Differences in the antigenic expression of immunomodulatory mannoprotein constituents on yeast and mycelial forms of *Candida albicans*

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The expression of a strongly immunomodulatory mannoprotein complex (GMP) in the different forms of growth of the human commensal and opportunistic pathogen *Candida albicans* was studied using a monoclonal antibody (mAb AF1) directed against an oligosaccharide epitope of GMP. Immunofluorescence revealed that the surface of the yeast cells was highly reactive with mAb AF1, but that the reactivity was greatly reduced or disappeared during mycelial conversion. This modulation was shared by a number of strains of *C. albicans*, and was not solely a temperature- or nutrition-dependent phenomenon. Hypha-deficient strains (A12 and CA2) did not show variations of surface fluorescence under environmental conditions which were permissive for hyphal conversion (incubation in *N*-acetylglucosamine or Lee's medium, at 37 °C). GMP extracts from yeast and mycelial forms of the fungus were separated into three chromatographically distinct, high molecular mass mannoprotein fractions (F1, F2 and F3), which were tested individually by indirect ELISA for mAb AF1 recognition. All yeast-derived constituents and two (F2 and F3) of the hyphal mannoproteins were recognized by the mAb. The low or absent reactivity of the F1 constituent from hyphal cells was confirmed by immunoblots. Irrespective of their source (yeast or mycelial), all fractions reacted to a similar extent with a polyclonal anti-*Candida* serum. Overall, the data suggest changes in epitope specificity and/or confinement of reactive constituents in the inner wall layers as possible mechanisms of modulated expression of mAb AF1-reactive epitope during mycelial conversion.

Introduction

Cell wall mannoproteins of *Candida albicans* play an important role in the host–parasite relationship. They are major cellular antigens, mediate adhesion of the micro-organism to host cell surfaces, and some of them are virulence enzymes (Odds, 1987; Domer *et al.*, 1988; Cassone, 1990). Recent studies have demonstrated remarkable changes in the surface expression of these molecules during fungal growth and morphogenesis (Poulain *et al.*, 1985). In particular, antigenic determinants exclusively or predominantly expressed on the yeast or hyphal form of growth have been found (Smail & Jones, 1984; Brawner & Cutler, 1986; Sundstrom *et al.*, 1988; Chaffin *et al.*, 1988; Casanova *et al.*, 1989). Modulation of antigen expression may provide the fungus with a potent means to evade the host's immune response or minimize its consequences. It is possible that the pathogenic value of this mechanism is proportional

to the importance that the modulated molecule has in eliciting an efficient immune response by the host. In this context, the aforementioned studies did not establish the immunological or pathogenic role, if any, of the molecules whose expression was modulated. Thus, we sought for possible modulations in the expression of antigenic constituents of a mannoprotein complex (GMP) of *C. albicans* that had been shown to have powerful immunogenic and immunomodulatory activities (Cassone *et al.*, 1987; Scaringi *et al.*, 1988; Ausiello *et al.*, 1989).

Methods

Organisms and growth conditions. All strains of *C. albicans* used in this study were from the collection of the Istituto Superiore di Sanità, Rome. Unless otherwise specified, experiments were performed with strain BP. Details of the origin and characteristics of this strain and of the two agerminative variants (CA2 and A12) used have been given elsewhere (Mattia *et al.*, 1982; Torosantucci *et al.*, 1984). Methods for isolation and characterization of *C. albicans*, including serotype determination, were as previously reported (Mondello *et al.*, 1986).

Abbreviations: mAb, monoclonal antibody; M-GMP, Y-GMP, glucomannan preparations from mycelial and yeast cells, respectively.

The organisms were maintained on malt-extract agar. Liquid, yeast-form cultures were grown in Winge medium (Torosantucci *et al.*, 1984) at 28 °C. Growth in the mycelial form was induced by incubating washed, stationary-phase yeast cells in *N*-acetylglucosamine (GlcNAc) or modified Lee's medium, at 37 °C (Lee *et al.*, 1975; Torosantucci *et al.*, 1984). In GlcNAc medium, germ-tubes were microscopically visible after 60–90 min of incubation, and more than 90% of the original yeast cells bore one or two hyphal elements after 240 min. Agerminative variants A12 and CA2, under the same cultural conditions, produced no germ-tubes, and continued to grow in the yeast form.

Preparation of cell wall mannoprotein complex (GMP) of *C. albicans*. A glucomannoprotein preparation, containing a major cell wall antigenic complex of the fungus, was obtained as previously reported (Ausiello *et al.*, 1986; Scaringi *et al.*, 1988), from both yeast (Y-GMP) and hyphal (M-GMP) cells. Yeasts were grown for 24 h at 28 °C in Winge broth, while hyphal cells were obtained from 24 h cultures in GlcNAc medium at 37 °C (see above). The two antigenic preparations contained comparable amounts of polysaccharides and slightly different amounts of protein, as determined by the phenol/sulphuric acid method of Dubois *et al.* (1956) and by Lowry's method, respectively (see also Table 2).

DEAE-Sephadex chromatography. Y-GMP and M-GMP were fractionated in a 16 × 1 cm column of DEAE-Sephadex A50 (Pharmacia), pre-equilibrated with 0.05 M-Tris/HCl buffer, pH 7.5. Ten milligrams of each antigenic preparation was loaded on the column and, after extensive washing with buffer, bound material was eluted with a linear gradient (0–2 M) of NaCl.

The eluate was collected as 1.5 ml fractions and assayed for total polysaccharide and for absorbance at 260/280 nm. Peak fractions were pooled and either dialysed against 0.2 M-sodium carbonate for use in the ELISA or dialysed against H₂O, lyophilized and resuspended at a suitable concentration for immunoblot analysis.

Antibodies. mAb AF1 was a murine monoclonal antibody of the IgM class raised against Y-GMP (Cassone *et al.*, 1988). Anti-*Candida* serum was obtained in rabbits after subcutaneous immunization with glutaraldehyde-inactivated cells of *C. albicans* (Scaringi *et al.*, 1988). Both antibodies were purified by ammonium sulphate precipitation or by affinity chromatography on a Protein A-Monoclonal Antibody Purification System (MAPS II), as reported elsewhere (Mariani *et al.*, 1989).

Antibody titres were determined as the highest dilution of the antibody giving at least twice the absorbance value of the negative control (irrelevant antibody) in an indirect ELISA (see below), employing Y-GMP (5 µg ml⁻¹) as solid-phase antigen.

ELISA. Antigenic fractions were diluted to different polysaccharide concentrations in 0.2 M-sodium carbonate buffer, pH 9.6. The solutions were dispensed into 96-well polystyrene microtitre plates (Dynatech), at 200 µl per well, and incubated overnight at 4 °C. Antigen solutions were then removed, the wells were saturated for 1 h at room temperature with 3% (w/v) bovine serum albumin (Sigma) and repeatedly rinsed with 0.2 M-phosphate-buffered saline (PBS) containing 0.05% Tween 20. Antibody (200 µl), diluted to the desired concentration in PBS, was subsequently added to the wells, and the plates were incubated for 1 h at room temperature. After several washings with PBS-Tween 20, antigen-bound antibody was revealed by adding goat anti-mouse IgM- or goat anti-rabbit IgG-alkaline phosphatase conjugates (Sigma) for 1 h, then *p*-nitrophenyl phosphate (1 mg ml⁻¹ in 1 M-diethanolamine/HCl, pH 9.8). The reaction was stopped after 20 min by adding 40 µl 3 M-NaOH. Plates were read with a Titertek Multiscan set at 405 nm. Readings of the ELISA were calculated as means of triplicate determinations; the SD values were

always less than 0.1% and have been omitted from the results. A_{405} values of negative control wells (i.e. wells coated with the highest concentration of the solid-phase antigen and reacted with an irrelevant antibody) were considered as the 'background reading' and subtracted from the results. A positive reaction was assumed when A_{405} values were more than twice the 'background reading' (usually 0.14–0.18).

Agglutination assay. The ability of purified mAb AF1 to agglutinate yeast cells of several *Candida* strains was assessed as reported elsewhere (Cassone *et al.*, 1988). Briefly, yeast cells were grown for 24 h in Winge medium at 28 °C, washed, then adjusted to a concentration of 3×10^7 cells ml⁻¹ in saline; 25 µl of this cell suspension were mixed with 25 µl of affinity-purified mAb AF1, at appropriate dilutions, on an agglutination slide, which was gently agitated for 5–10 min at room temperature. The results were assessed visually and expressed as end-point of antibody dilution giving clearly visible clusters of cells. Positive and negative controls were included in the test (see also Cassone *et al.*, 1988).

Indirect immunofluorescence. Yeast cells of *C. albicans* were induced to germinate in GlcNAc or Lee's medium (see above), and at different incubation times (up to 24 h), cells were harvested, resuspended in PBS and heat-fixed (30 min, 60 °C) on a microscope slide. The smears were covered for 30 min at 37 °C with the antibody (mAb AF1 or polyclonal rabbit antibody, each used at concentrations 1 and 5 times their previously established immunofluorescence titres), washed twice in PBS and allowed to react for 30 min at 37 °C with fluorescein isothiocyanate (FITC) conjugated to goat anti-mouse IgM or anti-rabbit IgG (Sigma). After extensive washing with PBS, the slides were mounted in 0.2 M-sodium carbonate buffer, pH 9.0, containing 90% (v/v) glycerol and examined by fluorescence microscopy (SM-LUX, Leitz).

Morphology control experiments were also performed with yeast cells of germinative strains incubated in GlcNAc medium at 28 °C (a germ-tube non-permissive temperature) or with cultures of the two agerminative variants, CA2 and A12 (Torosantucci *et al.*, 1984), in the same medium, at 37 °C.

Immunoblot assay. Antigenic fractions were subjected to SDS-PAGE according to Laemmli (1970), in a Mini-Protean II electrophoresis cell (Bio-Rad), using a 5–10% (w/v) acrylamide gradient slab, 0.75 mm thick, as the running gel and a 3.5% acrylamide as stacking gel. Twenty microlitres of each sample, at a polysaccharide concentration of 1 mg ml⁻¹, was run at a constant voltage of 200 V for approximately 45 min and subsequently transblotted for 1 h, at a constant voltage of 100V, onto a nitrocellulose paper sheet (Bio-Rad) in 0.025 M-Tris/0.192 M-glycine buffer, pH 8.3, containing 20% (v/v) methanol.

For immunological detection, the nitrocellulose was treated for 1 h with PBS containing 0.15% Tween 20, then incubated overnight at 4 °C with an appropriate dilution of mAb AF1 in PBS-Tween 20. After several washings in PBS, the bound mAb was revealed by treating the nitrocellulose sheet with an alkaline phosphatase-conjugated anti-mouse IgM antibody (Sigma), at 1:1000 strength, followed by Fast-Red/naphtholphosphate colour development solution (Bio-Rad) as the enzyme substrate.

Results

Expression of mAb AF1-reactive epitope on the surface of yeast and mycelial cells

Immunofluorescence was used to study the binding of mAb AF1 to yeast and mycelial forms of several strains of *C. albicans*. Control experiments were performed with

Fig. 1. Immunofluorescence pattern of yeast (*a, d*), germ-tube (*b, e*) and hyphal (*c, f*) cells of *C. albicans* after reaction with mAb AF1 (*a, b, c*) or polyclonal anti-*Candida* serum (*d, e, f*). Germ-tubes and hyphal cells were formed by incubating yeast cells in modified Lee's medium for 120 min (germ-tubes) and 24 h (hyphal cells). For other details, see text. Magnification $\times 380$.

irrelevant mAbs and polyclonal anti-*Candida* serum. Fig. 1 (*a, b, c*) shows a typical pattern of immunofluorescence obtained after reaction of mAb AF1 with yeast and hyphal forms of strain BP of *C. albicans*.

While the yeasts fluoresced strongly, the hyphal filaments were scarcely or not at all fluorescent. Any fluorescence associated with germ-tubes was usually detected only during the early stages of the morphogenesis while the surface of the mother cell remained stably fluorescent (Fig. 1*b*). Some fluorescence could be also observed at the hyphal tip (Fig. 1*c*). This pattern of fluorescence was not influenced by the medium used to obtain hyphal forms (GlcNAc or Lee's medium).

Both yeast and mycelial cell surfaces were constantly fluorescent, although to a slightly different extent, when the polyclonal anti-*Candida* serum was used (Fig. 1*d, e, f*). All other germinative strains examined showed a comparable, although quantitatively different pattern of fluorescence, with reproducible differences of reactivity between the yeast and the mycelial form. Table 1 shows the fluorescence intensities and mAb AF1 agglutination properties of all the strains tested. In only one case (*C. albicans* 195) was the fluorescence intensity roughly similar for yeast and hyphal cells. The most striking difference in fluorescence of the two morphological elements occurred with the isolate 221. Interestingly, this isolate had the highest agglutinability with mAb AF1 (Table 1).

Table 1. *Agglutination and indirect immunofluorescence (IF) reactivity with mAb AF1 of yeast and hyphal cells of C. albicans*

IF tests were performed using a 1:50 dilution of a mAb AF1 preparation with an ELISA titre of 1:2000. For other details on agglutination and IF test, see Methods.

Strain	Serotype	Agglutination titre	Fluorescence intensity*	
			Yeast cells	Hyphal cells
237	A	1:25	++	±
243	A	1:25	+++	+
195	A	1:25	+++	+++
213	A	1:25	+++	+
196	A	1:50	+++	+
200	A	1:75	+++	+
214	A	1:25	+++	+
189	A	1:25	+++	+
215	A	1:25	+++	±
BP	A	1:25	+++	±
3153	A	1:25	+++	++
239	B	1:25	+++	±
221	B	1:100	+++	—
170	B	1:25	+++	±
CA2	A	ND	+++	+++†
A12	A	ND	+++	+++†

ND, Not done.

* IF tests were performed with hyphal cells developed in GlcNAc medium at 37 °C for 4 h and with yeast cells kept at 28 °C for an equivalent time. —, Fluorescence totally absent; ±, barely detectable fluorescence; +, ++, ++++, different, arbitrary, scores of increasing fluorescence intensities.

† CA2 and A12 are agerminative variants which produced short, pseudomycelial-like filaments in GlcNAc medium at 37 °C.

Fig. 2. Chromatographic separation of GMP from yeast (Y-F, left) or mycelial (M-F; right) cells of *C. albicans*. —, Polysaccharide concentration; ----, A_{260} ; —, NaCl gradient, applied as indicated by the vertical arrow. For details of the chromatographic procedure, see Methods.

The simple incubation of yeast cells in buffer either at 28 °C or 37 °C (where no germination occurred) did not provoke any change in the pattern of fluorescence of yeast cells. Similarly, yeast cells of two agerminative, mAb AF1-reactive variants (CA2 and A12) were comparably fluorescent at 28 °C and 37 °C (Table 1).

Chromatographic separation of mannoprotein constituents

Mannoprotein preparations from yeast (Y-GMP) and mycelial (M-GMP) cells of *C. albicans* were subjected to ion-exchange chromatography with NaCl gradient elution (Fig. 2). In both cases, four major peaks were clearly separated, the most abundant of which were the unbound material (fraction F1: 9.6% and 10.5% recovery as sugar from Y-GMP and M-GMP, respectively) and fraction F2, eluted at 0.2–0.25 M-NaCl (39.3% and 39.9% recovery as sugar from Y-GMP and M-GMP, respectively). These fractions were followed by two sharply separated, minor fractions (F3 and F4). The latter, likely to consist mostly of nucleoprotein material (as shown by its strong absorbance at 260 nm and high relative phosphorus content; A. Torosantucci and others, unpublished data) was retained for use as a negative control in subsequent studies (see below). Fractions F1, F2 and F3 from both yeast and mycelial cells were essentially composed of mannoprotein material (see also Scaringi *et al.*, 1988), the hyphal fractions having a higher protein content relative to the polysaccharide than the yeast fractions (Table 2).

Table 2. Chemical composition of GMP fractions from yeast and mycelial cells of *C. albicans*

GMP was extracted from yeast (24 h growth in Winge medium at 28 °C) or hyphal (24 h growth in GlcNAc medium at 37 °C) cells. The analytical values are expressed as µg per mg cell dry weight.

Fraction	Yeast cells			Hyphal cells		
	Sugar	Protein	Protein/sugar ratio	Sugar	Protein	Protein/sugar ratio
GMP	4.50	0.41	0.09	4.96	0.63	0.13
F1	0.43	0.01	0.02	0.52	0.02	0.04
F2	1.77	0.09	0.05	1.98	0.16	0.08
F3	0.11	ND	—	0.09	ND	—
F4	0.60	0.06	0.10	0.57	0.09	0.16

ND, not done.

mAb AF1 reactivity with individual mannoprotein fractions

Mannoprotein fractions were tested for their antigenic reactivity with mAb AF1 and with the polyclonal serum raised against whole, chemically inactivated, cells of *C. albicans*. The reactivity was measured by indirect ELISA and, in the case of mAb AF1, by Western blotting (immunoblotting). Fig. 3 compares the reactivity of yeast and mycelial mannoprotein fractions in ELISA using a fixed amount of mAb AF1 and different concentrations of each fraction tested as coating antigen (within the range 0.2–1.0 µg ml⁻¹). The unfractionated mannoprotein extract (GMP) was also assayed. Although with

Fig. 3. mAb AF1 reactivity of individual GMP fractions from yeast (a) and mycelial (b) cells of *C. albicans* in ELISA using the indicated doses of each fraction as coating antigen. $\circ$, GMP; $\bullet$, F1; $\blacktriangle$, F2; $\blacksquare$, F3; $\square$, F4. The values represent the mean of triplicate wells. For other details, see text.

Fig. 4. Immunoblotting, with mAb AF1, of mannoprotein fractions from yeast (a) and mycelial (b) cells of *C. albicans*. The positions of molecular mass standards are indicated. For other technical details, see text.

apparently different intensity, all but fraction F4 from yeast cells strongly reacted with mAb AF1 (Fig. 3). Fractions F2 and F3 from the mycelial form also reacted strongly in ELISA with mAb AF1, although the F2 fraction was somewhat less reactive than the corresponding fraction from the yeast cells. In contrast, the mycelial fraction F1 gave a very low reaction, similar to that of the F4 nucleoprotein fraction taken as negative control (Fig. 3). In other experiments, using high doses of coating antigens ($5.0 \mu\text{g}$) and an inhibition-ELISA, it was confirmed that unlike the corresponding fraction from yeast cells, the F1 fraction of mycelial cells, up to a concentration of $2 \mu\text{g ml}^{-1}$, did not appreciably react with mAb AF1 (data not shown).

The mannoprotein fractions were also reacted in ELISA with the polyclonal anti-*Candida* serum (Table 3). The data showed no reactivity differences between F1 and F2 fractions from yeast or mycelial cells. F4 was not recognized (or only very weakly so) by the polyclonal serum.

Attempts to check the specificity of the ELISA reaction and identify the molecular constituents recognized by mAb AF1 were also made. Fractions were run in a 5–10% acrylamide gradient gel, transblotted and reacted with mAb AF1. As shown in Fig. 4, in all mAb AF1-reactive fractions the antibody recognized an epitope carried by an electrophoretically heterogeneous, polydisperse material, mostly distributed in the region of

Table 3. Indirect ELISA of yeast- and mycelial-derived GMP fractions with a polyclonal anti-*Candida* serum

Fraction	Dose*	A_{405} † using as coating antigen:	
		Y-GMP	M-GMP
Control‡	1.0	0.11	
Unfractionated GMP	1.0	0.76	0.81
	0.2	0.45	0.54
F1	1.0	0.71	0.61
	0.2	0.39	0.34
F2	1.0	0.81	0.83
	0.2	0.59	0.58
F3	1.0	0.58	0.79
	0.2	0.39	0.57
F4	1.0	0.17	0.20
	0.2	0.20	0.16

* Measured as μg polysaccharide ml^{-1} .

† Values are the means of triplicate wells obtained using the indicated fraction as the solid-phase antigen at the indicated dose.

‡ A GMP fraction from *C. parapsilosis*, prepared as for GMP from *C. albicans*, was used as coating antigen, as a negative control.

high molecular mass components. These components were practically absent in the F1 fraction of the mycelial extract, which showed a faint band at around 65 kDa. This latter component, or a constituent very close to it in molecular mass, was also present in all other fractions, regardless of their yeast or mycelial source.

Discussion

Several recent studies performed with polyclonal or monoclonal reagents have emphasized the antigenic variability of the cell surface in *C. albicans* (Poulain *et al.*, 1985; Brawner & Cutler, 1987; Chaffin *et al.*, 1988; Casanova *et al.*, 1989). However, it is not yet clear whether, and to what extent, this variability affects molecules which are relevant in the host-parasite relationship in candidosis. In this study, we measured the reactivity of a monoclonal antibody (mAb AF1) with constituents of a mannoprotein extract that has previously been shown to modulate a number of specific and non-specific immune responses in the experimental animal and in man (Cassone, 1990). Immunofluorescence showed that mAb AF1 binds extensively to the surface of yeast cells but scarcely, if at all, to that of the germ-tube (4 h) or hyphal (24 h) wall. This was verified with a number of fungal strains and hyphae induced in two media, and could not be accounted for solely by a decreased thickness of hyphal wall, as demonstrated by the fluorescence patterns observed with the polyclonal

serum (Fig. 1 and other data, not shown). Interestingly, two agerminative variants of *C. albicans* exhibited strong binding of mAb AF1 to their surface at both 37 °C and 28 °C, suggesting that the modulated expression of mAb AF1-reactive epitope did not merely depend on the temperature necessary for the development of each form. However, as in other instances of modulated epitope expression (Brawner & Cutler, 1986; Chaffin *et al.*, 1988), quantitative differences in binding of mAb AF1 to the yeast cell surface were detected, depending on the strain and also on growth conditions.

The results reported in this paper show that the oligosaccharide epitope recognized by mAb AF1 is shared by different chromatographically-separated mannoprotein fractions in the cell wall of *C. albicans*. Each fraction showed a heterogeneous distribution of mAb AF1 reactivity, much of which was of high molecular mass. The chromatographic profile of the mannoprotein extract in our reference strain (BP) was essentially similar whatever the form of growth from which the material was obtained, and the yield of the three major fractions was substantially the same in yeast and mycelial forms. However, the ratio between protein and polysaccharide was higher in the hyphal than in the yeast GMP, a difference reflected in the respective separated fractions. Also, the hyphal F2 fraction was eluted at a higher ionic strength than the corresponding yeast fraction, possibly due to the higher content of phosphorus and/or more charge on the protein moiety of mannoproteins in the hyphal fraction (A. Torosantucci and others, unpublished data). The evidence for qualitative and quantitative differences in the mannoprotein composition of the two forms of growth has been discussed elsewhere (Casanova *et al.*, 1989; Cassone, 1990). However, these chemical differences do not show a clear correlation to the differential mAb AF1 reactivity of the individual yeast and mycelial mannoprotein fractions. The most representative fractions (F1 and F2) were also similarly reactive with the polyclonal anti-*Candida* serum.

As two of the three mannoprotein constituents of the mycelial extract (F2 and F3) reacted with mAb AF1, the lack or the greatly diminished expression of mAb AF1-reactive epitope on the surface of germ-tube and hyphal cells is not due to the absence of relevant epitope-bearing mannoproteins in this form of growth. The lack of expression, however, would be explained if the F1 constituent were the only or the predominant mannoprotein constituent expressed on the yeast cell surface. Alternatively, either F2 or F3 (or both) might also be expressed on the surface of the yeast form together with F1 but remain confined in the inner layers of the cell wall in the hyphal cell. Recent, preliminary experiments on mannoprotein localization by immunogold electron

microscopic techniques have shown that mAb AF1-reactive epitopes are present in both external and internal wall layers in the yeast form but almost exclusively in the inner wall layers of the mycelial form (J. P. Latgé, personal communication).

Chemical and ultrastructural studies have demonstrated the shedding of surface constituents early in germ-tube development (Cassone *et al.*, 1973; Chattaway *et al.*, 1974; Brawner & Cutler, 1986), and we have recently observed that the released material contains mAb AF1-reactive, mannoprotein constituents, chromatographically similar to the F1 and F2 fractions reported here (A. Torosantucci and others, unpublished data). It could therefore be that some mAb AF1-reactive mannoproteins are shed into the external medium during initiation of the hyphal transition, while those that are still synthesized are no longer exported to the cell surface during hyphal growth. Finally, in order to be detected by immunofluorescence, a single epitope might need to be arranged in an ordered structure, and/or with an elevated density, at the cell surface. Thus, a scattered/disorganized arrangement could result in unreactivity. The three possibilities considered above are not mutually exclusive.

The F1 and F2 mannoprotein fractions have recently been obtained in sufficient quantity to allow their immunomodulatory properties to be assayed in both experimental and human models. Only F2 retained those immunological activities possessed by the crude extract (GMP), such as the antigenic induction of T-cell proliferation (Ausiello *et al.*, 1986) and interferon production (Spagnoli *et al.*, 1985; A. Torosantucci and others, unpublished data). In view of these properties, the lack or the greatly diminished expression of the F2 constituent from the hyphal cell surface may be relevant to the relationship between this form of growth and the pathogenicity of the fungus.

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