Heat-inducible ATP-binding proteins of *Candida albicans* are recognized by sera of infected patients

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Four proteins from *Candida albicans* extracts have been isolated by ATP affinity chromatography. These proteins were found to be at elevated levels in extracts of cells raised from 25 °C to 37 °C, but were present at low levels in cells grown at 25 °C. The molecular masses of the proteins (38–42 kDa, 66–68 kDa, 70–72 kDa and 74–76 kDa) correspond to the published sizes of *C. albicans* heat-shock proteins. Three of the four proteins were recognized by the sera of patients with oral and/or oesophageal *C. albicans* infections, with the 70–72 kDa protein reacting in all cases tested. Binding of antibodies to two of the other proteins (38–42 kDa and 74–76 kDa) differed from patient to patient. IgA antibodies were the dominant immunoglobulin class in these mucosal *C. albicans* infections. The IgA antibody titre may be of diagnostic value and seemed to be correlated to the severity of infections, with a higher level in oesophageal infections compared to oral infections. Antibody binding to these proteins was specific as the sera did not show the same enhanced recognition with bacterial or HeLa cell heat-shock proteins.

Introduction

*Candida albicans* is a major pathogen in immuno-compromised persons (Edwards *et al.*, 1978) and oral candidiasis serves as one indicator of HIV infection (Dodd *et al.*, 1991). To date, little is known about the molecules involved in pathogenesis, or which proteins are recognized by the immune system of the infected host. By preparing different cell-wall extracts it was shown that polysaccharides, especially mannans, were recognized as antigens by immune sera from animals (Summers *et al.*, 1964). Everall *et al.* (1974) discovered mannan precipitating antibodies in sera of humans without invasive candidiasis. This observation of polysaccharides or glycoproteins as antigens has been described by different groups (Saltarelli, 1989). More recently, cytoplasmic proteins have been identified as antigens (Strockbine *et al.*, 1984) and some have been cloned by antibody screening of expression libraries (Franklyn *et al.*, 1990; Shen *et al.*, 1991): at least one of these functions as an allergen (Shen *et al.*, 1991). Studying the antibody response to *C. albicans* is not only important for the determination of the immunogenic molecules of this pathogen, but it has also been suggested that antibodies may play a role in preventing disseminated candidiasis (Matthews *et al.*, 1988). Antibodies against a 47 kDa protein, which shows homology to hsp90 of *Saccharomyces cerevisiae*, are protective against systemic infections (Matthews & Burnie, 1992).

Over the past few years, heat-shock proteins have been shown to be major antigens in infectious diseases (Newport *et al.*, 1988; Polla, 1991). Antibodies against heat-shock proteins have been found in different parasitic infections, not only against invasive organisms living in the bloodstream like *Schistosoma mansoni* (Hedstrom *et al.*, 1987) or *Brugia* (Selkirk *et al.*, 1989), but also against intracellular parasites like *Plasmodium* or *Mycoplasma* (Bianco *et al.*, 1986; Young *et al.*, 1988; Jendoubi & Bonnefoy, 1988). In most cases the presence of antibodies reacting with heat-shock proteins has been demonstrated by sequencing cDNA clones isolated by antibody screening of expression libraries and observing sequence homology with heat-shock proteins (Bianco *et al.*, 1986; Hedstrom *et al.*, 1987; Jendoubi & Bonnefoy, 1988; Selkirk *et al.*, 1989). We aimed to determine
whether other heat-shock proteins of *C. albicans* are also recognized as antigens in human candidiasis, especially mucosal infections. Welch & Feramisco (1985) had previously purified heat-shock proteins of HeLa cells by ATP affinity chromatography. We used the same method with *C. albicans* protein extracts, and the isolated proteins were used as targets in Western blots and ELISA.

In this paper we show that heat-shock proteins of *C. albicans* can be isolated by ATP affinity chromatography and that they are recognized by sera of patients. The IgA antibody titre correlates with the severity of infection.

**Methods**

**Cells and culture conditions.** *C. albicans* strain SS was used throughout. The strain was originally isolated from a patient and characterized as *C. albicans* by Blagoyevich (1979). Cells were grown on YPD (2% w/v, yeast extract, 1% w/v, peptone, 2% w/v, dextrose) agar medium plates at 25 °C overnight and stored at 4 °C. For preparation of protein extracts, *C. albicans* was grown in YPD medium in a shaking water-bath at 25 °C overnight. Cells were then diluted 1:10 into fresh medium and incubated at 25 °C or at 37 °C with or without 5% (v/v) foetal calf serum at the higher temperature. After 4–6 h, cells were harvested and used for the preparation of protein extracts.

**Escherichia coli** LE392 was grown in LB medium at 37 °C in a shaking water-bath. To induce the expression of heat-shock proteins in *E. coli*, cells were incubated for 5 min at 44 °C followed by 1 h at 37 °C before cells were harvested and proteins extracted.

**Antibodies and sera.** Twenty sera of *C. albicans*-infected patients (5 oesophageal, 15 oral infections, mostly HIV positive) and 10 control sera from patients (HIV positive) without detectable oral *C. albicans* infection were obtained from the Oral AIDS Center at UCSF; 10 sera were obtained from healthy volunteers. Infections were confirmed by lesions in the mouth and/or swabs, which were cultured to determine the presence of *C. albicans*. Sera of volunteers were obtained by drawing 5 ml blood with an approved protocol of the human research committee, UCSF.

Monoclonal antibodies against human hsp72 and hsp73 (Ribiowol et al., 1988), and also rabbit serum specific for hsp58 (McCullin & Hallberg, 1987), which reacts with *E. coli* groEL protein (McCullin & Hallberg, 1988), were a gift from W. Welch, UCSF. The alkaline-phosphatase-labelled antibodies used as secondary antibodies in Western blotting and ELISA experiments were obtained from Zymed Laboratories. These antibodies recognized human, murine or rabbit immunoglobulin in general, or were specific for human IgM, IgG or IgA antibodies.

**Protein isolation.** Protein extracts were prepared by the method of Hostetter et al. (1990). In short, cell cultures were centrifuged at 3000 g, 4 °C, the cells washed with water twice and the final cell pellet resuspended in lysis buffer (30 mM-Tris/HC1 pH 8.0, 5 mM-EDTA, 1 mM-dithiothreitol, 1 mM-phenylmethylsulphonyl fluoride). An equal volume of glass beads was added. Cells were homogenized on a vortex mixer in five 2 min intervals with 2 min cooling on ice between. From the protein extracts, ATP-binding proteins were isolated by a slight modification of the method of Welch & Feramisco (1985). The protein preparations were centrifuged at 12000 g for 10 min and the supernatant loaded onto DEAE cellulose columns (Pharmacia). The columns were washed with buffer B (20 mM-Tris/acetate pH 7.5, 20 mM-NaCl, 0.1 mM-EDTA, 15 mM-β-mercaptoethanol) and bound proteins were eluted with buffer B containing 350 mM-NaCl. The eluate was applied directly onto an ATP agarose column (Sigma) and the column was washed with buffer D containing 0.5 M-NaCl followed by a buffer D wash. Buffer D is buffer B supplemented with 3 mM-MgCl₂. Bound proteins were eluted with buffer D containing 3 mM-ATP and the eluate was collected in 1 ml fractions. Initially, this step was preceded by developing the column with buffer D containing 1 mM-GTP as described by Welch & Feramisco (1985). This step was excluded in later experiments (see Results).

Portions (10 μl) of each fraction were electrophoresed on a 10% (w/v) SDS-polyacrylamide gel under reducing conditions (Laemmli, 1970). Protein-containing fractions were pooled, dialysed against water and, if necessary, concentrated by hophylisation. Protein isolation was identical for *C. albicans* and *E. coli* protein extracts.

**Gel electrophoresis and Western blotting.** Proteins were separated on 10% SDS-polyacrylamide gels under reducing conditions by the method of Laemmli (1970) using prestained marker proteins (Sigma or Rainbow marker, Amersham). Gels were either stained with Coomassie blue or the proteins were transferred onto nitrocellulose membranes by electroblotting under standard conditions (Burnette, 1981). The quality of each transfer was checked by reversible staining of the membranes with Ponceau S (Salinovich & Montelaro, 1988). Antibody reactions were done under conditions similar to published methods (Talbot et al., 1984) using TBS (50 mM-Tris/HC1 pH 8.0, 150 mM-NaCl) as the incubation and wash solution, and 5% (w/v) non-fat dry milk powder as blocking agent. Unspecific binding sites were blocked by incubating the filters with 1 x TBS and 5% non-fat dry milk powder for 3 h at room temperature. Tested sera were diluted 1:100 and incubated with the filters at 4 °C overnight. All further washes and incubations were done at 20 °C. Washes contained 0.05% Tween 20, except for the last two washes which were done in TBS alone. Secondary antibodies were diluted 1:500 in the cases of immunoglobulin class-specific antibodies, or 1:1000 in all other cases. The colour reaction was done in AP buffer (100 mM-Tris/HC1 pH 9.5, 100 mM-NaCl, 5 mM-MgCl₂) containing 132 μg Nitro blue tetrazolium ml⁻¹ and 72 μg 5-bromo-4-chloro-3-indolyl phosphate (Promega) ml⁻¹ until a clear signal was visible. The reaction was stopped by washing the filters in 20 mM-Tris/HC1 pH 8.0, 5 mM-EDTA.

**ELISA.** Bacterial and *C. albicans* ATP-binding proteins were tested under identical conditions using standard procedures (Engvall & Perlman, 1971). Portions (40 μl) of protein extract in 1 x PBS (0.137 mM-NaCl, 2.7 mM-KCI, 4 mM-Na₂HPO₄, 1.8 mM-KH₂PO₄, pH 7.2) were pipetted into each well of a 96-well microtitre plate and incubated overnight at 4 °C. Plates were then washed five times with 1 x PBS containing 0.05% Tween 20, and 200 μl of 1 x PBS containing 5% (w/v) BSA was added to each well. Plates were stored overnight at 4 °C. The contents of the plates were discarded and 40 μl of 1 x PBS containing 1% BSA was pipetted into each well. Portions (40 μl) of 1:25 diluted serum were added, mixed 10 times and 40 μl used for the next dilution. Three different final dilutions of sera (1:50, 1:100 and 1:200) were used and each reaction was done in duplicate. The plates were incubated for 3 h at 20 °C, washed five times with 1 x PBS containing 0.05% Tween 20, and then 50 μl of 1:500 diluted alkaline-phosphatase-labelled second antibody was added. The plates were incubated for 90 min at 20 °C, washed five times with 1 x PBS containing 0.05% Tween 20 followed by two washes with 10 mM-diethanolamine pH 9.5, 0.5 mM-MgCl₂. Aliquots (50 μl) of 1 mg ml⁻¹ p-nitrophenyl phosphate dissolved in 10 mM-diethanolamine, 0.5 mM-MgCl₂ were pipetted into each well and the plates were incubated for 1 h at 20 °C. The reaction was stopped by adding 50 μl 0.1 M-EDTA to each well. Colour formation was used as an indicator of the amount of
reactive antibodies in sera and was measured by absorbance at 405 nm versus 690 nm in a Biotec microplate reader. To compare the results of different experiments, the serum of one patient (oesophageal infection) was included as a positive control serum and the values adjusted to the readings of the control serum. The negative control was 1 x PBS. Throughout this paper the values of the 1:100 dilutions are used and all results are given as the mean with standard deviation. Statistics were done using Student’s t-test.

Results

Isolation of C. albicans heat-shock proteins by ATP affinity chromatography

To determine if the sera of patients with oral/oesophageal C. albicans infections possessed antibodies recognizing heat-shock proteins of C. albicans we isolated these proteins to be used as targets in Western blots and ELISA.

Using the method of Welch & Feramisco (1985) we were able to reproducibly isolate four ATP-binding proteins by eluting the ATP agarose column with 3 mM-ATP. In contrast to HeLa protein extracts (Welch & Feramisco, 1985), we found no protein in eluates of buffer containing 1 mM-GTP instead of 3 mM-ATP. In addition to these four C. albicans ATP-binding proteins, two further proteins were detected in the ATP eluate on gels or Western blots of highly concentrated preparations, but the isolation of these two proteins was variable.

The four ATP-binding proteins consistently isolated had molecular masses of 38–42 kDa, 66–68 kDa, 70–72 kDa and 74–76 kDa. The abundance of these proteins differed, with the 70–72 kDa protein being the most abundant and the 66–68 kDa protein being the least abundant. The four proteins were eluted in the same fractions of the ATP agarose columns which suggested that they have similar affinities for ATP.

The ATP-binding proteins were most abundant in cells whose culture temperature was shifted from 25 °C to 37 °C, and were barely detectable in cells grown at 25 °C (Fig. 1).
agarose columns. To elute the ATP-binding proteins, the same elution volume was used and identical volumes were loaded onto the gel. This was done to compare the amount of ATP-binding proteins in cells grown at different temperatures. By loading different amounts (10:1 ratio) of ATP eluate onto an SDS-polyacrylamide gel and comparing the intensity of the bands after Coomassie blue staining, we estimated a 50 to 100-fold difference in the amount of the ATP-binding proteins between cells grown at 25 °C compared to cells switched from 25 °C to 37 °C incubation temperature for 4–6 h (Fig. 1b). Based on this inducibility we suggest that the four isolated ATP-binding proteins are heat-shock homologues of \textit{C. albicans}.

Addition of foetal calf serum to cells grown at 37 °C did not affect the ATP-binding profile of protein extracts. Growing at 37 °C in the presence of serum always induced the morphological switch of \textit{C. albicans} from the yeast to the hyphal form.

**Reactivity of human sera with heat-shock proteins of \textit{C. albicans}**

The sera of patients suffering from different forms of oral and/or oesophageal \textit{C. albicans} infections and sera of uninfected persons, with or without HIV, recognized the heat-shock proteins when tested for all immunoglobulin classes (Fig. 2). The 70–72 kDa protein was recognized by all sera, although some of the infected patients showed a stronger reaction. The recognition of the other heat-shock proteins varied. Monoclonal antibodies which reacted with the HeLa 72 kDa heat-shock protein, the Hela 73 kDa heat-shock protein or polyclonal serum against the 58 kDa heat-shock protein did not bind to the four isolated \textit{C. albicans} proteins (data not shown).

To differentiate which immunoglobulin class bound to the heat-shock proteins of \textit{C. albicans} in human oral infections we used antibodies specific for the human IgM, IgG or IgA class as secondary antibodies. The data showed an increase in reactive IgA antibodies compared to IgM antibodies in the sera of infected persons, predominantly in oesophageal infections, which did not occur in the control sera (Fig. 3 and ELISA data, see below). In general, IgG antibodies were not elevated in these infections. This preference for IgA antibodies was in agreement with the mucosal nature of the infections analysed.

Three of the four heat-induced proteins (38–42 kDa, 70–72 kDa and 74–76 kDa) were recognized to differing extents by the sera from different patients, with the 70–72 kDa protein being recognized by all sera tested (Fig. 3). The other two proteins were not always recognized by the sera. There is no obvious correlation between disease status and the recognition pattern of the four proteins. Persons without \textit{Candida} infection also recognized heat-shock proteins, with the 70–72 kDa protein being the predominantly recognized protein.
Heat-shock proteins of Candida albicans

Bacterial ATP-binding proteins

Using the same method of ATP affinity chromatography we were able to isolate three proteins from heat-shocked E. coli cells. The approximate molecular masses of these proteins were 36 kDa, 58 kDa and 72 kDa (Fig. 4). That these ATP-binding proteins were bacterial heat-shock proteins was confirmed by the fact that the serum reacting with E. coli GroEL bound to our isolated 58 kDa protein. With the exception of the protein of about 72 kDa, the proteins of C. albicans and E. coli differed markedly in size, which excluded possible bacterial contamination of our preparations of C. albicans ATP-binding proteins.

ELISA

Based on the limited amount of purified heat-shock proteins available, only a restricted number of sera were tested, and IgG measurements were omitted as the Western blotting data suggested that their antibody levels were not enhanced during the tested type of infections. In the ELISA tests, comparable amounts of C. albicans and E. coli heat-shock proteins were used (Fig. 4). Although all sera tested showed a positive reaction, the sera of patients suffering from oesophageal candidiasis expressed on average fourfold higher IgA responses to C. albicans heat-shock proteins compared to the control sera (Table 1). This difference in the IgA response is statistically significant (P < 0.02). The increased abundance of antibodies reacting with C. albicans heat-shock proteins was not accompanied by an equal increase in antibodies against bacterial heat-shock proteins. HIV infection itself had no influence on the antibody level, as HIV patients without candidiasis showed no difference in antibody level compared to healthy volunteers (Table 1). HIV patients with oral C. albicans infections expressed a slightly enhanced titre (twofold) of IgA and IgM antibodies against C. albicans heat-shock proteins and also showed an increase in antibodies reacting with bacterial heat-shock proteins. Table 1 shows the mean values of the four groups of sera divided into immunoglobulin classes and recognized antigens.

To test the specificity of the sera, we incubated Western blots of HeLa heat-shock proteins and C. albicans heat-shock proteins with five sera from infected patients. Although all five sera reacted strongly with C.
albicans heat-shock proteins, only three reacted with HeLa heat-shock proteins and of those two were very weak and one comparable in strength to the reaction with heat-shock proteins of C. albicans (data not shown).

**Discussion**

A temperature shift from 25 °C to 37 °C was used in our experiments, as this is the normal temperature increase that C. albicans experiences during infection of a mammalian host. Using a slight modification of the method of Welch & Feramisco (1985) four ATP-binding proteins have been isolated consistently from C. albicans protein extracts of cells exposed to this temperature shift. In this study four proteins were isolated from C. albicans instead of the two proteins observed previously for HeLa extracts (Welch & Feramisco, 1985). This was most probably due to our use of the whole DEAE column eluates without prior fractionation, which was done by Welch & Feramisco (1985). A further difference was that we never detected any protein(s) released from the ATP column with GTP. In some cases, we detected more than four proteins in our eluates. These additional proteins were eluted in the same fractions as the four proteins observed in all experiments; they were eluted in lower concentrations than the four major proteins and were only detected by Coomassie blue staining when large amounts of protein extracts were used, or on some Western blots (Fig. 3). We assume that these additional proteins are degradation products, as they were only detectable in some preparations and gave no sharp bands on Western blots (Fig. 3).

The four ATP-binding proteins appear to be heat-shock proteins of C. albicans as the expression of these proteins was strongly increased in cells switched from

![Coomassie-blue-stained gel of bacterial and Candida ATP-binding proteins separated on a 10% SDS-polyacrylamide gel. Lane 1, E. coli proteins; lane 2, C. albicans proteins; lane 3, prestained marker.](image)

**Table 1. ELISA results of four groups of sera classified according to the type of Candida infection and HIV status**

The measured absorption values (405 nm) of ELISA reactions are shown. The data are differentiated into which heat-shock proteins were used as targets and which immunoglobulin class was detected with the second antibody.

<table>
<thead>
<tr>
<th>Type of Candida infection</th>
<th>No. of sera*</th>
<th>HIV</th>
<th>E. coli</th>
<th>Candidate</th>
<th>Candida</th>
<th>E. coli</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>IgM</td>
<td>IgA</td>
<td>IgM</td>
</tr>
<tr>
<td>Oesophageal</td>
<td>4 (4)</td>
<td>+</td>
<td>0.341 ± 0.127</td>
<td>1.049 ± 0.401§</td>
<td>0.266 ± 0.131</td>
<td>0.170 ± 0.070</td>
</tr>
<tr>
<td>Oral</td>
<td>10 (6)</td>
<td>+</td>
<td>0.467 ± 0.300</td>
<td>0.483 ± 0.299</td>
<td>0.228 ± 0.154</td>
<td>0.189 ± 0.110</td>
</tr>
<tr>
<td>Negative</td>
<td>8 (7)</td>
<td>+</td>
<td>0.189 ± 0.104</td>
<td>0.266 ± 0.162</td>
<td>0.124 ± 0.051</td>
<td>0.103 ± 0.066</td>
</tr>
<tr>
<td>Negative</td>
<td>7 (7)</td>
<td>-</td>
<td>0.281 ± 0.199</td>
<td>0.288 ± 0.183</td>
<td>0.128 ± 0.040</td>
<td>0.070 ± 0.036</td>
</tr>
</tbody>
</table>

*The numbers in parentheses show the number of patients used to obtain sera.
†Mean ± SD.
§P < 0.02 vs. control value (Candida negative, HIV negative) by Student's t-test.
25 °C to 37 °C. The molecular sizes of these proteins are comparable to the previously published sizes of heat-shock proteins in *C. albicans* (Zeuthen & Howard, 1989). Based on these in vivo labelling data (Zeuthen & Howard, 1989) and upon their different reactions with the sera the four proteins represent independent proteins and are not precursor(s) or degradation product(s). In contrast to the expression studies of heat-shock proteins by in vivo labelling (Zeuthen & Howard, 1989) where the 76 kDa protein was only detected after shifting the culture temperature from 37 °C to 45 °C, we detected the expression of the 74–76 kDa protein in cells switched from 25 °C to 37 °C. Assuming that our protein and the published 76 kDa protein (Zeuthen & Howard, 1989) are the same, the most likely explanation for this apparent discrepancy is that the ATP affinity chromatography method is more sensitive than in vivo labelling. Alternatively, we have identified a new *C. albicans* heat-shock protein. Further experiments are required to clarify this issue.

The heat-shock proteins were recognized more strongly, especially with IgA, by the sera of patients suffering from oral or oesophageal candidiasis than by the control sera. In contrast to the 70–72 kDa protein, the recognition of the other two heat-shock proteins was variable and they were not always recognized. Previously, mainly the hsp70 and hsp60 families have been recognized to be major immunogens in parasitic infections (Kaufmann, 1990). In *C. albicans* infections two additional heat-shock proteins are immunogens, the previously described hsp90 homologue (Matthews & Burnie, 1989) and the described 38–42 kDa protein. The existence of reactive antibodies in all sera is in agreement with the assumption that humans are constantly exposed to *C. albicans* and that a basal level of antibodies against *C. albicans* may usually be present, based perhaps on cross-reactivity (see below).

Depending upon the site of infection and the infective organism (Czerkinsky et al., 1987; Ogilvie, 1964), different immunoglobulin classes are predominant in the immune response. Although the control sera and sera of patients recognized the heat-shock proteins, there was a difference in the preference and amount of immunoglobulin classes used. Both groups showed little difference in the IgM levels, but the level of IgA antibodies compared to IgM antibodies was elevated in sera from infected patients, significantly so in patients suffering from oesophageal candidiasis. A comparable increase did not occur with IgG antibodies (Fig. 3) as judged by Western blotting data. The raised IgA antibodies in our study reflects the induction of IgA antibodies in mucosal infections (Heremans, 1974). The difference in the IgA level was most obvious in the ELISA data, where the values differed significantly (*P < 0.02*) between the sera from patients with oesophageal infections and the control group (Table 1). HIV infection alone did not influence the titre of reactive antibodies. There was only a slight difference (twofold) between control sera and sera of patients with oral *C. albicans* infection.

The recognition of *C. albicans* heat-shock proteins was not based purely on reactivity with heat-shock proteins in general, because the level of antibodies against *E. coli* heat-shock proteins did not correlate with the elevated titre of antibodies against *C. albicans* heat-shock proteins. Comparable amounts of proteins were used in the ELISA tests. hsp70 of *E. coli* has an amino acid homology of about 50% to eukaryotic hsp70 (Bardwell & Craig, 1984). This homology should be sufficient to explain reactivity if the detected increase was based on a general reactivity with common domains of hsp70. Western blotting data with HeLa heat-shock proteins also showed that antibody binding to *C. albicans* heat-shock proteins in the sera of patients was specific and not purely a reaction with heat-shock proteins in general. We could not totally exclude the possibility that some of the antibodies cross-react with heat-shock proteins of different organisms, as some sera showed reactivity with HeLa heat-shock proteins and all sera reacted with bacterial heat-shock proteins. In addition, it has been reported that patients with superficial candidiasis have elevated antibody titres to the 65 kDa heat-shock protein of *Mycobacterium* (Ivanyi & Ivanyi, 1990), which might also suggest a certain degree of cross-reactivity. Additional cross-reactivity with heat-shock proteins from other organisms cannot be excluded. A certain level of cross-reactivity may be beneficial as suggested by Kaufmann (1990). Nevertheless, antibodies reacting to *C. albicans* heat-shock proteins do exist in the sera of patients which do not bind heat-shock proteins of *E. coli* or HeLa cells and the titre of these antibodies increases with infection. The enhanced level of antibodies reacting with bacterial proteins in the sera of patients may be due to the fact that patients had inflammatory lesions in the mouth and/or oesophagus which led to secondary, opportunistic infections.

Taken together, our data support the conclusion that heat-shock proteins of *C. albicans*, other than the hsp90 homologue, are recognized during infections and induce an immune response in the host. Additionally, the data show a mucosal infection is sufficient to induce an antibody response against heat-shock proteins of the infectious organism.

The induction of antibodies binding heat-shock proteins of *C. albicans* may be of diagnostic value, at least in the case of oesophageal infections and seems to be correlated to the severity of the infection. In order to analyse the diagnostic value of the recognition of these proteins by antibodies, it will be necessary to clone the
relevant genes for large-scale production of these proteins. Unfortunately an insufficient amount of protein was purified by ATP agarose affinity chromatography to carry out large-scale studies. The detection of antibodies against the three described heat-shock proteins in the sera of patients with oral and/or oesophageal infection, who rarely get disseminated candidiasis (Matthews & Burnie, 1992), supports the suggestion that protective antibodies play a role in preventing disseminated candidiasis (Matthews et al., 1988). This hypothesis is supported by data with squirrel monkeys infected by Plasmodium falciparum. Immunization of squirrel monkeys with hsp90 of P. falciparum induces a protective humoral immunity against a future infection with P. falciparum (Dubois et al., 1984; Jendoubi & Bonnefoy, 1988). The question of whether these antibodies against the heat-shock proteins of C. albicans really have a role in the host immune defence, as with antibodies to the 47 kDa protein (Matthews & Burnie, 1992), has to be studied in more detail.

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