, 20]. Blastospores exposed to papain release glycoproteins of low molecular weight, which bind to vaginal epithelial cells and competitively block attachment of intact *Candida* [16].

In contrast, Segal et al. [17] (on the basis of blocking studies using amino sugars related to chitin) suggested that chitin, localized to inner layers of the cell wall of *Candida*, may be involved in the *Candida*–epithelial cell interaction. A chitin-soluble extract inhibited attachment of yeasts to vaginal mucosa of mice pretreated with the extract [33]. The aforementioned greater adherence of logarithmic- than of stationary-phase organisms [17] is intriguing in light of the transmission electron microscopic studies of Howlett and Squier [34], which showed intimate contact between the epithelial cell surface and the deeper layers of the cell wall of *Candida*. Accumulation and cross-linking of superficial mannoproteins during the stationary phase [35] might conceivably limit exposure of internally situated cell wall components, such as chitin. However, amino sugars are components of glycocalyx of host cell surfaces and of candidal mannoprotein [36] as well as chitin. Furthermore, mannose and *N*-acetyl glucosamine share a common three-dimensional structure except at the C-2 position, and *N*-acetyl glucosamine competes with mannan for uptake by hepatic mannan-binding proteins [37]. Therefore, the evidence supporting an adhesive role for a deeply situated cell-wall constituent (chitin or glucan) is less than compelling and is in conflict with a growing number of observations favoring more superficially disposed mannoprotein as the primary adhesin of *Candida*.

Adherence of germinated *C. albicans* has been reported to be enhanced by two- to 50-fold over that of yeast-phase organisms [18, 20, 22, 23], and a germ tube–negative mutant was found to be relatively avirulent in the rat model of candidal vaginitis [38]. Factors possibly contributing to enhanced germ-tube adherence include qualitative changes in adhesin, the expression of a germ tube–specific adhesin [39–41], changes in exposure to adhesin, a potential for multisite interactions as conferred by the increased surface area of germinated yeasts, and the greater tendency of germinated organisms to clump if relatively high inocula of *Candida* are used.

The influence of antibodies to *Candida* on mucosal adherence has been examined by several investigators. The effect of salivary antibodies to *Candida* on buccal adherence is unclear. Specific antibodies of the IgG and IgA classes are present in saliva of patients with oral candidal infection but do not protect against relapse following therapy with antifungal agents [42]. Kimura and Pearsall [22] cited

preliminary data which suggested that antibodies to *Candida* inhibit in vitro adherence to buccal cells. In a subsequent report Epstein et al. [42] noted an inverse correlation between the titer of salivary IgA and the adherence of *Candida* in the presence of IgA-bearing saliva. However, adherence increased in only four of 13 experiments following partial removal of salivary antibodies to *Candida* by immunoprecipitation of IgA and in only four of 20 experiments following immunoprecipitation of all antibody classes. Evidence for modest inhibition of the adherence of *Candida* to buccal cells by breast milk IgA has been reported [43]. This finding may be relevant to the lower incidence of thrush in breast-fed than in bottle-fed infants [44], but this association requries confirmation.

An influence of resident bacteria on colonization by *Candida* has been known for many years. In the animal model, the indigenous bacterial flora suppresses mucosal colonization by *Candida*, and the suppression of the normal flora by antibiotics renders mice and rats more susceptible to early and prolonged gastrointestinal candidiasis [45–48]. Mixed human salivary bacteria and strains of *Streptococcus salivarius* and *Streptococcus miteor* suppress oral colonization by *Candida* in germ-free mice [45]. In contrast to adult mice, infant mice are readily colonized by *C. albicans* in the absence of antibiotic exposure or immunocompromising modalities [49, 50]. The role of adherence in these interactions is unclear, as colonization by *Candida* is probably influenced not only by competition for, or modification of, mucosal receptors but also by competition for nutrients, bacterial elaboration of antifungal metabolites, and changes in redox potential that are unfavorable to fungal proliferation. In vitro systems have more precisely defined the role of bacterial-fungal competition for mucosal receptors. *S. salivarius* and *S. miteor* reduced attachment of *Candida* to HeLa cell monolayers [28]; this effect suggested that competition for receptors is indeed a likely factor underlying the in vivo observations. In a similar fashion, preattachment of *Lactobacillus* diminished adherence of *Candida* to vaginal epithelial cells [20]. The complexity of bacterial-fungal interactions is evident in studies showing enhanced adherence of *Candida* to uroepithelial cells preincubated with mannose-sensitive, piliated, gram-negative rods [25, 51]. Microscopic studies confirmed that the enhancement resulted from adherence of *Candida* to the preattached bacteria.

Penetration of mucosal epithelial cells. Mechanisms of mucosal invasion following adherence of *Candida* have been studied in vitro [26, 34, 52–55] and in the animal model [47, 50, 56, 57]. By transmission electron microscopy Marrie and Costerton [52] demonstrated (*1*) loose attachment of *Candida* to exfoliated mucosal cells that was mediated by a ruthenium red-staining matrix; (*2*) a more intimate type of contact, with no matrix material evident between the yeast and the host cell; and (*3*) host cell invasion by hyphal elements. In the aforementioned study Howlett and Squier [34] showed close contact between mucosal cells and deep layers of the cell wall of *Candida*. Hyphal invasion is thought to result both from enzymatic lysis and from mechanical force, although direct evidence of either process is lacking. Pugh and Cawson [53] demonstrated the extracellular localization of phospholipases A and C of *Candida* at the surface of blastospores and hyphal tips that were invading the chorioallantoic membrane in chicks, but the role of such phospholipases in the invasive process was not established.

Hyphal invasion is accompanied by disruptive changes in host cells that may vary from minimal [34] to severe [52, 55, 58]. Since the majority of superficial buccal cells may be nonviable [29], it is difficult to attribute the observed host cell damage entirely to invasion by *Candida*.

In the animal model, mucosal penetration by *Candida* has resulted from intestinal persorption of blastospores [50] or hyphal invasion of oral [34, 47, 56, 57], gastric [46], and intestinal mucosa [59]. The depth of hyphal invasion in the rat tongue mucosa model parallels the virulence of the *Candida* species studied. Hyphal and pseudohyphal invasion by *C. albicans* is most extensive, whereas penetration by less virulent species appears to be limited by keratinized layers [60].

In a murine model, when the mucosa is damaged because of the action of antineoplastic agents, gastrointestinal invasion by *Candida tropicalis* exceeds that by *C. albicans*; this difference may be related to the recent emergence of *C. tropicalis* as a pathogen in the setting of chemotherapy for cancer [61, 62]. However, studies focusing specifically on adherence of *Candida* to mucosa damaged by cytoreductive therapy have not been published.

Adherence to fibrin-platelet matrices and endocardial adherence. The rabbit model of endocarditis has been utilized to identify factors important in the induction of candidal endocarditis [63,

64]. Circulating yeast cells readily attach to fibrin-platelet deposits that form on the aortic and mitral valves following transaortic catheterization (left-sided endocarditis). The vegetation enlarges because of the deposition of phagocytic cells, platelets, fibrin, and erythrocytes. Platelets contribute to the pathogenesis of this infection in two ways: (*1*) their cationic proteins stimulate germination of yeast cells, and (*2*) cell wall fragments of *Candida*, through complement-fixation mechanisms, cause platelet aggregation, which, in turn, may promote the clotting cascade on the valve surface [65]. *Candida* elaborates a procoagulant material, fungal plasmacoagulase [66], though the role of this enzyme in the development of cardiac vegetations and disseminated intravascular coagulation remains speculative.

With the use of fibrin-platelet matrices as a model of traumatized valvular endothelium, the adherence of yeasts has been studied in vitro [67, 68]. Matrices are made by the combination of human or rabbit platelet-rich plasma with $CaCl_2$ and thrombin. Adherence of radiolabeled yeast cells to the fibrin platelets is reduced if yeast cells are heat-killed or pretreated with proteolytic enzymes or immune serum (or purified γ globulin to *Candida*). Antiserum to *Candida* absorbed with mannan or mannoprotein of *C. albicans* loses its adherence-blocking activity [67]. The reduction of adherence by antiserum to *Candida* is also observed in vivo, since valvular colonization in immunized animals is significantly reduced even though the extent of clearance of yeast cells in these animals is the same as in unimmunized animals [69].

A characterization of the surface component that promotes adherence of *Candida* has been undertaken. A mannoprotein extract of the cell wall of *Candida*, when conjugated to sheep red blood cells, promotes adherence of the latter to the fibrin-platelet matrix [68]. In contrast to the aforementioned diminution in adherence to epithelial cells, proteolysis of the cell wall mannoprotein does not alter the adherence of conjugated sheep red blood cells to the fibrin-platelet matrix. However, cleavage of the mannan backbone by acetolysis and cleavage of oligosaccharide side chains with α-mannosidase do alter adherence of sheep red blood cells; these results suggest that an intact polysaccharide moiety is essential for adherence.

More recently, two spontaneous mutants of *C. albicans* that are unable to adhere to fibrin platelets in vitro have been isolated (R. A. C., unpublished observations). It is of interest that both mutants are avirulent in the rabbit model of endocarditis when they are compared with wild-type *C. albicans*. Such mutants should be useful in the characterization of surface determinants that promote adherence to damaged endocardium.

The fibrin-platelet receptor for *C. albicans* yeast cells has not been characterized. However, fibronectin, a major surface glycoprotein of mammalian cells, is found in large quantities in fibrin clots [70] and binds in vitro to *C. albicans* (as opposed to nonpathogenic *Candida* species) [71]. Adherence is enhanced in the presence of calcium and is reduced if yeast cells are nonviable or are pretreated with one of several proteases or if adherence is measured at 4°C. The fibronectin must be fixed on glass cover slips or tissue culture dishes, as soluble fibronectin does not bind to yeast cells. This observation is analogous to in vivo conditions under which fibronectin is associated with fibrin clots. Studies examining attachment of *Candida* to fibrin-platelet matrices prepared from fibronectin-depleted plasma may help to clarify the role of fibronectin in these interactions.

Adherence to endothelial cells. Several investigators have examined the role of adherence of *Candida* to endothelial cells—both in vivo and in vitro—in the pathogenesis of hematogenously disseminated infection. Endothelial attachment and subsequent penetration seem intuitively to be requisite stages in the induction of such infections and may possibly contribute to the rapid clearance of candidal antigenemia. The earliest demonstration of yeast adherence to endothelial cells was in the studies of Johnston and Latta [72, 73]. Direct attachment of blastospores of *Saccharomyces* to renal endothelium was shown to be mediated by a polysaccharide-rich material on the yeast cell surface. Subsequent studies [16, 74] using *C. albicans* have shown similar ultrastructural features. Mechanical lodgement of blastospores in the microvasculature on the basis of size or sludging was deemed unlikely because of the careful selection of organisms with a diameter of <5 μm and the extensive postinfection perfusion by blood and fixative. In addition, the majority of tissue sections showed close apposition of organisms to the capillary endothelium without evidence of capillary occlusion or retention of nonadherent *Candida*. In other studies renal localization of *C. albicans* was more than four times that of inert microspheres [16] or nonpathogenic *Candida* species of similar size

[75]. Adherence of yeast to sinusoidal endothelium may play a role in the clearance of *Candida* by hepatic tissues [76]. However, the relative importance of endothelial attachment in these studies is difficult to assess, since photomicrographs suggest that intralumenal sludging and yeast clumping probably contribute to hepatic trapping [77].

Although it did not specifically address the role of endothelial adherence, a frequently cited study of clearance of *Candida* deserves mention. Stone et al. [59] studied clearance of *Candida* by skeletal muscle and the pulmonary, renal, and hepatic vascular beds. Clearance ratios ranged from 100:1 to 1,000:1, varying according to the vascular bed studied. However, interpretation of these data is complicated since concentrations of *Candida* reportedly infused at the upper end of the range (10^2–10^{20}/ml) are unattainable.

Mechanisms of attachment of *Candida* to endothelial cells have been explored in vivo in the perfused rabbit kidney [74] and in vitro with cultured monolayers of human endothelial cells [75] and fresh, porcine large-vessel explants [78]. In vitro studies revealed a hierarchy of adherence that correlated well with the relative virulence of the *Candida* species tested. The viability of *Candida* was not essential for endothelial adherence, though formalin- and periodate-killed *Candida* retained adherence properties to a greater extent than did heat-killed and ultraviolet-irradiated organisms.

Adherence to cultured endothelial cells is not blocked by mannose or by other simple sugars or purified mannan of *C. albicans* [75]. In contrast, in a previously mentioned report [76], hepatic trapping of *Candida* was found to be mannose sensitive and to be diminished by a mannoprotein with a low molecular weight. Prior exposure of *C. albicans* blastospores to proteolytic enzymes (a treatment which rendered them nonadherent to mucosal epithelial cells) did not alter vascular attachment in the perfused rabbit kidney [16] and modestly increased adherence to cultured endothelial monolayers [75]. These results suggest that the mechanism of endothelial adherence differs from that which mediates attachment of *Candida* to other host cells. Similar distinctions have been noted in bacterial attachment to oral mucosal and uroepithelial cells [79]. Attachment of *Candida* to cultured endothelial cells was blocked by antibodies to mannan of *C. albicans* [80], an effect suggesting a role for mannan as a component of the adhesin of *Candida*. Endothelial cell adherence of germinated *C. albicans* exceeded that of *Candida* blastospores, but only modest increases in adherence were noted [75].

Three studies address the mechanism of endothelial cell penetration following attachment of *Candida* [74, 78, 80]. In the perfused rabbit kidney, germ tubes penetrated endothelial cells in the absence of apparent host cell damage [74]. Fungal elements were bounded by endothelial plasma membrane; this observation suggested that penetration occurred by either endothelial cell phagocytosis or deep invagination of the endothelial cell membrane by elongating germ tubes. Endothelial phagocytosis was deemed unlikely, as pseudopodia were not observed and as the organism's diameter greatly exceeded the thickness of the attenuated capillary endothelium. In contrast, cultured endothelial cells phagocytosed the organisms, as evidenced by attachment of *Candida* to endothelial pseudopodia and the morphologically identical incorporation of viable or prekilled germ tubes into endothelial phagosomes [80]. Furthermore, elongating germ tubes failed to penetrate cytochalasin B–treated endothelial cells. In contrast, using relatively high inocula of *Candida*, Klotz et al. [78] showed the apparent lytic digestion of porcine endothelial explants by viable blastospores of *Candida*, independent of germ-tube formation. These intriguing and important results will stimulate investigation of blastospore exoenzymes if confirmed by use of techniques that preclude spontaneous endothelial cell death following explantation and endothelial injury–related perturbation of the milieu occurring at high inocula of *Candida*.

Adherence to plastic surfaces. Comparatively few studies on adherence of *Candida* to plastic surfaces have been conducted despite the potential relevance of such adherence for denture stomatitis and infections involving plastic prosthetic devices and intravascular and urinary catheters. Chronic atrophic candidiasis is a common complication in elderly denture wearers. Usually, *C. albicans* is recovered from the fitting surface of the denture; this pattern suggests that denture acrylic acts as a reservoir of infection [81]. Growth in a variety of sugars has been studied to determine whether dietary constituents influence adherence of *Candida* to dental acrylic. Adherence is unaffected by growth of the organism in lactose or xylitol but increases linearly after incubation in varying concentrations of other dietary sugars (sucrose, galactose, glucose, maltose, and fructose) [82]. Incubation in 500 m*M* sucrose or galactose

enhances production of a floccular cell surface material with the staining characteristics of an anionic polysaccharide [30]. Only limited studies address the in vivo relevance of these findings. The increased adherence and acquisition of cell surface floccular material with certain *C. albicans* strains grown in selected sugars have been correlated with increased virulence in a mouse model of disseminated candidiasis [31]. Dietary carbohydrate intake is considered an etiologic factor in patients with denture stomatitis [83], and sucrose rinses may initiate or aggravate the condition [84].

Palatal trauma probably results in the adsorption of serum components to the denture-fitting surface [82] and may be a factor promoting colonization of dentures in vivo. In vitro the adherence of *Candida* is diminished in the presence of normal saliva [30, 82]; this finding is in accord with the observation that colonization of prostheses by *Candida* increases with a decreased salivary flow in both experimental animals [85] and patients [86].

Minagi et al. [13] correlated adherence of *Candida* species to dental resin materials of varying hydrophobicity. *C. tropicalis* bound preferentially to surfaces of increasing hydrophobicity, while *C. albicans* favored less hydrophobic resins. The roughly 100-fold greater adherence of *C. tropicalis* to all surfaces tested and the pronounced differences in the adherence of *C. albicans* to materials of similar hydrophobicity suggest that factors other than hydrophobicity alone are important.

Limited studies have been performed on the adherence of *Candida* to plastics used for intravenous catheters. *Candida* species attached in vitro more readily to polyvinyl chloride catheters, which are generally used for central venous access, than to Teflon catheters, which are more commonly used at peripheral sites [87]; in contrast to the relative binding of *Candida* species to host tissues, the adherence of *C. tropicalis* to catheters greatly exceeded that of *C. albicans*. Scanning electron microscopy of naturally infected catheters revealed adherent *Candida* enmeshed in fibrin-like strands [88]; factors that promote persistent colonization possibly include fibrin deposition and decreased microbicidal activity of phagocytes in the microenvironment of the foreign body [89]. Staphylococci degrade and metabolize superficial catheter constituents in vitro [90], a process that may enhance the ability of these organisms to colonize catheter surfaces; similar properties have not been explored among *Candida* species.

Designing Laboratory Models of the Adherence of *Candida*

Host cell preparations and receptor expression. Most studies of the adherence of *Candida* to mucosal epithelium have relied on exfoliated cells. The resulting cell populations are not uniform with respect to a number of properties that are potentially relevant to adherence of *Candida*, including loss of host cell viability [29]; contamination by commensal bacteria, yeasts, and food debris; and adsorption of immunoglobulins and other components of salivary or vaginal secretions [21, 27, 28]. Other host variables that may have an impact on adherence include donor age, phase of the menstrual cycle, use of oral contraceptives or spermicidal creams, and antecedent bacterial or viral infection [20, 91]. In general, most laboratories have used healthy donors for routine studies and have minimized by extensive washing the influence of bacterial and yeast contamination. Lack of bacterial contamination may be confirmed by visual assays, and the influence of low-level yeast contamination may be minimized by subtraction of background counts. In addition, the impact of donor-to-donor variability can be eliminated by the pooling of exfoliated cells or the restriction of comparative studies to a single donor. Despite the use of such precautions, significant donor-to-donor variability [14, 20, 24], as well as day-to-day variability in studies that have been restricted to a single donor [14], have been demonstrated.

Demonstration and quantitation of adherence of *Candida* to exfoliated cells in vitro may be misleading if significant attachment occurs to the serosal surface. Aside from an isolated preliminary report [92], we are unaware of attempts to localize adherence of *Candida* to the lumenal or serosal surface of exfoliated cells.

Adherence of *Candida* has been quantitated by radiolabel and visual assays, both of which necessitate cautious interpretation. Separation of host cells from nonadherent *Candida* has generally been accomplished by collection on filters of 10- to 12-μm pore size, which trap epithelial cells but which should allow passage of single yeasts. Extensive washing with agitation may be important, since at high epithelial cell densities a significant proportion of the filter surface may be occluded. Furthermore, particle trapping by filters may involve factors more complex than relative particle and pore size; in one study 10%–15% of an inoculum of *Candida* (10^6 cfu/ml)

was retained (presumably via electrostatic or hydrophobic forces) by filters of the pore sizes routinely used in adherence studies [93].

Visual assays that quantitate adherence in terms of the percentage of host cells with a minimal number of attached *Candida* may reflect either the expression of host receptors or the tendency of the organisms to coadhere. In contrast, methods that avoid clumping and measure the mean number of organisms attached to the entire host cell population provide a more accurate estimate of adhesiveness [2].

As a means of circumventing some of these difficulties, models of the adherence of *Candida* to cultured cell monolayers have been developed. Cell culture provides a more homogeneous population of host cells, free of contamination, in which organism attachment is restricted to the "lumenal" surface. Nonadherent organisms are easily removed by washing, and adherence can be quantitated by visual methods, radiolabeling, or colony counts after agar overlay. Use of monolayer models should permit systematic studies of receptor modulation by viral infection and by endocrine and pharmacologic modalities. However, cell surface characteristics may be altered in vitro with transformed or multiply passaged cells, and one cannot assume that cultured cells will retain receptors functionally analogous to those expressed by the parent cell in vivo. Models that utilize fresh tissue or whole organ explants may resemble more closely the complex mucosal microenvironment of the intact host but may be poorly acclimated to the ex vivo state and, in general, are not as amenable to experimental manipulation as are cultured or exfoliated cells.

Preparation of Candida and adhesin expression. Expression of bacterial adhesin may be influenced by host factors in vivo and by growth conditions of the organism in vitro [3]. Media constituents and optimal conditions favoring expression of adhesins of *Candida* have as yet been only partially defined. *C. albicans* strain-related differences in adhesiveness may be either minor [20, 24] or substantial [22, 57]. Although several studies have demonstrated augmented adherence of germinated blastospores of *Candida*, the factors leading to their increased adherence have not been delineated.

With rare exceptions the adherence of *Candida* has been studied with the use of blastospores or germ tubes suspended at concentrations ($>10^6$ cfu/ml) that favor clumping of the organism. Radiometric assays may be particularly susceptible to these effects since suspensions of $0.5\text{–}3.0 \times 10^8$ cfu/ml are routinely employed. Interpretation of such studies may be difficult because coadherence is expected to alter determinations of host cell adherence and because quantitative changes in adherence due to soluble inhibitors and experimental modification of the cell surface of *Candida* may result from an effect on coadherence of *Candida* as opposed to *Candida*–host cell interactions.

Care must be exercised to avoid manipulations or excessively high inocula of *Candida* that adversely affect the physiochemical properties of the system. Host cells in tissue culture may be sensitive to shifts in pH, proteolytic enzymes, changes in ionic strength, or concentrations of divalent cations. Both use of inhibitors (especially antibody and lectin) with potential for agglutination and cross-linking of the organism to the host cell surface may also influence results. Blocking studies using antibody (or lectin) provide only indirect evidence that implicates the target antigen as the adhesin of *Candida*. Whereas diminished adherence may result from specific interaction of antibody with adhesin, surface-bound antibody may conceivably block adherence by steric hindrance or alteration in charge.

Several laboratories have shown diminished adherence after experimental manipulation of the cell surface of *Candida*. These data must be interpreted with the recognition that relatively little is known (in comparison with available information on piliated bacteria) regarding the selective degradation of the cell surface of *Candida* by physiochemical and enzymatic means. On the basis of selective stepwise cleavage of cell wall components, Cassone and coworkers [94] concluded that the layering of the cell wall of *Candida* as seen by electron microscopy, reflects the distribution of a rigid, insoluble glucan-chitin inner matrix and the organization of mannoproteins that extend throughout the cell wall. The mannan of *Candida* is a polymer of α-1,6-linked mannose residues, with antigenic specificity residing in the configuration of single, or closely situated, oligosaccharide side chains composed of $\alpha(1\rightarrow2)$- and $\alpha(1\rightarrow3)$-linked mannose residues [95]. Recent studies suggest a "branched-tree" (as opposed to a "comb-like") structure for the mannoprotein molecule, with a central protein core supporting the mannan backbone and oligosaccharide side chains [96]. During mannan purification by the method of Peat et al. [97], antigenically important acid- and alkali-labile oligomannosyl residues may be lost, and pro-

tein constituents may be degraded or released [98]. In contrast, extraction by cetyltrimethylammonium bromide yields a mannan polysaccharide with intact oligosaccharide side chains and protein moieties covalently bound through *o*-glycosidic linkage to serine and threonine [98].

Heat and alkaline extraction release mannan from the wall of *Candida* [94], and, as noted by Chattaway et al. [99, 100], the activity of hydrolytic enzymes is appreciably enhanced by reduction of disulfide bonds in the outer glycoprotein layer. Prolonged ultraviolet irradiation releases a glycoprotein with a low molecular weight that is implicated in the attachment of *Candida* to phagocytes [101]. The effects of detergents and salts on the mannoproteins of *Candida* and possible steric changes secondary to periodate oxidation and formalin fixation remain largely unexplored.

Elucidation of adherence mechanisms on the basis of morphologic studies. Morphologic studies may contribute to the recognition of attachment mechanisms but are best viewed in the context of data obtained through other approaches. Demonstration of particular cell surface constituents or histochemical evidence of enzyme release at the point of contact does not, in the absence of more direct proof, indicate participation in attachment or tissue invasion by the organism. Clustering of adherent organisms may indeed reflect distribution of host cell receptors [78], but it is equally likely that it reflects clumping of the organism when high inocula are used. Difficulties in distinguishing the cross-sectional profiles of blastospores and germ tubes may lead to erroneous conclusions regarding their relative invasive potential [34]. These examples illustrate some limitations of morphologic studies; nevertheless, such studies can complement techniques that are more quantitative, illustrate specificity of attachment for a particular host cell type [102], and suggest mechanisms of subsequent invasion or incorporation by the host cell.

Remaining Areas of Investigation and Future Developments

Evidence that a particular cell-surface constituent functions as an adhesin is most compelling when the purified material competitively inhibits attachment of the intact organism. It is interesting that less-than-complete inhibition by purified constituents of *Candida* has been shown despite numerous attempts to demonstrate complete inhibition. One explanation may be that degradation or changes in steric configuration occur during extraction and purification of the putative adhesin. Until more profound competitive inhibition is attained, characterization of the adhesin of *Candida* will depend on less direct evidence such as blocking by antibody, lectin, and simple sugars and selective modification of putative adhesins and receptors.

In many instances discrepant results have been obtained among laboratories. Such discrepancies may reflect differences in mechanisms of attachment to divergent host cell types or in methodologies used to measure adherence, interstrain differences, or day-to-day variability in a nonhomogeneous host cell population. Undoubtedly, the field would benefit from a systematic identification of the conditions for the growth of *Candida* that influence adherence and from interlaboratory standardization of assay techniques. Evolving concepts of the cell wall structure of *Candida* and the application of modern technologies to the purification and characterization of antigens of *Candida* [39, 40, 103, 104] will probably influence our understanding of adherence mechanisms, as will the increasing availability of serologic probes, particularly monoclonal antibodies to well-defined cell surface domains [105–107]. Characterization of nonadherent mutants [104] and identification of mannan- or mannoprotein-deficient mutants will undoubtedly aid in the elucidation of attachment mechanisms. Current assays of the adherence of *Candida* are, in general, laborious and time-consuming. Simple screening techniques that are anologous to bacterial hemagglutination would be useful. Currently, only limited information is available in this area [108].

At present, the importance of adherence of *Candida* in pathogenesis is only partially defined. The contention that virulence of the *Candida* depends on adherence is based on association alone. Whether adhesin expression renders the organism more susceptible to phagocytosis has not been explored, nor has the possibility that released adhesin blocks phagocyte receptors. Similarities between leukocyte recognition factors and putative adhesins suggest these possibilities [101, 109, 110]. Adhesin expression of bacteria may be modulated as infection progresses from superficial to deep tissues [3]; aside from an isolated report describing the loss of surface flocculer material that follows the penetration of host cells

[55], the possibility of tissue-dependent modulation of the adhesin of *Candida* remains unexplored.

Other areas awaiting investigation include the identification of host tissue alterations that render patients more susceptible to candidiasis. Exposure to antibiotics, chemotherapeutic agents, and endogenous and exogenous hormones may ultimately be shown to influence receptor expression.

Similarly, modulation of adhesin expression and surface potential or hydrophobicity by chemotherapeutic agents, antibiotics, and antifungal agents deserves attention. The demonstration of progesterone and estradiol receptors of *Candida* [111] will undoubtedly stimulate consideration of the hormonal effects of adhesin expression.

Specificity of attachment (tropism for a particular host cell type) has been considered a characteristic of bacterial ligand-receptor interactions. The ready adherence of *Candida* to divergent host cells, noncellular biologic substrates, and plastic and glass surfaces has been viewed as evidence of nonspecific (nonligand) adherence [112]. However, ligand adherence cannot be excluded solely on the basis of adherence to divergent tissues and synthetic materials. Furthermore, though substrate specificity may be lacking, adherence mechanisms may still be operative in the colonization and invasion of host tissues. The major question, as summarized by Freter and Jones [2], is whether or not a particular form of adherence observed in vitro plays a role in colonization or infection in vivo. Initial studies [31, 113] suggest that adherence is correlated with virulence. If so, inhibitors or other modalities that block adherence in vitro should be evaluated in the animal model. On the basis of our present understanding, it seems likely that knowledge of the mechanisms of adherence of *Candida* will ultimately find application in attempts to modify or prevent disease caused by this emerging pathogen.

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