***Candida albicans* expresses a fibronectin receptor antigenically related to $\alpha 5\beta 1$ integrin**

Giorgio Santoni,¹ Angela Gismondi,² Jin Hong Liu,³ Antonello Punturieri,² Angela Santoni,² Luigi Frati,² Mario Piccoli² and Julie Y. Djeu³

Author for correspondence: Giorgio Santoni. Tel: +39 737 2538. Fax: +39 737 2538.

¹ Chair of General Pathology, University of Camerino, Camerino, Italy

² Department of Experimental Medicine, University of Rome 'La Sapienza', Rome, Italy

³ Moffitt Cancer Center, University of South Florida, Tampa, FL, USA

Cell adhesion molecules, by regulating host–micro-organism interaction, play a major role in the pathogenesis of infectious diseases. The present study was undertaken to investigate the expression of the fibronectin (FN) receptor prototype, $\alpha 5\beta 1$ integrin, on *Candida albicans* and its involvement in the adhesion to FN. By immunofluorescence and fluorescence activated cell sorter (FACS) analysis, several monoclonal antibodies (mAbs) directed against human $\alpha 5$ or $\beta 1$ integrin subunits, or two different antisera to FN receptor positively stained *C. albicans* yeast and germ tube phases, this immunoreactivity increasing upon germ tube transition. Twenty-five to thirty per cent of [³H]glucose-labelled *Candida* yeasts specifically adhered to FN and this adhesion was increased upon germ tube transition. *C. albicans* yeast and germ tube forms bound to an RGD-containing 120 kDa tryptic fragment of FN and adhesion to FN was markedly inhibited by GRGDSP, but not GRGESP peptides. Moreover, binding of both *C. albicans* phases to FN was strongly inhibited by anti- $\alpha 5$ SAM-1 mAb, or both anti-fibronectin receptor (FNr) antisera. Overall these results indicate that *C. albicans* yeast and germ tube phases express a receptor antigenically related to $\alpha 5\beta 1$ integrin which mediates their adhesion to FN. The $\alpha 5\beta 1$ integrin-like receptor expression on *C. albicans* could be relevant for fungus–host interaction and in the dissemination process of *Candida* infection.

Keywords: *Candida albicans*, fibronectin, adhesion, integrins

INTRODUCTION

Candida albicans is a dimorphic fungus normally carried as a commensal organism in man but capable of causing serious disease in the immunocompromised host (Whelan, 1987; Odds, 1988). Secondary candidal infection frequently occurs in patients undergoing immunosuppressive therapy for organ transplantation or chemoradiotherapy for tumour load reduction (Bach *et al.*, 1973; Bodey, 1984; Whimbey *et al.*, 1987). In addition, those suffering from primary immunodeficiencies or human immunodeficiency virus (HIV)-associated acquired im-

mune deficiency syndrome (AIDS) often succumb to persistent candidal infections (Klein *et al.*, 1984; Armstrong, 1987).

C. albicans shows a wide range of infectious capabilities. It normally exists in the mucosal tissues of man as yeast, whereas the mycelium is usually considered the tissue-invading form (Sobel *et al.*, 1984; Odds, 1985). Thus, infections can vary from superficial mucosal lesions to multiple visceral organ involvement and are potentially fatal.

The hallmark of disseminated candidiasis is the presence of metastatic sites of infection throughout the body. The establishment of these metastatic sites presumably occurs following yeast adherence to the endothelial basement membrane and/or subendothelial extracellular matrix (ECM). Adherence to ECM components therefore represents a crucial step in the development of candidiasis and because the ability of *C. albicans* strains to bind ECM

Abbreviations: ECM, extracellular matrix; FITC, fluorescein isothiocyanate; FN, fibronectin; FNr, fibronectin receptor; GaM, goat anti-mouse; GaR, goat anti-rabbit; GaFNr, goat anti-fibronectin receptor; iC3b, inactivated complement receptor type 3; LM, laminin; mAb, monoclonal antibody; MFI, mean fluorescence intensity; NK, natural killer cell; RaFNr, rabbit anti-fibronectin receptor; RaG, rabbit anti-goat.

proteins correlates with the rank order of their relative pathogenicity, it is considered a significant virulence factor for these fungi (Calderone & Braun, 1991; Tronchin *et al.*, 1991; Klotz, 1992).

Micro-organism adherence has become one of the most exciting areas of study in the field of infectious diseases (Finlay, 1990). It implies the recognition of the host tissues by the pathogen and requires the expression of complementary molecules on both yeast and host cell surfaces.

Among the molecules involved in cell adhesion, integrins are a superfamily of highly conserved receptors that mediate cell-matrix and cell-cell interactions in embryogenesis, haemostasis, wound healing, tumour invasion, immune response and inflammation (Albenda & Buck, 1990; Ruoslahti, 1991; Hynes, 1992). They are heterodimers composed of noncovalently linked α and β subunits. At least eight different integrin subfamilies have been characterized on the basis of the association of the distinct α chain with common or related β subunits. In the $\beta 1$ integrin family there are at least four receptors capable of binding fibronectin FN: $\alpha 3\beta 1$, $\alpha 4\beta 1$, $\alpha 5\beta 1$ and $\alpha v\beta 1$ (Hemler, 1990). $\alpha 5\beta 1$ is the first antigen identified as an FN receptor and binds only FN, recognizing the RGD sequence in the central region of the molecule (Takada *et al.*, 1987). Recently, several micro-organisms have been shown to express integrin-like molecules. *Mycobacterium avium*-*Mycobacterium intracellulare* expresses $\alpha 3\beta 1$ and $\alpha 5\beta 1$ integrins which mediate its adhesion to FN (Rao *et al.*, 1992). A $\beta 1$ integrin-like gp120 surface glycoprotein which functions as an FN-binding protein and is required for attachment to alveolar epithelial cells has been described on *Pneumocystis carinii* (Pottratz & Martin, 1990; Pottratz *et al.*, 1991).

C. albicans is known to bind different ECM proteins such as FN, laminin (LM), or collagens (Skerl *et al.*, 1984; Bouchara *et al.*, 1990; Klotz & Smith, 1991; Klotz, 1992). However, the molecular mechanisms involved in these adhesive interactions are still poorly defined. The presence of a specifically cross-reactive protein of a size similar to $\beta 1$ has been reported on *C. albicans* yeast by the use of an antibody to the conserved cytoplasmic domain of this integrin subunit (Marcantonio & Hynes, 1988).

The present study was undertaken to investigate the expression of the FN receptor prototype, $\alpha 5\beta 1$, on *C. albicans* and its involvement in the adhesive interaction with FN. Moreover, we investigated whether changes in the expression and function of this receptor might occur during yeast-mycelial transition, a process which is closely related with increased invasiveness and pathogenicity.

METHODS

Candida albicans. Organisms used in this study were four clinical isolates, kindly provided by Professor A. Cassone (Istituto Superiore di Sanità, Rome, Italy). Yeasts were maintained by weekly subculture into Sabouraud's dextrose agar plates (Unipath) at 24 °C. Before each experiment, a loopful of

yeast cells was removed from the agar slant, washed twice with cold PBS by centrifugation at 3000 r.p.m. for 10 min, and grown to exponential phase at 24 °C in Sabouraud's broth. Growth curves were determined spectrophotometrically by OD₄₂₀ measurements and simultaneous counts. Germ tubes were obtained by plating yeast cells suspended in RPMI 1640 (Flow Laboratories) supplemented with 20 mM D-glucose, 0.5 mM CaCl₂ and MgCl₂ (D-glucose medium) on six-well tissue cultured plates (Costar) for 2 h at 37 °C in 5% (v/v) CO₂. Yeast-mycelium transition was verified microscopically and germ tubes were not less than 95% of total cells. All isolates were tested for their ability to adhere to FN and found to be equivalent. Therefore, the results with a single isolate are presented.

Antibodies. The following mouse mAbs were used: anti-human $\alpha 5$, clone P1D6 (IgG₃) and SAM-1 (IgG_{2b}) (Velde *et al.*, 1988) were purchased from Telios Pharmaceuticals and AMAC, respectively; anti-human $\beta 1$ (4B4) (IgG₁) and A1A5 (IgG_{2b}) (Hemler *et al.*, 1987) were obtained from Coulter 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 (RaFNr) was purchased from Calbiochem; a goat polyclonal antibody, raised against affinity purified human placenta FNr (GaFNr) was provided by Dr G. Tarone (University of Turin, Turin, Italy) (Conforti *et al.*, 1989). F(ab')₂ fragment and Ig fraction of goat polyclonal antibody against human FN were purchased from Sigma and Cappel Laboratories. A polyclonal antibody against LM was purchased from Bethesda Research Laboratories. A mouse mAb to a cell surface glucomannoprotein constituent of *C. albicans* was kindly provided by A. Cassone (Istituto Superiore di Sanità, Rome, Italy) (Tosantucci *et al.*, 1990). Purified fluorescein isothiocyanate (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 and its 120 kDa fragment were purchased from Gibco Laboratories and Calbiochem, respectively. LM was obtained both from a murine Engelbreth-Holm-Swarm sarcoma (Gibco Laboratories) and from human placenta (Telios Pharmaceuticals). Substrate preparations were > 95% pure as checked by SDS-PAGE. BSA was purchased from Sigma. The GRGDSP and GRGESP peptides were obtained from Telios Pharmaceuticals.

Immunofluorescence and flow cytometry. Aliquots of 1×10^6 *C. albicans* yeasts and germ tubes were incubated with the first antibody, appropriately diluted, for 30 min at 4 °C, and then washed twice with cold PBS. Cells were then 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.

The cell populations were analysed for relative fluorescence intensity and the percentage of positive stained cells, determined over 10000 events, was analysed on a FACScan cytofluorimeter (Becton Dickinson). Fluorescent intensity is expressed in arbitrary units on a logarithmic scale. The results are presented both as mean fluorescence intensity and as percentage of positive cells.

Candida labelling. *C. albicans* yeast and germ tube phases were labelled with [³H]glucose (NET-807, D-[5,6-³H]glucose; specific activity 66.6 Ci mmol⁻¹, 2464 GBq mmol⁻¹; Dupont) as previously described (Djeu *et al.*, 1986). Yeast cells (1×10^7) were labelled with [³H]glucose (5 μ Ci) in 0.5 ml PBS supple-

Fig. 1. Reactivity of antibody directed against human $\alpha 5$, $\beta 1$ or $\alpha 5\beta 1$ integrins with *C. albicans* yeasts (a) and germ tubes (b), evaluated by flow cytometric analysis. Dotted areas indicate negative controls. G anti-FNr, goat anti-fibronectin receptor; R anti-FNr, rabbit anti-fibronectin receptor.

Table 1. *C. albicans* yeast and germ tube express an Fnr related to $\alpha 5\beta 1$ integrin

The results are means of four separate experiments, $\pm$ SD.

Antibody*	Yeast		Germ tube	
	Mean fluorescence intensity	Percentage positive cells	Mean fluorescence intensity	Percentage positive cells
GaM	5.4 $\pm$ 0.8	2.0 $\pm$ 0.5	9.6 $\pm$ 1.4	3.3 $\pm$ 0.2
SAM-1	15.8 $\pm$ 2.3	26.6 $\pm$ 1.9	56.1 $\pm$ 2.4	76.5 $\pm$ 3.4
P1D6	11.4 $\pm$ 2.3	7.0 $\pm$ 1.4	49.3 $\pm$ 3.3	34.1 $\pm$ 1.6
4B4	7.7 $\pm$ 1.3	6.0 $\pm$ 1.3	39.3 $\pm$ 3.3	41.3 $\pm$ 1.8
A1A5	9.9 $\pm$ 1.4	6.2 $\pm$ 0.8	73.2 $\pm$ 4.0	58.9 $\pm$ 2.6
RaG	7.5 $\pm$ 0.1	4.0 $\pm$ 0.3	8.5 $\pm$ 1.5	5.7 $\pm$ 0.3
GaFnr	17.1 $\pm$ 0.1	32.3 $\pm$ 3.5	45.0 $\pm$ 0.9	80.0 $\pm$ 6.8
GaR	6.7 $\pm$ 1.6	4.8 $\pm$ 0.1	8.9 $\pm$ 1.6	5.0 $\pm$ 0.6
RaFnr	57.5 $\pm$ 4.2	50.6 $\pm$ 3.5	146.3 $\pm$ 3.6	96.6 $\pm$ 3.2

*GaM, goat anti-mouse; RaG, rabbit anti-goat; GaR, goat anti-rabbit; GaFnr, goat anti-fibronectin receptor; RaFnr, rabbit anti-fibronectin receptor.

mented with 0.5 mM CaCl_2 and MgCl_2 for 3 h at room temperature; germ tubes were radiolabelled with [^3H]glucose diluted at 20 μCi per 10^7 cells in D-glucose medium for 1 h at 37 $^\circ\text{C}$, 5% CO_2 . After labelling, micro-organisms were washed twice in cold PBS and resuspended at $5 \times 10^6 \text{ ml}^{-1}$ in PBS supplemented with CaCl_2 and MgCl_2 .

Adhesion assay. Stock preparations of human plasma FN, its proteolytic 120 kDa fragment LM or BSA were diluted in PBS pH 7.4, at a concentration of 75 $\mu\text{g ml}^{-1}$. Protein solution (100 μl) was distributed into 96-well tissue culture flat-bottomed plates (Costar). After overnight incubation at 4 $^\circ\text{C}$, coated plates were washed three times with PBS to remove nonimmobilized protein. Cell suspensions (100 μl) containing $5 \times 10^5 \text{ ml}^{-1}$ and $2.5 \times 10^5 \text{ ml}^{-1}$ radiolabelled *C. albicans* yeast and germ tube cells,

respectively, were allowed to adhere to the protein-coated surface and incubated for 30 min at 37 $^\circ\text{C}$, 5% CO_2 . Optimal cell concentrations for both phases were used as determined by dose-response experiments (data not shown). Adhesion was also 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 trypsin/EDTA (Flow Laboratories) solubilized in 0.5 M NaOH for 10 min at 37 $^\circ\text{C}$. Bound c.p.m. as well as c.p.m. from nonadherent cells plus washes were quantified in a β -scintillation counter. Quadruplicate wells were assayed for each sample.

To rule out the contribution of *C. albicans* binding to plastic or BSA 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 binding to plastic, BSA or LM.

Cell adhesion was calculated as follows:

$$[(\text{adherent cells c.p.m.})/(\text{total c.p.m.})] \times 100$$

where total c.p.m. indicates the sum of nonadherent cells, washes and adherent cells c.p.m. This value routinely exceeded 93% of total cell counts.

Specific binding to FN was calculated as the difference between the total percentage adhesion and the percentage adhesion not inhibited by anti-FN antibody (nonspecific adhesion). Non-specific adhesion of yeast and germ tube never exceeded 10–15%.

Inhibition assay. For inhibition of *C. albicans* adhesion to FN, the binding assay was performed following pretreatment of [³H]glucose radiolabelled *C. albicans* yeast and germ tube cells at 4 °C for 30 min with a RaFNr or GaFNr antiserum, anti- $\alpha 5$ mAb, control antibodies including normal goat and rabbit serum, an anti-glucomannoprotein mAb or different concentrations of GRGDSP and GRGESP peptides. In some experiments, the inhibition of *C. albicans* yeast binding to FN was performed by adding antibodies to the adhesion assay.

Statistical analyses. Statistical analysis by Student's *t* test was performed by the STATPAC Computerized Program; a *P* value of less than 0.05 was used as the significance criterion.

RESULTS

Expression of a receptor antigenically related to $\alpha 5\beta 1$ integrin by *C. albicans* yeast and germ tube phases

Candida albicans has been shown to bind to FN (Skerl *et al.*, 1984; Klotz & Smith, 1991); nevertheless, the molecular basis of this interaction has not yet been clearly defined. In an attempt to investigate whether the FN receptor prototype, $\alpha 5\beta 1$ integrin, could be expressed by this organism, *C. albicans* yeast and germ tube phases were stained with a panel of mAbs directed against the $\alpha 5$ (SAM-1, P1D6) or $\beta 1$ (A1A5, 4B4) subunits of human FNr, or with a rabbit or a goat polyclonal antibody raised against the FNr purified from human placenta, and analysed by flow cytometry. As shown in Fig. 1(a) and Table 1, anti- $\alpha 5$ clone SAM-1 reacted with yeast cells; some reactivity, although marginal, was detected with another anti- $\alpha 5$ clone (P1D6). Positive staining of yeasts was also observed with both anti-FNr polyclonal antibodies, while both mAbs directed against $\beta 1$ chain (A1A5, 4B4) were negative.

We then investigated whether the expression of $\alpha 5$ and $\beta 1$ integrin subunits was affected upon germ tube transition. Germ tube transformation resulted in a marked increase of $\alpha 5$ or $\beta 1$ integrin expression, and anti- $\beta 1$ A1A5 and 4B4 mAbs, which did not react with *C. albicans* yeast, positively stained the germ tube form (Fig. 1b, Table 1). Since cell size increases during yeast–mycelial transition, reactivity of a mAb directed against a cell surface glucomannoprotein constituent was tested as control; decreased expression on germ tubes was found as previously described by Torosantucci *et al.* (1990) (data not shown).

A time-course study to follow the changes of integrin subunit expression during germ tube transition (Fig. 2) showed that an increase in both $\alpha 5$ and $\beta 1$ was already evident at 1 h, and continued throughout the 3 h of the experiment.

Overall these results suggest that *C. albicans* expresses an $\alpha 5\beta 1$ -like integrin receptor that is upregulated during germ tube transition.

C. albicans adhesion to FN: dose response and time course

The ability of *C. albicans* yeast or germ tube phases to bind to FN was evaluated by incubating [³H]glucose-labelled micro-organisms on FN-coated plates for 30 min at 37 °C. As shown in Fig. 3, adhesion to FN increased with the increase of protein concentration; at an FN concentration of 75 $\mu\text{g ml}^{-1}$ a total of 32% yeasts and 50% germ tubes bound to FN. Because no further increase in adhesion was observed at higher substrate concentrations (100 $\mu\text{g ml}^{-1}$) (data not shown), 75 $\mu\text{g ml}^{-1}$ was the concentration chosen for all the subsequent experiments.

The ability of *C. albicans* yeasts and germ tubes to adhere to immobilized FN (75 $\mu\text{g ml}^{-1}$) as a function of time was

Fig. 2. Enhancement of $\alpha 5$ and $\beta 1$ integrin expression on *C. albicans* upon germ tube transition: a time-course study. Reactivity of SAM-1 (●) and P1D6 (○) anti- $\alpha 5$ mAb or 4B4 (▲) and A1A5 (□) anti- $\beta 1$ mAb was evaluated by indirect immunofluorescence and flow cytometric analysis at different times during germ tube transition. Control samples (△) were stained with second-step reagent alone (GaM). Data are expressed as the mean of mean fluorescence intensity (MFI) (a) or of the percentage positive cells (b) $\pm$ SD (four separate experiments).

Fig. 3. Adhesion of *C. albicans* yeasts and germ tubes to different doses of FN. The adhesion of [³H]glucose labelled *C. albicans* yeast (○) and germ tubes (●) was evaluated after 30 min incubation at 37 °C. The results are presented as the mean of percentage adhesion $\pm$ SD (five separate experiments).

also investigated (data not shown) and was found to be maximal at 30 min for both the *Candida* forms.

C. albicans yeasts and germ tubes specifically bind to FN

To determine whether *C. albicans* cells specifically bind to FN, their ability to adhere to FN-coated plates in the presence of an anti-FN serum was analysed (Fig. 4). A polyclonal antibody against human plasma FN, or its F(ab')₂ fragment (data not shown), completely inhibited the adhesion of *C. albicans* yeasts and germ tubes to FN in a dose-dependent manner, but did not affect the adhesion to LM- or BSA-coated plates. By contrast, a polyclonal

Fig. 5. Total (■) and specific (▨) binding of *C. albicans* yeast and germ tube forms to FN and its 120 kDa proteolytic fragment (a) Specific adhesion of [³H]glucose-labelled *C. albicans* yeasts and germ tubes to FN was evaluated in the presence of a saturating dose (1:25) of an anti-FN antiserum. The percentage cell adhesion was determined after 30 min of incubation at 37 °C. The results are presented as the mean percentage adhesion $\pm$ SD (six separate experiments). All values are significantly different (* P < 0.0005) when calculated by comparing both total and specific binding of yeasts to FN with those of germ tubes respectively. (b) Specific adhesion of [³H]glucose-labelled *C. albicans* yeasts and germ tubes to 120 kDa FN proteolytic fragment (75 µg ml⁻¹) was tested in the presence of a saturating dose (1:25) of an anti-FN antiserum. The percentage cell adhesion was determined after 30 min incubation at 37 °C. The results are presented as the mean percentage adhesion $\pm$ SD of three separate experiments. All values are significantly different (* P < 0.01; ** P < 0.05) when calculated by comparing both total and specific binding of yeasts to 120 kDa FN proteolytic fragment with those of germ tubes, respectively.

antibody anti-human LM that specifically inhibited the adhesion of *C. albicans* yeasts to LM, did not affect adhesion to FN. Overall these results indicate that *C.*

Fig. 4. *C. albicans* yeasts and germ tubes specifically bind to FN. The adhesion of [³H]glucose-labelled *C. albicans* yeasts (a) and germ tubes (b) to 75 µg ml⁻¹ of FN, LM or BSA was tested in the presence of different doses of an anti-FN or anti-LM serum (1:25) after 30 min incubation at 37 °C. The results are presented as the mean of quadruplicate determinations and are representative of three separate experiments. C, control (no antiserum).

Fig. 6. Inhibition of *C. albicans* yeast or germ tube adhesion to FN by an RGD-containing peptide. Adhesion of [³H]glucose-labelled *C. albicans* yeasts and germ tubes to FN (75 µg ml⁻¹) was tested in the presence of different concentrations of GRGDSP (□, yeast; ○, germ tube) or GRGESP (■, yeast; ●, germ tube) synthetic peptides. The percentage cell adhesion was determined after 30 min incubation at 37 °C. The results, presented as percentage inhibition of specific adhesion, are the mean of quadruplicate determinations and are representative of three separate experiments. Specific cell adhesion calculated in the presence of a saturating dose (1:25) of an anti-FN antiserum was 91% for the yeast and 85% for the germ tube phase.

albicans yeasts specifically adhere to FN and this binding is increased during germ tube transition (Fig. 5a).

Adhesion of *C. albicans* yeasts and germ tubes to FN is mediated by an $\alpha 5 \beta 1$ -like integrin receptor

Inhibition of *C. albicans* yeast and germ tube adhesion to FN by RGD-containing peptides. Studies on mammalian cells have shown that $\alpha 5 \beta 1$ recognizes the RGDS binding site in the central region of the FN molecule (Takada *et al.*, 1987). To investigate the possible involvement of $\alpha 5 \beta 1$ in the interaction of *C. albicans* with FN, the adhesion assay was performed in the presence of an RGD-containing peptide (Fig. 6). Pretreatment of *C. albicans* yeasts and germ tubes with different doses of GRGDSP markedly, although not completely, inhibited the adhesion of both phases of *C. albicans* to FN in a dose-dependent manner. No inhibition was observed in the presence of different doses of the control peptide GRGESP.

***C. albicans* yeasts and germ tubes adhere to the 120 kDa fragment of FN.** To further investigate whether the binding of *C. albicans* yeasts and germ tubes was RGD-dependent, we tested their ability to adhere to the 120 kDa FN fragment that contains the RGDS cell-binding site (Pierschbacher *et al.*, 1981).

As shown for FN (Fig. 5a), specific binding to the 120 kDa fragment of FN (Fig. 5b) also increased upon germ tube transition, although to a lesser extent.

Fig. 7. Effect of anti- $\alpha 5$ or anti $\alpha 5 \beta 1$ antibody on *C. albicans* yeast and germ tube adhesion to FN. [³H]Glucose-labelled *C. albicans* yeasts (a) and germ tubes (b) were pretreated for 30 min at 4 °C with different dilutions of anti- $\alpha 5$ (●) (SAM-1), goat anti-FNr antiserum (GaFNr) (□), rabbit anti-FNr antiserum (RaFNr) (△) and a normal goat or rabbit Ig or a mouse mAb directed against a glucomannoprotein as control antibodies (■), and then tested for binding to FN (75 µg ml⁻¹). The percentage cell adhesion was determined after 30 min incubation at 37 °C. The results, presented as percentage inhibition of specific adhesion, are the mean of quadruplicate determinations and are representative of three separate experiments. Specific cell adhesion calculated in the presence of a saturating dose (1:25) of an anti-FN antiserum was 90% for yeast and 85% for germ tube phase. None of the control antibodies inhibited adhesion and, for simplicity, the results of only normal rabbit Ig are presented.

Inhibition of *C. albicans* yeast and germ tube adhesion to FN by $\alpha 5 \beta 1$ or anti-FNr antibodies. The involvement of an $\alpha 5 \beta 1$ -like integrin in adhesion of *C. albicans* to FN was further investigated by testing the ability of antibodies directed against $\alpha 5$ chain or the $\alpha 5 \beta 1$ heterodimer to inhibit yeast and germ tube binding to FN (Fig. 7). Pretreatment of *C. albicans* cells with different doses of anti- $\alpha 5$ SAM-1 mAb completely blocked the adhesion of both yeasts and germ tubes to FN. Moreover, a GaFNr or RaFNr antiserum strongly inhibited both yeast and germ tube binding to FN. A slight inhibition (27%) was found in the presence of the anti- $\beta 1$ 4B4 mAb (data not shown), whereas no inhibition was observed with normal rabbit Ig, normal goat Ig or an anti-glucomannoprotein mAb used as control. In some experiments the inhibition of *C. albicans* binding to FN was evaluated by directly adding the antibodies to the adhesion assay and similar results were found (data not shown).

DISCUSSION

The present study is the first description of a surface receptor on yeast and germ tube phases of *C. albicans* that shares antigenic homology with the $\alpha 5 \beta 1$ integrin receptor. Flow cytometric analysis indicates that certain antibodies that recognize human $\alpha 5$, $\beta 1$ or $\alpha 5 \beta 1$ hetero-

dimer, specifically bind to *C. albicans* yeasts or germ tubes. Moreover, germ tube transition results in increased expression of $\alpha 5$ or $\beta 1$ integrin subunit. We tend to rule out the possibility that up-regulation of receptor expression may be attributable to changes of *C. albicans* cell size occurring during hyphal transformation; a decreased expression of a glucomannoprotein constituent of *C. albicans* cell surface was observed in the germ tube phase as previously described (Torosantucci *et al.*, 1990).

A specifically cross-reactive protein with similar size to the $\beta 1$ integrin was previously detected in *C. albicans* by immunoblotting with an antibody to the conserved cytoplasmic domain of this subunit (Marcantonio & Hynes, 1988). No evidence was provided, however, on the expression of α chains which could possibly be associated with the $\beta 1$ chain. At least eight different α integrin subunits have been described so far which may associate with $\beta 1$ to form a variety of functionally different ECM receptors (Hynes, 1992). Our results suggest that in *C. albicans* $\beta 1$ might associate with an $\alpha 5$ -like chain to create a functional FN receptor.

The presence of a member of the $\beta 1$ integrin family in *C. albicans* is not surprising. $\beta 1$ integrins are evolutionarily conserved receptors whose expression is not restricted to eukaryotes. Recently, several micro-organisms have been shown to express integrin-like $\beta 1$ molecules (see Introduction). Moreover the expression of other receptors antigenically related to integrins has been already described in *C. albicans*. Heidenreich & Dierich (1985), Edwards *et al.* (1986) and Mayer *et al.* (1990) demonstrated the expression of a receptor antigenically identical or similar to mammalian cell iC3b receptor that belongs to the $\beta 2$ integrin family (Kishimoto *et al.*, 1989). An α chain similar to the human neutrophil α_M (CD11b) has also been identified both in *C. albicans* blastoconidia (Hostetter *et al.*, 1990) and on pseudohyphae (Eigentler *et al.*, 1989). Interestingly, as in our study, expression of iC3b receptor was increased upon germ tube transition (Gustafson *et al.*, 1991).

The $\alpha 5\beta 1$ -like receptor we found in *C. albicans* is functional, being capable of mediating adhesion to immobilized FN. Indeed 30% of *C. albicans* yeasts specifically adhered to FN and this adhesion increased during germ tube transition. Both yeasts and germ tubes bound to a 120 kDa tryptic fragment of FN that contains the RGDS sequence recognized by $\alpha 5\beta 1$ (Pierschbacher *et al.*, 1981); in addition, the adhesion to FN was RGD-dependent, being inhibited by the GRGDSP, but not GRGESP peptides. A more direct demonstration that an $\alpha 5\beta 1$ -like integrin is involved in the interaction of *C. albicans* with FN was obtained by using functional antibodies directed against $\alpha 5$, or $\alpha 5\beta 1$ FNr that completely blocked specific adhesion to FN of both yeast and germ tube phases.

Since *C. albicans* strongly binds to plastic surfaces that are commonly used in adherence assays (Rotrosen *et al.*, 1986), the specificity of binding to FN was evaluated by performing the adhesion assay in the presence of saturating doses of anti-FN antibody, which inhibited binding to FN, but not to LM or BSA.

Our results indicate that a receptor antigenically related to $\alpha 5\beta 1$ is the primary mediator of adhesion to FN in both *C. albicans* cell phases. Expression of $\alpha 5$ and $\beta 1$ subunits increases during germ tube transition, and this increase is associated with enhanced adhesion.

Binding of *C. albicans* to FN has been previously evaluated using FN in either a soluble or immobilized form. In the reports in which a solid-phase assay was used, an appropriate specificity control was not always present (Skerl *et al.*, 1984; Scheld *et al.*, 1985; Klotz & Smith, 1991).

A FNr with a dissociation constant of 1.1×10^{-8} has been described and the number of binding sites for FN per yeast cells was ~ 8000 , with more receptors being expressed by the germ tube form. RGD, GRGESP and GRGDTP were capable of inhibiting ^{125}I -labelled FN binding to the yeasts whereas GRGDSP had no activity; when immobilized FN was used as substrate, only soluble FN and GRGESP caused significant inhibition of yeast adherence, while RGD, GRGDSP and GRGDTP peptides were ineffective. These observations are inconsistent with the functional characteristics of any of the mammalian integrin FNr previously described. GRGESP is usually inactive in inhibiting the adherence of mammalian cells to FN and therefore often serves as negative control (Pierschbacher *et al.*, 1981). Also in our study GRGESP failed to inhibit both yeast and germ tube adhesion to FN. Different experimental conditions may account for the discrepancies with the previous report (Klotz & Smith, 1991).

Expression of an FNr antigenically related to $\alpha 5\beta 1$ on *C. albicans* yeasts and germ tubes appears to be particularly significant with respect to its interaction with host tissues. *C. albicans* binds to epithelial and endothelial cells both *in vivo* and *in vitro* (Douglas, 1987; Klotz, 1987; Calderone & Braun, 1991; Klotz, 1992; Bendel & Hostetter, 1993; Hostetter, 1994). Adhesion to epithelial cells appears to involve either a lectin-like protein (Douglas, 1987) or a cell surface mannan; recently, however, the involvement of FN in the interaction of *C. albicans* with genital mucosal surface has been reported (Kalo *et al.*, 1988). Binding of *C. albicans* to endothelium is RGD-dependent (Gustafson *et al.*, 1991) and most likely involves one or more integrin receptors. In this regard, the possibility that iC3b may function as *Candida* adhesin for human endothelium has been suggested (Gustafson *et al.*, 1991).

A role for integrin receptor(s) in the development of infection is also suggested by *in vivo* experiments. Thus, a peptide which contains the RGD sequence of FN (PepTite-2000) has been reported to affect the distribution and the fate of intravenously administered *C. albicans* yeast cells (Klotz *et al.*, 1992). The heart, kidney and liver of peptide-treated animals had significantly fewer c.f.u. of fungus; moreover, the RGD-containing peptide reduced the number of renal abscesses that were formed. The mechanisms by which RGD affects the pathogenesis of disseminated candidiasis are unclear. Inhibition of fungal adherence to vasculature may be a critical event to prevent dissemination. However, involvement of natural immun-

ity effector mechanisms should be also considered. It has been recently shown that FN or RGD-containing peptides increase hepatic killing of *C. albicans* in the isolated perfused mouse liver model (Sawyer *et al.*, 1992); thus RGD peptides, by binding to a $\alpha 5\beta 1$ -like receptor on the surface of *C. albicans*, may serve as opsonins promoting fungus killing by Kupffer cells.

C. albicans interacts also with natural killer (NK) cells, enhancing their cytotoxic activity and inducing the release of tumour necrosis factor α and gamma interferon, which activate the fungicidal activity of neutrophils and macrophages (Marconi *et al.*, 1985; Djeu & Blanchard, 1987). The receptor(s) mediating this interaction have not yet been identified. Expression of both FN and $\alpha 4\beta 1$ and $\alpha 5\beta 1$ integrin FN receptors on the NK cell surface has been described (Santoni *et al.*, 1989; Gismondi *et al.*, 1991). It can be hypothesized that *C. albicans*-NK cell interaction may involve the $\alpha 5\beta 1$ -like integrin receptor on *Candida* which binds either FN associated on NK cell surface or soluble FN functioning as an opsonic bridge.

Altogether, this study suggests an important role for an $\alpha 5\beta 1$ -like integrin receptor as a mediator of *Candida*-host cell interaction. The understanding of the mechanisms mediating *C. albicans* adherence to host structures could be of potential benefit in 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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