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Binding of Plasma Proteins to *Candida* Species *in vitro*

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The ability of purified human albumin, fibrinogen and transferrin to bind to *Candida* species was measured by immunofluorescence. The proteins all bound with high avidity to germ-tubes formed by *Candida albicans*, but did not bind to blastospores of *C. albicans* or other pathogenic *Candida* species, not even to parent blastospores bearing germ-tubes. The extent of binding of the proteins to *C. albicans* germ-tubes varied between growth media and from germ-tube to germ-tube. Strains of *C. albicans* that did not form germ-tubes were incapable of binding any of the proteins. There was evidence that purified fibrinogen bound to germ-tubes with higher avidity than albumin and transferrin. When germ-tubes were treated with whole human plasma or serum, indirect immunofluorescence revealed that proteins were bound all over the surface of *C. albicans* blastospore–germ-tube units, indicating behaviour different from that seen with the purified proteins tested alone or in mixtures. *C. albicans* cells grown in the presence of azole antifungal agents bound purified plasma proteins in the same way as cells untreated with the drugs. The results of this study suggest that binding of host proteins to the surface of *C. albicans* may not be a property related directly to virulence of the fungus *in vivo*.

INTRODUCTION

Adhesion of *Candida albicans* to host surfaces is one of a number of factors involved in the pathogenesis of *Candida* infections. The ability of *Candida* species to bind to host tissues correlates with the rank order of their relative pathogenicity (Critchley & Douglas, 1985), which suggests that adhesion may be a significant virulence factor for these fungi. It has been suggested that the host cell receptor for *Candida* adhesion is the mammalian cell surface glycoprotein, fibronectin (Rotrosen *et al.*, 1986). Tronchin *et al.* (1987) have proposed that adhesion to fibronectin is mediated by the human plasma protein, fibrinogen. Purified human fibrinogen binds specifically to *C. albicans* hyphal germ-tubes *in vitro*, but not to the parent blastospores from which the germ-tubes emerge (Bouali *et al.*, 1986; Annaix *et al.*, 1987), an observation that accords with the known superior propensity of *C. albicans* germ-tubes to adhere to epithelial cells *in vitro* (Kimura & Pearsall, 1980; Rotrosen *et al.*, 1985). Fibrin has been suggested as the receptor for *C. albicans* cells that adhere to blood clots *in vivo* (Bouali *et al.*, 1987).

While these observations are all compatible with the concept of fibrinogen binding by *C. albicans* as a component of adhesion mechanisms in this fungus, it should be noted that other host proteins also bind to *C. albicans*; notably lactoferrin and ovotransferrin (Valenti *et al.*, 1986), a serum mannan-binding protein (Bull & Turner, 1984; Kawasaki *et al.*, 1985) and complement components C3d and iC3b (Edwards *et al.*, 1986; Heidenreich & Dierich, 1985). It is therefore possible that the binding of proteins to *C. albicans* cell surfaces is more of a nonspecific phenomenon than a fibrinogen-specific event. The present study was undertaken to evaluate the propensity of three plasma proteins, albumin, fibrinogen and transferrin, to bind to *C. albicans* and other *Candida* species and to determine various conditions that affect the extent of protein binding by the fungus. Indirect immunofluorescence assays of protein binding were chosen to allow detection of variations in the degree of protein binding between fungal cell units.

Abbreviations: EMEM, Eagle's minimal essential medium; NAG, imidazole-buffered *N*-acetylglucosamine medium; AAS, amino acids/salts medium; MSAB, modified Sabouraud's broth.

METHODS

Fungi and growth conditions. *C. albicans* isolates 73/055, 73/079, 83/008, 84/031, 85/1395, 85/787, *C. glabrata* isolate 82/004, *C. guilliermondii* isolate 84/003, *C. kefyr* isolate Holt, *C. krusei* isolate 81/014, *C. parapsilosis* isolate 81/034, *C. tropicalis* isolate 73/071 and *Saccharomyces cerevisiae* isolate 83/001 were all originally obtained from clinical specimens. *C. albicans* isolates 610, H12, Sh8 and Sh27 were kindly provided by Dr D. A. Stevens, Infectious Diseases, Santa Clara Valley Medical Center, San Jose, CA 95128, USA. The latter two of these isolates are highly attenuated in lethality for mice by intravenous or intraperitoneal injection (Loose *et al.*, 1983). All isolates were maintained on Sabouraud's glucose agar (Oxoid) and inocula were prepared from overnight cultures of the yeasts on this medium at 37 °C. The cells were suspended in sterile, deionized water, concentrations were estimated from haemocytometer counts, and the fungi were added to prewarmed culture media to an initial concentration of 10^6 cells ml^{-1}.

Several growth media known to favour hypha development in *C. albicans* were used in the experiments: Eagle's minimal essential medium (EMEM; Odds *et al.*, 1985), imidazole-buffered *N*-acetyl-D-glucosamine (NAG; Shepherd *et al.*, 1980), amino acids/salts medium (AAS; Lee *et al.*, 1975) and modified Sabouraud's broth (MSAB; Evans *et al.*, 1975). Cultures in EMEM were incubated statically at 37 °C under 5% (v/v) CO_2 in air; those in AAS and NAG were shaken at 160 r.p.m. and 37 °C in air; and MSAB cultures were shaken at 160 r.p.m. and 40 °C in air. Yeast forms of *C. albicans* were grown in MSAB or in yeast nitrogen base (Difco) supplemented with 50 mM- or 500 mM-galactose. All yeast cultures were shaken at 160 r.p.m. and 26 °C or 37 °C in air. They were all incubated for 2·5 h after which growth was harvested on membrane filters, washed twice with water *in situ* and resuspended to a concentration of approximately 10^7 cells (blastospores or blastospore–germ-tube units) per ml.

Proteins and detector antisera. Purified human albumin, fibrinogen and transferrin (substantially iron free) were purchased from Sigma and dissolved in phosphate-buffered saline (PBS), pH 7·0, at concentrations intended to reflect approximately those that occur naturally in human blood (Putnam, 1975); i.e. 10, 4, and 2·5 mg ml^{-1}, respectively. Protein solutions were sterilized by membrane filtration and stored at −20 °C. Lyophilized goat antisera to albumin, fibrinogen and transferrin (Sigma) were used as detectors for the proteins. They were reconstituted with PBS and diluted to concentrations predetermined to be optimal for indirect fluorescence work: these were 1/320 for anti-albumin, 1/160 for anti-fibrinogen and 1/10 for anti-transferrin. At these concentrations none of the detector antisera bound nonspecifically to untreated *C. albicans* cells at the level of detection of the indirect immunofluorescence assay (see below). Solutions of proteins and antisera were sterilized by membrane filtration and stored at 4 °C.

Human serum and plasma. Samples of blood donated by healthy volunteers were collected in plain glass bottles for preparation of serum and in medical citrated tubes for preparation of plasma. Individual samples of serum and plasma were pooled, sterilized by membrane filtration, and stored in small lots at −20 °C. In some experiments portions of the pooled serum were heat inactivated at 56 °C for 30 min. The pooled serum was negative in counter-immunoelectrophoresis tests for *Candida* precipitins, but it was not tested for antibodies to *Candida* by more sensitive methods.

Antifungal drugs. Samples of clotrimazole (Bayer), fluconazole (Pfizer) and ketoconazole (Janssen Pharmaceutica) were kindly supplied by the manufacturers. The drugs were dissolved in dimethyl sulphoxide to give stock solutions with concentrations of 10^{-5}, 10^{-7} and 10^{-9} M. These solutions were stored at −20 °C. They were diluted 100-fold into EMEM cultures of *C. albicans* in experiments designed to evaluate the effects of the agents on subsequent binding of proteins to *C. albicans* surfaces.

Saturation of transferrin with iron. To test the possibility that iron saturation of transferrin might alter the avidity of this protein to bind to *C. albicans* surfaces, one lot of a stock unsaturated transferrin solution was divided into two aliquots. One was mixed with 0·1 M-ammonium ferric citrate in PBS, the other with 0·1 M-ammonium citrate in PBS to give protein concentrations of 2·5 mg ml^{-1}. These solutions were then dialysed separately against three changes of PBS to remove unbound iron.

Indirect immunofluorescence assay for protein binding to C. albicans. Fluorescein-conjugated rabbit anti-(goat immunoglobulins) antiserum (SeraLab) was diluted 1/40 in PBS (pH 7·0) for the assay. This concentration was the highest at which the fluorescent antiserum showed no nonspecific binding to *C. albicans* yeasts or germ-tubes. The fluorescent antiserum was stored at −20 °C.

To measure protein binding, *Candida* cell suspensions (0·1 ml) were mixed with test protein solutions (0·1 ml) in glass vials and rotated at 30 r.p.m. and room temperature for 30 min. The cells were pelleted in a microcentrifuge and resuspended in PBS three times to remove unbound proteins then resuspended in 50 µl PBS. Duplicate 20 µl samples of the suspension were placed on glass microscope slides and allowed to dry in air. To each dried spot, 20 µl of the appropriate detector anti-protein was added and the slides were incubated in moist chambers at 37 °C for 30 min. The slides were next washed for 3, 5 and 7 min periods in fresh lots of PBS, incubated as before with fluorescent anti-(goat immunoglobulins), washed for 1 h in PBS then allowed to dry in air.

The slides were labelled with codes assigned randomly by an independent person so they could be read blind. All were examined for fluorescence by a single operator to minimize subjective variations in assessment of fluorescent

intensity. A Leitz ultraviolet microscope and a 50× water-immersion objective were used for all assays. One hundred cell units were routinely counted for each sample. Cells or portions of cells exhibiting fluorescence judged to be at or above an arbitrary '2+' level of brightness were scored as 'positive'. Cells that were non-fluorescent or only weakly fluorescent were scored as 'negative'. Percentages of 'positive' cells were determined for each experiment. In preliminary experiments it was found that percentages of 'positives' varied substantially from run to run when the fungi were grown in glass containers, so that plastic containers were used routinely for all the experiments described below.

RESULTS

Binding of proteins to C. albicans parent yeasts and germ-tubes

To test the specificity of the indirect immunofluorescence assay for protein binding to *C. albicans* 73/055, fungal cells grown in EMEM were treated with solutions of albumin, fibrinogen, transferrin or PBS, washed with PBS as described above, then each protein-treated or -untreated cell sample was incubated with anti-albumin, anti-fibrinogen and anti-transferrin. Detector antisera that bound to the cells were revealed by indirect immunofluorescence with fluorescein-conjugated anti-(goat immunoglobulins). With the reagent dilutions specified above, each detector antiserum bound only to *C. albicans* cells that had been coated with the homologous protein. There was no apparent cross-reactivity between any of the three test proteins and heterologous detector antisera, and no nonspecific binding of detector antisera to cells unpretreated with albumin, fibrinogen or transferrin.

Fluorescence, indicating binding of the purified proteins to *C. albicans*, was seen only with the germ-tube portion of the cell units: the parent blastospores appeared not to bind albumin, fibrinogen or transferrin (Fig. 1). Not all germ-tubes fluoresced with the same intensity: approximately 5–10% showed only a weak fluorescence in tests with all three proteins (Fig. 1). Because of this apparent cell-to-cell variation in protein binding, results of tests of protein binding were expressed in terms of percentages of fungal cells that showed strong fluorescence in subsequent experiments.

Variation in binding of plasma proteins to C. albicans 73/055 with culture conditions and morphological form

Quantitative determination of the mean percentages of fluorescence-positive *C. albicans* cells in tests for plasma protein binding showed that the ability of the fungus to bind proteins varied with the growth medium used, as well as with morphological form of the fungus. The data in Table 1 show that no blastospores bound any of the three plasma proteins tested to their

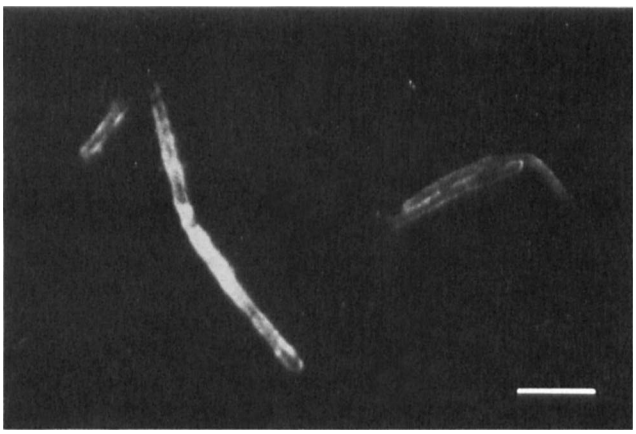

Fig. 1. *C. albicans* 73/055 germ-tubes in indirect fluorescence assay for fibrinogen binding. The fungi were grown for 2·5 h in AAS medium. No fluorescence was seen associated with the parent blastospores and the intensity of fluorescence varied between individual hyphae. Bar, 10 μm.

Table 1. *Mean percentages of fluorescence-positive C. albicans 73/055 when cells were grown for 2·5 h under several different conditions*

Growth conditions	Morphological form	Mean percentage of fluorescence-positive cells after exposure to: Albumin No.*	Albumin Mean ± SD	Fibrinogen No.*	Fibrinogen Mean ± SD	Transferrin No.*	Transferrin Mean ± SD
EMEM/37 °C/CO_2	Germ-tubes	6	96·7 ± 9·0	8	84·9 ± 8·5	6	60·8 ± 37·5
NAG/37 °C/air	Germ-tubes	3	90·0 ± 16·2	3	89·3 ± 7·6	3	70·3 ± 7·5
AAS/37 °C/air	Germ-tubes	3	63·8 ± 29·5	3	33·7 ± 52·3	3	50·7 ± 38·7
MSAB/40 °C/air	Germ-tubes	11	9·2 ± 8·5	2	15·5 ± 0·5	2	0
MSAB/26 °C/air	Blastospores	6	0	5	0	5	0
YNB50†/37 °C/air	Blastospores	1	0	1	0	1	0
YNB500‡/37 °C/air	Blastospores	1	0	1	0	1	0

* No. of replicate runs.
† Yeast nitrogen base + 50 mM-galactose.
‡ Yeast nitrogen base + 500 mM-galactose.

Table 2. *Binding of purified plasma proteins by ten C. albicans isolates and single isolates of other yeast species as measured by indirect immunofluorescence*

All isolates were grown for 2·5 h in EMEM at 37 °C under 5% CO_2 in air. Data for *C. albicans* isolate 73/055 are repeated from Table 1. Those for all other isolates are means ± SD for duplicate experiments.

Candida isolate (and serotype)	Binding (% positive cells) of: Albumin	Fibrinogen	Transferrin
C. albicans 73/055 (A)	97 ± 9	85 ± 9	61 ± 38
C. albicans ATCC 28366 (A)	68 ± 7	59 ± 3	85 ± 11
C. albicans 83/008 (A)	72 ± 4	79 ± 18	93 ± 6
C. albicans 610 (A)	85 ± 3	84 ± 4	95 ± 0
C. albicans SH8* (A)	100 ± 0	100 ± 0	100 ± 0
C. albicans 84/0031 (B)	72 ± 2	53 ± 7	76 ± 7
C. albicans 85/0787 (B)	49 ± 2	37 ± 18	37 ± 27
C. albicans 85/1395 (B)	62 ± 4	55 ± 21	42 ± 5
C. albicans H12† (A)	0	0	0
C. albicans SH27*† (A)	0	0	0
C. glabrata 82/004	7 ± 6	18 ± 10	43 ± 4
C. guilliermondii 84/003	0	0	17 ± 17
C. kefyr Holt	0	0	69 ± 13
C. krusei 81/014	0	0	30 ± 6
C. parapsilosis 81/034	0	7 ± 1	4 ± 2
C. tropicalis 73/071	15 ± 4	0	18 ± 10
S. cerevisiae 83/001	1 ± 2	0	43 ± 11

* Strain with attenuated lethality for mice.
† Strain did not form germ-tubes in EMEM.

surfaces, regardless of the nature of the culture medium in which the yeasts were grown. Most *C. albicans* germ-tubes bound albumin and fibrinogen when the fungi were grown in EMEM or NAG. Somewhat fewer of the germ-tubes gave strong indirect fluorescence in tests to detect transferrin binding to the cells. Only 33–64% of the germ-tube populations bound all three test proteins when the fungi were grown in AAS. There was no binding of transferrin by germ-tubes grown in MSAB, and fewer than 20% of the fungi grown in this medium bound albumin and fibrinogen. Under all conditions tested, only the germ-tube portion of each cell unit bound plasma proteins.

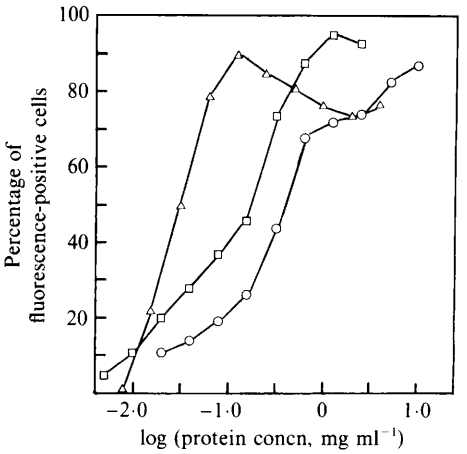

Fig. 2. Binding affinities of purified human albumin (○), fibrinogen (△), and transferrin (□) to germ-tubes of *C. albicans* 73/055. The fungi were treated with different concentrations of the proteins in the standard indirect fluroescence assay then scored for percentages of germ-tubes that fluoresced with 2+ or greater intensity.

Binding of plasma proteins by different strains and species of Candida

The plasma-protein-binding ability of ten *C. albicans* isolates and of seven other yeast species was measured by indirect immunofluorescence. The *C. albicans* isolates contained at least two examples of serotype B strains (the less common type in clinical material), strains of reduced virulence and strains unable to form germ-tubes in EMEM. The results (Table 2) showed that all *C. albicans* strains that formed germ-tubes bound albumin, fibrinogen and transferrin but neither of the strains that did not form germ-tubes bound the proteins. A *C. albicans* strain that did form germ-tubes in EMEM but which had low lethality for mice consistently gave the highest extent of plasma protein binding with 100% of its germ-tubes fluorescing strongly with all three proteins in each of two test runs.

Among species other than *C. albicans*, all of which grew as blastospores in EMEM, binding of albumin and fibrinogen was noted only occasionally and always at a low level. However, the isolates of *C. kefyr*, *C. glabrata* and *S. cerevisiae* bound transferrin to some of their blastospores.

Determination of relative affinities of plasma proteins for C. albicans

Germ-tubes of *C. albicans* 73/055 grown for 2·5 h in EMEM were suspended in different concentrations of albumin, fibrinogen and transferrin (in a doubling dilution series) to assess the extent to which the concentrations of the proteins influenced the percentage of fluorescence-positive cells (Fig. 2). For albumin and transferrin there was an approximately direct proportionality between protein concentration and percentage of fluorescence-positive germ-tubes. For these two proteins there was apparent saturation of the binding effect at concentrations of the order of 0·5 mg ml^{-1}. Fibrinogen showed a different binding effect with an apparent optimum binding concentration of approximately 0·1 mg ml^{-1} and some reduction in the percentage of fluorescence-positive germ-tubes at concentrations up to the 4 mg ml^{-1} used in routine tests. The data suggested that fibrinogen had a higher propensity than the other two proteins for binding to *C. albicans* germ-tubes.

In terms of concentrations that caused 50% of the germ-tubes to fluoresce, fibrinogen was still clearly the protein with the highest binding affinity. It can be seen that 50% of the germ-tubes fluoresced when exposed to fibrinogen concentrations of 30 μg ml^{-1}. The equivalent figures for the other proteins were 180 μg ml^{-1} (transferrin) and 380 μg ml^{-1} (albumin).

Table 3. *Relative affinities of albumin and fibrinogen for C. albicans germ-tubes as shown by indirect immunofluorescence tests with fungi exposed to mixtures of the two proteins at different relative concentrations*

Data are means ± SD of percentages of fluorescence-positive germ-tubes calculated from duplicate experiments.

Fibrinogen : albumin ratio	Detector antiserum specific for: Albumin	Fibrinogen
1:0	0	90 ± 2
1:1	4 ± 2	61 ± 1
1:3	24 ± 4	75 ± 3
1:7	46 ± 2	51 ± 3
1:15	65 ± 4	15 ± 1
1:30	73 ± 2	1 ± 1
1:60	89 ± 1	1 ± 1
0:1	91 ± 3	0

Binding of mixtures of plasma proteins to C. albicans

To examine further the relative binding affinities of the three plasma proteins for germ-tubes of *C. albicans* 73/055, the fungal cells were exposed to combinations of pairs of the proteins and to all three mixed together at their standard test concentrations. The percentages of fluorescent cells determined in these experiments confirmed that fibrinogen had a particularly high affinity for the fungus. When fibrinogen was paired with albumin or transferrin, 69 ± 2% and 78 ± 1% of germ-tubes fluoresced with anti-fibrinogen as detector antiserum, while 8% or fewer germ-tubes fluoresced with anti-albumin or anti-transferrin detector antisera. Paradoxically, when the fungi were exposed to a mixture of all three proteins, the highest proportion of fluorescent germ-tubes was seen in tests with anti-albumin as detector: 95 ± 2% fluoresced with anti-albumin, 69 ± 2% with anti-fibrinogen and 7 ± 1% with anti-transferrin.

As a final test of the high affinity of fibrinogen for germ-tubes of *C. albicans* 73/055, the fungi were treated with mixtures of fibrinogen and albumin over a range of different relative concentrations. The total (albumin + fibrinogen) concentration was 4 mg ml^{-1} in every case. Even when the concentration of albumin in the mixture exceeded that of fibrinogen by a ratio of 7:1, 50% of the germ-tubes were still fluorescence positive with an anti-fibrinogen detector serum (Table 3). Only when the albumin concentration was at least 15 times greater than the fibrinogen concentration did the anti-albumin detector serum produce more than 50% fluorescence-positive germ-tubes.

Binding of native serum and plasma proteins to C. albicans

The proteins in pooled human serum and plasma did not bind qualitatively to *C. albicans* in the same manner as purified albumin, fibrinogen and transferrin. Germ-tubes of *C. albicans* 73/055 suspended for 30 min in serum or plasma then probed for protein binding with detector antisera specific to albumin, fibrinogen and transferrin showed fluorescence over the entire cell surface – the germ-tubes and their parent blastospores (Fig. 3).

Quantitative assessment of percentages of *C. albicans* cells that were fluorescence positive after exposure to serum and plasma also revealed patterns of protein binding unlike those seen with the purified plasma proteins (Table 4). (The results for plasma resembled those in the experiment in which germ-tubes were treated with a mixture of purified albumin, fibrinogen and transferrin). However, in serum, the fluorescence assay results suggested that fibrinogen was bound to more germ-tubes than were albumin or transferrin. This result was highly paradoxical because serum should contain no more than negligible residual quantities of fibrinogen. Heat pretreatment of the serum at 56 °C for 30 min appeared to enhance the binding of albumin and transferrin to the germ-tubes, but more than half the fungal cells continued to fluoresce when tested with an anti-fibrinogen detector serum.

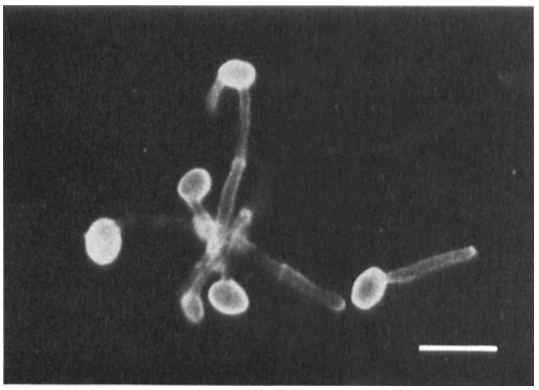

Fig. 3. *C. albicans* 73/055 germ-tubes treated with whole human plasma then tested for the presence of bound fibrinogen by indirect fluorescence. All parts of the fungal surface fluoresced. Bar, 10 μm.

Table 4. *Binding of proteins in human serum, plasma and heat-inactivated serum to C. albicans germ-tubes as shown by indirect immunofluorescence tests with detector antisera specific to albumin, fibrinogen or transferrin*

Results shown are means ± SD of the percentage of fluorescence-positive *C. albicans* 73/055 germ-tubes grown for 2·5 h in EMEM.

Sample tested	No. of tests	Detector antiserum specific to: Albumin	Fibrinogen	Transferrin
Plasma	2	79 ± 16	76 ± 1	2 ± 2
Serum	4	21 ± 19	80 ± 20	32 ± 23
Heat-inactivated serum (56 °C/30 min)	4	91 ± 7	63 ± 32	86 ± 4

Effects of azole antifungals on protein binding to C. albicans 73/055

When *C. albicans* 73/055 was grown for 2·5 h in the presence of clotrimazole or ketoconazole at 10^{-5} M the cells produced were pleomorphic, stunted, unseparated growths, only a minority of which were barely recognizable as germ-tubes. At 10^{-7} M the two antifungals caused some apparent reduction in the length of germ-tubes and increased the proportion of blastospore-shaped cells, and at 10^{-9} M they had no noticeable effect on germ-tube development. Fluconazole had less pronounced effects on the morphology of the germ-tubes: its effects at 10^{-5} M were similar to those of clotrimazole and ketoconazole at 10^{-7} M. The effects of these compounds on hyphal development in *C. albicans* have been described in detail elsewhere (Odds *et al.*, 1985). The binding of purified albumin, fibrinogen and transferrin to *C. albicans* cells grown in the presence of azole antifungals appeared to be unaltered. Despite the morphological differences clearly evident in cells grown at the highest azole concentrations, fluorescence, indicating binding of all three plasma proteins, was noted on most of the 'daughter' outgrowths and no fluorescence was observed on the parent blastospores.

DISCUSSION

This study has confirmed the work of Bouali *et al.* (1986) and Annaix *et al.* (1987) in showing that purified fibrinogen binds specifically to *C. albicans* germ-tubes and not to the parent blastospores from which germ-tubes grow out. Two other purified human plasma proteins, albumin and transferrin, bound to the fungus in the same manner. Fibrinogen was bound more readily to *C. albicans* than were albumin or transferrin. Thus, even if there is a common binding

receptor for all three proteins, the higher avidity of the binding for fibrinogen indicates some degree of specificity in the mechanism of attachment. The nature of the surface-protein-binding receptor(s) in *C. albicans* remains to be elucidated.

Brawner & Cutler (1986) have shown that at least two surface antigenic epitopes in *C. albicans* are expressed variably from cell to cell and from time to time in germ-tube cultures. Protein-binding receptors in *C. albicans* may be similar examples of molecules that appear at the surface in a variable fashion.

The percentage of germ-tubes that bound the three purified proteins was influenced notably by the growth medium: for all *C. albicans* isolates that formed germ-tubes readily, those grown in EMEM and NAG bound proteins more often than cells grown in AAS or MSAB. The developmental processes and growth rates of *C. albicans* in NAG differ substantially from those in the other media (Sevilla & Odds, 1986), but the medium-induced differences in plasma protein binding cannot be explained in terms of gross differences in rates of growth or of incubation temperature since the culture conditions studied represent a thorough cross-selection of such parameters and no clear correlations were found.

The phenomenon of protein binding was almost entirely specific to *C. albicans* germ-tubes. Blastospores of *C. albicans* and of other *Candida* species did not bind albumin or fibrinogen at all, and in the occasional instances where some yeasts bound transferrin, fewer than 50% of the cell population did so with sufficient avidity to score as fluorescence positives in the assay used. In all experiments with germ-tubes the parent blastospore portions of the cells failed to bind the purified plasma proteins.

The concept of physiological behaviour specific to *C. albicans* germ-tubes is not new. There are numerous reports of germ-tube-specific antigens (Ponton & Jones, 1986; Smail & Jones, 1984; Sundstrom & Kenny, 1984; Umenai & Chiba, 1977); *C. albicans* pseudohyphae express more surface receptors than blastospores for the complement cleavage product C3d (Calderone *et al.*, 1988); and *C. albicans* germ-tubes adhere better than blastospores to epithelial cells *in vitro* (Kimura & Pearsall, 1980; Rotrosen *et al.*, 1985). Tronchin *et al.* (1987) have proposed that fibrinogen bound to germ-tubes acts as a ligand in binding to fibronectin on mammalian cell surfaces and/or as a mechanism for providing the fungus with a protein coat that could mask it from host immune defences. Our work has shown that at least two other plasma proteins, albumin and transferrin, can bind in a similar manner and may therefore act similarly. However, we believe that adhesion via plasma protein intermediates is more likely to be a secondary mechanism than a primary one, since Calderone & Scheld (1987) have shown that fibronectin binds directly to blastospores *in vitro* and McCourtie & Douglas (1985) have shown that surface mannoproteins on blastospores directly mediate adhesion of blastospores to epithelia, whereas blastospores cannot bind purified albumin, fibrinogen or transferrin *in vitro*.

The results of our experiments with purified human plasma proteins did not correlate with the behaviour of the proteins in their native form. When pooled samples of whole human plasma or serum were used to treat *C. albicans* germ-tubes and the binding of albumin, fibrinogen or transferrin was assessed with antisera specific to these proteins, fluorescence, indicating binding of all three proteins, was seen over the surface of germ-tubes and their parent blastospores. Experiments with serum revealed levels of apparent fibrinogen binding equivalent to those seen with plasma-treated fungi. Since fibrinogen should have been removed from the serum by the clotting process this result suggests that the effects seen were nonspecific. They may have resulted from low levels of anti-*Candida* antibodies in the pooled serum cross-reacting with the fluorescent detector serum or from nonspecific binding of the detector serum mediated by some other serum or plasma constitutent: possible candidates for this role are complement components iC3b and C3d, since both of these have been shown to bind to *C. albicans* surfaces *in vitro* (Heindenreich & Dierich, 1985; Edwards *et al.*, 1986; Calderone *et al.*, 1988), and a yeast mannan-binding protein described by Bull & Turner (1984) and Kawasaki *et al.* (1985).

Our data on protein binding to strains of *C. albicans* with reduced lethality for mice suggest that plasma protein binding *per se* is not an essential attribute for virulence since one of the attenuated strains bound all three test proteins more avidly than most other isolates of the species. The lack of protein binding in the second attenuated strain appears to relate more to its

inability to form germ-tubes in EMEM than to its inherently low virulence. Even if the ability of germ-tubes to bind plasma proteins is not an essential prerequisite for virulence of the fungus, it remains a property of interest in the context of virulence for two reasons. First, it is possible that the protein for which the putative surface receptor has greatest avidity has not yet been discovered; secondly, the specific correlation of the property with germ-tube formation and its variable expression in different environments may prove to be an experimentally useful marker in future research on the pathogenesis of candidosis.

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