Detection of Antibodies to Candida albicans Germ Tubes during Experimental Infections by Different Candida Species

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Identification and characterization of Candida albicans germ tube-specific antigens may be of relevance for the serodiagnosis of invasive candidiasis since they could be the basis for the development of new diagnostic tests. In this study, we have identified two antigens of 180 and >200 kDa in the cell wall of C. albicans germ tubes which are responsible for the induction of antibodies to C. albicans germ tubes. Antigens of similar molecular masses have been demonstrated in the cell walls of the Candida species C. stellatoidea, C. parapsilosis, C. guilliermondii, C. tropicalis, and C. krusei, but not C. glabrata. The kinetics of the antibody responses to C. albicans germ tubes were studied in rabbits infected with different Candida species. Although these antibodies were detected in rabbits infected with all Candida species except C. glabrata, the kinetics of the antibody responses to C. albicans germ tubes induced by the Candida species studied were different. Both the highest titer and the earliest response of antibodies to C. albicans germ tubes were observed in rabbits infected with either of the two serotypes of C. albicans used. However, the time needed to elicit the antibodies to C. albicans germ tubes could be reduced as the result of an anamnestic antibody response. The results presented in this study show that a test designed to detect antibodies against C. albicans germ tube antigens may be suitable for the diagnosis of infections caused by most of the medically important Candida species.

Candida albicans is a dimorphic opportunistic pathogen involved in a wide range of infections, from transient mucocutaneous candidiasis to life-threatening invasive candidiasis in immunocompromised patients. Diagnosis of invasive candidiasis usually requires a high index of suspicion and is difficult because the infection lacks pathognomonic signs, blood cultures are often negative, and in many instances, it is not possible to obtain specimens for histology (18). Serology could be an aid in the diagnosis of candidiasis provided specific markers which distinguish between superficial and invasive infections are identified.

Among the different antigens used in the serodiagnosis of invasive candidiasis, those expressed in the mycelial phase have been particularly studied in the hope that they could be specific for the diagnosis. A variety of germ tube-specific antigens with molecular masses of 155, 200, and >200 kDa (31–33), 62 and 70 kDa (3), 180 and 260 kDa (5), 19 and 235 to 250 kDa (22, 23), 35 and 27 kDa (17), 43, 47, and 80 kDa (4), 20 to 67 kDa (19), 110 to 170 kDa (20), 43 kDa (2), and 30 kDa (1) have been described. However, the complexity of the antigenic extracts, the variation of antigen expression depending on culture conditions, and the variation in the antisera used have not allowed a complete characterization of the number, structure, and function of the various antigens. Identification and characterization of germ tube-specific antigens may be of relevance for the serodiagnosis of invasive candidiasis. An indirect immunofluorescence technique based on the detection of antibodies to C. albicans germ tubes has been used for the diagnosis of invasive candidiasis (6, 24–26, 28, 34). Antibodies to C. albicans germ tubes can be found not only in patients with invasive C. albicans infections but also in patients with infections caused by C. tropicalis, C. parapsilosis, and C. krusei (6, 24, 26, 28). If other members of the genus Candida are also able to induce antibodies to C. albicans germ tubes, a test designed to detect antibodies against C. albicans germ tube antigens may be suitable for the diagnosis of infections caused by most of the medically important Candida species. In this study, we have identified the cell wall antigens from C. albicans and other Candida species responsible for the induction of antibodies to C. albicans germ tubes. Furthermore, we have studied the kinetics of induction of these antibodies in rabbits infected with different Candida species.

MATERIALS AND METHODS

Organisms. Most strains used in this work were obtained from the National Collection of Pathogenic Fungi (Bristol, United Kingdom) or the American Type Culture Collection (Rockville, Md.) and included C. albicans serotype A NCPF 3153, C. albicans serotype B NCPF 3156, C. tropicalis NCPF 3111, C. stellatoidea ATCC 20408, C. parapsilosis NCPF 3104, C. guilliermondii NCPF 3099, C. glabrata NCPF 3203, C. krusei NCPF 3109, Aspergillus fumigatus NCPF 2109, and Scedosporium prolificans NCPF 2799. C. albicans CA2, which is a germ tube-deficient strain obtained from strain NCPF 3153, was kindly provided by A. Casone (Rome, Italy). A Trichosporon beigelii clinical isolate was obtained from the Universidad del País Vasco strain collection.

Culture conditions. For most experiments, C. albicans blastospores and germ tubes were grown in medium 199 (Sigma Chemical Co., St. Louis, Mo.) as previously described (21). Briefly, 48-h-old blastospores grown in glucose-yeast extract-agar plates were transferred at 5 × 10⁷ blastospores/ml to Erlenmeyer flasks containing medium 199, and they were incubated at 25°C for 18 h in a rotatory shaker set at 200 rpm. After incubation, blastospores were harvested by centrifugation, inoculated into new medium, and incubated at 21°C for 24 h at 200 rpm to obtain blastospores or at 37°C for 4 h at 200 rpm to obtain germ tubes. To obtain the cells to inoculate the rabbits, the Candida species and T. beigelii were grown in Sabouraud agar (Difco, Detroit, Mich.) for 48 h at 25°C. A. fumigatus and S. prolificans were grown in Sabouraud broth at 25°C for 24 h at 200 rpm.

Preparation of antigens. Cell walls of C. albicans blastospores and germ tubes were extracted in the presence of dithiothreitol (DTT) (Sigma) as reported previously (21). Cell wall extracts from other Candida species were obtained after incubation in the same conditions used for germ tubes. In some experiments, C. albicans germ tube cell wall antigens were oxidized with sodium meta-
periodate. Briefly, germ tube DTT-antigenic extracts were placed in a dialysis membrane and treated with 0.05 M sodium metaperiodate in 0.05 M acetate buffer for 18 h at 4°C. The sample was then dialyzed against distilled water and lyophilized.

**Experimental infection of rabbits.** Female New Zealand White rabbits, each with an initial weight of 2 to 2.5 kg, were intravenously inoculated with 10⁷ blastospores of C. albicans serotype A, C. albicans serotype B, C. tropicalis, C. stellatoidea, C. parapsilosis, C. guilliermondii, C. glabrata, or C. krusei. Infections were repeated at monthly intervals. These rabbits were inoculated with T. beigelii, A. fumigatus, or S. prolificans and used as controls. In the infection with T. beigelii, we followed the immunization schedule described above for *Candida* species. The other rabbits were inoculated subcutaneously with a suspended A. fumigatus or S. prolificans filament containing the equivalent of 10 µg of protein in 0.5 ml of saline and 0.5 ml of complete Freund adjuvant. Preimmune serum was obtained from each rabbit, and samples of immune serum were extracted weekly from the ear marginal veins of the rabbits. An additional rabbit antiserum to C. albicans germ tubes was prepared by weekly subcutaneous inoculations of 5 × 10⁸ formalin-killed germ tubes in a 1:1 emulsion with 0.5 ml of saline and 0.5 ml of complete Freund adjuvant. In one experiment, two rabbits were inoculated intravenously with 10⁷ blastospores of C. albicans, treated with 0.6 mg of amphotericin B (Fungizona; Squibb Industria Farmaceutica S.A., Madrid, Spain)/kg of body weight during 20 days, and finally inoculated with 10⁷ blastospores of C. glabrata. The animal studies were performed in accord with the guidelines of the Department of Agriculture of the Basque Government.

**Adsorption of sera.** Two types of antiserum were obtained from every serum sample taken from the infected rabbits: (i) the unadsorbed antiserum (labeled as *C. albicans* blastospore antiserum) and (ii) the adsorbed antiserum, obtained by adsorption of the blastospore antiserum with C. albicans blastospores as previously described (21). Briefly, sera were mixed with an equal volume of a 10¹⁰ heat-killed blastospores/ml suspension in saline and incubated for 2 h at room temperature. After incubation the suspension was centrifuged, and the supernatant was dialyzed against phosphate-buffered saline and incubated at 4°C for 18 h. This adsorbed serum was labeled as the germ tube antiserum. Additionally, an antiserum containing antibodies reacting with the germ tube wall cell surface was obtained by a previously described method (27) from the rabbit immunized subcutaneously with formalin-killed germ tubes. Briefly, 0.5 ml of the rabbit's hyperimmunized serum was adsorbed with blastospores as described above, and this adsorbed antiserum was incubated with 10¹⁸ live germ tubes/ml suspension in saline and incubated for 2 h at room temperature. After incubation the suspension was centrifuged, and the supernatant was dialyzed against phosphate-buffered saline (PBS) buffer containing 0.1% sodium azide. Unadsorbed serum proteins were removed by three washes in PBS, and attached antibodies were then eluted by treatment with 2.5 M NaCl in PBS for 1 h at room temperature. Germ tubes were spun down, and the supernatant was dialyzed against PBS for 48 h at 4°C. This antiserum was labeled as *C. albicans* germ tube surface-specific antiserum.

**Immunofluorescence.** Indirect immunofluorescence assays were carried out as previously described (25). Briefly, C. albicans NCPF 3153 germ tubes were fixed to Teflon-coated wells of immunofluorescence slides. Germ tubes were incubated with serial dilutions of the rabbit sera and washed, and the reacting antibodies were revealed by an incubation with biotin-conjugated goat anti-rabbit immunoglobulin G (whole molecule) antibodies (Sigma). Concanavalin A staining was used as a control for the oxidation, and the oxidized DTT extracts showed no reactivity with the lectin (data not shown).

**Immunochemistry.** Western blot analysis (SDS-PAGE and blotting) was performed by the method of Laemmli (16) in a minigel system (Bio-Rad Laboratories, Richmond, Calif.). Electrophoresis was done in 10 and 4 to 15% acrylamide gradient slab gels at 200 V. Subsequently, the gels were transferred electrophoretically to a nitrocellulose membrane and treated with 0.05 M sodium metaperiodate in 0.05 M acetate buffer for 18 h at 4°C. The sample was then dialyzed against distilled water and lyophilized.

**RESULTS**

**Identification of C. albicans antigens reactive with antibodies to C. albicans germ tubes.** The presence of germ tube-specific antigens in the cell wall of C. albicans strains was studied by both indirect immunofluorescence and immunoblotting for two C. albicans strains. Strain NCPF 3153 yielded germ blastospores in both culture conditions. When cells from both strains grown in the different conditions were studied by indirect immunofluorescence with the *C. albicans* germ tube antiserum and the *C. albicans* germ tube surface-specific antiserum, only germ blastospores produced by *C. albicans* NCPF 3153 stained (data not shown). As shown by immunoblotting, the germ tube antiserum reacted with antigens in DTT extracts from both strains spanning a wide range of molecular masses (Fig. 1A). However, the *C. albicans* germ tube surface-specific antiserum reacted predominantly with an antigen with a molecular mass of >200 kDa. The >200-kDa antigen was expressed in *C. albicans* germ tubes, but not in blastospores, and it was not expressed in strain Ca2 regardless of the culture conditions (Fig. 1B).

In one experiment, DTT extracts from strain NCPF 3153 were oxidized with sodium metaperiodate prior to electrophoresis and blotting (Fig. 1C). Oxidation of the >200-kDa antigen yielded a 180-kDa antigen, showing that the antigen is a glycosylated protein. Concanavalin A staining was used as a control for the oxidation, and the oxidized DTT extracts showed no reactivity with the lectin (data not shown).

**Induction of antibodies against C. albicans cell wall surface antigens in experimental infections.** The kinetics of the antibody response to *C. albicans* cell wall antigens was studied by indirect immunofluorescence in rabbits experimentally infected with different *Candida* species. In a typical experiment, antibodies against antigens expressed on the blastospore cell wall were detected shortly after infection of the rabbits with *C. albicans* NCPF 3153 serotype A (mean, 5.6 ± 1.16 days) (Fig. 2 and Table 1), and the titers peaked around day 10 postinfection and were maintained with small variations until the end of the experiment. The induction of antibodies to *C. albicans* blastospores was studied by both indirect immunofluorescence and immunoblotting, with respect to the blastospore antibody response, since antibodies to *C. albicans* germ tubes were detected between 16 and 38 days postinfection (mean, 25.2 ± 4.03 days) (*P* < 0.0001). The kinetics of the anti-*C. albicans* antibody responses in rabbits experi-
mentally infected with \textit{C. albicans} serotype B or \textit{C. stellatoidea} were very similar to those observed in rabbits infected with \textit{C. albicans} serotype A (Table 1). In fact, when the times needed to detect antibodies to the \textit{C. albicans} germ tubes and blastospores for these two animal models were compared with those for the \textit{C. albicans} serotype A infection, the differences were not statistically significant. Infection with \textit{C. tropicalis}, \textit{C. parapsilosis}, \textit{C. guilliermondii}, or \textit{C. krusei} induced a \textit{C. albicans} blastospore antibody response which was similar, in most cases, to that shown by the rabbits infected with \textit{C. albicans} (Fig. 3 and Table 1). However, the antibody response against \textit{C. albicans} germ tubes showed a statistically significant delay in comparison to that shown by the animals infected with \textit{C. albicans} (Table 1) \((P < 0.0001)\). Some variability in the antibody response was observed in the groups of animals infected with the same \textit{Candida} species, the highest being observed in the rabbits infected with \textit{C. tropicalis} or \textit{C. parapsilosis} (Table 1). Infection with \textit{C. glabrata} elicited a \textit{C. albicans} blastospore antibody response which was similar to that shown by rabbits infected with \textit{C. albicans}. However, no antibodies against \textit{C. albicans} germ tubes were detected in these rabbits during the 250 days of study. The specificity of the antibody response to \textit{C. albicans} germ tubes was studied in rabbits infected with \textit{T. beigelii}, \textit{A. fumigatus}, or \textit{S. prolificans}. Infection with these fungi induced a low \textit{C. albicans} blastospore antibody response, but no antibodies to \textit{C. albicans} germ tubes were detected (data not shown).

Since antibodies to \textit{C. albicans} germ tubes are occasionally detected in patients with \textit{C. glabrata} infections (11), an additional experiment was performed to study the induction of antibodies to \textit{C. albicans} germ tubes in rabbits which had been previously exposed to \textit{C. albicans} antigens. In this experiment, two rabbits were initially infected with \textit{C. albicans} and then were treated with amphotericin B for 20 days to clear the infection and reinfected with \textit{C. glabrata} (Fig. 4). The infection of the rabbits with \textit{C. albicans} induced the production of antibodies to \textit{C. albicans} blastospores after 9 and 6 days, and the antibody response to \textit{C. albicans} germ tubes was observed after 18 and 16 days, respectively. After the initial rise in the titers of both types of antibodies, there was a decrease in the titer of antibodies to \textit{C. albicans} blastospores and a disappearance of the antibody response to \textit{C. albicans} germ tubes. The infection with \textit{C. glabrata} boosted the antibody response to \textit{C. albicans}

\begin{table}[h]
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\begin{tabular}{|c|c|c|c|c|}
\hline
Strain & Rabbit no. & Day on which first positive serum was detected for: & & \\
\hline
 & & Antiblastospore antibodies & Anti-germ tube antibodies & \\
\hline
\textit{C. albicans} serotype A & 1 & 7 & 30 & \\
 & 2 & 7 & 18 & \\
 & 3 & 7 & 16 & \\
 & 4 & 1 & 38 & \\
 & 5 & 6 & 24 & \\
\textit{C. albicans} serotype B & 6 & 7 & 30 & \\
 & 7 & 9 & 20 & \\
\textit{C. stellatoidea} & 8 & 8 & 21 & \\
\textit{C. tropicalis} & 9 & 1 & 227 & \\
 & 10 & 1 & 36 & \\
\textit{C. parapsilosis} & 11 & 1 & 205 & \\
 & 12 & 28 & 36 & \\
\textit{C. guilliermondii} & 13 & 67 & 178 & \\
 & 14 & 7 & 110 & \\
\textit{C. glabrata} & 15 & 1 & — & \\
 & 16 & 6 & — & \\
\textit{C. krusei} & 17 & 7 & 71 & \\
 & 18 & 1 & 70 & \\
\hline
\end{tabular}
\caption{Detection of antibodies against \textit{C. albicans} cell wall antigens by indirect immunofluorescence during experimental infections with different \textit{Candida} species}
\end{table}

\[^{a}\text{—}, \text{no antibodies were detected in the 250 days of the study.}\]

\begin{figure}[h]
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\includegraphics[width=\textwidth]{figure2.png}
\caption{Kinetics of antibody responses to \textit{C. albicans} blastospores (○) and to \textit{C. albicans} germ tubes (●), detected by indirect immunofluorescence, in a rabbit infected with \textit{C. albicans} serotype A.}
\end{figure}

\begin{figure}[h]
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\includegraphics[width=\textwidth]{figure3.png}
\caption{Kinetics of antibody responses to \textit{C. albicans} blastospores (○) and to \textit{C. albicans} germ tubes (●), detected by indirect immunofluorescence, in a rabbit infected with \textit{C. guilliermondii}.}
\end{figure}

\begin{figure}[h]
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\includegraphics[width=\textwidth]{figure4.png}
\caption{Kinetics of antibody responses to \textit{C. albicans} blastospores (○) and to \textit{C. albicans} germ tubes (●), detected by indirect immunofluorescence, in a rabbit infected with \textit{C. albicans} serotype A, treated with amphotericin B, and infected with \textit{C. glabrata}.}
\end{figure}
C. glabrata (lane 7), or C. tropicalis (lane 3), are listed to the left of the gel.

C. tropicalis antisera it was possible to identify a well-defined antigen of infected with activity which was similar to that shown by the sera from rabbits.

persed components with a molecular mass of.

from the rabbit infected with C. glabrata C. parapsilosis with both serotypes of C. albicans from by immunoblotting with a germ tube cell wall DTT extract move the antimannan antibodies, and then they were studied.

Candida species were first adsorbed with C. albicans cell wall antigens in experimental infections. Since the infection by Candida species other than C. albicans induced an antibody response to C. albicans germ tubes, it was of interest to identify the antigens eliciting such an antibody response. The antisera from rabbits infected with different Candida species were first adsorbed with C. albicans blastospores to remove the antimannan antibodies, and then they were studied by immunoblotting with a germ tube cell wall DTT extract from C. albicans NCPF 3153. Antiseria from rabbits infected with both serotypes of C. albicans recognized predominantly antigens with molecular masses of >60 kDa (Fig. 5). With both antisera it was possible to identify a well-defined antigen of >200 kDa. Antiseria from rabbits infected with C. stellatoidea, C. parapsilosis, C. guilliermondii, and C. krusei showed a reactivity which was similar to that shown by the sera from rabbits infected with C. albicans. The serum from the rabbit infected with C. tropicalis stained only two antigens of >200 and 35 kDa present in extracts from C. albicans germ tubes. The antisera from the rabbit infected with C. glabrata reacted with polydispersed components with a molecular mass of >100 kDa but failed to stain the well-defined component of >200 kDa observed with the other antisera (Fig. 5).

In a different experiment, the reactivities of a C. albicans germ tube antiserum and a C. albicans germ tube surface-specific antiserum against cell wall extracts from different Candida species were studied. The C. albicans germ tube antiserum reacted with antigens in all the Candida species studied, spanning a wide molecular mass range (Fig. 6A), but only a few antigens were stained by the germ tube surface-specific antiserum (Fig. 6B). Interestingly, components of >200 kDa were recognized in extracts from both serotypes of C. albicans and from C. stellatoidea, C. tropicalis, C. krusei, and C. guilliermondii. Conversely, a component of 150 kDa was observed in the C. parapsilosis extract, and no bands were observed in the C. glabrata cell wall extract.

DISCUSSION

The ability of C. albicans to alter its cell morphology from blastospores to hyphae helps the fungus to adhere to the host epithelium (30) and to penetrate the host tissues (14). During this morphological transition important changes in the antigenic composition of the fungus occur which may be useful for serodiagnosis of invasive candidiasis. Different groups have demonstrated that the sera from patients with invasive candidiasis may have antibodies which react with antigens expressed specifically or predominantly on the germ tube cell wall surface (6, 10, 25, 29, 34). Using an antiserum that reacts with antigens expressed on the cell wall surface we have identified in this study a mannoprotein of >200 kDa which was only present in extracts from C. albicans germ tubes. This antigen is likely to be germ tube specific because it was not detected in an agerminative mutant grown at 37°C. Different groups have described high-molecular-weight antigens specifically expressed in C. albicans germ tubes. Using polyclonal antibodies, Sundstrom and coworkers (31–33) identified three antigens of 155, 200, and >200 kDa and Ponton and Jones (21, 22) identified an antigen of 235 to 250 kDa, all of which were specifically expressed in the C. albicans germ tube cell wall. Using monoclonal antibodies, Casanova et al. (5) identified two antigens of 180 and 260 kDa and Marot-Leblond et al. (20) identified an antigen of 110 to 170 kDa, all of which were specific to the C. albicans germ tube cell wall. Although the molecular masses of these antigens are different from the molecular mass of the antigen we describe here, it is likely that they correspond to the same mannoprotein and that the differences result from differences in glycosylation as a consequence of the extraction and separation methods used. In fact, the C. albicans germ tube surface-specific antiserum reacted with a >200-kDa antigen in untreated germ tube extracts, but it reacted with an antigen of 180 kDa when the extracts had been oxidized with sodium metaperiodate. Interestingly, a monoclonal antibody produced against a C. albicans germ tube cell wall antigen of 260 kDa also recognized a 180-kDa antigen (5).
The study of the kinetics of *C. albicans* germ tube antibody response in patients with invasive candidiasis is complicated by the difficulty in establishing the onset of the infection. However, in animal models the kinetics of the antibody response to *C. albicans* infection can be studied in detail. Although there are significant differences between human and rabbit infection, the rabbit model provides important insights about antibody response to *Candida* infection. We chose a rabbit model since there exist similarities in the antibody responses of rabbits and humans to antigens of *C. albicans* (12, 13).

The results presented in this study show that both serotypes of *C. albicans* and *C. tropicalis*, *C. stellatoidea*, *C. parapsilosis*, *C. guillermondii*, and *C. krusei* are able to induce antibodies to *C. albicans* germ tubes. These results are in agreement with previously published data regarding the detection of antibodies to *C. albicans* germ tubes in patients infected with *C. tropicalis*, *C. parapsilosis*, or *C. krusei* (25, 26, 28, 34). As expected, the kinetics of the antibody response to *C. albicans* germ tubes induced by the different *Candida* species studied were different. The highest antibody titer to *C. albicans* germ tubes and the earliest antibody response were detected in rabbits infected with serotypes A and B of *C. albicans*. The antibody response to *C. albicans* germ tubes induced by *C. stellatoidea* type II, which is considered to be a sucrose-negative mutant of *C. albicans* serotype A (15). However, the antibody response to *C. albicans* germ tubes was detected late in the course of the infections by *C. krusei*, *C. parapsilosis*, *C. tropicalis*, and *C. guillermondii*. Two possibilities may explain these delayed antibody responses: (i) these species are less able than *C. albicans* to establish an infection and therefore they may produce a lower antigenic stimulus, and (ii) in these species there was a low expression of antigens capable of eliciting an antibody response to *C. albicans* germ tubes. Both possibilities are in agreement with the lower or nonexistent antibody titers to *C. albicans* germ tubes reported in patients infected with non-*C. albicans* species (24, 26).

The rabbits infected with *C. glabrata* did not induce an antibody response to *C. albicans* germ tubes during the 250 days of study. Three possibilities could explain this observation: (i) this species lacks antigens capable of inducing such an antibody response, (ii) *C. glabrata* has the antigen but it is found in such low amounts that it fails to induce an antibody response, and (iii) *C. glabrata* infection actually suppresses the antibody response to this antigen. If the second possibility is true, the antibodies to *C. albicans* germ tubes could be induced in rabbits infected with *C. glabrata* as the result of an anamnestic antibody response stimulated by a previously expanded B-cell pool that can recognize the antigen even if found in low amounts. This possibility has been confirmed in this study since the rabbits which had had an antibody response to *C. albicans* germ tubes as a result of a previous *C. albicans* infection induced an antibody response to *C. albicans* germ tubes shortly after the infection with *C. glabrata*. This anamnestic antibody response may also explain the difference in the time needed to induce an antibody response to *C. albicans* germ tubes between the two rabbits infected with *C. tropicalis* and *C. parapsilosis*. If this anamnestic response occurs in humans, it could result in a more rapid antibody response to *C. albicans* germ tubes. The kinetics of the antibody response to *C. albicans* germ tubes induced in rabbits infected by different *Candida* species presented in this study suggested that there was a cross-reactivity between the >200-kDa antigen of *C. albicans* and antigens from other *Candida* species. Cross-reactivity among mannans from most of the medically important *Candida* species has been demonstrated in several studies (7–9). However, the immunoblot analyses presented in this report suggest the existence of antigens similar to the >200-kDa antigen present in the germ tube of *C. albicans* in all the non-*C. albicans* species studied, with the exception of *C. glabrata*. In this regard, similar high-molecular-mass components reactive with the *C. albicans* germ tube surface antisera were found in extracts of cells of *C. albicans*, *C. stellatoidea*, *C. guillermondii*, *C. tropicalis*, *C. parapsilosis*, and *C. krusei* grown at 37°C. Although a complete characterization of these antigens is needed to confirm their relatedness, it is tempting to speculate that they represent the same protein, with different degrees of glycosylation, which is expressed during growth as mycelium or pseudomycelium by the different *Candida* species. The *C. albicans* germ tube surface antisera failed to reveal any high-molecular-mass component in *C. glabrata* extracts from cells grown at 37°C. Interestingly, *C. glabrata* is the only species studied which is not able to produce pseudomycelium (14).

In conclusion, our results suggest that the antibodies to *C. albicans* germ tube react with protein epitopes of an antigen of 180 or >200 kDa which is expressed specifically on the *C. albicans* germ tube cell wall surface. This antigen or other antigens showing cross-reactivity with it are expressed on the cell wall of several *Candida* species but not *C. glabrata*. Our results show that a serological test to detect antibodies to *C. albicans* germ tube antigens can be developed for the diagnosis of *Candida* infections. The >200-kDa antigen appears to be a good candidate for continued studies.

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