MYCOLOGY

An $\alpha 5\beta 1$ -like integrin receptor mediates the binding of less pathogenic *Candida* species to fibronectin

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Summary. The present study was undertaken to investigate whether less pathogenic *Candida* species (*C. tropicalis*, *C. stellatoidea*, *C. krusei* and *C. glabrata*) express a fibronectin receptor (FNr) antigenically related to $\alpha 5\beta 1$ integrin, which mediates their binding to fibronectin (FN). By flow cytometric analysis, a monoclonal antibody (MAb) directed against human $\alpha 5$ integrin subunit (clone SAM-1) and two different antisera to FNr positively stained *C. tropicalis*, *C. stellatoidea* and *C. glabrata*, with the greatest expression observed for *C. tropicalis*. No or only marginal immunoreactivity was found on *C. krusei*. *C. tropicalis*, *C. stellatoidea*, *C. glabrata*, but not *C. krusei* yeasts specifically adhered to FN; higher levels of adhesion were found for *C. tropicalis* and *C. stellatoidea* with respect to *C. glabrata*. Less pathogenic *Candida* spp. bound to the Arg-Gly-Asp (RGD) containing 120-kDa fragment of FN and adhesion to intact FN was markedly inhibited by Gly-Arg-Gly-Asp-Ser-Pro (GRGDSP), but not by Gly-Arg-Gly-Glu-Ser-Pro (GRGESp) peptides. In addition, anti- $\alpha 5$ SAM-1 MAb and both anti-FNr antisera strongly blocked binding of less pathogenic *Candida* spp. to FN. Overall, these results indicate that less pathogenic *Candida* spp., including *C. tropicalis*, *C. stellatoidea* and *C. glabrata*, express a receptor antigenically related to $\alpha 5\beta 1$ integrin which mediates their adhesion to FN.

Introduction

Candida spp. have emerged as important causes of nosocomial infections.^{1–5} The rise in life-threatening opportunist mycoses reflects the increase in high-risk immunocompromised hosts, including immunodeficiency virus-infected individuals,^{6–8} patients undergoing kidney or bone marrow transplantation,^{9–11} or those with cancer.^{12–15} Among opportunist mycoses, *Candida* spp. are now the fourth most commonly isolated organisms in blood cultures. *C. albicans* accounts for 60–80% of isolates, whereas less pathogenic *Candida* spp. including *C. stellatoidea*, *C. tropicalis*, *C. krusei* and *C. glabrata* account for the remaining clinical specimens.^{5, 10, 16} The importance of non-*albicans* *Candida* spp. is due to their increasing frequency, the emerging tolerance or resistance to antimycotic agents, as well as the higher mortality associated with their infections.^{11, 17–19}

Candida spp. show a wide range of infectious capabilities. Thus, the infections can vary from superficial mucosal lesions to multiple visceral organ in-

volvement. The gravest complication of candida infections is disseminated disease resulting in clinical entities such as endocarditis, pyelonephritis, endophthalmitis, osteomyelitis or chronic mucocutaneous infections.^{20–25}

The pathogenesis of these lesions is not completely understood, but is known to involve adherence to endothelial and epithelial cells as well as to extracellular matrix (ECM) components.^{16, 26–35} Adherence to ECM represents a crucial step in the development of candidosis and is considered a significant virulence factor for these fungi, as the ability of *Candida* spp. to bind ECM proteins correlates with their relative pathogenicity.^{36–38}

Among the molecules involved in cell adhesion to ECM proteins, integrins are a supergene family of highly conserved receptors that mediate cell-matrix and cell-cell interactions in embryogenesis, haemostasis, wound healing, tumour invasion, immune responses, inflammation and infectious diseases.^{39, 40} Integrins are a gene family of 15 α chains and 8 β chains expressed on a wide variety of mammalian cells, including epithelial and endothelial cells and leucocytes. Integrin receptors are heterodimers composed

of non-covalently linked α and β subunits. Both α and β chains have cytoplasmic tails that mediate intracellular signalling *via* interaction with cytoskeleton components. At least eight different integrin subfamilies have been characterised on the basis of the association of distinct α chains with common or related β subunits. In the $\beta 1$ family, $\alpha 5\beta 1$ is the first integrin identified as the fibronectin (FN) receptor (FNR) and binds only FN recognising the Arg-Gly-Asp (RGD) sequence in the central region of the molecule.⁴¹

Candida spp. adhere to FN,^{30, 31, 35, 42, 43} but the mechanisms of interaction with FN are poorly defined. We have recently demonstrated an FNR in *C. albicans* antigenically related to the $\alpha 5\beta 1$ integrin that is involved in the adherence of both yeast and germ-tube phases to FN.⁴³ An FNR belonging to the $\beta 1$ integrin subfamily has been identified also on *C. albicans* by Klotz *et al.*;⁴² binding to FN was inhibited by RGD-containing peptides whereas carbohydrates including D-mannose did not affect adhesion.

The present study was undertaken to investigate the expression of the $\alpha 5\beta 1$ integrin and its functional role in mediating the adhesive interaction of FN with less pathogenic *Candida* spp.

A better understanding of the mechanisms by which *Candida* spp. adhere to ECM proteins may be useful in the development of new therapeutic strategies to prevent disseminated infections.

Materials and methods

Candida spp.

C. tropicalis (two clinical isolates), *C. stellatoidea* (two isolates), *C. glabrata* (two isolates) and *C. krusei* (two isolates) were identified by the API 20C system in the Microbiology and Immunology Laboratory of the University of South Florida, Tampa, FL, USA. Yeasts were cultured in Sabouraud's Dextrose Agar (Unipath) and frozen at -80°C in small volumes in Sabouraud broth containing glycerol 5% until use. For assays, a loopful of yeasts was placed in 50 ml of Sabouraud dextrose broth and incubated with shaking at 26°C for 20 h. Yeast cells were washed twice with cold PBS by centrifugation at 3000 rpm for 10 min, pelleted and examined microscopically to exclude the presence of germ-tube or pseudohyphal phases. All isolates were tested for their ability to adhere to FN and found to be equivalent. Therefore, one isolate of each *Candida* spp. was used in all the experiments.

Antibodies

The following mouse monoclonal antibodies (MAbs) were used: anti- $\alpha 5$, clone P1D6 (IgG3) was purchased from Telios Pharmaceuticals Inc. (San Diego, CA, USA); anti- $\alpha 5$, clone SAM-1 (IgG2b) from AMAC Inc. (Westbrook, ME, USA); anti- $\beta 1$, clone 4B4 (IgG1) and clone A1A5 (IgG2b) were obtained from Coulter (Hialeah, FL, USA) and as

a gift from Dr M. Hemler (Dana-Farber Cancer Institute, Boston, MA, USA), respectively. A rabbit polyclonal antibody raised against affinity-purified human placenta FNR (R α FNR) was purchased from Calbiochem Corp. (La Jolla, CA, USA). A goat polyclonal antibody reactive with the $\beta 1$ subunit, raised against affinity-purified human placenta FNR (G α FNR) was provided by Dr G. Tarone (University of Turin, Turin, Italy).⁴⁴ A MAb to a cell surface glucomannoprotein constituent of *C. albicans* (clone AF1) was kindly provided by Dr A. Cassone (Istituto Superiore di Sanita, Rome, Italy).⁴⁵ F(ab')₂ fragment and Ig fraction of goat polyclonal antibody against human FN were purchased from Sigma and Cappel Laboratories (Cooper Biomedical Inc., Malvern, PA, USA). Purified FITC-conjugated goat F(ab')₂ fragment anti-mouse (GaM), and anti-rabbit Ig (GaR), rabbit F(ab')₂ fragment anti-goat Ig (RaG) were purchased from Cappel Laboratories.

Adhesive proteins and peptides

Human plasma FN was purchased from Gibco Laboratories (Grand Island, NY, USA). The 120-kDa FN proteolytic fragment was obtained from Calbiochem Corp. Substrate preparations were > 95% pure as checked by SDS-PAGE. The GRGDSP and GRGESP peptides were purchased from Telios Pharmaceuticals Inc.

Immunofluorescence and flow cytometry

Aliquots of 10^6 yeast cells were incubated with a saturating dose of the first antibody for 30 min at 4°C and then washed twice with cold PBS. Cells were incubated with the FITC-conjugated second antibody for 30 min at 4°C , washed twice and resuspended in PBS. Second-step reagents as well as irrelevant isotype-matched MAb plus GaM were used as negative controls. Cells were analysed for relative fluorescence intensity and the percentage of positively stained cells was determined over 10000 events on a FACScan cytofluorimeter (Becton Dickinson, Mountain View, CA, USA). Fluorescence intensity is expressed in arbitrary units on a logarithmic scale. The results are presented both as mean fluorescence intensity (MFI) and percentage of positive cells. Positive yeast cells exhibited an increase in fluorescence intensity above autofluorescence, and the signal from negative cells was not greater than that of the autofluorescence control.

Labelling of *Candida* spp.

Yeast cells (10^8) were labelled with 25 μCi of ^3H -glucose (Glucose D-(5,6- ^3H), specific activity 66.6 Ci/mmol; Dupont Comp. Biotechnology, Wilmington, DE, USA) in 2.5 ml of PBS supplemented with 0.5 mM CaCl_2 and MgCl_2 for 3 h at room temperature as described previously.⁴⁶ After labelling,

yeast cells were washed three times in cold PBS to remove free isotope, counted and adjusted to a concentration of 5×10^6 /ml in PBS supplemented with 0.5 mM CaCl_2 and MgCl_2 .

Adhesion assay

Stock preparations of human plasma FN and its proteolytic 120-kDa fragment were diluted in PBS, pH 7.4, at a concentration of 25 $\mu\text{g}/\text{ml}$, which has been determined previously to be optimal (data not shown). Protein solution (100 μl) was distributed in 96-well tissue culture flat-bottomed plates (Costar, Cambridge, MA, USA). After overnight incubation at 4°C, the coated plates were washed three times with PBS to remove unbound protein. Labelled yeast cells (5×10^5) of each representative isolate of *Candida* spp. were allowed to adhere to the protein-coated surface for 30 min at 37°C in CO_2 5%. Optimal cell concentrations for all *Candida* spp. were determined by dose-response experiments (data not shown). Adhesion was verified microscopically; yeast phase was maintained throughout the adhesion assay and agglutination was not observed.

Unattached cells were removed by extensive washing with warm PBS and adherent cells were harvested by adding 100 μl of Trypsin-EDTA (Flow Laboratories) solubilised in 0.5 M NaOH for 10 min at 37°C. Bound cpm as well as cpm from non-adherent cells plus washes were quantitated in a Wallac Pharmacia 1410, β scintillation counter. Quadruplicate wells were assayed for each sample.

To rule out the contribution of yeast cell binding to plastics in the adhesion to FN, assays were always performed in the presence of saturating doses of anti-FN antibodies, which completely abolished cell binding to FN, while not affecting attachment to the plastic surface as described previously.⁴³

Total adhesion was calculated according to the following formula: $\text{cpm (adherent cells)}/\text{total cpm} \times 100$, where total cpm indicates the sum of non-adherent cells, washes and adherent cells cpm. This value routinely exceeded 93% of total cell counts.

Specific binding to FN was calculated as the difference between total percentage adhesion and percentage adhesion not inhibited by anti-FN antibody (non-specific adhesion). Non-specific cell adhesion never exceeded 10%.

Inhibition assay

For inhibition of the adhesion of *Candida* spp. to FN, binding assays were performed following pre-treatment of radiolabelled yeast cells for 30 min with a rabbit or a goat anti-FNr antiserum, anti- $\alpha 5$ SAM-1 or PID6 MAb, control antibody including normal goat IgG, rabbit IgG, and an anti-glucomannoprotein MAb or with different concentrations of GRGDSP and GRGESD peptides.

Statistical analysis

Statistical analysis by Student's *t* test was performed by the STATPAC computerised program.

Results

Expression of a receptor antigenically related to $\alpha 5 \beta 1$ integrin by different *Candida* spp.

Adherence of *Candida* spp. to FN has been reported previously,^{30, 31, 35, 42, 43} but the molecular basis of this interaction has not yet been clearly defined. In an attempt to investigate the receptors involved in the specific adhesion of less pathogenic *Candida* spp. to FN, the expression of the FNr prototype, $\alpha 5 \beta 1$ integrin, was evaluated on *C. tropicalis*, *C. stellatoidea*, *C. glabrata* and *C. krusei* by immunofluorescence and flow cytometric analysis with a panel of MAbs directed against the $\alpha 5$ (SAM-1, PID6) or $\beta 1$ (A1A5, 4B4) subunit of human FNr, or with a rabbit or goat polyclonal anti-FNr antibody purified from human placenta. As shown in fig. 1 and the table, *C. tropicalis*, *C. glabrata* and *C. stellatoidea* were positively stained by the anti- $\alpha 5$ SAM-1 MAb, with the greatest expression observed in *C. tropicalis*. In contrast, no immunoreactivity with the anti- $\alpha 5$ SAM-1 MAb was observed in *C. krusei*. Marginal reactivity was detected with another anti- $\alpha 5$ MAb, clone PID6, in all *Candida* spp. analysed. Both rabbit and goat anti-FNr antisera positively stained all *Candida* spp. *C. tropicalis* and *C. glabrata* showed higher percentages of fluorescent cells and average MFI with rabbit and goat anti-FNr antiserum, respectively. As observed with the anti- $\alpha 5$ SAM-1 MAb, *C. krusei* reacted poorly with both anti-FNr antisera. No major reactivity was found with MAb directed against the $\beta 1$ integrin chain (A1A5, 4B4) in any *Candida* spp. tested.

These results indicate that the yeast forms of *C. tropicalis*, *C. stellatoidea* and *C. glabrata*—but not *C. krusei*—express an FNr antigenically related to $\alpha 5 \beta 1$ integrin as reported previously for *C. albicans*.⁴³

Candida spp. yeast cells specifically binding to FN

The ability of the different *Candida* spp. to bind FN was evaluated by incubating ³H-glucose-labelled organisms on FN-coated plates for 30 min at 37°C in the presence or absence of an anti-FN antibody or its F(ab')₂ fragment (data not shown). More than 90% of yeast cell adhesion to FN was specific as indicated by the ability of anti-FN antibody to completely inhibit binding to FN, in a dose-dependent manner (fig. 2a). Higher levels of specific adhesion to FN were found for *C. tropicalis* and *C. stellatoidea* than for *C. glabrata* (fig. 2b). *C. krusei* bound to FN only marginally; adhesion was not increased by higher FN doses (25–100 $\mu\text{g}/\text{ml}$) or longer adhesion times (30–60 min) (data not shown).

Fig. 1. Reactivity of antibody directed against human $\alpha 5$, SAM-1 (■), P1D6 (□), $\beta 1$ 4B4 (■), A1A5 (▨) or $\alpha 5 \beta 1$ G α FNr (▤), R α FNr (▥) integrins with *C. stellatoidea*, *C. glabrata*, *C. krusei* and *C. tropicalis* yeasts evaluated by flow cytometric analysis. The results are presented as percentage of positive stained cells and represent one of four separate experiments.

Table. Expression of a $\alpha 5 \beta 1$ integrin-related fibronectin receptor on *Candida* spp. yeast cell

<i>Candida</i> spp.	Mean fluorescence intensity (SD)* with antibody					
	SAM-1	P1D6	A1A5	4B4	R α FNr	G α FNr
<i>C. tropicalis</i>	6.15 (0.9)	2.07 (0.3)	1.32 (0.2)	2.82 (0.3)	114.03 (2.3)	3.54 (0.3)
<i>C. stellatoidea</i>	3.06 (0.7)	1.39 (0.2)	1.25 (0.1)	1.51 (0.3)	7.35 (0.7)	8.14 (1.1)
<i>C. glabrata</i>	3.73 (0.8)	1.38 (0.1)	0.92 (0.1)	0.97 (0.1)	5.89 (0.7)	9.42 (1.0)
<i>C. krusei</i>	1.25 (0.3)	0.79 (0.1)	0.66 (0.2)	0.98 (0.1)	1.83 (0.1)	1.86 (0.3)

Reactivity of anti- $\alpha 5$ (clones SAM-1 and P1D6), anti- $\beta 1$ (clones 4B4 and A1A5) MAbs, goat (G α FNr) and rabbit (R α FNr) anti-FNr antisera with *C. tropicalis*, *C. stellatoidea*, *C. glabrata* and *C. krusei* was evaluated by indirect immunofluorescence and flow cytometric analysis.

*Mean fluorescence intensity (MFI) data are the means of four separate experiments and SD. MFI was calculated as the ratio between the MFI of the experimental group and MFI of the control (second-step reagent alone).

Adhesion of *Candida* spp. yeast cells to the 120-kDa proteolytic fragment of FN

The mechanisms involved in the adhesion of different *Candida* spp. to FN were investigated further by testing the ability of *C. tropicalis*, *C. stellatoidea*, *C. glabrata* and *C. krusei* to specifically adhere to the 120-kDa FN proteolytic fragment which contains the RGDS cell binding site⁴⁷ (fig. 3). *C. tropicalis*, *C. glabrata* and *C. stellatoidea* bound to FN as well as its 120-kDa fragment, *C. tropicalis* being the most adhesive species. As observed with the entire FN molecule, *C. krusei* did not significantly bind to the 120-kDa FN fragment.

Inhibition of *Candida* spp. adhesion to FN by RGD-containing peptides

Studies on mammalian cells have shown that $\alpha 5 \beta 1$ recognises the RGDS binding site in the central region

of the FN molecule.⁴¹ To investigate the possible involvement of $\alpha 5 \beta 1$ in these interactions, the adhesion assay was performed in the presence of an RGD-containing peptide (fig. 4). Pre-treatment of *C. tropicalis*, *C. stellatoidea* and *C. glabrata* yeast cells with different doses of GRGDSP peptide partially inhibited the adhesion to FN in a dose-dependent manner, with the highest inhibition found for *C. tropicalis*. No inhibition was observed in the presence of different doses of the control peptide GRGESp. These results indicate that adhesion of less pathogenic *Candida* spp. to FN is mediated by RGD-containing peptides.

Inhibition of *Candida* spp. adhesion to FN by anti- $\alpha 5$, or anti-FNr antibodies

The possible involvement of $\alpha 5 \beta 1$ integrin in the adhesion of *C. tropicalis*, *C. stellatoidea* and *C.*

Fig. 2. *C. tropicalis*, *C. stellatoidea*, *C. glabrata*, but not *C. krusei*, specifically bind to FN. **a**, the adhesion of ^3H -glucose-labelled *Candida* spp. to FN 25 $\mu\text{g}/\text{ml}$ was tested in the absence (■) or presence of different concentrations (▨ 1 in 25; ■ 1 in 50; □ 1 in 100) of an anti-FN antiserum. The results are presented as the mean percentage adhesion and SD of three separate experiments. **b**, total (■) and specific (▨) binding of *Candida* spp. to FN were evaluated in the presence of a saturating dose (1 in 25) of an anti-FN antiserum; the results are presented as the mean percentage adhesion and SD of five separate experiments.

Fig. 3. Total (■) and specific (▨) binding of *Candida* spp. yeast cells to the 120-kDa FN proteolytic fragment. Specific adhesion of ^3H -glucose-labelled *Candida* spp. yeast cells to the 120-kDa FN fragment (25 $\mu\text{g}/\text{ml}$) was tested in the presence of a saturating dose (1 in 25) of an anti-FN antiserum. The results are presented as the mean percentage adhesion and SD of five separate experiments.

glabrata to FN was further analysed by testing the ability of antibodies directed against $\alpha 5$ or $\alpha 5\beta 1$ heterodimer to inhibit yeast binding to FN (fig. 5). Pre-treatment of yeast cells with different doses of both goat and rabbit anti-FNr antisera or with anti- $\alpha 5$ SAM-1 and P1D6 MAb strongly blocked the adhesion of *Candida* spp. to FN in a dose-dependent manner. No inhibition was found in the presence of an anti-

mannan MAb or normal rabbit and goat IgG used as controls.

Discussion

Adherence of *Candida* spp. to ECM components, endothelial or epithelial cells represents an important

Fig. 4. Inhibition of (a) *C. tropicalis*, (b) *C. stellatoidea* and (c) *C. glabrata* adhesion to FN by an RGD-containing peptide. Adhesion of ^3H -glucose-labelled *Candida* spp. yeast cells to FN (25 $\mu\text{g}/\text{ml}$) was tested in the presence of different concentrations of GRGDSP (○) or GRGESp (●) synthetic peptides. The results are the mean of quadruplicate determinations and are representative of three separate experiments. Specific cell adhesion calculated in the presence of a saturating dose (1 in 25) of an anti-FN antiserum was 90% of total adhesion.

step in the development of candidosis. Both yeast and germ-tube phases of *C. albicans* express a functional FNr antigenically related to $\alpha 5\beta 1$, that is up-regulated during germ-tube transition and mediates adhesion to FN of both *Candida* phases.⁴³

The results of the present study indicate that yeast forms of less pathogenic *Candida* spp., including *C. tropicalis*, *C. stellatoidea*, *C. glabrata*, but not *C. krusei*, also express a surface receptor that shares antigenic homology with the $\alpha 5\beta 1$ integrin and mediates adhesion to FN. Flow cytometric analysis indicates that certain antibodies directed against the human $\alpha 5$ integrin subunit or $\alpha 5\beta 1$ heterodimer differentially

bind to *Candida* spp. with *C. tropicalis*, *C. stellatoidea* and *C. glabrata* exhibiting high reactivity and *C. krusei* reacting only marginally. The expression of $\alpha 5\beta 1$ -like integrin directly correlated with the extent of *Candida* spp. adhesion to FN; 20–30% of *C. tropicalis*, *C. stellatoidea* and *C. glabrata* yeasts specifically bound to FN, whereas *C. krusei* failed to adhere to this ECM glycoprotein.

The $\alpha 5\beta 1$ -like receptor expressed by *C. tropicalis*, *C. stellatoidea* and *C. glabrata* is the primary mediator of adhesion to immobilised FN. The less pathogenic *Candida* spp. bound to the 120-kDa proteolytic fragment of FN which contains the RGDS sequence recognised by $\alpha 5\beta 1$ ⁴¹ and their binding to the entire FN molecule was partially inhibited by GRGDSP, but not GRGESp peptides. More direct evidence that an $\alpha 5\beta 1$ -like integrin is involved in the interaction of *Candida* spp. with FN was obtained by the use of functional antibodies directed against $\alpha 5$ or $\alpha 5\beta 1$ FNr that blocked specific adhesion to FN.

Expression of an FNr antigenically related to $\alpha 5\beta 1$ on *Candida* spp. appears to be particularly significant for their interaction with host tissues such as epithelial or endothelial cells. Involvement of FN in the interaction of *C. albicans* or *C. tropicalis* with buccal and vaginal epithelial cells, epidermal keratinocytes or endothelium has been reported.^{48–55} Adhesion of *C. albicans* germ tubes to cultured human epidermal keratinocytes was partially inhibited by PepTite-2000, an RGD-containing synthetic peptide.⁵⁰ Similarly, binding of *C. tropicalis* yeasts to epithelial cells was significantly inhibited by RGD peptides derived from FN but not from inactivated complement receptor type 3 (iC3b), while inverse results were obtained for *C. albicans*.⁴⁹ Adhesion of *C. albicans* to endothelium is also RGD-dependent but probably involves more than one integrin receptor as suggested by the possible involvement of iC3b.⁵¹

It is possible that $\alpha 5\beta 1$ -like receptors play a major role in the development of candida infection. A FN-derived RGD-containing peptide affects the distribution and the fate of intravenously administered *C. albicans* yeast cells:⁵⁶ after administration of RGD-containing peptides, the heart, kidney and liver of treated animals had significantly fewer cfu of fungus and the number of renal abscesses was reduced.

Several animal and experimental models of infection have provided conclusive evidence for a hierarchy of relative virulence among the pathogenic *Candida* spp. These differences in virulence are closely paralleled by the varying abilities of *Candida* spp. to bind to epithelial and endothelial cells and complement components, as well as to ECM proteins. Thus, *C. albicans* attaches to exfoliated vaginal or buccal cells in much greater numbers than *C. tropicalis* (the next most adherent species), whereas *C. krusei* shows little or no adhesion.⁵² Similar species differences have been reported for adhesion to epidermal corneocytes,⁵³ and vascular endothelium.^{54, 55} In addition, mutant strains of *C. albicans* with reduced ability to adhere to human

Fig. 5. Effect of anti- $\alpha 5$ or anti- $\alpha 5\beta 1$ antibody on (a) *C. tropicalis*, (b) *C. stellatoidea* and (c) *C. glabrata* adhesion to FN. ^3H -glucose-labelled *Candida* spp. yeast cells were pre-treated for 30 min at 4°C with different dilutions of anti- $\alpha 5$ SAM-1 (○) and PID6 (●) MAb, goat anti-FN antiserum (□), rabbit anti-FN antiserum (■) and normal goat or rabbit Ig or a mouse MAb directed against a glucomannoprotein as control antibodies (△), and then tested for binding to FN (25 $\mu\text{g}/\text{ml}$). The results are the mean of quadruplicate determinations and are representative of three separate experiments. Specific cell adhesion calculated in the presence of a saturating dose (1 in 25) of an anti-FN antiserum was 90%. None of the control antibodies inhibited adhesion, the results of normal rabbit Ig only are presented for simplicity.

vaginal epithelial cells *in vitro* were less virulent in animal models of vaginal candidosis.⁵⁷ With regard to the ability of *Candida* spp. to bind complement components, *C. albicans* and *C. stellatoidea*, but not *C. tropicalis* or *C. krusei*,⁵⁸ adhere to iC3b- and C3d-coated sheep erythrocytes. Interestingly, adhesion to iC3b involves a receptor antigenically identical or similar to a mammalian integrin belonging to the $\beta 2$ subfamily,^{59,60} and its expression on *Candida* spp. directly correlates with *Candida* iC3b rosetting.

Adhesion of different *Candida* spp. to FN³⁰ directly correlates with the ability to cause endocarditis in rabbits,^{36,38} moreover, *Candida* spp. with a high isolation frequency from endocarditis cases, such as *C. tropicalis* and *C. albicans*, show significantly higher binding to FN *in vitro*, whereas *Candida* spp. that do not adhere *in vitro* to FN (e.g., *C. krusei*) are rarely implicated in this disease.

These results show a correlation between the expression and function of $\alpha 5\beta 1$ integrin-like receptor on *Candida* spp. and the hierarchy of relative virulence

among the pathogenic *Candida* spp. and suggest that the $\alpha 5\beta 1$ analogue may be a significant virulence factor for these fungi. The highest infection capability showed by *C. tropicalis* directly correlated with the expression and function of $\alpha 5\beta 1$ integrin-like receptor. Conversely, the poor infection capability exhibited by *C. krusei* may be attributable to the failure to express this receptor.

In conclusion, *Candida*-host cell interaction, is a multifactorial phenomenon involving different adhesion pathways. An understanding of the molecular basis of these adhesive interactions would enable the development of new therapeutic strategies; invasive infections may be prevented by specific blockade of fungal adherence receptors with adhesion molecule-based drugs.

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