Expression of Surface Hydrophobic Proteins by *Candida albicans* In Vivo

PATI M. GLEE,¹ PAULA SUNDSTROM,² AND KEVIN C. HAZEN^{1,3*}

Departments of Pathology¹ and Microbiology,³ University of Virginia Health Sciences Center, Charlottesville, Virginia 22908, and Department of Medical Microbiology and Immunology, Ohio State University, Columbus, Ohio 43210²

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***Candida albicans* modulates cell surface hydrophobicity during growth and morphogenesis in vitro. To determine if surface hydrophobicity is expressed during pathogenesis, we generated a polyclonal antiserum against yeast hydrophobic proteins. The antiserum was then used for indirect immunofluorescence analysis of tissues from mice colonized and chronically infected with *C. albicans*. Results demonstrated that yeast hydrophobic proteins are exposed on fungal cells present in host tissues. The polyclonal antiserum distinguished between hydrophobic and hydrophilic cell surfaces in vitro and gave similar staining patterns and intensities for *C. albicans* cells in vivo. Of the yeast forms present within tissue lesions, approximately half exhibited moderate to intense immunofluorescence with the antiserum. Immunoblot analysis indicated that antigens recognized by the antiserum are predominantly low-molecular-mass hydrophobic proteins that are expressed by different *C. albicans* isolates and are expressed regardless of growth temperature. Taken together, the immunofluorescence and immunoblot analyses of antigens indicate that *C. albicans* displays surface hydrophobic proteins during pathogenesis and these proteins are available for hydrophobic interactions with host tissues. The effect of hydrophobic protein exposure on the virulence of *C. albicans* is discussed.**

As the primary agent of mucocutaneous and disseminated candidiasis, the opportunistic pathogen *Candida albicans* causes significant morbidity and mortality among immunocompromised and other patient populations (for a review, see reference 34). The *C. albicans* cell wall is regarded as an important site that influences the organism's virulence (8, 9, 36). For example, the cell wall surface determines fungal adhesion to host substrata, presents immunomodulatory components to the host, and affects phagocytic events (6, 10, 28). Biochemical studies have demonstrated that the wall is a complex molecular structure, which is composed of proteins, polysaccharides (chitin, glucans, and mannans), and lipids arranged in electron-dense layers (6, 40). However, the number and relative composition of cell wall layers are affected by environmental and physiologic factors (12, 40). Such variation complicates interpretation of studies which compare cell wall structure with function in pathogenesis. Characterization of the cell wall changes during pathogenesis is needed for understanding how *C. albicans* promotes disease progression.

Recent investigations have demonstrated that alterations to *C. albicans* cell wall components can lead to changes in cell surface hydrophobicity (CSH), which would then result in a shift in the free energy of interaction of the cell with host tissues. Such alterations in CSH occur during hyphal and yeast growth of the organism in vitro (16, 18). Freeze-fracture analyses have shown that the CSH shifts are correlated with changes in the length and concentration of fibrils in the exterior layer of the cell wall (21, 22). CSH shifts are affected by various factors, including temperature (16, 18). For example, stationary-phase yeast cells in complex broth media are hydrophobic when grown below 28°C, whereas higher growth temperatures (e.g., 37°C) result in hydrophilic stationary-phase

cells. Growth temperature, however, does not independently determine surface hydrophobicity status. When incubated at 37°C, actively growing yeast cultures express moderate levels of CSH, pseudohyphal cells are variably hydrophobic, cells grown on solid media exhibit a wide range of surface hydrophobicity, yeast cells become hydrophobic prior to germination, and young hyphal cell walls are highly hydrophobic (16, 18). Thus, while temperature may influence expression of CSH, physiologic and morphologic factors and environmental conditions also affect expression of cell surface attributes.

Growth temperature has been used by us and others (for example, see references 1, 5, 19, and 27) to obtain hydrophobic and hydrophilic cell populations in vitro to investigate the pathogenic significance of hydrophobicity. The hydrophobic *C. albicans* cells grown in vitro demonstrated increased virulence compared with the hydrophilic cells. Hydrophobic cells also exhibited greater adherence to epithelial cells and extracellular matrix proteins, decreased susceptibility to killing by phagocytic cells, and the ability to attach to multiple sites in kidney, spleen, and lymph node tissues (1, 13, 17, 19, 28, 37, 41). These observations concerning *C. albicans* CSH expression at 37°C in vitro suggest the potential for hydrophobic surface exposure during infection by yeast cells and hyphae.

We have previously compared the cell wall proteins exposed on the surface of hydrophobic and hydrophilic *C. albicans* cells and have isolated hydrophobic protein fractions by hydrophobic interaction chromatography (HIC) (21–23). Here we describe the preparation of an antiserum that distinguishes hydrophobic from hydrophilic cell wall surfaces and that is directed against a limited number of hydrophobic proteins. Using this antiserum, we determined that hydrophobic proteins are exposed on the surfaces of both yeast and hyphal cells of *C. albicans* in vivo.

MATERIALS AND METHODS

Yeast isolates and culture conditions. *C. albicans* LGH1095, our standard laboratory isolate, was cultured for cell wall protein isolation and in vitro immu-

* Corresponding author. Mailing address: Department of Pathology, Box 214, University of Virginia Health Sciences Center, Charlottesville, VA 22908. Phone: (804) 924-8067. Fax: (804) 924-8060. Electronic mail address: khazen@virginia.edu.

no fluorescence analysis (IFA) as previously described (1, 17, 19, 21). Briefly, cells were grown to stationary phase in phosphate-buffered yeast nitrogen base (plus amino acids [Difco]) containing 0.5% glucose (in 0.055 M NaPO₄ [pH 7.2]) at either 23 or 37°C to obtain hydrophobic (CSH $\geq$ 95%) or hydrophilic (CSH $\leq$ 5%) cells, respectively. CSH levels were assessed by the hydrophobic microsphere assay as described elsewhere (16), except that different suppliers were used for low-sulfate microspheres (catalog no. P0008251PN; Bangs Laboratories, Carmel, Ind.) and polypropylene tubes (catalog no. T3311; Marsh Biomedical Products, Rochester, N.Y.). Culture conditions were similar for *C. albicans* LGH870 (24), SC5314 (30), and B311 (7). For some experiments, yeast cells cultivated at 23 and 37°C were germinated (2 h, 37°C) in Auto-Pow minimal essential medium with Earle's salts (pH 6.8) (Flow Laboratories, McLean, Va.) supplemented with biotin (250 μ g/ml), glucose (9 g/liter), glycine (1 g/liter), and HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid) (6 g/liter) as previously described (20).

Cell wall protein preparation. Stationary-phase yeast cells were harvested, and cell wall proteins were removed by limited B1-3 glucanase (lyticase; Sigma, St. Louis, Mo.) digestion in the presence of a protease inhibitor cocktail containing 1 mM EDTA, 0.2 mM phenylmethylsulfonyl fluoride, 1 μ M leupeptin, and 1 μ M pepstatin. The *C. albicans* cells remained viable after lyticase treatment. The digestion was done as previously described (21, 22), except that 1×10^9 to 5×10^9 cells per ml were digested with 500 U of lyticase per ml and the cell wall protein solutions were not dialyzed prior to electrophoresis or chromatography. Cell wall protein samples prepared with these modifications are herein referred to as rapid crude digests (RCD). Protein concentrations were determined by the Coomassie Plus Assay (Pierce Chemical Company, Rockford, Ill.) which is a modification of the Bradford method (4).

RCD samples were immediately subjected to either HIC-high-pressure liquid chromatography (HPLC) or sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) as described elsewhere (21). Briefly, separation of hydrophilic and hydrophobic cell wall proteins by HIC-HPLC was carried out in a seven-step discontinuous gradient of phosphate-buffered ammonium sulfate to water (21). Fractions, designated HICF1 through -F7, contain hydrophilic proteins in early fractions (HICF1 to -F3) and more-hydrophobic proteins in later fractions (HICF5 to -F7). HICF1 also contains the column void volume. All fractions were concentrated and dialyzed at pH 7.0 in the presence of EDTA and phenylmethylsulfonyl fluoride. The final concentrate was replenished with the protease inhibitor cocktail (described above), unless the sample was used for immunizing mice. Protein samples were mixed with SDS-PAGE sample buffer, heated, and electrophoresed in large-format 12.5% polyacrylamide gels (dimensions, 170 by 150 by 0.75 mm). Prestained protein standards (BioRad, Hercules, Calif.) were included for molecular mass estimates of *C. albicans* proteins. Separate SDS-PAGE calibrations of the Coomassie blue-bound prestained standards against nonprestained protein standards indicated that the prestained proteins migrated within 3 kDa of reported values.

Anti-HICF6 antiserum production. Mice (female A/J, 6 to 8 weeks old, maintained in an American Association for the Accreditation of Laboratory Animal Care approved facility) were immunized with HICF6 hydrophobic proteins from *C. albicans* (30 μ g delivered subcutaneously in complete Freund's adjuvant) and given booster 4 injections and 8 weeks later (30 and 10 μ g, respectively, in incomplete Freund's adjuvant). Two weeks after the final booster injection, mice were bled through the tail vein to obtain the anti-HICF6 polyclonal antiserum (PAb).

Preparation and analysis of Western blots (immunoblots). Protein transfer onto polyvinylidene fluoride (PVDF) membranes (Immobilon-P; Millipore, Bedford, Mass.) was carried out with a semidry blotting system (MilliBlot-SDE, Millipore) with electrical ramping conditions that were optimized for the transfer of hydrophobic proteins. These conditions were tested in a comparison with wet-blot preparations (samples blotted to both PVDF and nitrocellulose membranes) to ensure similar transfer efficiencies for other cell wall components including high-molecular-mass hydrophilic proteins. PVDF membranes were soaked briefly in transfer buffer (12.5 mM Tris, 96 mM glycine, 5% methanol [44]) and placed with the gel and buffer-soaked filter paper according to the manufacturer's instructions. Electrotransfer conditions were 0.5 mA/cm² membrane for 30 min, 1.25 mA/cm² for 15 min, and 1.5 mA/cm² for a final 15 min. For visualization of total blotted proteins in HICF1 through -F7, membrane strips were stained with colloidal gold (BioRad, Hercules, Calif.).

For immunoblotting, membranes were rehydrated, washed for 10 min in Dulbecco's phosphate-buffered saline (pH 7.2) (DPBS) (Sigma) and incubated for 1 h (37°C) in fresh blocking solution containing 5% (wt/vol) nonfat milk and 0.2% (vol/vol) Tween 20 in DPBS (BLOTTO/DPBST). BLOTTO/DPBST was used for all antibody dilutions and wash steps. Blocked membranes were incubated for 1 h (37°C) with 1:4,000 to 1:20,000 anti-HICF6 PAb or preimmune mouse serum and washed three times (10 min each). Blots were incubated for 1 h (37°C) in 1:500 biotin- or alkaline phosphatase-conjugated goat F(ab')₂ anti-mouse immunoglobulin G (IgG) (Fc specific or whole) (Jackson Immuno-Research Laboratories, West Grove, Pa.). Blots receiving biotin-conjugated antibodies were washed three times (10 min each) and incubated for 40 min in streptavidin-conjugated alkaline phosphatase (1 μ g/ml; Jackson Immuno-Research or Zymed Laboratories, San Francisco, Calif.). All immunoblots were washed three times (15 min each) in 0.1 M Tris-0.1 M NaCl-1 mM MgCl₂ (pH 9.5), and reactive

bands were visualized with nitroblue tetrazolium and 5-bromo-4-chloro-3-indolylphosphate (BCIP) as described elsewhere (38).

Indirect IFA of *C. albicans* cells in vitro. Stationary-phase yeast cells (23 and 37°C) and germinated *C. albicans* LGH1095 were harvested, washed three times in cold deionized H₂O, and 6×10^6 cells (0.17 ml of 3.5×10^7 cells per ml of DPBS) were incubated for 10 min on ice with 1:200 anti-HICF6 antiserum diluted in DPBS. Cells were washed three times in cold DPBS and incubated 5 min on ice with fluorescein isothiocyanate (FITC)-conjugated goat F(ab')₂ anti-mouse IgG antibody (1:1,000 in DPBS; Cappel-Organon Teknika, Durham, N.C.). Control samples were incubated with the FITC conjugate only. Samples were washed twice in cold DPBS and suspended in 0.1% phenylenediamine (free base; Sigma)-45% glycerol in DPBS prior to microscopic observation. Alternately, germinated cells were fixed in 0.5% formalin and stained for IFA as previously described (42). The fluorescence intensities of the samples were scored on a scale of 0 (negative) to 4+ (strong). Samples were photographed (Olympus model BH2, epifluorescence) with Kodak Tmax Professional film (ASA800) and developed per the manufacturer's instructions.

IFA of tissue sections. Paraffin-embedded tissues from an 11-month-old male *bg/bg nu/+* mouse following 9 months of monoassociation with *C. albicans* B311 were generously provided by Edward Balish. Procedures for producing *C. albicans* monoassociated immunodeficient mice have been previously described (7). Sections (10 μ m) of kidney, stomach, esophagus and tongue were removed from paraffin in three changes of xylene and rehydrated in a graded series of ethanol to water (twice in 100% ethanol and once each in 95, 70, and 50% ethanol, followed by deionized H₂O). Tissue sections were blocked in 1% bovine serum albumin (BSA) in phosphate-buffered saline (PBS) (1 h, 37°C), washed three times in PBS (5 min each; at room temperature) and placed in a moist chamber to incubate for 1 h (37°C) with 1:100 anti-HICF6 antisera or preimmune mouse serum diluted in 1% BSA in PBS. Slides were washed three times (5 min each) in PBS and incubated (30 min, 37°C) with a 1:100 dilution of FITC-conjugated goat F(ab')₂ anti-mouse IgG F(ab')₂ antibody (Cappel-Organon Teknika). After three washes in PBS, a coverslip was placed onto the tissue sections with a drop of 0.1% phenylenediamine-90% glycerol in PBS. Total apparent yeast forms in five different microscopic fields (magnification of $\times 200$) were counted and scored for fluorescence intensity as either negative to weak (0 to 1+) or moderate to intense (2+ to 4+).

RESULTS

Antiserum reactivity against HIC-fractionated *C. albicans* cell wall proteins. Immunoblot analysis was utilized to assess the murine response to immunization with hydrophobic cell wall proteins from *C. albicans*. Western blot strips were prepared from HIC-HPLC-fractionated cell wall proteins from hydrophobic *C. albicans* LGH1095 yeast cells and stained with colloidal gold (Fig. 1A) or probed with the anti-HICF6 PAb (Fig. 1B). In HIC separations with the conditions described in Materials and Methods, the hydrophilic proteins elute first from the column (HICF1 to F3) whereas the more-hydrophobic cell wall proteins elute in later fractions (HICF5 to F7). Colloidal gold stained-PVDF strips indicate that most cell wall proteins elute prior to hydrophobic fractions HICF6 and HICF7 (Fig. 1A). Similar step-gradient, HIC elution profiles were observed in previous studies of *C. albicans* yeast cell wall proteins (21).

The anti-HICF6 PAb primarily recognized hydrophobic proteins in fractions HICF5 through -F7, with the strongest reactivity against multiple components between 34 and 44 kDa, plus 25-, 50-, and 56-kDa cell wall proteins (Fig. 1B). The strength of the immune response elicited by the 34- to 44-kDa hydrophobic proteins was demonstrated by positive Western blot signals for these proteins with as little as 1:50,000 anti-HICF6 PAb against 1.5 μ g of total F6 protein. Titer by enzyme-linked immunosorbent assay (ELISA) was 50,000 against 0.25 μ g of total F6 protein. The antisera raised against HICF6 proteins also recognized three proteins in more-hydrophilic HIC fractions, a 90-kDa protein (Fig. 1B, HIC F2 and F3) and two proteins with masses between 50 and 54 kDa (Fig. 1B, a 50-kDa protein in HICF2 to -F7, and a 54-kDa protein in HICF2).

Immunoblot analysis of other *C. albicans* isolates and growth temperatures. The anti-HICF6 PAb reactivity was compared for Western blots of RCD samples from hydropho-

FIG. 1. Immunoblot analysis of PAb reactivity to HIC-HPLC fractions from hydrophobic *C. albicans* yeast cells. Western blot strips containing approximately 1.25 μ g of total protein from HIC fractions F1 through F7 (lanes 1 to 7, respectively) were stained with colloidal gold (A) or incubated with anti-HICF6 PAb (B) followed by biotin-conjugated anti-mouse IgG (Fc specific). The molecular masses of protein standards are indicated in kilodaltons to the left of the strips. Control strips from each fraction were negative when incubated with preimmune mouse serum (data not shown). philic, hydrophilic; phobic, hydrophobic.

FIG. 2. Anti-HICF6 PAb reactivity to different *C. albicans* isolates and growth temperatures. Cell wall protein (RCD) samples were electrophoresed from hydrophobic (23°C, lane 1) and hydrophilic (37°C, lane 2) LGH1095 yeast cells and hydrophobic (23°C, lane 3) LGH870 yeast cells. Western blot strips were incubated with anti-HICF6 PAb followed by alkaline phosphatase-conjugated anti-mouse IgG (whole). The molecular masses of protein standards are indicated in kilodaltons to the left of the strips.

bic (Fig. 2, lane 1) and hydrophilic (Fig. 2, lane 2) LGH1095 yeast cells and hydrophobic LGH870 yeast cells (Fig. 2, lane 3). In each case, the PAb recognized a similar antigenic profile, especially for the main reactive bands contributed by hydrophobic proteins between 34 to 44 kDa and 56 kDa (Fig. 2). Anti-HICF6 PAb reactivity in immunoblots with other *C. albicans* isolates, SC5314 and B311, were similar to those shown in Fig. 2 (data not shown). The intensities of some anti-HICF6 positive bands in total RCD blots (e.g., the 90-, 50-, and 25-kDa bands; Fig. 2, lane 1) appeared weaker than blots of HIC fractions (Fig. 1B) because of differences in relative protein concentrations in the samples.

Immunofluorescence of *C. albicans* yeast cells and hyphae in vitro. The anti-HICF6 antiserum distinguished between hydrophobic and hydrophilic yeast cell surfaces of *C. albicans* LGH1095 in indirect IFA analysis (Fig. 3). All cells in hydrophobic yeast cultures exhibited moderate to high levels (3+ to 4+) of homogeneous cell wall fluorescence (Fig. 3A). Stationary-phase hydrophilic (37°C) yeasts were negative or displayed weak (1+), punctate staining intensity of the cell wall (Fig. 3D). Control experiments performed without primary antibody were negative for yeast cells cultivated at the two growth temperatures (data not shown). The efficacy of the anti-HICF6 antiserum in differentiating between hydrophobic and hydrophilic yeast cultures was consistent with other *C. albicans* isolates (LGH870 and SC5314, data not shown).

Under the germination conditions described in Materials

and Methods, hydrophobic yeast cultures primarily produced hyphal forms (Fig. 3B) whereas hydrophilic cultures evidenced pseudohyphal and budding forms (Fig. 3E). The anti-HICF6 PAb stained the mother cell walls of germinated cells intensely (4+), and hyphal cell walls moderately (2+) (Fig. 3B). Moderate staining intensity (2+ to 3+) was observed on growing pseudohyphal tips and emergent buds with the anti-HICF6 PAb (Fig. 3F). Control samples of hyphal and pseudohyphal cells displayed negligible fluorescence when incubated with only the FITC-conjugated antibody (data not shown).

IFA detection of hydrophobic cell wall antigens in vivo. Tissue sections examined by immunofluorescence displayed both yeast and hyphal forms of *C. albicans* in the mucosal infections of stomach, esophagus, and tongue tissues, as was previously described for the *bg/bg nu/+* mice (7). Strongly immunofluorescent fungal cells, especially budding yeast cells, were observed in tissue sections stained with the anti-HICF6 PAb (Fig. 4D and F, bright-field photographs in Fig. 4C and E, respectively). Yeast forms with weak cell wall staining were also observed (Fig. 4D). Homogeneous cell wall staining was present on some yeast forms, whereas other cells appeared to have punctate staining (Fig. 4D and F). These staining patterns and intensity differences in vivo (Fig. 4) were similar to the anti-HICF6 PAb reactivity of hydrophobic and hydrophilic *C. albicans* cells in vitro (Fig. 3A and C). The estimated percentages of yeast forms exhibiting moderate-to-intense cell wall

FIG. 3. Immunofluorescence of *C. albicans* yeast and hyphal cells in vitro. Cells were stained with anti-HICF6 PAb and FITC-conjugated anti-IgG antibody. Hydrophobic yeast cells (A) placed in 37°C Auto-Pow medium (2 h) produced true hyphal forms (B), whereas hydrophilic yeast cells (C) make predominantly short pseudohyphae and buds (E). Fluorescence photomicrographs (D and F) correspond to bright-field views of cells in panels C and E, respectively. Cell wall regions of the short pseudohyphae and buds which reacted with the anti-HICF6 PAb are noted with arrows in E and F. Control samples, incubated with FITC conjugate only, were negative.

staining ranged from 50 to 67% (at least 36 yeast cells per field) in five additional fields similar to Fig. 4D. Hyphal cell walls exhibited weak to moderate (1+ to 2+) staining in vivo with the anti-HICF6 PAb, whereas lateral blastoconidia stained more intensely than adjacent hyphal cell walls (Fig. 4F). Control tissue sections, incubated with normal mouse serum instead of primary antibody, demonstrated no apparent fungal fluorescence (Fig. 4A and B). These results indicate that hydrophobic and hydrophilic cell wall surfaces are displayed by *C. albicans* in vivo.

DISCUSSION

Investigation of *C. albicans* CSH expression in vivo required development of an immunologic reagent that would be similar in performance to the hydrophobic microsphere binding assay in distinguishing cell surfaces with hydrophobic interaction potential (16). A second requirement for the reagent was recognition of hydrophobic cell wall proteins, especially those iden-

tified in previous work as potential contributors to CSH (21, 22). Our results, as discussed below, demonstrate that the anti-HICF6 polyclonal antiserum fulfills these two qualifications.

The anti-HICF6 PAb recognized hydrophobic proteins previously detected by surface radioiodination of *C. albicans* yeast cells (21, 22). The radiolabelled hydrophobic proteins ranged in size between 34 and 40 kDa and were seen on hydrophobic yeast surfaces of different isolates and on exponential-phase yeast cells at 37°C. In this study, the anti-HICF6 PAb detected similar hydrophobic proteins in Western blots among these isolates of *C. albicans* and from yeast cells cultivated at different growth temperatures. The observations that anti-HICF6 positive-hydrophobic proteins were present in lyticase digests of hydrophilic cells and that hydrophilic cells were either negative or weakly fluorescent in IFA analysis agree with previous indications that the 34- to 40-kDa hydrophobic cell wall proteins are buried or masked within hydrophilic cell walls (21, 22). Overall, the anti-HICF6 PAb reacted to a limited number

FIG. 4. Immunofluorescence of *C. albicans* in vivo, stained with the anti-HICF6 PAb. Bright-field (A, C, and E) and immunofluorescence photographs (B, D, and F, respectively) of the same microscopic field are shown for stomach tissue sections incubated with normal mouse serum (A and B) or anti-HICF6 PAb (C, D, E, and F) followed by FITC-conjugated anti-mouse IgG. Similar results were observed for *C. albicans* lesions in esophagus and tongue tissue sections. Magnification, $\times 200$ for panels A to D and $\times 400$ for panels E and F.

of cell wall proteins and predominantly recognized proteins having molecular masses less than 60 kDa.

Three hydrophilic proteins were also recognized on Western blots by the anti-HICF6 PAb (Fig. 1B). For the 90- and 54-kDa reactive bands in HICF2 to -F3, no similar-sized antigens are apparent in HICF6. One explanation for the recognition of hydrophilic proteins by an anti-hydrophobic protein antiserum may be that the hydrophilic proteins are more glycosylated forms of proteins present in HICF6. Alternatively, the hydrophilic proteins may contain cross-reactive epitopes with HICF6 proteins. For the 50-kDa protein, the presence of reactive bands in multiple fractions (HICF2 to -F7) suggests that the protein may behave similarly to other proteins separated by HIC chromatography (26, 46). These proteins displayed surface regions with differing hydrophobic interaction strengths,

such that interactions between the protein regions and the HIC ligand are broken at various ratios of ammonium sulfate to water (early to late fractions, respectively). The 50-kDa protein may undergo these variable HIC interactions. It appears to be the only anti-HICF6 PAb-reactive antigen to elute in more than three fractions.

Immunofluorescence results demonstrate that the anti-HICF6 PAb readily distinguished hydrophobic from hydrophilic cell wall surfaces in vitro, especially yeast cell walls and growing pseudohyphal tips. In vivo-grown *C. albicans* yeast forms and hyphae had the same spectrum of cellular reactivities to PAb as that of cells grown in vitro: staining patterns were homogeneous or punctate, yeast cell intensities ranged from 0 to 4+, and hyphal cell walls stained moderately. These patterns were not affected by formalin fixation. Initial studies

demonstrated that formalin treatment did not alter the CSH status of hydrophobic and hydrophilic cells (unpublished results). The *in vivo* immunofluorescence results suggest that both hydrophilic and hydrophobic cell wall surfaces are available during pathogenesis.

The levels of immunofluorescence intensity for *in vitro*-grown *C. albicans* were directly comparable to the densities of hydrophobic microspheres on hydrophobic versus hydrophilic surfaces, with the exception of hyphal cell walls. Previous studies demonstrated that *C. albicans* hyphal cell wall surfaces are more hydrophobic and bind microspheres at a greater density than hydrophobic yeast cell surfaces (for a review, see reference 18). The moderate-to-weak reactivity to hydrophobic hyphal surfaces was likely the result of preparing the HICF6 immunogens from yeast cell wall samples. Our results suggest that although some hydrophobic antigens may be shared between yeast and hyphal surfaces, the components of hyphal surface hydrophobicity may be predominantly different than the yeast cell wall. Further studies will be necessary to identify the components responsible for hyphal CSH expression.

The anti-HICF6 PAb recognition of the three hydrophilic proteins indicates that some of the observed immunofluorescence of fungal surfaces could be contributed by these proteins. Two observations suggest that their contribution is likely minimal, especially for hydrophobic yeast cell surfaces. First, most of the high-molecular-mass surface-iodinated proteins in HICF2 produce broad bands on electrophoresis (21, 22), in contrast to the tight, 90-kDa band seen in Fig. 1 (<2-kDa band spread). If the 90-kDa protein is surface exposed, its labelled signal is possibly indistinct or covered by a broad signal band, and its surface exposure is likely less than the heavily labelled hydrophobic proteins (21–23) including the 30- to 40-kDa proteins that are strongly recognized by the anti-HICF6 antiserum in this study. Second, hydrophobic cell surfaces display minimal labelling of proteins between 40 and 60 kDa, though growth at 37°C permits transient labelling of a hydrophilic 52-kDa protein (21, 22), which is similar in size and HIC elution to the 54-kDa antigen in this study (Fig. 1B). Thus, antiserum recognition of the hydrophilic proteins may contribute more to the punctate immunofluorescence pattern seen on hydrophilic cell surfaces than to the intense, homogeneous staining of hydrophobic yeast surfaces (Fig. 3).

Whether the hydrophobic proteins recognized by the anti-HICF6 PAb have been previously described is unknown. Five secreted *C. albicans* enzymes have been identified in the mass range of 34 and 44 kDa, the region with the most intense and broad signal on anti-HICF6 immunoblots (Fig. 1B and 2). Reported proteins include the 40- to 43-kDa secreted aspartyl proteinase isoenzymes (for a review, see reference 45), a 41-kDa lysophospholipase-transacylase (43), a 40-kDa allergen identified as an alcohol dehydrogenase (39), a 38-kDa 1,3- β -D-exoglucanase which is also found wall associated (32), and a 34-kDa β -glucan branching enzyme that displayed hydrophobic interaction tendencies (11, 14, 15). Our preliminary experiments (data not shown) using antibody to the 1,3- β -D-exoglucanase (kindly provided by Patrick Sullivan, University of Otago, Dunedin, New Zealand) and four monoclonal antibodies to aspartyl proteinase (Sap2, kindly provided by Tom Ray, University of Iowa, Iowa City) were negative against RCD protein preparations in both ELISA and Western blot analysis, suggesting that the proteinase and exoglucanase are not present in the cell wall protein samples.

The specific molecules participating in the pathogenic roles for *C. albicans* CSH, especially those contributing to adhesion mechanisms (13, 17, 19), have yet to be fully characterized. The degree to which CSH participates in adhesion events may differ

depending on both the fungal surface and the host tissue involved; indeed, hydrophobic yeast and hyphal surfaces appear enriched for extracellular matrix-binding proteins (2, 3, 29, 33). Investigators of specific adhesion molecules have identified a growing list of lectins, integrin-like molecules, carbohydrate epitopes, and extracellular matrix binding receptors on *C. albicans* (for reviews, see references 6, 9, and 25). It is not known whether the receptors themselves are hydrophobic or if hydrophobic forces facilitate initial contact and stability for receptor interactions (17, 19). However, recent studies suggest that a 58-kDa fibrinogen-binding protein on the *C. albicans* cell surface is hydrophobic and is distributed in a punctate fashion on yeast cells (31, 35). Further investigation is under way to assess the relationship between anti-HICF6 PAb-reactive proteins and other *C. albicans* cell wall proteins including the above-mentioned enzymes, a 37-kDa laminin-binding protein (2), and the 58-kDa fibrinogen-binding protein (31, 35).

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