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Altered Hepatic Clearance and Killing of *Candida albicans* in the Isolated Perfused Mouse Liver Model

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The adherence of *Candida albicans* was studied in situ by using the perfused mouse liver model. After exhaustive washing, 10^6 *C. albicans* were infused into mouse livers. At the time of recovery, 62 ± 5% (mean ± standard error of the mean) of the infused *C. albicans* were recovered from the liver and 14 ± 3% were recovered from the effluent for a total recovery of 76 ± 4%. This indicates that 86 ± 3% of the original inoculum was trapped by the liver and that 24 ± 4% was killed within the liver. Chemical pretreatment of *C. albicans* with 8 M urea, 12 mM dithiothreitol, 2% β-mercaptoethanol, 1% sodium dodecyl sulfate, 10% Triton X-100, or 3 M potassium chloride or enzyme pretreatment with α-mannosidase, α-chymotrypsin, subtilisin, β-*N*-acetyl-glucosaminidase, pronase, trypsin, papain, or lipase did not alter adherence of *C. albicans* to hepatic tissue. By contrast, pepsin pretreatment significantly decreased hepatic trapping. Simultaneous perfusion with either 100 mg of *C. albicans* glycoprotein per liter or 100 mg of *C. albicans* mannan per liter also decreased trapping. Furthermore, both substances eluted previously trapped *C. albicans* from hepatic tissue. Chemical pretreatment with 8 M urea, 12 mM dithiothreitol, or 3 M KCl or enzymatic pretreatment with α-mannosidase, subtilisin, α-chymotrypsin, or papain increased killing of *C. albicans* three- to fivefold within hepatic tissue. The data suggest that mannose-containing structures on the surface of *C. albicans*, for example, mannans or glucomannoproteins, mediate adherence of *C. albicans* within the liver. Indirectly, chemical and enzymatic pretreatment renders *C. albicans* more susceptible to hepatic killing.

There is an increasing spectrum of *Candida albicans* infections associated with a variety of clinical conditions predisposing the host to systemic invasion (4, 17, 18, 20, 27). Despite a voluminous literature concerning the in vitro adherence of *C. albicans* to plastic surfaces, epithelial cells, endothelial cells, and other cell types (11, 29), relatively little is understood concerning the molecular interaction of *C. albicans* with the plasma membranes of the host. An understanding of this interaction would be valuable, since the adherence of *C. albicans* to host cells or tissues may be important to the pathogenesis of candidal infections (29).

Microorganisms entering the portal venous circulation via the gastrointestinal tract are, for the most part, removed from the bloodstream by the liver (26). Bloodstream clearance within the liver occurs in two distinct ways. The major portion of microbes passing through the liver is cleared from the bloodstream by adherence to hepatic sinusoidal endothelium (endothelial trapping). A proportionately smaller number of microbes is removed from the bloodstream by adherence to Kupffer cells, which reside within the hepatic sinusoidal lumen (26, 31). This latter event may be followed by the phagocytosis and intracellular destruction of the microbes (32). Each type of adherence has been demonstrated with *Salmonella typhimurium* (26) and with *C. albicans* (31, 32). In an effort to extend our understanding of this initial interaction of *C. albicans* with host tissues, we employed the isolated perfused mouse liver model. In the present study, we evaluate the effects of chemical and enzymatic pretreatment and the effects of fungal extracts on the ability of the liver to trap and kill *C. albicans*.

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MATERIALS AND METHODS

Animals. Female Hsd/ICR mice (Harlan Sprague-Dawley, Indianapolis, Ind.) 8 to 12 weeks old were maintained under standard laboratory conditions with food and water available ad libitum.

Organism and culture conditions. *C. albicans* 20A was a gift of Judith E. Domer, Tulane University Medical School, New Orleans, La. Cultures were maintained on Sabouraud dextrose agar (SDA; Difco Laboratories, Detroit, Mich.) slants at 24°C. Fresh transfers were made into 100 ml of tryptic soy broth (Difco) plus 0.5 M galactose (TSB-GAL), and the pH was adjusted to 5.9. Liquid cultures were incubated at 37°C in a shaking water bath for 18 h. Cultures were harvested and washed three times in ice-cold sterile saline by centrifugation at 1,700 × *g* for 5 min at 4°C. Hemacytometer counts of 10-fold serial dilutions in ice-cold saline were used to adjust the concentration of inocula to approximately 10^6 yeast cells per ml of RPMI 1640 plus glutamine (Fisher Scientific Co., Atlanta, Ga.). Approximately 10^7 yeast cells in 10 ml of RPMI 1640 were homogenized in a sterile ice-cold tube with an ice-cold Teflon pestle, aseptically transferred to a sterile test tube, and held on ice. One milliliter of the stock inoculum was withdrawn with a 1-ml syringe and warmed to 37°C, providing the inoculum for infusion of yeast cells into perfused livers. Quantitative pour plates of 10-fold serial dilutions of the inoculum were made in SDA and incubated overnight at 37°C, and the number of CFU in the inoculum was counted on an Artek 880 colony counter (Artek Corp., New York, N.Y.). Less than 10% variability between hemacytometer counts and the number of CFU in the inoculum was noted throughout the study. In control studies, there was no decrease in yeast number or a decrease in yeast viability due to killing of yeast cells by homogenization, aggregation of yeast cells within the inoc-

ulum (as determined by visual inspection), or adhesion of yeast cells to glassware or to cellular debris.

Mouse liver perfusion. The methods for mouse liver perfusion have been described in detail (26). In brief, mice were anesthetized by intraperitoneal injection of pentobarbital and then heparinized. A midline incision was made, and the intestines were reflected to expose the portal vein. Ligatures were placed around the portal vein, above and below the splenic vein, and above the renal veins. A cannula was inserted into the portal vein, the ligatures were secured, and perfusion medium was run out through a nick in the inferior vena cava to wash the liver free of blood. The rib cage was removed, and a ligature was placed around the inferior vena cava, above the diaphragm. A cannula was inserted through a nick in the right atrium into the inferior vena cava and secured with the ligature. The ligature above the renal veins was closed. Perfusion medium was allowed to flow through the portal vein into the liver and was collected aseptically from the efferent cannula in a sterile bottle. The liver does not swell during perfusion.

Before *C. albicans* infusion, the liver was washed with approximately 20 to 30 ml of perfusion medium. Approximately 10^6 CFU of *C. albicans* in one ml of RPMI 1640 was infused through a three-way valve placed on the efferent cannula. After infusion of *C. albicans*, the three-way valve was switched to the perfusion buffer reservoir, the liver was washed with 50 ml of RPMI 1640, and the perfusion buffer was collected aseptically from the efferent cannula in a sterile bottle. At the end of the perfusion, the liver was disconnected from the perfusion apparatus, excised, and transferred to 9 ml of sterile saline. The inoculum, the liver, and 10 ml of the effluent were homogenized in ice-cold tubes with ice-cold Teflon pestles. Tenfold serial dilutions of the inoculum, liver homogenate, and effluent were made in ice-cold saline, and quantitative pour plates and SDA were used to determine the number of CFU. One hundred percent of the viable CFU was recovered from the inoculum control taken at the finish of the perfusion in comparison to the inoculum control taken before the infusion of 10^6 *C. albicans*. This shows that there was no decrease in the number of CFU infused due to a loss in yeast viability, aggregation of yeast cells, or adhesion of yeast cells to glassware.

The percentage of *C. albicans* CFU killed was calculated as 100% minus the percentage recovered from the liver homogenate and the percentage in the effluent. The percentage of *C. albicans* CFU trapped by the liver was calculated as the percentage recovered in the liver homogenate plus the percentage killed by the liver. Based upon previous studies (26, 31, 32), we assumed that killing resulted from the phagocytic clearance and killing of microbes within Kupffer cells.

Chemical pretreatment. All chemicals were purchased from Sigma Chemical Co., St. Louis, Mo. *C. albicans* was chemically pretreated by the method of Lee and King (21). *C. albicans* was incubated and harvested as described above, and the number of yeasts was adjusted to approximately 10^8/ml by hemacytometer counts. Quantitative SDA pour plates were made to determine the number of viable CFU before chemical treatment. Inoculum controls were performed before chemical treatment, after chemical pretreatment, and on the pretreated inoculum at the completion of the experiment. The 10^8 CFU were incubated at 37°C for 1 h on a tipping platform in 10-ml solutions of the following: 8 M urea, 1% sodium dodecyl sulfate, 10% Triton X-100, or 3 M KCl contained in sterile distilled water; 2% β-mercaptoethanol in 0.1 M Tris hydrochloride buffer (pH 9.3); 12 mM dithiothreitol in 0.05 M Tris hydrochloride buffer (pH 7.5) containing 0.8 M mannitol. After incubation, pretreated *C. albicans* were washed three times in sterile saline by centrifugation at 1,700 × *g* for 5 min at 4°C. The concentration of the inoculum was adjusted to approximately 10^6 yeast cells per ml of RPMI 1640, based on hamocytometer counts, and the inoculum was homogenized as above. Quantitative SDA pour plate counts of 10-fold serial dilutions of the inoculum were made after chemical pretreatment. Comparison of the number of viable CFU made before and after chemical treatment were used to determine whether chemical pretreatment resulted in a reduction in *C. albicans* viability. In most instances, there were significant reductions in yeast viability as determined by comparison of these two inoculum controls. Reductions in viability were expressed as a percentage of the control viability (before treatment). During the perfusion experiment the pretreated inoculum was held on ice and homogenized as above, and quantitative SDA pour plates were made at the end of the experiment. Comparison of the number of CFU recovered from the pretreatment inoculum control, taken before the infusion of yeast cells, with the inoculum control, taken after completing the perfusion experiment, showed no significant reduction in yeast number or viability. Visual inspection of the inoculum after chemical treatment indicated no aggregation of yeast cells. This suggests that the reduction in yeast viability noted between the inoculum controls taken before versus after chemical treatment were most probably explained by effects of the chemicals directly on the yeast cells and less likely due to increased susceptibility of yeast cells to homogenization, aggregation of yeast cells after treatment, or adhesion of yeast cells to glassware after treatment.

Enzymatic pretreatment. All enzymes were purchased from Sigma; catalog identification numbers are given within parentheses. Inoculum control samples were taken and processed as described above before enzyme treatment, after enzyme treatment, and after the perfusion experiment was completed. Before infusion of *C. albicans*, approximately 10^8 CFU were incubated at 37°C for 1 h on a tipping platform in 2-ml solutions of the following: 1 mg of α-mannosidase (M 7257) per ml of 0.04 M sodium acetate buffer (pH 4.5); 0.4 M trypsin (T 8003) in 0.01 M phosphate buffer (pH 8.0); 0.1 U of β-*N*-acetyl-glucosaminidase (A 2415) per ml of 0.1 M citrate buffer (pH 5.5); 3 μM papain (P 3125) in 0.01 M phosphate buffer plus 5 mM *d*-cysteine hydrochloride and 2 mM EDTA (pH 8.0); 1 mg of lipase (L 9767) per ml of 0.1 M acetate buffer (pH 5.0); 0.3 mM pepsin (P 7012) in 0.07 M phosphate buffer (pH 5.7); 0.4 mM α-chymotrypsin (C 7762) in 0.01 M phosphate buffer (pH 8.0); 7.5 U of protease XXVII (P 4789; subtilisin) in 0.01 M phosphate buffer (pH 7.5); 0.1 mg of protease XIV (P 5147; pronase) per ml of 0.05 M Tris hydrochloride (pH 7.5) and 0.01 M $CaCl_2 \cdot H_2O$. After digestion, the inoculum was harvested and washed by centrifugation, and the dilutions were prepared as above. Enzyme pretreatment resulted in no reduction in *C. albicans* viability with any of the enzymes tested. There was no reduction in yeast number or viability in comparison of the inoculum control taken after enzyme treatment and before infusion with the inoculum control taken at the completion of the perfusion experiment. Visual inspection of yeast cells after enzyme treatment indicated no aggregation of yeast cells. This suggests that there was no artificial loss of yeast cells in the inoculum due to the possibility of loss in viability from homogenization, aggregation of treated yeast cells, or the adhesion of yeast cells to glassware.

TABLE 1. Effect of chemical pretreatment on the hepatic clearance and killing of *C. albicans* 20A[a]

Pretreatment	% of control viability	% Recovery			% Killing	% Trapping
		Liver	Effluent	Total		
None	100	62 ± 5	14 ± 3	76 ± 4	24 ± 4	86 ± 3
8 M urea	2	47 ± 7[b]	15 ± 4	62 ± 10	38 ± 10[b]	86 ± 3
12 mM dithiothreitol	49	30 ± 1[b]	5 ± 1[b]	35 ± 2[b]	65 ± 2[b]	95 ± 3
2% β-mercaptoethanol	75	64 ± 3	7 ± 2	71 ± 5	29 ± 5	93 ± 2
1% sodium dodecyl sulfate	16	49 ± 5[b]	28 ± 5	77 ± 2	23 ± 2	72 ± 4
10% Triton X-100	76	66 ± 2	16 ± 3	82 ± 3	18 ± 3	84 ± 4
3 M potassium chloride	100	47 ± 3[b]	17 ± 4	64 ± 3	36 ± 3[b]	83 ± 3

[a] Each value represents the mean ± standard error of the mean of at least five separate experimental determinations.
[b] $P = 0.01$; White rank order test.

Isolation and purification of fungal substances. Mannan was isolated and purified from 6 liters of *C. albicans* 20A cultivated in TSB-GAL at 37°C for 18 h under continuous agitation by a modification (15) of the method of Peat et al. (28). It was dialyzed against distilled water and lyophilized. Mannan prepared by this method contains 6 to 8% protein (15) and has been shown by DiLuzio (14) to be free of glucan. Glucomannoprotein (GP) was a generous gift of Judith E. Domer, Tulane University Medical School, New Orleans, La. (5).

The effects of either mannan or GP on the hepatic clearance and killing of *C. albicans* were evaluated by inclusion of either substance in the pre- and postperfusion media at 0.1 mg of substance per ml of RPMI 1640. Control perfusions were performed in the absence of substance, with the addition of 0.01 mg of β-glucan (Accurate Chemical Co., St. Louis, Mo.) per ml of RPMI 1640 or 0.1 mg of *Saccharomyces cerevisiae* mannan (Sigma; M 7504) per ml of RPMI 1640. β-Glucan is a particulate, glycosyl polymer 3 to 4 μm in diameter. In a second series of experiments, extracts were present only in the postperfusion medium at 0.1 mg/ml, to test the ability of either mannan or GP to elute trapped yeast cells from the liver. Control perfusions were performed with the addition of 0.01 mg of β-glucan per ml.

Statistics. Statistical analysis of data was performed by the White rank order test (35). Probability values equal to or less than 0.01 were considered significant.

RESULTS

Effects of chemical pretreatment on hepatic clearance and killing of *C. albicans*. Hepatic tissue was washed with RPMI 1640, and 10^6 CFU of untreated *C. albicans* were infused (Table 1). Approximately 62% of the inoculum was recovered from the liver homogenate with 14% in the effluent for a total recovery of 76%. This suggests that approximately 86% of the inoculum was trapped by the liver and that 24% was killed by the liver.

Pretreatment with 8 M urea or 1% sodium dodecyl sulfate reduced *C. albicans* viability to 2 and 16% of the pretreatment controls, respectively. With the exception of 3 M KCl pretreatment, all other chemical pretreatments reduced the viability of *C. albicans* in comparison to the corresponding pretreatment control. Chemical pretreatment did not significantly change the ability of the liver to trap *C. albicans*. Pretreatment with urea, dithiothreitol, or KCl significantly increased hepatic killing of *C. albicans*.

Effects of enzymatic pretreatment on hepatic clearance and killing of *C. albicans*. Enzymatic pretreatment did not alter the viability of *C. albicans*. Only enzymatic pretreatment with pepsin significantly decreased hepatic trapping of *C. albicans* (Table 2). Pretreatment with α-mannosidase, subtilisin, α-chymotrypsin, or papain significantly increased hepatic killing of *C. albicans*.

Blocking of hepatic clearance and killing of *C. albicans* with fungal extracts. In comparison to the control groups (no treatment, or β-glucan), the addition of either *C. albicans* mannan or GP to either pre- or postperfusion medium blocked hepatic trapping of *C. albicans* without altering hepatic killing ability (Table 3). *S. cerevisiae* mannan also blocked the ability of the liver to trap *C. albicans*.

Elution of trapped *C. albicans* from hepatic tissue. Either *C. albicans* mannan or GP eluted previously trapped *C. albicans* from the liver when these fungal molecules were

TABLE 2. Effect of enzymatic pretreatment[a] on the hepatic clearance and killing of *C. albicans* 20A[b]

Pretreatment	% Recovery			% Killing	% Trapping
	Liver	Effluent	Total		
None	73 ± 2	18 ± 3	91 ± 2	9 ± 2	82 ± 3
α-Mannosidase	47 ± 3[c]	8 ± 2	55 ± 1[c]	45 ± 2[c]	92 ± 2
Protease XXVII	50 ± 4[c]	16 ± 4	66 ± 1	34 ± 1[c]	84 ± 4
Protease XIV	63 ± 6	13 ± 3	76 ± 4	24 ± 4	87 ± 2
α-Chymotrypsin	50 ± 4[c]	19 ± 2	69 ± 3[c]	31 ± 3[c]	81 ± 3
Trypsin	59 ± 5	21 ± 6	80 ± 2	20 ± 2	79 ± 6
Papain	65 ± 6	7 ± 2[c]	72 ± 7[c]	28 ± 7[c]	93 ± 2
Lipase	64 ± 4	16 ± 3	80 ± 2	20 ± 1	84 ± 3
Pepsin	60 ± 5	32 ± 3[c]	92 ± 2	8 ± 2	68 ± 3[c]
β-*N*-Acetyl-glucosaminidase	69 ± 4	17 ± 4	86 ± 2	14 ± 2	83 ± 4

[a] No enzymatic treatment tested had any effect on *C. albicans* viability.
[b] Each value represents the mean ± standard error of the mean of at least five separate experimental determinations.
[c] $P = 0.01$; White rank order test.

TABLE 3. Effect of fungal extracts on the hepatic clearance and killing of *C. albicans* 20A[a]

Treatment	% Recovery			% Killing	% Trapping
	Liver	Effluent	Total		
None	53 ± 7	24 ± 2	77 ± 6	23 ± 6	76 ± 2
S. cerevisiae β-glucan	54 ± 2	11 ± 2	65 ± 3	35 ± 3	89 ± 2
C. albicans 20A mannan	30 ± 4[b]	48 ± 5[b]	78 ± 4	22 ± 4	52 ± 5[b]
S. cerevisiae mannan	34 ± 3[b]	53 ± 6[b]	87 ± 4	13 ± 4	47 ± 5[b]
C. albicans 20A GP	35 ± 5[b]	38 ± 7[b]	73 ± 8	27 ± 8	62 ± 6[b]

[a] Each value represents the mean ± standard error of the mean of at least five separate experimental determinations.
[b] $P = 0.01$; White rank order test.

included in postperfusion medium (Table 4). β-Glucan had no effect on elution of *C. albicans* from the liver.

DISCUSSION

The isolated perfused liver model has been used in a variety of experimental approaches investigating hepatic clearance of microbes. Using the perfused liver model, Moon and his co-workers distinguished between the trapping and killing abilities of the liver; they demonstrated that hepatic clearance of *S. typhimurium* is due to phagocytic uptake and to trapping of bacteria on the surface of hepatic sinusoidal endothelium (26). In rats, the adherence of *C. albicans* to sinusoidal endothelium plays a significant role in hepatic clearance of yeast cells in vivo and in the perfused liver (31). Scanning electron microscopy shows that a portion of the infused inoculum is trapped by adhesion of yeast cells to sinusoidal endothelium or to Kupffer cells. When the number of yeast cells in the inoculum exceeds 10^6, yeast cells trapped by adhesion within the sinusoids begin to clog the lumen of these vessels. Clogging backs up yeast cells, similar to logjamming, into larger portal venules and veins. In this instance, scanning electron microscopy reveals that yeast cells adhere to each other within the lumen of larger vessels. Thus, at high yeast numbers one component of trapping includes the coadhesion of yeast cells within the lumen of a larger venous structures. When numbers of yeast cells below 10^6 are infused into livers, this logjamming effect is not observed by scanning electron microscopy. At lower numbers, yeast cells appear to be trapped primarily by endothelial and Kupffer cell adherence (31). This suggests that coadhesion of yeast cells within the lumen of hepatic venous structures is most likely an artifact of using numbers in excess of 10^6 yeast cells in the inoculum, a pitfall avoided in the present study. More recently, Saywer (30) suggested that mannose-containing cell wall surface molecules may play an important role in the hepatic trapping of *C. albicans*. However, the precise mechanism(s) mediating adherence remains ill defined.

A significant decrease in the ability of the liver to trap *C. albicans* was only observed after pepsin pretreatment of yeast cells. Otherwise, both chemical and enzymatic pretreatment of *C. albicans* did not alter the trapping ability of the liver. A possible explanation is that the high carbohydrate content of the yeast cell wall protected proteins within the cell wall from proteolytic attack. The acidic pH used in pepsin digestion might allow pepsin to attack susceptible peptide bonds not exposed at the neutral pH used in the incubation of yeast cells with the other proteases. Alternatively, proteins altered by pretreatments may not function as adhesins. This suggests that adhesins on the surface of *C. albicans* are pepsin sensitive at acidic pH and that tertiary protein structure within the cell wall may be necessary for maintenance of adhesin integrity. In contrast, Lee and King (21) found that both chemical and protease pretreatment of yeast cells decreased the adherence of *C. albicans* to human vaginal mucosal epithelium. Adherence of *C. albicans* was not blocked by pretreatment of human vaginal mucosal epithelium cells either with mannan or with a cell wall mannoprotein extract. Variation in the results between the present study and those conducted with epithelial cells may reflect the fact that *C. albicans* adheres to different cells via different adhesins. It is also possible that variations in the chemical composition of the outer cell wall, depending upon the strain or *C. albicans* used or upon the conditions employed for cultivation, might alter the ability of *C. albicans* to adhere to different cells and tissues. For example, Douglas and her co-workers (16, 23) found that incubation in the presence of 0.5 M galactose increased adherence of *C. albicans* to epithelial cells. Increased epithelial cell adherence was attributed to an increased amount of a mannoprotein adhesin isolated from the surface of *C. albicans* (24). Recent studies show that the various morphological forms of *C. albicans* express different surface molecules and that *C. albicans* may modulate the expression of surface molecules during its life cycle (2, 6). It seems reasonable to conclude that *C. albicans* may display a variety of adhesive mechanisms that permit its initial adherence to different cells and tissues.

When *C. albicans* mannan or GP was included in perfusion medium, significantly fewer yeast cells were trapped by

TABLE 4. Elution of *C. albicans* 20A from hepatic tissue by postperfusion in the presence of either mannan or GP[a]

Treatment	% Recovery			% Killing	% Trapping
	Liver	Effluent	Total		
S. cerevisiae β-glucan	58 ± 5	23 ± 4	81 ± 3	19 ± 3	77 ± 4
C. albicans 20A mannan	52 ± 4	41 ± 4[b]	93 ± 1	7 ± 1[b]	58 ± 3[b]
C. albicans 20A GP	45 ± 5	56 ± 10[b]	87 ± 8	13 ± 8	57 ± 5[b]

[a] Each value represents the mean ± standard error of the mean of at least five separate experimental determinations.
[b] $P = 0.01$; White rank order test.

the liver. Both molecules eluted previously trapped *C. albicans* from the liver. Mannan from *S. cerevisiae*, but not β-glucan, blocked *C. albicans* trapping by the liver. Together, the data show that mannose-containing structures, either mannans or GPs, on the surface of *C. albicans* act as adhesins mediating initial adherence to the liver and that the host cell receptor may nonspecifically recognize mannose-containing adhesins on *C. albicans*. At present, it is unknown whether these adhesins mediate endothelial trapping, Kupffer cell trapping, or the adherence of *C. albicans* to both cell types. Employing an ex vivo adhesion model, Cutler et al. (7) recently showed that *C. albicans* adhere to areas rich in mononuclear phagocyte populations in lymph node and splenic tissue, whereas they adhere to endothelial aspects within kidney tissue. Diamond et al. (12, 13) found that *C. albicans* adhere to human polymorphonuclear neutrophils in vitro and that adherence is mediated by a mannoprotein. Tronchin et al. (34) studied the role of the fibrillar surface in adherence of *C. albicans* to plastic surfaces. It is not known whether GP, which was used in this study, is related to the mannoprotein adhesins described in previous studies (12, 13, 24, 34). The receptor molecules on host cells for *C. albicans* adhesins are also unknown. Sawyer (30) showed that mannose and α-methyl mannoside, but not other monosaccharides, block *C. albicans* trapping by the liver, suggesting that the mannose receptor within the liver may bind mannose-containing adhesins on *C. albicans*. Bullock and Wright (3) showed that *Histoplasma capsulatum* adheres to peripheral blood monocyte-macrophages through interactions with the α chains of the CR3/LFA-1/p150,95 integrin family and that adhesion was not mediated by FC receptors, macrophage C3 secretion, or down regulation of integrin α chains. The isolated perfused liver model may provide a way to determine whether *C. albicans* adheres to liver cells via the CR3/LFA-1/p150,95 integrins. Recent studies show that *C. albicans* expresses surface molecules that bind the C3d complement fragment (33), laminin (1), and a surface protein that may be similar to the cytoplasmic domain of the integrin β-1 subunit (T. C. White and N. Agabian, J. Cell. Biochem. **14A**[Suppl.]:173, 1990). The role of these binding proteins in adhesion is also unknown.

The release of mannan or GP from the surface of *C. albicans* trapped within deep tissues may play an important role in the pathogenesis of systemic infection in the compromised host. Mannanemia has been demonstrated both in patients and in experimental models of animal with candidiasis (10). Deep tissue invasion may be accompanied by the appearance of mannan, mannan-immune complexes, or mannoproteins in the peripheral blood (8, 9, 22, 25). Locally released mannans or mannoproteins might block adherence or elute trapped *C. albicans* from vascular membranes or from the surface of phagocytes. By this mechanism, *C. albicans* could disseminate to anatomical sites, such as the kidneys or brain, where difficulty in eliminating the fungus might be more pronounced.

A surprising finding in the present study was the increased ability of the liver to kill *C. albicans* after either chemical or enzymatic pretreatments. Extraction of *C. albicans* with urea, treatment with the reducing agent dithiothreitol, and treatment with high salt concentrations increased the ability of the liver to kill *C. albicans*. Both urea and dithiothreitol pretreatments killed large numbers of *C. albicans* before their infusion into the liver. Further, enzyme pretreatments significantly increased the ability of the liver to kill *C. albicans* without altering the viability of the yeast cells in the inoculum. The data suggest that chemical and enzyme pretreatments of *C. albicans* altered the ability of hepatic tissue to eliminate *C. albicans*. Increased killing within the liver was observed with enzyme pretreatments of *C. albicans* specific for both carbohydrate (α-mannosidase) and protein (subtilisin, papain, α-chymotrypsin). Increased killing within the liver may have been due to Kupffer cells; alternatively, some of the ability of the liver to kill *C. albicans* might have been due to the candidacidal activity of polymorphonuclear neutrophils remaining trapped within the hepatic sinusoids despite thorough prewashing of the liver. Approximately 1 to 2% of the nonparenchymal cells isolated after enzymatic digestion of the perfused mouse liver were polymorphonuclear neutrophils, which are probably marginated within the hepatic sinusoids (unpublished observations). These issues may be resolved by evaluating the adherence of *C. albicans* to Kupffer cells and their candidacidal activity in vitro. Houston and Douglas (19) showed that the production of the fibrillar layer by *C. albicans* enhanced virulence by increasing resistance to intracellular killing by polymorphonuclear neutrophils. From the data, it is possible to conclude that chemical or enzymatic pretreatments may have altered the cell wall of *C. albicans*, rendering yeast cells susceptible to killing by phagocytes within the liver.

In summary, the data suggest that mannan and GP function as adhesins, mediating the adherence of *C. albicans* to the liver. As previously suggested (30), the mannose receptor, located on either Kupffer cells or sinusoidal endothelium, may mediate hepatic clearance of *C. albicans*.

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LITERATURE CITED

1. **Bouchara, J.-P., G. Tronchin, V. Annaix, R. Robert, and J.-M. Senet.** 1990. Laminin receptors on *Candida albicans* germ tubes. Infect. Immun. **58**:48–54.
2. **Brawner, D. L., J. E. Cutler, and W. L. Beatty.** 1990. Caveats in the investigation of form-specific molecules of *Candida albicans*. Infect. Immun. **58**:378–383.
3. **Bullock, W. E., and S. D. Wright.** 1987. Role of the adherence-promoting receptors, CR3, LFA-1, and p150,95, in binding of *Histoplasma capsulatum* by human macrophages. J. Exp. Med. **165**:195–210.
4. **Burnie, J. P., F. C. Odds, W. Lee, C. Webster, and J. D. Williams.** 1985. Outbreak of systemic *Candida albicans* in intensive care unit caused by cross infection. Br. Med. J. **290**:746–748.
5. **Carrow, E. W., and J. E. Domer.** 1985. Immunoregulation in experimental murine candidiasis: specific suppression induced by *Candida albicans* cell wall glycoprotein. Infect. Immun. **49**:172–181.
6. **Chaffin, W. L., J. Skudlarek, and K. J. Morrow.** 1988. Variable expression of a surface determinant during proliferation of *Candida albicans*. Infect. Immun. **56**:302–309.
7. **Cutler, J. E., D. L. Brawner, K. C. Hazen, and M. A. Jutila.** 1990. Characteristics of *Candida albicans* adherence to mouse tissues. Infect. Immun. **58**:1902–1908.
8. **DeRepentigny, L., R. J. Kuykendall, F. W. Chandler, J. R. Broderson, and E. Reiss.** 1984. Comparison of serum mannan, arabinitol, and mannose in experimental disseminated candidiasis. J. Clin. Microbiol. **19**:804–812.
9. **DeRepentigny, L., L. D. Marr, J. W. Keller, A. W. Carter, R. J. Kuykendall, L. Kaufman, and E. Reiss.** 1985. Comparison of enzyme immunoassay and gas-liquid chromatography for the rapid diagnosis of invasive candidiasis in cancer patients. J.

Clin. Microbiol. **21**:972–979.
10. **DeRepentigny, L., and E. Reiss.** 1984. Current trends in immunodiagnosis of candidiasis and aspergillosis. Rev. Infect. Dis. **6**:301–312.
11. **Diamond, R. D.** 1988. Fungal surfaces: effects of interactions with phagocytic cells. Rev. Infect. Dis. **10**(Suppl. 2):S428–S431.
12. **Diamond, R. D., and R. Krzesicki.** 1978. Mechanisms of attachment of neutrophils to *Candida albicans* pseudohyphae in the absence of serum, and of subsequent damage to pseudohyphae by microbicidal processes of neutrophils *in vitro*. J. Clin. Invest. **61**:360–369.
13. **Diamond, R. D., F. Oppenheim, Y. Nakagawa, R. Krzesicki, and C. C. Haudenshild.** 1980. Properties of a product of *Candida albicans* hyphae and pseudohyphae that inhibits contact between the fungi and human neutrophils *in vitro*. J. Immunol. **125**:2797–2804.
14. **DiLuzio, N. R.** 1983. Immunopharmacology of glucan: a broad spectrum enhancer of host defense mechanisms. Trends Pharmaceut. Sci. **4**:344–347.
15. **Domer, J. E., R. E. Garner, and R. N. Befidi-Mengue.** 1989. Mannan as an antigen in cell-mediated immunity assays and as a modulator of mannan-specific cell-mediated immunity. Infect. Immun. **57**:693–700.
16. **Douglas, L. J., J. G. Houston, and J. McCourtie.** 1981. Adherence of *Candida albicans* to human buccal epithelial cells after growth on different carbon sources. FEMS Microbiol. Lett. **12**:241–243.
17. **Evans, E. G. V.** 1975. The incidence of pathogenic yeasts among open-heart surgery patients-the value of prophylaxis. J. Thorac. Cardiovasc. Surg. **70**:466–470.
18. **Ezdinli, E. Z., D. D. O'Sullivan, L. P. Wasser, U. Kim, and L. Stutzman.** 1979. Oral amphotericin for candidiasis in patients with hematologic neoplasms. J. Am. Med. Assoc. **242**:258–260.
19. **Houston, J. G., and L. J. Douglas.** 1989. Interaction of *Candida albicans* with neutrophils: effects of phenotypic changes in yeast cell-surface composition. J. Gen. Microbiol. **135**:1885–1893.
20. **Krause, W., H. Matheis, and K. Wulf.** 1969. Fungaemia and funguria after oral administration of *Candida albicans*. Lancet **i**:598–599.
21. **Lee, J. C., and R. D. King.** 1983. Characterization of *Candida albicans* adherence to human vaginal epithelial cells in vitro. Infect. Immun. **41**:1024–1030.
22. **Lew, M. A., G. R. Siber, D. M. Donahue, and F. Maiorca.** 1982. Enhanced detection with an enzyme-linked immunosorbent assay of *Candida* mannan in antibody-containing serum after heat extraction. J. Infect. Dis. **145**:45–56.
23. **McCourtie, J., and L. J. Douglas.** 1981. Relationship between cell surface composition of *Candida albicans* adherence to acrylic after growth on different carbon sources. Infect. Immun. **32**:1234–1241.
24. **McCourtie, J., and L. J. Douglas.** 1985. Extracellular polymer of *Candida albicans*: isolation, analysis and role in adhesion. J. Gen. Microbiol. **131**:495–503.
25. **Meckstroth, K. L., E. Reiss, J. W. Keller, and L. Kaufman.** 1981. Detection of antibodies and antigenemia in leukemic patients with candidiasis by enzyme-linked immunosorbent assay. J. Infect. Dis. **144**:24–32.
26. **Moon, R. J., R. A. Vrable, and J. A. Broka.** 1975. In situ separation of bacterial trapping and killing functions of the perfused liver. Infect. Immun. **12**:411–418.
27. **Myerowitz, R. L., G. J. Pazin, and C. M. Allen.** 1977. Disseminated candidiasis: changes in incidence, underlying diseases, and pathology. Am. J. Clin. Pathol. **68**:29–38.
28. **Peat, S., W. J. Whelan, and T. E. Edwards.** 1961. Polysaccharide of baker's yeast. IV. Mannan. J. Chem. Soc. **1**:29–34.
29. **Rotrosen, D., R. A. Calderone, and J. E. Edwards, Jr.** 1986. Adherence of *Candida* species to host tissues and plastic surfaces. Rev. Infect. Dis. **8**:73–85.
30. **Sawyer, R. T.** 1988. The effect of monosaccharides on *in situ* trapping of *Candida albicans*. Mycopathology **104**:81–85.
31. **Sawyer, R. T., R. J. Moon, and E. S. Beneke.** 1976. Hepatic clearance of *Candida albicans* in rats. Infect. Immun. **14**:1248–1355.
32. **Sawyer, R. T., R. J. Moon, and E. S. Beneke.** 1981. Trapping and killing of *Candida albicans* by *Corynebacterium parvum*-activated livers. Infect. Immun. **32**:945–950.
33. **Saxena, A., and R. Calderone.** 1990. Purification and characterization of the extracellular C3d-binding protein of *Candida albicans*. Infect. Immun. **58**:309–314.
34. **Tronchin, G., J.-P. Bouchara, R. Robert, and J.-M. Senet.** 1988. Adherence of *Candida albicans* germ tubes to plastic: ultrastructural and molecular studies of fibrillar adhesins. Infect. Immun. **56**:1987–1993.
35. **Wilcoxon, F., and R. A. Wilcox.** 1949. Some rapid approximate statistical procedures. American Cyanamide Co., New York.