

## ORIGINAL ARTICLE

# Tissue invasiveness and non-acidic pH in human candidiasis correlate with "in vivo" expression by *Candida albicans* of the carbohydrate epitope recognised by new monoclonal antibody 1H4

C Monteagudo, A Viudes, A Lazzell, J P Martinez, J L Lopez-Ribot

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**Background:** The morphogenetic conversion between yeast and hyphal growth forms appears to be crucial in the pathogenesis of invasive candidiasis, and can be regulated by environmental signals such as extracellular pH.

**Aims:** To characterise the epitope recognised by monoclonal antibody 1H4, and to evaluate the expression of its corresponding epitope in *Candida albicans* cells under different conditions of pH and temperature, and "in vivo", in tissue samples from patients with human candidiasis.

**Methods:** Monoclonal antibody 1H4 was generated against the 58 kDa cell wall mannoprotein of *C albicans* (mp58), and was further characterised by immunoblot analysis, periodate treatment of the antigenic preparations, and agglutination experiments of *C albicans* strains 3153A, SC5314, and 412, cultured under different environmental conditions (growth media and pH). An immunohistochemical study was performed in 24 human tissue samples from patients with mucocutaneous and systemic candidiasis.

**Results:** 1H4 recognises a pH sensitive carbohydrate epitope on the surface of *C albicans* cells, and this epitope is not restricted to mp58, but is shared with other cell wall mannoproteins. Immunohistochemical findings indicated that expression of the 1H4 epitope on *C albicans* cells in tissue sections from human candidiasis correlates with tissue invasion and pH of the niche. 1H4 immunoreactivity was also found in candida remnants within macrophages.

**Conclusions:** The fact that 1H4 epitope expression selectively identifies invasive forms of *C albicans*, in addition to candida remnants within macrophages, supports its potential value in the diagnosis and management of human candidiasis.

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The yeast *Candida albicans* is the most common fungal pathogen of humans, and the third or fourth most common microorganism isolated from blood cultures in the USA.<sup>1,2</sup> In normal individuals, this organism colonises the gastrointestinal tract, vagina, and some cutaneous areas. Opportunistic superficial and systemic *C albicans* infections develop in premature newborns, patients with AIDS, and debilitated patients with cancer, and are particularly frequent and severe after bone marrow transplantation. These opportunistic infections are believed to have an endogenous origin.<sup>2</sup>

Most authors agree that the ability of *C albicans* to invade host tissues is largely dependent on morphogenetic conversion between the yeast and the filamentous forms.<sup>2–4</sup> Yeast cells and hyphae may encounter different microniches within the host. In addition to temperature and serum, extracellular pH is an important environmental cue that regulates the transition between the yeast and the hyphal growth forms.<sup>5–8</sup>

"Most authors agree that the ability of *Candida albicans* to invade host tissues is largely dependent on morphogenetic conversion between the yeast and the filamentous forms"

Here, we report the generation and characterisation of a monoclonal antibody (1H4), which recognises an epitope within the carbohydrate moiety of the immunodominant 58 kDa cell wall mannoprotein of *C albicans* (mp58), and evaluate the expression of this epitope in *C albicans* cells in

culture, under different conditions of pH and temperature. In addition, we have studied by immunohistochemistry the differential expression of this epitope "in vivo" in a variety of human tissues from patients with superficial and systemic candidiasis.

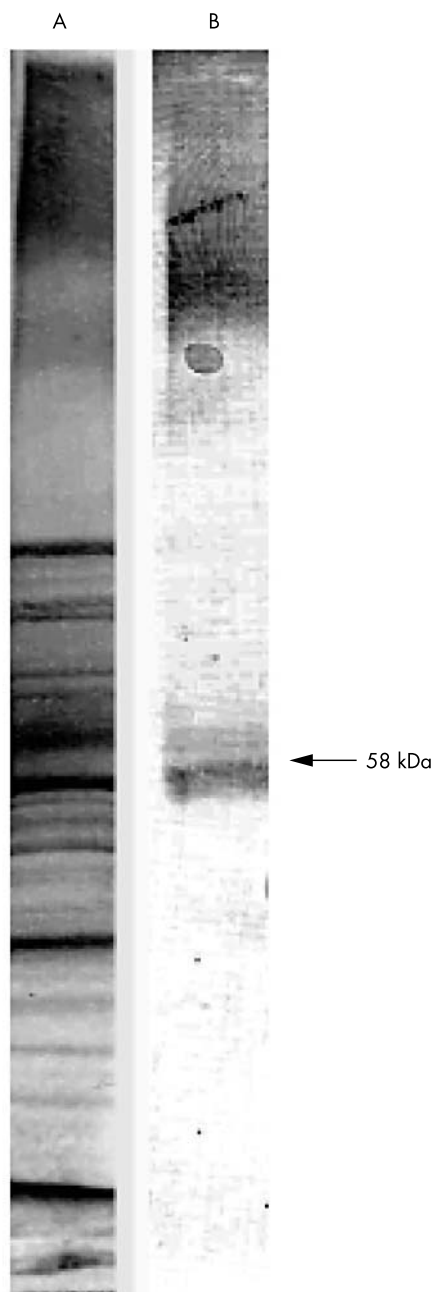
## MATERIAL AND METHODS

### Organisms, culture conditions, and preparation of cell wall extracts

*Candida albicans* strain 3153A was maintained on Sabouraud medium containing 2% (wt/vol) agar. Yeast cells were grown in suspension culture in the medium of Lee *et al* at 22°C.<sup>9</sup> Germ tubes were induced from stationary phase yeast cells by incubating at 37°C in the same medium for four to six hours. Cell wall extracts were prepared from intact cells (germ tubes) by treatment with  $\beta$  mercaptoethanol ( $\beta$ ME), as described previously.<sup>10</sup> The total sugar content in the extract was determined colorimetrically, with mannose as the standard.

In another series of experiments (agglutination, see below), liquid cultures of *C albicans* strains 3153A, SC5314, and 412<sup>11</sup> were obtained by overnight incubation at different temperatures in different media, including yeast peptone dextrose (YPD; 1% wt/vol yeast extract, 2% wt/vol peptone, 2% wt/vol dextrose; US Biological, Swampscott,

**Abbreviations:**  $\beta$ ME,  $\beta$  mercaptoethanol; BSA, bovine serum albumin; ELISA, enzyme linked immunosorbent assay; mp58, 58 kDa cell wall mannoprotein; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; TBS, Tris buffered saline; YPD, yeast peptone dextrose



**Figure 1** Immunoblot analysis to assess the reactivity of monoclonal antibody 1H4 with *Candida albicans* cell wall materials. (A) Reactivity of a rabbit polyclonal antiserum containing antibodies against most moieties present in the *C. albicans*  $\beta$  mercaptoethanol extract. (B) Reactivity of monoclonal antibody 1H4 against materials present in the cell wall extracts.

Massachusetts, USA), Lee, and RPMI 1640 (Angus Buffers and Chemicals, Niagara Falls, New York, USA) that had been previously adjusted to neutral (6.8–7.2) or acidic (4.0) pH.

#### Purification of *C. albicans* mp58

For purification of mp58, components in the ME were separated by preparative sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) under denaturing conditions. Briefly, about 10 mg (based on total sugar content) of the corresponding ME extract was applied to a 13 cm wide, 20 cm high 5–15% polyacrylamide slab gradient

gel. Prestained molecular weight standards (Gibco-BRL, Life Technologies Inc, Gaithersburg, Maryland, USA) were run in parallel in a single reference well formed to one side of the resolving gel slab. The transverse section of the gels corresponding to mp58 (as identified by Coomassie staining) were excised, crushed, and the polypeptide moieties electroeluted.<sup>12</sup>

#### Generation of 1H4 monoclonal antibody

Two BALB/c mice were immunised with 25  $\mu$ g of mp58 purified by preparative electrophoresis and subsequent electroelution from the gel slice (see above). Immunisation protocols consisted of a first injection (using complete Freund's adjuvant), two subsequent booster injections (with incomplete Freund's adjuvant) at three week intervals, and one final booster injection without adjuvant three days before fusion (all injections were subcutaneous). For hybridoma production, mice were sacrificed and their spleens removed aseptically. Antibody secreting cells were isolated and mixed with myeloma cells (NS1) using dropwise addition of polyethylene glycol. After the fusion, cells were diluted in selective medium and plated at low densities in multiwell tissue culture dishes. Hybridoma cell lines secreting antibodies against *C. albicans* mp58 were screened by enzyme linked immunosorbent assay (ELISA) and immunoblot procedures and single cell subcloned by the limiting dilution method. A hybridoma cell line producing a monoclonal antibody designated 1H4 (an IgG1 as determined by an isotyping kit; Zymed, South San Francisco, California, USA) was established. Ascitic fluid for this 1H4 cell line was prepared by intraperitoneal injection of the hybridoma cells in pristane primed mice.

#### Immunoblot analysis

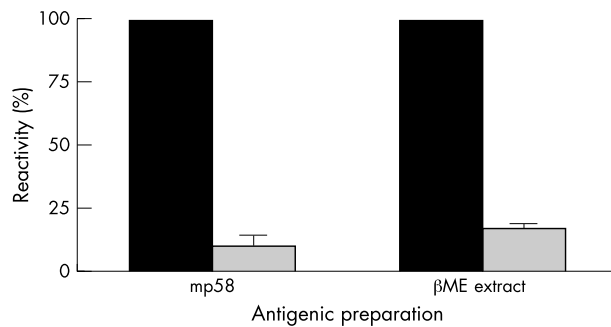
For immunoblotting, materials present in the *C. albicans* 3153A ME extract were separated by SDS-PAGE using precast 4%–15% gradient minigels (Bio-Rad, Hercules, California, USA) and transferred to nitrocellulose membranes. After blocking the membranes in Tris/HCl buffer plus 0.9% (wt/vol) NaCl (Tris buffered saline; TBS) containing 3% bovine serum albumin (BSA; wt/vol), they were incubated in the presence of monoclonal antibody 1H4 (diluted 1/1000 in TBS with 0.05% Tween 20 and 1% BSA) or with a rabbit polyclonal antiserum generated against *C. albicans* cell wall extracts.<sup>13</sup> Peroxidase labelled goat antimouse or antirabbit immunoglobulins (Bio-Rad) were used as secondary antibodies. Coloured reactive bands were developed with  $H_2O_2$  and 4-chloro-1-naphthol as the chromogenic reagent.

#### Periodate treatment and agglutination experiments

Periodate treatment of the antigenic preparations initially reactive with monoclonal antibody 1H4 was carried out using a modified ELISA assay.<sup>14</sup> Slide agglutination tests were performed by directly mixing 15  $\mu$ l of overgrown tissue culture supernatants from the hybridoma secreting monoclonal antibody 1H4 with 30  $\mu$ l of yeast cell suspensions (approximately  $2 \times 10^8$  cells/ml). These preparations were mixed for one minute at room temperature and agglutination determined with the unaided eye and by low power bright field microscopy. Agglutination reactions were scored as very strong (+++), strong (++) , medium (+), weak (+/-), or no agglutination (-).

#### Tissues

Twenty four human tissue samples from patients with mucocutaneous and systemic candidiasis were retrieved from the files of the department of pathology, Hospital Clinico Universitario, Valencia, Spain. Fourteen samples were obtained during postmortem examination, and 10 were



**Figure 2** Effect of periodate oxidation of *Candida albicans* mp58 and a *C. albicans*  $\beta$  mercaptoethanol ( $\beta$ ME) extract on monoclonal antibody 1H4 reactivity examined by a modified enzyme linked immunosorbent assay. Values are expressed as per cent reactivity compared with control (untreated) samples (100%). Error bars indicate standard deviations. Black bars, untreated samples; shaded bars, 10mM sodium periodate treatment.

incisional or endoscopic biopsies. These cases have been extensively characterised.<sup>15 16</sup>

### Immunohistochemistry

The immunohistochemical study was performed on 4  $\mu$ m thick, paraffin wax embedded tissue sections by the avidin–biotin immunoperoxidase technique, with diaminobenzidine as chromogen, as described previously.<sup>15</sup> 1H4 culture supernatant (1/5 dilution) was used as the primary antibody (45 minutes). All incubations were performed at room temperature. Antigen retrieval techniques were not applied. Incubation of adjacent tissue sections with sialyl-I monoclonal antibody culture supernatant (a gift from Dr Hakomori, Biomembrane Institute, Seattle, USA) as the primary antibody served as negative control.<sup>16</sup>

## RESULTS

### Establishment of a monoclonal antibody producing hybridoma and characterisation of 1H4

We established a hybridoma cell line producing a monoclonal antibody designated 1H4. This antibody was an IgG1 class antibody, which was reactive with *C. albicans* cell wall extracts and purified mp58 in ELISA experiments. Immunoblotting experiments indicated that monoclonal antibody 1H4 recognised mp58 (used to immunise the mice) but also a higher molecular mass polydispersed material present in the cell wall extracts of *C. albicans* (fig 1).

Two early observations led us to believe that the epitope recognised by monoclonal antibody 1H4 may be a carbohydrate. First, in epitope mapping experiments this antibody recognised none of the overlapping peptides encompassing the entire sequence of the protein portion of *C. albicans* mp58

(not shown). Second, as shown in fig 1, immunoblot analysis with monoclonal antibody 1H4 demonstrated its cross-reactivity with high molecular mass, highly polydispersed material, which is made up mainly of carbohydrate. As shown in fig 2, the antigenic determinants that reacted with the monoclonal antibody were sensitive to periodate treatment, and 10mM sodium periodate treatment almost completely abolished binding of monoclonal antibody 1H4 to both mp58 and cell wall extracts. These results indicate that the epitope recognised by 1H4 is carbohydrate in nature and that it is not specific for *C. albicans* mp58 but is found in other glycoproteins in the cell wall of the fungus.

### Expression of the epitope recognised by 1H4 under different in vitro growing conditions: pH dependency

Initial observations established that incubation of *C. albicans* yeast cells with monoclonal antibody 1H4 results in agglutination. We took advantage of this property to investigate the expression of the epitope recognised by this monoclonal antibody on the surface of *C. albicans* cells grown under different environmental conditions (growth media and pH). Table 1 summarises results from these agglutination experiments with three different *C. albicans* strains grown as yeast cells on YPD at different temperatures and pHs. Monoclonal antibody 1H4 agglutinated yeast cells from all strains grown on YPD at different temperatures at neutral pH, but failed to agglutinate those grown at acidic pH. These observations indicated the pH dependency of the expression of the epitope recognised by this monoclonal antibody. However, this pH dependency is not absolute, because the antibody agglutinated (although to a much lesser extent) the same *C. albicans* strains when grown as yeast cells in different media (Lee and RPMI).

### Immunohistochemistry in human superficial and systemic candidiasis

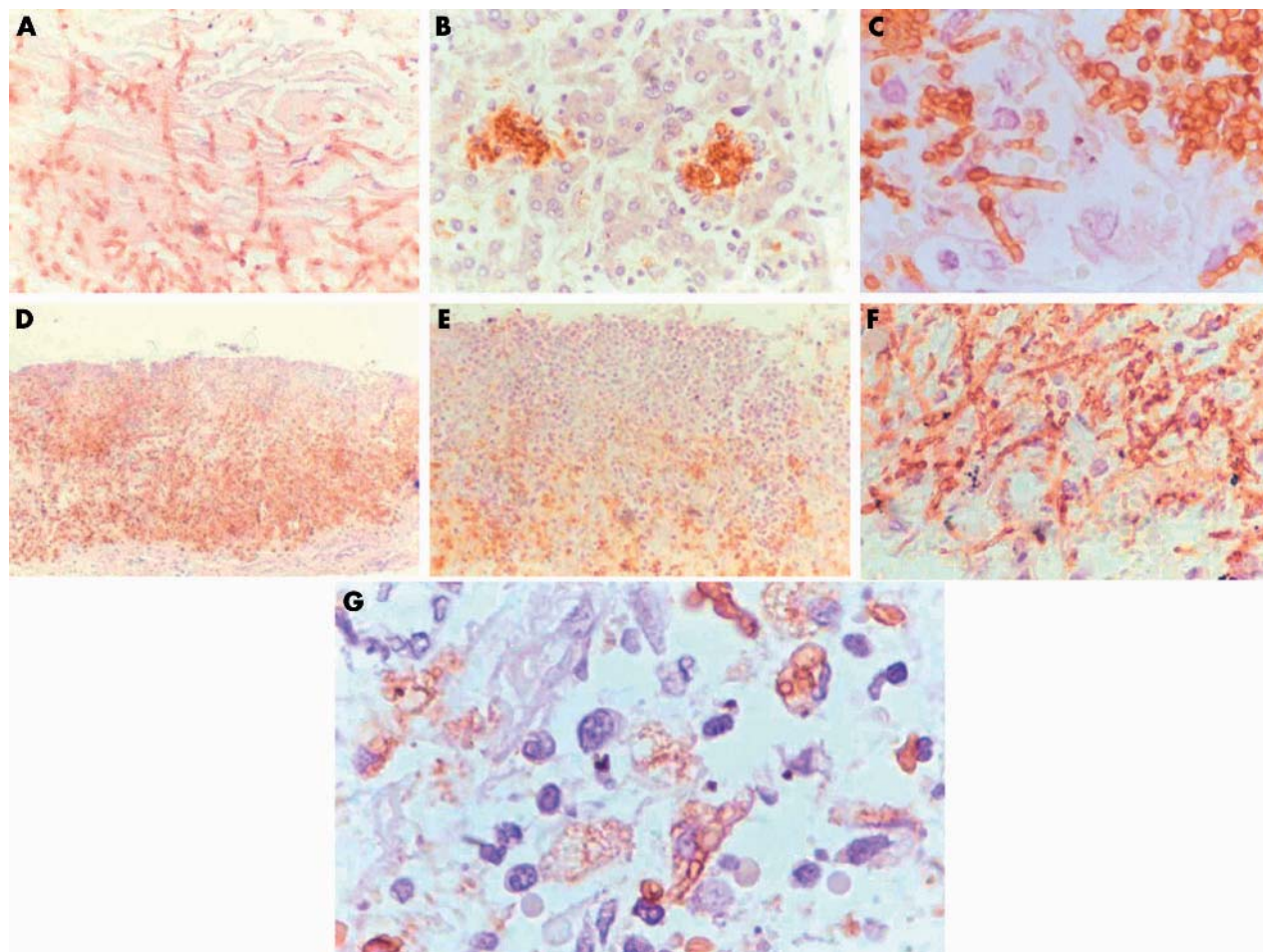
Table 2 shows the immunohistochemical results. In tissue samples from mucosal surfaces with a non-acidic pH, such as the tongue, oesophagus, intestine, and most skin areas, filamentous forms of *C. albicans* predominated, and most of them exhibited both 1H4 immunostaining and an invasive phenotype (fig 3A). In internal organs having a non-acidic pH (liver, lung, heart, and thyroid) from patients with systemic candidiasis, variable numbers of yeast cells were found, together with hyphae or pseudohyphae in virtually all cases. In these tissues, both yeast and filamentous forms showed strong 1H4 immunoreactivity (fig 3B, C). In contrast, in those tissues with an acidic pH, such as the stomach and collecting ducts of the kidney, the predominant form of *C. albicans* was the blastospore (yeast). Interestingly, in these locations yeast cells essentially showed no 1H4 immunoreactivity (fig 3D, E). However, when adjacent tissue invasion

**Table 1** Agglutination tests to examine the expression of the epitope recognised by monoclonal antibody 1H4 on the surface of *Candida albicans* propagated as yeast forms under different growth conditions

Medium	Temperature (°C)	3153A		SC5314		412	
		Neutral pH	Acidic pH	Neutral pH	Acidic pH	Neutral pH	Acidic pH
YPD	24	++	–	+++	–	+	–
	30	++	–	+++	+/-	+++	–
	37	+++	–	+++	+/-	+++	–
RPMI	24	++	+	++	++	++	–
	30	+	+	++	++	+	+
Lee	24	+	+/-	+++	++	++	+/-

Agglutination reactions were scored as follows: +++, very strong; ++, strong; +, medium; +/-, weak; –, no agglutination.

YPD, yeast peptone dextrose.



**Figure 3** 1H4 immunoreactivity in human candidiasis. (A) Invasive candidiasis of the oesophagus, showing positive mycelial cells. (B, C) Systemic candidiasis; positive yeast cells in the liver (B); both mycelial and yeast cells showing immunoreactivity in the thyroid (C). (D–F) Invasive gastric candidiasis; 1H4 negative yeasts are the predominant form in the superficial part (D and E); numerous 1H4 positive invading mycelial cells are seen in the deep part (F). (G) Systemic candidiasis; 1H4 immunostaining is detected in fungal remnants within the cytoplasm of tissue macrophages (immunoperoxidase, haematoxylin counterstain).

**Table 2** Tissue distribution of 1H4 immunoreactivity of *Candida albicans* in human superficial and systemic candidiasis

Tissue	No of cases	1H4 immunoreactivity			
		Epithelial surface		Invasive	
		Yeast	Hyphae	Yeast	Hyphae
Skin	3	–	–	+	+
Tongue	2		+	+	+
Oesophagus	4		+	+	+
Stomach	4	–	+	+/-	+
Intestine	2		+	+/-	+
Urethra	1	+/-	+	+	+
Kidney	2				
Glomeruli				+	+
Tubules				+	+
Collecting ducts				–	+
Bronchus	1	–	+	+	+
Lung	2			+	+
Myocardium	1			+	+
Thyroid	1			+	+
CNS	1			+	+

1H4 immunoreactivity: –, negative; +/-, <50% positive cells; +, >50% positive cells; empty spaces, fungal elements were not present in this form or location.  
CNS, central nervous system.

was present, hyphae or pseudohyphae were the predominant form. Strong 1H4 cell surface immunostaining was seen in most of these filamentous forms of *C albicans* (fig 3F). In some cases of invasive candidiasis, we found 1H4 immunostaining in cellular remnants of degenerated candida organisms within the cytoplasm of phagocytic cells, particularly macrophages (fig 3G). No immunostaining was found using anti-sialyl-I culture supernatant as the primary antibody.

## DISCUSSION

The ability to undergo transition from the yeast to the hyphal form appears to be crucial in the pathogenesis of invasive candidiasis.<sup>4-6</sup> Both yeast cells and hyphae are found in infected tissues and contribute to pathogenesis. Yeast cells are better suited for rapid haematogenous dissemination, but together with hyphal elements they are also capable of breaching epithelial and endothelial barriers to cause extensive organ damage.<sup>4</sup> During the infectious process, yeast cells and hyphae may encounter different microenvironments within the host. At acidic pH, *C albicans* grows mostly in the yeast form; at an alkaline pH, it grows primarily in the filamentous form.<sup>2 6 7</sup> Gastric acid provides an effective barrier to most microorganisms (normal gastric pH values are 1–3.5). In contrast, achlorhydria and the use of H2 antagonists, which raise gastric pH, have been found to be associated with a higher proportion of invasive gastric candidiasis.<sup>17</sup> Similarly, although the skin is relatively inhospitable to fungal growth,<sup>18</sup> the experimental increase of skin surface pH yields more pronounced cutaneous candidiasis in human volunteers.<sup>19</sup>

Alkaline induced filamentation is associated with changes in gene expression. Three genes are known to participate in this alkaline induced process: PRA1/FBP1, PHR1, and RIM101.<sup>7 20 21</sup> Other genes (EFG1, CPH1, TUP1) are involved in non-pH dependent yeast to filament transition.<sup>7</sup> The product of PRA1/FBP1 is mp58, a cell wall mannoprotein, which was initially identified by its binding affinity for fibrinogen.<sup>21-24</sup> The carbohydrate moiety of mp58 (*N* and *O* linked sugar residues) is known to play an important role in fibrinogen binding affinity.<sup>22</sup> PRA1/FBP1 gene expression is regulated by RIM101,<sup>7</sup> a gene for which a role in host cell damage has been suggested.<sup>5</sup> In addition, although the involvement of the protein moiety in the immunogenicity of mp58 has received much attention, the carbohydrate moiety is also expected to play a role.<sup>25</sup> We have shown that the epitope recognised by 1H4 is carbohydrate in nature, and although it is present in mp58 it is not restricted to this glycoprotein, but is also found in other cell wall mannoproteins.

It has recently been hypothesised, with regard to fungal infection, that the type of disease and host response depend on the invasiveness of the particular strain of *C albicans* involved.<sup>3</sup> Because most strains that cause disease are commensals from the patient, unknown regulatory events

are believed to trigger the switch to an invasive state.<sup>3</sup> The main virulence factors of *C albicans* involved in the pathogenesis of candidiasis are: adhesion to host tissues, phase transition (that is, the conversion of yeast cells to filamentous forms), enzymatic activity, and phenotypic switching.<sup>3 26</sup> The morphogenesis of *C albicans* is a highly complex response of the fungal cells to their environment, with no obligate correlation between the strain's ability to form filaments in vivo and in vitro.<sup>6</sup> Moreover, the virulence of *C albicans* is tissue specific,<sup>27</sup> and one of the local factors that greatly influences the virulence of candida organisms is tissue pH.<sup>5 7 26 28</sup> In addition, immunoprotection against candida infection is also site specific.<sup>26</sup>

*“Candida albicans cell surface expression of the epitope recognised by monoclonal antibody 1H4 is significantly increased during invasion”*

Our results support an association of the 1H4 epitope, which is in part linked to the PRA1/FBP1 gene product in the *C albicans* cell wall, with host cell invasion and tissue damage, in addition to the pH dependent phenotype. When the gastrointestinal barrier is iatrogenically altered by diagnostic explorations and treatment, particularly in immunodepressed patients, candida organisms produce germ tubes and invade tissues. We found that *C albicans* cell surface expression of the epitope recognised by monoclonal antibody 1H4 is significantly increased during invasion. As shown in agglutination experiments using different strains and culture conditions, expression of the 1H4 epitope is not exclusively dependent upon pH conditions or the phase form of the candida organisms. The “in vivo” study shows that it can be detected in filamentous forms in niches with an acidic pH, and in invasive yeast forms in internal organs, but not in non-invasive yeast cells.

In conclusion, we have generated and characterised a new monoclonal antibody (1H4) which recognises a pH sensitive carbohydrate epitope on the surface of *C albicans* cells in culture. The in vivo expression of the 1H4 epitope on the surface of *C albicans* cells in tissue sections obtained from cases of human candidiasis correlates mainly with tissue invasion, and secondarily with pH of the niche and morphology. Indeed, our data show a strong correlation between invasiveness and expression of this specific epitope, more so than between invasion and morphology. In addition, the fact that candida remnants within macrophages are also labelled with 1H4 may be helpful to confirm invasive candidiasis in difficult cases. Therefore, this monoclonal antibody may be a useful tool in the evaluation and management of human candidiasis.

## ACKNOWLEDGMENTS

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## Take home messages

- We have developed and characterised a new monoclonal antibody, 1H4, which recognises a pH sensitive carbohydrate epitope on the surface of *Candida albicans* cells, and this epitope is not restricted to mp58, but is shared with other cell wall mannoproteins
- 1H4 epitope expression selectively identifies invasive forms of *C albicans*, and candida remnants within macrophages, and therefore has potential value in the diagnosis and management of human candidiasis

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## REFERENCES

- Odds FC. Pathogenic fungi in the 21st century. *Trends Microbiol* 2000;**8**:200–1.
- El Barkani A, Kurzai O, Fonzi WA, *et al*. Dominant active alleles of RIM101 (PRR2) bypass the pH restriction on filamentation of *Candida albicans*. *Mol Cell Biol* 2000;**20**:4635–47.
- Bernhardt J, Herman D, Sheridan M, *et al*. Adherence and invasion studies of *Candida albicans* strains, using in vitro models of esophageal candidiasis. *J Infect Dis* 2001;**184**:1170–5.
- Gow NA, Brown AJ, Odds FC. Fungal morphogenesis and host invasion. *Curr Opin Microbiol* 2002;**5**:366–71.
- Davis D, Edwards JE Jr, Mitchel AP, *et al*. *Candida albicans* RIM101 pH response pathway is required for host–pathogen interactions. *Infect Immun* 2000;**68**:5953–9.
- Odds FC, Van Nuffel L, Gow NAR. Survival in experimental *Candida albicans* infections depends on inoculum growth conditions as well as animal host. *Microbiology* 2000;**146**:1881–9.
- Davis D, Wilson RB, Mitchell AP. RIM101-dependent and independent pathways govern pH responses in *Candida albicans*. *Mol Cell Biol* 2000;**20**:971–8.
- Mitchel AP. Dimorphism and virulence in *Candida albicans*. *Curr Opin Microbiol* 1998;**1**:687–92.
- Lee KL, Buckley HR, Campbell CC. An amino acid liquid synthetic medium for the development of mycelial and yeast forms of *Candida albicans*. *Sabouraudia* 1975;**13**:148–53.
- Casanova M, Lopez-Ribot JL, Martinez JP, *et al*. Characterization of cell wall proteins from yeast and mycelial cells of *Candida albicans* by labelling with biotin: comparison with other techniques. *Infect Immun* 1992;**60**:4898–906.
- Lopez-Ribot JL, McAtee RK, Lee LN, *et al*. Distinct patterns of gene expression associated with development of fluconazole resistance in serial *Candida albicans* isolates from human immunodeficiency virus-infected patients with oropharyngeal candidiasis. *Antimicrob Agents Chemother* 1998;**42**:2932–7.
- McDonald CSF, Pappin D, Higgins S. Electroelution of proteins from SDS gels. *Trends Genet* 1986;**2**:35.
- Lopez-Ribot JL, Chaffin WL. Binding of the extracellular matrix component entactin to *Candida albicans*. *Infect Immun* 1994;**62**:4564–71.
- Woodward MP, Young WW Jr, Bloodgood RA. Detection of monoclonal antibodies specific for carbohydrate epitopes using periodate oxidation. *J Immunol Methods* 1985;**78**:143–53.
- Monteagudo C, Marcilla A, Mormeneo S, *et al*. Specific immunohistochemical identification of *Candida albicans* in paraffin-embedded tissue with a new monoclonal antibody (1B12). *Am J Clin Pathol* 1995;**103**:130–5.
- Monteagudo C, Lopez-Ribot JL, Murgui A, *et al*. Immunodetection of CD45 epitopes on the surface of *Candida albicans* cells in culture and human infected tissue. *Am J Clin Pathol* 2000;**113**:59–63.
- Goenka MK, Kochhar R, Chakrabarti A, *et al*. *Candida* overgrowth after treatment of duodenal ulcer. A comparison of cimetidine, famotidine, and omeprazole. *J Clin Gastroenterol* 1996;**23**:7–10.
- Wagner DK, Sohnle P. Cutaneous defenses against dermatophytes and yeasts. *Clin Microbiol Rev* 1995;**8**:317–35.
- Runeman B, Faergemann J, Larko O. Experimental *Candida albicans* lesions in healthy humans: dependence on skin pH. *Acta Dermatol Venereol* 2000;**80**:421–4.
- Saporito-Irwin SM, Birse CE, Sypherd PS, *et al*. PHR1, a pH-regulated gene of *Candida albicans*, is required for morphogenesis. *Mol Cell Biol* 1995;**15**:601–13.
- Sentandreu M, Elorza MV, Sentandreu R, *et al*. Cloning and characterization of PRA1, a gene encoding a novel pH-regulated antigen of *Candida albicans*. *J Bacteriol* 1998;**180**:282–9.
- Casanova M, Lopez-Ribot JL, Monteagudo C, *et al*. Identification of a 58-kilodalton cell surface fibrinogen-binding mannoprotein from *Candida albicans*. *Infect Immun* 1992;**60**:4221–9.
- Lopez-Ribot JL, Monteagudo C, Sepulveda P, *et al*. Expression of the fibrinogen binding mannoprotein and the laminin receptor of *Candida albicans* in vitro and in infected tissues. *FEMS Microbiol Lett* 1996;**142**:117–22.
- Lopez-Ribot JL, Sepulveda P, Cervera AM, *et al*. Cloning of a cDNA fragment encoding part of the protein moiety of the 58-kDa fibrinogen-binding mannoprotein of *Candida albicans*. *FEMS Microbiol Lett* 1997;**157**:273–8.
- Viudes A, Perea S, Lopez-Ribot JL. Identification of continuous B-cell epitopes on the protein moiety of the 58-kilodalton cell wall mannoprotein of *Candida albicans* belonging to a family of immunodominant fungal antigens. *Infect Immun* 2001;**69**:2909–19.
- Calderone RA, Fonzi WA. Virulence factors of *Candida albicans*. *Trends Microbiol* 2001;**9**:327–35.
- Taylor BN, Fichtenbaum C, Saavedra M, *et al*. In vivo virulence of *Candida albicans* isolates causing mucosal infections in people with the human immunodeficiency virus. *J Infect Dis* 2000;**182**:955–9.
- De Bernardis F, Muhlschlegel FA, Cassone A, *et al*. The pH of the host niche controls gene expression in and virulence of *Candida albicans*. *Infect Immun* 1998;**66**:3317–25.



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