New Approach for Diagnosis of Candidemia Based on Detection of a 65-Kilodalton Antigen

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Nosocomial candidiasis is a major concern in tertiary care hospitals worldwide. This infection generally occurs in patients with degenerative and neoplastic diseases and is considered the fourth most frequent cause of bloodstream infections. Diagnosis of candidemia or hematogenous candidiasis has been problematic because clinical signs and symptoms are nonspecific, leading to delays in diagnosis and, consequently, delays in appropriate antifungal therapy. We developed an inhibition enzyme-linked immunosorbent assay (ELISA) for detection of a 65-kDa antigen in an experimental model of candidemia and for diagnosis of patients in intensive care units (ICUs) with suspected candidemia. An anti-65-kDa monoclonal antibody was tested for detection of the 65-kDa antigen produced by Candida albicans, Candida tropicalis, and Candida parapsilosis in murine candidemia models. The 65-kDa antigen was detected in sera at concentrations ranging from 0.012 to 3.25 µg/ml. A total of 20 human patients with candidemia were then evaluated with the inhibition ELISA using sequential sera. Sixteen (80%) patients had the 65-kDa antigen in concentrations ranging from 0.07 to 5.0 µg/ml. Sequential sera from patients with candidemia presented three different patterns of antigenemia of the 65-kDa molecule: (i) total clearance of antigenemia, (ii) initial clearance and relapse of antigenemia, and (iii) partial clearance of antigenemia. Our results indicate detection of the 65-kDa protein may be a valuable tool for the diagnosis of candidemia by C. albicans, C. tropicalis, and C. parapsilosis.

Matthews and Burnie developed an immunobinding method for detection of a 47-kDa cytoplasmatic protein antigen in patients with systemic candidiasis (34). An immunoassay detecting a 48-kDa antigen of Candida, subsequently recognized as enolase (13, 33), is available for the diagnosis of invasive candidiasis (54). Latex agglutination tests are based on the detection of mannan, a cell wall component that is the most widely studied antigen in patients with candidiasis (5, 20). Commercial tests (Pastorex Candida assay and Cand-Tec assay) have been used to detect this molecule in sera. Colorimetric assays (Fungitec G and Fungitec G MT) detect β-glucan, a major structural component of the fungal cell wall, in serum and have been used for diagnosis of fungal infections. Studies show the concentration of β-glucan is increased in experimental models of fungal infections (35–38), as well as in the plasma of patients with mycosis (20, 36).

Various tests have been developed based on detection of antibodies, antigens, and metabolites, although, they are all time-consuming and lack either specificity or sensitivity (50). In C. albicans, a 65-kDa mannoprotein (Mp65) is a structural and secreted component of the fungus. This mannoprotein is particularly observed in extracellular fractions of hyphal cells (1, 8, 17, 44–46). Mp65 is also present in both the structural and secretory mannoprotein material and is recognized by peripheral blood T cells of practically all healthy individuals (quasi-universal antigen) (1, 44, 45), making it a potential target for immunodiagnosis of patients with suspected candidemia. Arancia et al. (1) used real-time PCR to detect and quantify C. albicans in sera from patients with invasive candidiasis. This assay was specific for a DNA fragment containing the gene for the 65-kDa mannoprotein of C. albicans (CaMP65). The assay...
was shown to be sensitive and specific for *C. albicans*, allowing quantitative detection of this fungus in clinical samples.

The inhibition enzyme-linked immunosorbent assay (inh-ELISA) is an enzyme immunoassay (EIA) to detect circulating antigens in sera of patients with invasive fungal infections. This test uses a species-specific murine monoclonal antibody (MAb) with high sensitivity and specificity and is useful in diagnosis and follow-up of paracoccidioidomycosis patients. It is also useful for antigen detection in the cerebrospinal (28) and bronchoalveolar (27, 29–31) fluids of these patients.

In this study, our intention was to demonstrate that inh-ELISA is a sensitive detection method able to detect nanograms of antigen in serum samples from patients with candidemia and not to compare it or show its superiority to other assays. Na and Song previously compared three serological methods of detecting *C. albicans* secreted aspartyl proteinase antigen (39); they found inh-ELISA had 93.9% sensitivity and 96.0% specificity and detected concentrations ranging from 6.3 to 19.0 ng/ml. The sensitivity and specificity for standard ELISA were 69.7 and 76.0%, respectively; while for capture ELISA, the sensitivity and specificity were 93.9 and 92.0%, respectively. The results of Na and Song showed inh-ELISA with MAb CAP1 effectively detected circulating secreted aspartyl proteinase antigen and suggest it may be useful for the diagnosis and treatment monitoring of invasive candidiasis.

The aim of this study was to standardize an alternative inh-ELISA for detection of a 65-kDa antigen, present in *C. albicans*, *C. tropicalis*, and *C. parapsilosis*, using a MAb specific for the 65-kDa *Candida* protein. The assay could be used for diagnosis and follow-up of patients with candidemia. The present study involved five different stages: (i) identification of an immunodominant 65-kDa antigen of *C. albicans* that is common to *C. tropicalis* and *C. parapsilosis*, (ii) production of an anti-*C. albicans* 65-kDa-molecule MAb for detection of the immunodominant antigen mentioned above, (iii) application of the MAb to the inh-ELISA, (iv) characterization of antigenemia in an animal model, and (v) evaluation of the developed inh-ELISA with sera from patients with candidemia.

**MATERIALS AND METHODS**

**Fungal isolates.** Isolates of *C. albicans* (ATCC 90028), *Candida parapsilosis* (ATCC 22019), *Candida tropicalis* (ATCC 750), and *Candida glabrata* (ATCC 90030) were obtained from the yeast stock collection of the Special Mycology Laboratory, Federal University of São Paulo.

**Candida exoantigens.** Each Candida species was grown on Sabouraud agar (three tubes) for 3 days at 36°C. All growth was transferred to a 250-ml Erlenmeyer flask containing 50 ml modified Lee’s medium without amino acids (MLMwAA) (23) under agitation (50 rpm). MLMwAA, as modified by Tronchin et al. (43), contains 2.0 g/liter K2HPO4, 5.0 g/liter NaCl, 10.0 g/liter glucose, and 0.04 g/liter biotin at pH 6.8.

**Paracoccidioides brasiliensis.** *Paracoccidioides brasiliensis* cultures underwent sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (22) and Western blotting as previously described (47). Briefly, after transferring proteins to nitrocellulose membrane, the membrane was blocked with Tris-buffered saline (20 mM Tris- HCl, 150 mM NaCl, pH 7.2) containing 5% nonfat dry milk for 2 h at room temperature. The membranes were then incubated with previously obtained rabbit anti-*C. albicans* hyperimmune sera (1:50 dilution) for 1 h at room temperature. Next, the membranes were washed three times with phosphate-buffered saline (PBS)–Tween 20 and incubated with a 1:1000 dilution of peroxidase-conjugated anti-rabbit immunoglobulin G (IgG) (Sigma-Aldrich) for 1 h at 37°C. The reaction was developed in a solution containing 5 mg of 3,3’-diaminobenzidine (DAB; Sigma) and 10 μl of H2O2 in 50 ml of Tris buffer, pH 7.4. The membranes were washed, dried, and exposed to autoradiography.

**Electroelution of Candida albicans 65-kDa protein.** Various SDS-PAGE gels of *C. albicans* exoantigens were run and stained with 0.3 M copper chloride (CuCl2). The region containing the 65-kDa molecule was excised from gels and electroeluted in an electroelution chamber (Isoelectric model 1750 electrophoretic concentrator; Iso, Inc., Lincoln, NE). Electroelution was performed for 3 to 5 h in 192 mM glycine and 25 mM Tris-HCl, pH 8.3, at a 2-A constant current. Proteins were eluted and dialyzed exhaustively against distilled water. The concentration was determined by the method of Bradford (4).

**MAb production of the anti-65 kDa C. albicans molecule.** The MAb was produced by the method of Lopes and Alves (26). Of note, the production of MAb was only initiated after a pilot study showed the antigen was recognized by polyclonal antibody. Six-week-old BALB/c mice were immunized every week for 3 weeks subcutaneously with 50 μg of *C. albicans* 65-kDa protein in PBS incorporated into Freund's complete adjuvant for the first injection and in incomplete Freund's adjuvant for the subsequent ones. Injectons were always made at four different sites in the axillary and inguinal regions at final volumes of 100 μl per site. Before each immunization, mice were bled through the ocular plexus and the serum was separated by centrifugation and stored at −20°C. Final immunizations (50 μg of 65-kDa protein in 100 μl of PBS intravenously) were performed 2 days before cell fusion, following the method of Lopes and Alves (26).

**Antigenemia in sera of patients with invasive fungal infections.** To determine the developed inh-ELISA with sera from patients with candidemia and not to compare it or show its superiority to other methods of detecting *C. albicans*, we followed a method described by Go´mez et al. (16) and involving the following steps: (i) antigenemia in an animal model, (ii) application of the MAb to the inh-ELISA, (iii) characterization of antigenemia in an animal model, and (iv) evaluation of the developed inh-ELISA with sera from patients with candidemia.

**Antibody screening by EIA.** The EIA was performed as described by Pucía and colleagues (41). Briefly, polystyrene microplates (Corning Costar; Corning, NY) were coated with 50 μg of a 2-μg/ml solution of purified 65-kDa protein in PBS for 1 h at room temperature. After blocking free sites with PBS containing 5% nonfat milk for 2 h at room temperature, 50 μl of culture supernatant or purified MAb was added to each well. After 1 h of incubation at 37°C, wells were thoroughly washed with PBS containing 0.5% gelatin (Difco) and 0.05% Tween 20 (Sigma) (PBS-T-G) and treated with affinity-purified peroxidase-conjugated goat anti-mouse Ig (Bio-Rad) for 1 h at 37°C. This was followed by three washes with PBS-T-G. Reactions were developed by the addition of o-phenylenediamine in 0.1 M acetate-phosphate buffer, pH 5.8, stopped with 4 N sulfuric acid, and read in a Titertek Multiskan EIA reader at 492 nm.

**Specificity of MAb against 65-kDa protein.** The exoantigens from *C. albicans*, *C. tropicalis*, *C. parapsilosis*, *H. capsulatum*, *A. fumigatus*, *P. brasiliensis*, *S. schenckii*, and *T. rubrum* were subjected to SDS-PAGE (22) and Western blotting (47). Briefly, the membrane was blocked with Tris-buffered saline (20 mM Tris-HCl, 150 mM NaCl, pH 7.2) containing 5% nonfat dry milk for 2 h at room temperature. The membrane was then incubated with anti-65 kDa MAb (10 μg/ml) for 1 h at room temperature. Then, the membrane was washed three times with PBS-Tween 20 and incubated with a 1:1,000 dilution of peroxidase-conjugated anti-mouse IgG (Sigma) for 1 h at 37°C. The reaction was developed in a solution containing 5 mg of DAB (Sigma) plus 10 μl of H2O2 in 50 ml of Tris buffer, pH 7.4. The membrane was washed, dried, and stored for documentation.

**SDS-PAGE and Western blot for determination of immunodominant antigen.** SDS-PAGE and Western blot for determination of immunodominant antigen involved five different stages: (i) identification of an immunodominant 65-kDa antigen of *C. albicans* that is common to *C. tropicalis* and *C. parapsilosis*, (ii) production of an anti-*C. albicans* 65-kDa-molecule MAb for detection of the immunodominant antigen mentioned above, (iii) application of the MAb to the inh-ELISA, (iv) characterization of antigenemia in an animal model, and (v) evaluation of the developed inh-ELISA with sera from patients with candidemia.
FIG. 1. Scheme 1. (A) Serum samples were mixed with an equal volume of 0.1 M EDTA and boiled at 100°C to separate antigen/antibody complexes. (B) The plate was blocked, and samples from panel A were transferred to the inhibition plate and mixed with MAb anti-65-kDa protein. The MAb was linked to free 65-kDa protein in serum. (C) The MAb/65-kDa protein complex from panel B was transferred to a plate previously sensitized with the 65-kDa protein (reaction plate). The free MAb was linked to the 65-kDa protein on the plate, and an IgG-peroxidase conjugate anti-mouse IgG was added. The reaction was developed using chemiluminescent solution, and the degree of inhibition of MAb binding was shown to be reciprocal to the concentration of circulating antigen in the sample.

Purified anti-65 kDa MAb was used (10 mg/ml), and all samples were diluted 1:2 in diluting buffer. The method is illustrated in detail in Fig. 1.

Pretreatment of immune sera for use in inh-ELISA. Aliquots of immune serum (200 µl) were mixed with an equal volume of 0.1 M EDTA (Sigma), pH 7.2, and boiled at 100°C for 3 to 5 min. The tubes were cooled and centrifuged at 13,000 × g for 30 min. The supernatant was used for the test.

Inhibition plates. An inhibition standard curve was constructed by adding different concentrations of C. albicans 65-kDa protein (from 1 ng/ml to 30 µg/ml) to 100 µl of pooled NHS and then adding 100 µl of the standardized concentration of anti-65 kDa protein MAB. NHS (diluted 1:2 in diluting buffer) was used as a negative control. All standards, samples, and controls were tested in triplicate. Samples were plated onto 96-well flat microtiter plates (Corning Costar), previously blocked with 200 µl of 5% nonfat milk per well, made up in PBS-Tween, for 2 h at 37°C. Plates were mixed in a shaker for 30 min at room temperature and then incubated overnight at 4°C.

Reaction plates. Maxisorp polystyrene plates (Corning Costar), previously blocked with 200 µl of 5% nonfat milk per well, made up in PBS-Tween and blocked with 200 µl per well of 1% bovine serum albumin in PBS for 1 h at 37°C. After three more washes, 100 µl from each inhibition plate well (containing a mixture of the MAb circulating complexes and free MAB) was transferred to the respective wells in the reaction plate and incubated for 2 h at 37°C. After being washed as described above, 100 µl of goat anti-mouse IgG-peroxidase (Sigma) was added, and the plates were incubated for 1 h at 37°C. After further washings, the reaction was developed with SuperSignal West Pico Chemiluminescent solution (1:10) (Pierce biotechnology). Optical densities (ODs) were measured at 470 nm with a Chemiluminescent ELISA reader (SpectraMax). The OD at 470 nm was then plotted on a standard curve constructed from the data derived from MAb titration with NHS containing known quantities of 65-kDa protein, as described above. The degree of inhibition of MAb binding was shown to be reciprocal to the concentration of circulating antigen in the sample. The cutoff point was established as the receiver operator characteristic curve.

Neutropenic model of disseminated Candida infection for detection of 65-kDa protein. For determination of circulating antigens in an experimental model of candidemia, 6-week-old, 22- to 24-g BALB/c female mice (n = 21) were rendered neutropenic (absolute neutrophil count of <100/µl) by administration of 150 mg/kg of intraperitoneal (i.p.) cyclophosphamide 4 days prior to infection. This was followed by a second dose of 100 mg/kg i.p. cyclophosphamide 1 day prior to infection and then 100 mg/kg i.p. cyclophosphamide every other day thereafter. Twenty-four hours after the second dose of cyclophosphamide, three groups of mice (n = 7/group) were intravenously inoculated with 1 × 10^6 cells of C. albicans, 1 × 10^6 cells of C. parapsilosis, or 1 × 10^6 cells of C. tropicalis. A pool of serum was collected daily for each group for up to 7 days or until the group died. Animal experiments were approved by the Institutional Animal Care and Use Committee of the Federal University of São Paulo.

Human clinical samples. A total of 20 candidemic patients, diagnosed by a positive blood culture processed by the Bactec 9240 system, were sequentially enrolled in this study. Volunteers were selected among patients who signed the informed consent form and were admitted to the Hospital São Paulo, São Paulo, Brazil, between February 2006 and July 2007. Serum samples for antigen detection were obtained at the time of diagnosis of candidemia and after different intervals. Stocked serum samples collected from candidemia patients before the onset of fungemia were also evaluated when available. A pool of serum samples from healthy volunteers (n = 20, blood donors) were included as negative controls. To verify cross-reactions, sera from patients with active histoplasmosis (n = 3), active paracoccidioidomycosis (n = 3), active Jorge Lobo’s disease (n = 3), and active aspergillosis (n = 3) were also tested.

RESULTS

Composition of the immunodominant antigen. After SDS-PAGE of the exoantigens from C. albicans, C. parapsilosis, C. tropicalis, and C. glabrata, a common protein band of 65 kDa was found among the three species C. albicans, C. tropicalis, and C. parapsilosis. C. glabrata did not show this molecule (Fig. 2). In the pilot study, the rabbit anti-C. albicans hyperimmune serum recognized the 65-kDa antigen present in the exoantigens of C. albicans, C. tropicalis, and C. parapsilosis by immunoblotting.

Specificity of the MAb against the 65-kDa protein. After detecting the presence of the 65-kDa antigen in C. albicans, C. tropicalis, and C. parapsilosis exoantigens and its detection in the sera of experimental models and candidiasis human sera using rabbit polyclonal antibodies (data not shown), MAbs
were produced to detect the 65-kDa protein. A panel of different hybridoma lines reactive to *C. albicans* 65-kDa antigen was obtained. MAbs belonged to the IgG1 subclass and recognized an antigenic determinant of *C. albicans* with a relative mass of 65 kDa by Western blotting (Fig. 3). This MAb also recognized the 65-kDa molecule present in *C. tropicalis* and *C. parapsilosis*. This MAb showed no reactivity with exoantigens of *H. capsulatum*, *S. schenckii*, *T. rubrum*, *A. fumigatus*, and *C. glabrata* and was selected to develop the inh-ELISA test. This MAb was designated “IgG1-2.”

**Dynamics of 65-kDa antigenemia in a murine model of invasive candidiasis by MAb inh-ELISA.** The inh-ELISA was first tested in a murine model of invasive candidiasis before validation in the human system. Three groups of mice were infected with *C. albicans*, *C. tropicalis*, and *C. parapsilosis*, respectively. Animals were not challenged with *C. glabrata* because the 65-kDa protein was not detected in this species. All animals presented the 65-kDa protein in serum samples collected daily for up to 7 days. In all three groups, the final serum sample collection occurred when the infected mice were dead. In the murine model infected with *C. albicans*, the 65-kDa molecule was detected in the range of 0.019 to 2.6 µg/ml; in the *C. tropicalis* model, the molecule was detected in the range of 0.012 to 3.25 µg/ml; and in the *C. parapsilosis* model, the molecule was detected in the range of 0.019 to 1.68 µg/ml. Figure 4 shows typical curves obtained from the groups of animals infected with different *Candida* species. Antigen concentrations higher than 0.23 µg/ml (maximum concentration obtained with 10 normal mouse sera) were considered positive.

**Dynamics of 65-kDa antigenemia in patients with invasive candidiasis.** Figure 5 illustrates the standard inhibition curve constructed with known quantities of purified 65-kDa protein.
obtained with known quantities of \textit{C. albicans} 65-kDa protein. This curve was used to determine the concentration of 65-kDa antigen in each sample tested. Antigenemia was present in 20 candidemia patients, from 10 to 87 years of age (median, 47.1 years): 45% were male, and all had been exposed to multiple risk factors before developing candidemia. All patients were treated with antifungal drugs 1 to 4 days after the incidence of candidemia. A total of 16 of the 20 candidemic patients exhibited the 65-kDa protein in concentrations ranging from 0.072 μg/ml to 5 μg/ml of antigen per ml of serum. Four patients (no. 4, 7, 9, and 10) that had sera collected before the onset of candidemia presented the 65-kDa protein. Sequential sera presented three different patterns of antigenemia due to the 65-kDa molecule: (i) total clearance of antigenemia (Fig. 6A), (ii) initial clearance and relapse of antigenemia (Fig. 6B), and (iii) partial clearance of antigenemia (Fig. 6C). An antigen concentration higher than 0.02 μg/ml (normal human serum) was considered positive. Table 1 summarizes the 65-kDa antigen

FIG. 6. Dynamics of 65-kDa antigenemia in patients with invasive candidiasis by MAb inh-ELISA. (A) Total clearance of antigenemia; (B) initial clearance and relapse of antigenemia; (C) partial clearance of antigenemia. The value 0.02 represents NHS. Crosses indicate death; smiling faces indicate the patient survived, and a dotted line represents the beginning of treatment.
the system was tested in a murine experimental model of disseminated candidiasis infection. During the experiments, 65-kDa antigen was detected in concentrations ranging from 0.07 to 5.0 μg/ml. Three dynamics of 65-kDa antigenemia were observed among these patients. Four patients experienced a total clearance of 65-kDa antigenemia after treatment with antifungal drugs: the concentration of 65-kDa antigen in the last sample tested was less than 0.11 μg/ml. Five patients experienced an initial clearance after treatment with antifungal drugs (with the exception of patient 1) and a relapse of antigenemia (patient 7 presented an initial clearance, a relapse of antigenemia, total clearance of antigenemia, and survival after treatment). Seven patients experienced a partial clearance of antigenemia in which the concentration of 65-kDa antigen in the last sample analyzed was greater than 0.25 μg/ml. Patients with persistent 65-kDa antigenemia after antifungal treatment did not survive, with the exception of patient 20. During patient follow-up, some sequential sera showed “clearance” of the antigen which may be explained by rapid renal clearance or the host’s immune response. Otherwise, the inh-ELISA was able to detect the 65-kDa protein in serum samples collected before the onset of candidemia, indicating its possible use for early diagnosis of disseminate candidiasis. Although five species of Candida—C. albicans, C. tropicalis, C. kefyr, and C. glabrata—were isolated from the blood of these patients, the 65-kDa antigen was detected only in sera from those patients infected with C. albicans, C. parapsilosis, or C. tropicalis.

It is extremely complex to evaluate the cause of mortality in patients with candidemia. Considering all underlying conditions present at the moment a patient develops candidemia, it is possible a significant proportion of patients will have mortality related to candidemia but not attributable to it. Consequently, it is hard to establish a direct connection between antigen levels and risk of mortality. Data obtained from animal models suggest that transient clearance of antigen from blood may occur in animals that will die of systemic infection. In humans, no positive results were found among the sera from healthy people.

Available data suggest the inh-ELISA has good sensitivity and specificity for the diagnosis of candidemia. However, more data are needed to clarify whether this test may or may not be used for monitoring clearance of Candida infections from blood or tissues. Based on our preliminary data, negative inh-ELISA results were not correlated with survival. In addition, we observed transient clearance of antigenemia may occur with further increase of antigen after an interval. Taken together, this suggests the level of antigen in serum may not correlate in time with the burden of infection.

Hypothetically, the clearance can be explained either by the immune response of the host, where phagocytes ingest the molecule of 65-kDa antigen, decreasing the levels in the serum of patients at the moment of sample collection, or by filtration of blood in the kidneys, where the molecule could also be

<table>
<thead>
<tr>
<th>Patient</th>
<th>Conc (μg/ml) of 65-kDa protein</th>
<th>Species isolated from blood culture</th>
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<tbody>
<tr>
<td>1</td>
<td>2.24</td>
<td>C. tropicalis</td>
</tr>
<tr>
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<td>ND</td>
<td>C. kefyr</td>
</tr>
<tr>
<td>3</td>
<td>ND</td>
<td>C. glabrata</td>
</tr>
<tr>
<td>4</td>
<td>ND</td>
<td>C. albicans</td>
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<td>5</td>
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</tr>
<tr>
<td>6</td>
<td>ND</td>
<td>C. glabrata</td>
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<td>7</td>
<td>2.56</td>
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</tr>
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<tr>
<td>20</td>
<td>1.98</td>
<td>C. parapsilosis</td>
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</tbody>
</table>

*Shown is the concentration of 65-kDa protein in sera from patients at the moment of diagnosis (Bactec positive). ND, not detected.

The system was tested in a murine experimental model of detection of the 65-kDa antigen in the inh-ELISA system, always considered low.

During pilot studies analyzing exoantigens from the more prevalent Candida species, isolated from Brazilian patients with candidemia, we found an intense 65-kDa molecule common to C. albicans, C. tropicalis, and C. parapsilosis. We hypothesized the presence of this molecule could be used as a marker of invasive candidiasis. The 65-kDa molecule from C. albicans was used to produce a hyperimmune serum (in rabbits) that was able to recognize this antigen in C. albicans, C. tropicalis, and C. parapsilosis blot tests. To verify the efficiency of detection of the 65-kDa antigen in the inh-ELISA system, the system was tested in a murine experimental model of disseminated candidiasis before its application in a human system. The results showed detection of this antigen was possible in murine candidiasis evoked by the three species of Candida.

To increase the sensitivity of the inh-ELISA test, MAb against 65-kDa C. albicans was produced and tested in C. albicans, C. tropicalis, and C. parapsilosis murine models of disseminated candidiasis infection. During the experiments, the 65-kDa antigen was detected in concentrations ranging from 0.012 to 3.25 μg/ml of serum and the inh-ELISA was sensitive enough to detect 100% of the sera tested. The dynamics of 65-kDa antigenemia was different in each group; but in each group, “clearance” of the 65-kDa antigen was observed in some serum samples collected after infection and the clearance was not dependent on antifungal treatment. The drop in the level of 65-kDa antigen in mice infected with C. tropicalis does not necessarily indicate the mice were cleared, because on the last day sample sera were collected, the group of mice was dead. The levels in control sera from uninfected animals were always considered low.

**DISCUSSION**

During pilot studies analyzing exoantigens from the more prevalent Candida species, isolated from Brazilian patients with candidemia, we found an intense 65-kDa molecule common to C. albicans, C. tropicalis, and C. parapsilosis. We hypothesized the presence of this molecule could be used as a marker of invasive candidiasis. The 65-kDa molecule from C. albicans was used to produce a hyperimmune serum (in rabbits) that was able to recognize this antigen in C. albicans, C. tropicalis, and C. parapsilosis blot tests. To verify the efficiency of detection of the 65-kDa antigen in the inh-ELISA system, the system was tested in a murine experimental model of disseminated candidiasis before its application in a human system. The results showed detection of this antigen was possible in murine candidiasis evoked by the three species of Candida.

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eliminated. However, in this study it was not possible to collect urine from our patients because they were in the ICU with renal failure. In the future, we will do this analysis.

In this study, some patients presented with the 65-kDa antigen in sera collected before the onset of candidiasis (diagnosed by the Bactec system). Therefore, in patients suspected of disseminated candidiasis, we suggest serum samples be collected as early as possible in an attempt to detect this marker antigen before candidiasis is diagnosed by the Bactec system. Early diagnosis of candidiasis would allow for appropriate antifungal treatment and prevention of Candida dissemination, reducing the rate of mortality among these patients. Blood cultures are still considered the “gold standard” for the diagnosis of candidiasis, as no single molecular method has been proved superior. This may be confirmed by a recent review of the criteria for diagnosis of invasive fungal infections published by NIH (2).

In conclusion, we propose the use of this inh-ELISA system, using a species-specific MAb, as an additional test for detection of circulating antigen in patients with candidiasis. The use of the 65-kDa antigen detection system described here will provide a rapid and specific means of diagnosis. Moreover, this test has the potential to follow the course of antifungal therapy by monitoring the reduction in fungal load, as evidenced by sequential reduction in antigen detection. This will be addressed in future studies.

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