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Characterization of a Monoclonal Antibody (RJ5) against the Immunodominant 41-kD Antigen of *Candida albicans*

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Abstract. A 41-kD component of *Candida albicans* was identified to be the major antigen radioimmunoprecipitated by antibodies with increased titers in the sera of patients with invasive candidiasis. A mouse monoclonal antibody (RJ5) was generated which, by immunoblotting, showed positive reactivity to the immunoprecipitated 41-kD component. By two-dimensional gel electrophoresis and immunoblotting, MoAb RJ5 was shown to react with different isoforms of the 41-kD component with pI values from 6.1 to 6.9. Furthermore, MoAb RJ5 showed positive reactivity to cytoplasmic antigens of *C. albicans* by frozen section and immunoperoxidase staining. By SDS-polyacrylamide gel electrophoresis and immunoblotting, MoAb RJ5 showed no cross-reactivity to antigens of *Candida tropicalis* and *Candida parapsilosis*. The epitope of the 41-kD molecule recognized by MoAb RJ5 was susceptible to treatment of proteinase K at concentrations of ≥ 5 μ g/ml, and was relatively resistant to periodate oxidation with concentration of NaIO₄ up to 20 mM. This MoAb may be useful in the purification and characterization of the immunodominant 41-kD antigen of *C. albicans*, and as a probe in the detection of *Candida* antigens in the sera of patients with invasive candidiasis.

Introduction

Recently, with the use of potent antibacterial, immunosuppressive, and cytotoxic drugs, lethal invasive candidiasis in compromised hosts has been described with increasing frequency [1-4]. However, accurate diagnosis of invasive candidiasis is not without problems. Few distinctive clinical pictures have been observed in such patients [5-7]. Blood cultures known to be of high diagnostic value in bacterial septicemia are positive in only less than half of such cases [1, 5, 6] and numerous serological tests have been described to facilitate the diagnosis of *Candida* infections [7, 8]. Since *Candida albicans* is a component of the normal flora, low titers of specific antibodies are also present in many healthy individuals [7, 9, 10]. Detection of serum antibodies may be unable to distinguish invasive candidiasis from colonization or superficial infection by *Candida* species [7, 10, 11]. Therefore, development of sensitive and specific tests based on direct detection of antigen(s) seems to be an approach worthy of investigation for differential diagnosis [7]. To achieve

this goal, it is necessary to identify the major antigens associated with invasive candidiasis and develop sensitive probes for the detection of such antigens.

In the present study, immunodominant antigens associated with invasive candidiasis were identified by a radioimmunoprecipitation test. A monoclonal antibody (MoAb RJ5) against a *Candida* antigen which demonstrated the greatest serodiagnostic potential was also generated. *Candida* antigens recognized by MoAb RJ5 were characterized in pilot studies.

Materials and Methods

Patient Sera

All the serum samples were collected from patients treated at the Veterans General Hospital, Taipei. Seventeen serum samples were collected from 16 patients with invasive candidiasis who showed one or more sets of positive blood cultures for *Candida* species taken at least 24 h apart. Most of them had malignancy, others had diabetes mellitus, burns, pancreatitis, acute renal failure or septic shock. Thirteen of these patients were infected with *C. albicans*, 2 with *Candida parapsilosis*, and 1 with *Candida tropicalis*. Paired sera taken 26 days apart were available from a pa-

tient infected with *C. albicans*. In addition, 2 serum samples obtained from patients with positive urine cultures of *C. albicans* and *C. tropicalis* each were also collected. Another 2 serum samples from patients with *Cryptococcus neoformans* infection were also available. As controls, 7 serum samples from healthy laboratory personnel were also included in this study.

Antigen Preparation

Gamma-radiation-treated, freeze-dried *C. albicans* was obtained from Allergon AB (Engelholm, Sweden). Three *Candida* species, *C. albicans* (ATCC 20511), *C. tropicalis* (ATCC 20521) and *C. parapsilosis* (ATCC 20515) were also included in the present study. The crude extract of *C. albicans* was prepared by extracting 5 g of the freeze-dried *C. albicans* with 50 ml of phosphate-buffered saline (PBS, containing 85 mM NaCl, 2.6 mM KH_2PO_4 , 49 mM Na_2HPO_4 , pH 8.0) essentially as described previously [12]. Extracts of 3 *Candida* species, used for the analysis of antigenic cross-reactivity, were prepared by directly solubilizing test organisms grown on Sabouraud dextrose agar (Difco, Detroit, Mich., USA) in the sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) sample buffer (see below).

Radioimmunoprecipitation

In order to analyze *Candida* antigens recognized by antibodies in the sera of the patients, proteins of the crude extract of *C. albicans* were labeled with ^{125}I (Amersham, UK) by the chloramine T method [13]. The labeled antigen (1×10^6 cpm) was mixed with 50 μl of individual serum and incubated at room temperature for 1 h. Fifty microliters of a 50% solution of protein A-Sepharose CL-4B beads (Pharmacia, Uppsala, Sweden) was added to each tube and incubated for another hour. The beads were washed with PBS (150 mM, pH 7.2) containing 0.5% NP-40. One hundred microliters of SDS-PAGE sample buffer (containing 2% SDS and 5% 2-mercaptoethanol) was added to each tube and cooked in boiling water for 5 min. Radiolabeled antigens precipitated by antibodies were analyzed by 12.5% SDS-PAGE according to Laemmli [14]. After gel drying, autoradiography was performed at -70°C for 2 days with X-ray film (Fuji, Japan).

Development of Monoclonal Antibody

Monoclonal antibodies against *C. albicans* were prepared essentially as described previously [15]. Antibody-producing hybrid cells were screened by enzyme-linked immunosorbent assay (ELISA) and SDS-PAGE immunoblotting using the crude extract of *C. albicans*. The isotype of the MoAb was determined by ELISA with class-specific antisera (Zymed, San Francisco, Calif., USA).

SDS-PAGE, Two-Dimensional (2D) Gel Electrophoresis and Immunoblotting

The SDS-PAGE was carried out essentially as described by Laemmli [14]. The molecular weight standards used were obtained from Pharmacia. For screening the hybrid cells, crude extract containing an equivalent of 20 μg proteins in the SDS-PAGE sample buffer was boiled for 5 min and loaded onto a SDS-polyacrylamide gel. For studies on the cross-reactivity among antigens of different *Candida* species, supernatants containing antigens solubilized in SDS-PAGE sample buffer were also boiled for 5 min and were loaded on the SDS-polyacrylamide gels. The 2D gel electrophoresis was carried out essentially as described previously

[12]. After gel electrophoresis, immunoblotting was performed to identify proteins in the unstained gels recognized by MoAbs as described previously [12].

Immunoperoxidase Staining

The *C. albicans* cells grown on the Sabouraud dextrose agar plate were harvested and washed. The cells were then coated onto glass slides by either direct cytospinning of the yeast suspension or after frozen section of the yeast pellet. After fixation, the slides were blocked by incubation with 2% horse serum. Following washing procedures, the slides were incubated with culture supernatants of hybridoma cells at room temperature for 1.5 h. After washing, the slides were incubated with biotin-labeled horse anti-mouse IgG (H+L; Vector, Burlingame, Calif., USA) and then with avidin-biotin-peroxidase complex (Vector) for 30 min each. Finally, the substrate solution containing 3-amino-9-ethyl-carbazole (Sigma, St. Louis, Mo., USA) was added to the slides. After washing with water, the result was observed under light microscope and recorded by photography.

Periodate Oxidation and Proteinase K Treatment

The effect of periodate oxidation and proteinase K treatment of antigens of *C. albicans* on the binding of MoAbs was analyzed by ELISA. Periodate oxidation of antigens was performed as described by Woodward et al. [16]. The antigens of *C. albicans* coated onto microtiter plates were exposed individually to varying concentrations of sodium m-periodate (0.1–20 mM May & Baker, Dagenham, UK) and proteinase K (0.005–100 $\mu\text{g}/\text{ml}$, Merck, Hohenbrunn, FRG). The activity of MoAbs to treated antigens was then determined by ELISA.

Results

The autoradiographic profile of immunoprecipitation of antigens of *C. albicans* by antibodies in different serum samples is shown in figure 1. Increased antibody activities against *Candida* antigens in the sera of patients with invasive candidiasis (fig. 1, lanes 1–17) were observed and compared with those in the sera of control samples (fig. 1, lanes 18–28). Fifteen of the seventeen serum samples obtained from patients with invasive candidiasis showed strong antibody activities to the 41-kD component of *C. albicans*. Increased antibody activities to the component with a molecular weight of about 37 kD was also observed in 8 of the 17 serum samples. Furthermore, relatively weak antibody activities to *Candida* components with molecular weights of about 26 and 20 kD were also observed in some of the 17 serum samples as shown in figure 1.

For the preparation of MoAbs, hybridomas secreting MoAbs were first screened by ELISA using the crude extract of *C. albicans*. By SDS-PAGE and immunoblotting, one hybridoma (RJ5) producing MoAb against the 41-kD component of *C. albicans* (fig. 2)

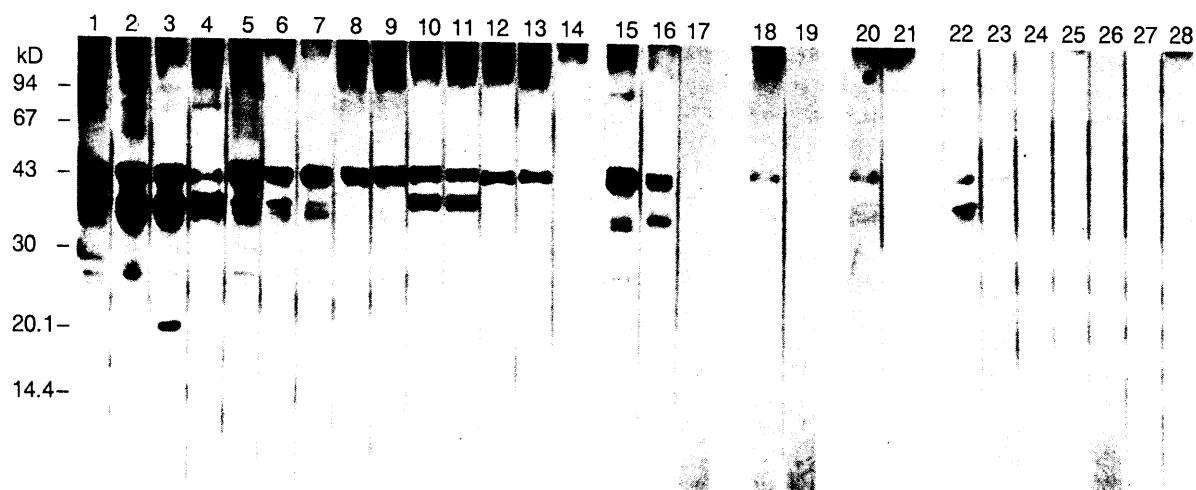


Fig. 1. The autoradiographic profile of immunoprecipitation of antigens of *C. albicans* by antibodies in different serum samples. Lanes 1–14 represent serum samples from 13 patients with invasive candidiasis due to *C. albicans*. Lanes 8 and 9 are paired serum samples obtained from the same patient taken 26 days apart. Lanes 15–17 represent sera from patients with invasive candidiasis due to *C. parapsilosis* (lanes 15 and 17) and *C. tropicalis* (lanes 16). Lanes 18 and 19 are sera from patients with candidaurea due to *C. tropicalis* (lane 18) and *C. albicans* (lane 19). Lanes 20 and 21 are sera from 2 patients with infections due to *Cryptococcus neoformans*. Lanes 22–28 are sera from 7 healthy laboratory personnel.

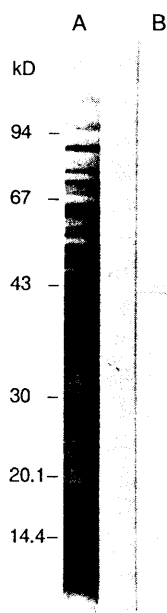


Fig. 2. The Coomassie blue-stained protein profile of the crude extract of *C. albicans* separated by 12.5% SDS-PAGE (lane A). Lane B shows the immunoblot profile of MoAb RJ5 to the crude extract of *C. albicans*.

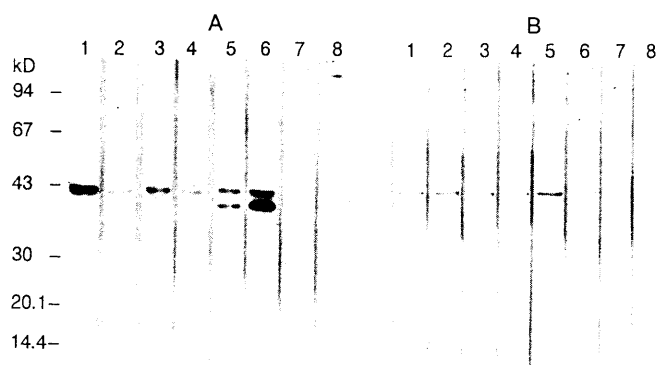


Fig. 3. The immunoblot profile of MoAb RJ5 to antigens of *C. albicans* immunoprecipitated by antibodies in the sera from patients with invasive candidiasis due to *C. albicans*. **A** Autoradiographic profile of the antigens immunoprecipitated and transferred onto the PVDF membrane and then immunoassayed using MoAb RJ5 as shown in **B**. **A** Lanes 1–6 are serum samples from 6 patients with invasive candidiasis due to *C. albicans*; lanes 7–8 are the sera from 2 healthy laboratory personnel. **B** Lanes 1–8 represent the same serum samples as those shown in **A**.

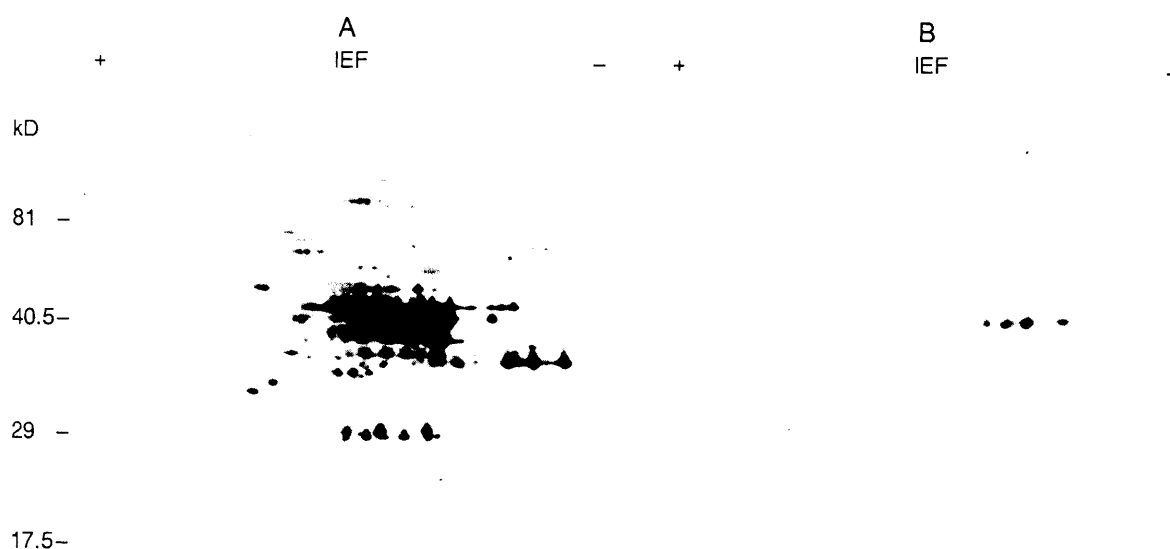


Fig. 4. The 2D protein profile of the crude extract of *C. albicans* (A). The molecular weight markers (Pierce, Rockford, Ill., USA) used are: transketolase (yeast), 81 kD; creatine phosphokinase (rabbit muscle), 40.5 kD; phosphoglycerate mutase (rabbit muscle), 29 kD, and myoglobin (horse), 17.5 kD. **B** The immunoblot profile of MoAb RJ5 to the 2D gel-separated protein components.

was chosen for further studies. The isotype of MoAb RJ5 is IgG1, k. Furthermore, our results also showed that MoAb RJ5 reacted with the radiolabeled 41-kD component of *C. albicans* immunoprecipitated by antibodies in the sera of 6 patients with invasive candidiasis (fig. 3). MoAb RJ5 showed no activity to the radiolabeled 37-kD component of *C. albicans* immunoprecipitated by antibodies in 2 of the 6 serum samples (fig. 3, lanes 5 and 6). To further characterize the antigens of *C. albicans* recognized by MoAb RJ5, 2D gel electrophoresis and immunoblotting were performed. Figure 4A demonstrates the 2D protein profile of the crude extract of *C. albicans*. MoAb RJ5 reacted with different isoforms of the 41-kD component of *C. albicans* with pI values ranging from 6.1 to 6.9 as shown in figure 4B.

To examine the cross-reactivity between antigens of different *Candida* species, the reactivity of MoAb RJ5 to antigens of two other *Candida* species associated with invasive candidiasis was also analyzed by SDS-PAGE and immunoblotting. Figure 5A shows the protein profiles of these three *Candida* species. MoAb RJ5 showed positive reactivity only to the antigen of *C. albicans* (fig. 5B, Lane 1).

The cellular localization of antigens recognized by MoAb RJ5 was identified by immunoperoxidase staining of whole cells and frozen sections of *C. albi-*

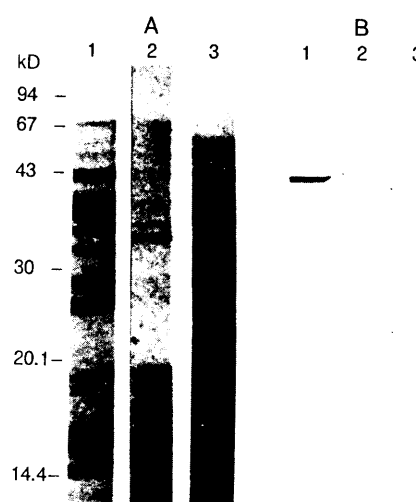


Fig. 5. The SDS-PAGE protein profiles of the extracts of 3 different *Candida* species. **A** Lane 1 *C. albicans*, lane 2 *C. tropicalis*, and lane 3 *C. parapsilosis* prepared by dissolving cellular pellets of each yeast directly in sample buffers and cooked for 5 min. **B** The immunoblot profile of MoAb RJ5 to these antigen preparations. Lanes 1–3 represent the same yeast species as shown in A.

cans. No reactivity was detected when whole cells of *C. albicans* cytospinned onto slides were reacted with MoAb RJ5 (data not shown). However, positive color reaction in the cellular cytoplasm of *C. albicans* was observed when frozen sections were reacted with the

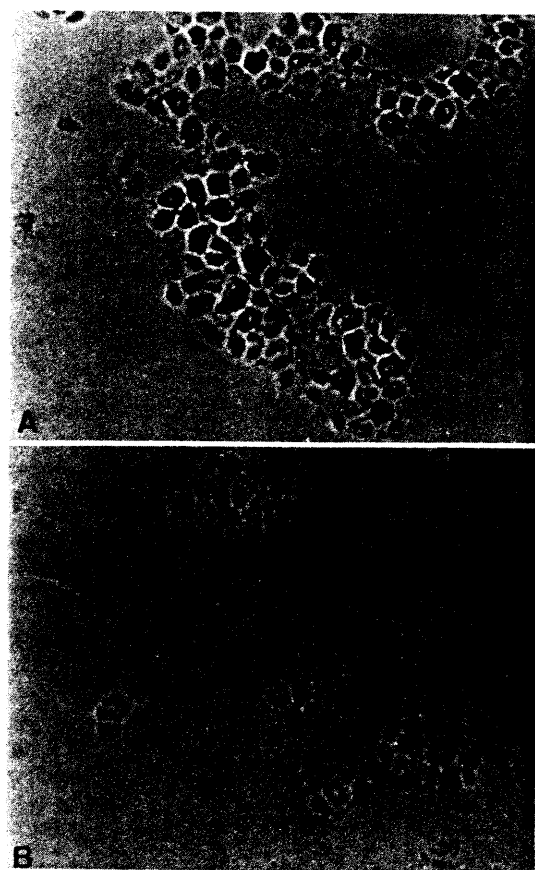


Fig. 6. **A** The immunoperoxidase staining of frozen sections of *C. albicans* using MoAb RJ5. **B** The negative control of the staining using the culture supernatant of NS-1 cells.

Table 1. Effect of periodate oxidation and proteinase K treatment of *C. albicans* antigens on the binding of MoAb RJ5 as assayed by ELISA

Periodate oxidation		Proteinase K treatment	
periodate mM	ELISA A_{405}	proteinase K $\mu\text{g/ml}$	ELISA A_{405}
–	1.100	–	1.355
0.1	1.076	0.005	1.387
0.5	1.009	0.050	1.316
1.0	0.959	0.500	1.168
5.0	0.985	5.000	0.293
10.0	0.937	50.000	0.228
20.0	0.930	100.000	0.224

culture supernatant of hybridoma RJ5 (fig. 6A). Figure 6B shows a negative control of the immunoperoxidase staining using the culture supernatant of NS-1 cells.

To characterize properties of the epitope of the 41-kD component recognized by MoAb RJ5, antigens of *C. albicans* coated onto a microtiter plate were treated with different concentrations of sodium metaperiodate or proteinase K. Our results showed that the epitope recognized by RJ5 was susceptible to the treatment by proteinase K at concentrations of $\geq 5 \mu\text{g/ml}$ (table 1), and was relatively resistant to the treatment of periodate oxidation with a NaIO_4 concentration of up to 20 mM (table 1).

Hybridoma RJ5 is still producing antibodies since its establishment 2 years ago, and is available for other researchers.

Discussion

The identification and characterization of serologically important antigens of *C. albicans* would greatly facilitate the development of a valuable serodiagnostic test. In the present study, a 41-kD component was identified to be the immunodominant antigen of *C. albicans* most frequently recognized by antibodies in the sera of patients with invasive candidiasis. By immunoblot analysis, Matthews et al. [17, 18] demonstrated the immunodominance of an antigen of 47 kD, with 74% of patients producing antibody to this component. Similar immunodominant antigens have also been reported by Strockbine et al. [19] (44–52 kD), Greenfield and Jones [20] (48.9–59.7 kD), Au-Young et al. [21] (45 kD), and Gattermann et al. [22] (43 kD). The antigens described by these authors may be the same or similar antigens with the molecular weight variations due to different methods of antigen preparation and antibody detection being used. Analyzed by immunoblot analysis, antibodies to these antigens were detected in the majority ($\geq 70\%$) of serum samples from patients with invasive candidiasis. It was rare in uninfected controls and infrequent in superficially infected patients [17, 19, 21, 22].

For the characterization of the 41-kD immunodominant antigen of *C. albicans*, a hybridoma stably producing MoAbs (RJ5) was generated and described in this study. Results from the radioimmunoprecipitation and immunoblot analysis showed that the protein recognized by MoAb RJ5 was the same immunodomi-

nant protein recognized by antibodies in the sera of patients with invasive candidiasis. A MoAb (F19) against the proteins recognized by antibodies in the sera of patients during an invasive *Candida* infection has also been reported by Strockbine et al. [23]. By immunoblot analysis, MoAb F19 was identified to be reactive with proteins of 42, 43 and 50 kD in the mycelial cytoplasmic extract of *C. albicans* [23]. In the present study, by 2D-gel electrophoresis and immunoblotting, RJ5 was identified to be reactive with proteins of 41 kD and pI values of 6.1–6.9 in the crude extract of *C. albicans*. Although it is possible that the same antigen was recognized by both MoAbs RJ5 and F19, different epitopes may have been involved. It has also been reported that the immunodominant antigen in invasive candidiasis does not appear to be present on the cell surface [21, 24]. In the present study, the antigens recognized by RJ5 were also identified to be located in the cytoplasm by frozen section and immunoperoxidase staining.

Our results also showed that increased antibody activity against the 41-kD antigen of *C. albicans* was detected in the sera of patients with infection by *C. tropicalis* and *C. parapsilosis*. Strockbine et al. [23] also reported a similar finding that serum from a patient with endocarditis due to *C. parapsilosis* contained antibodies against the 35- to 38-, 44- to 52-, and 120- to 135-kD antigens of *C. albicans*. However, by the SDS-PAGE immunoblot analysis, RJ5 showed positive reactivity to antigens of *C. albicans* only, but not to proteins of the two other *Candida* species, as has also been reported by Matthews and Burnie [25]. They found that when total unfractionated hyperimmune serum to *C. albicans* was used in the detection of *Candida* antigens in the sera of patients, all 6 patients with *C. parapsilosis* infections gave positive results by both the dot immunobinding assay and the reverse passive latex agglutination. However, when the 47-kD antigen-specific probe against *C. albicans* was used, relatively poor detection of invasive candidiasis caused by *Candida* species other than *C. albicans* was observed [25].

In conclusion, a mouse monoclonal antibody (RJ5) against the 41-kD immunodominant antigen of *C. albicans* associated with invasive candidiasis was generated in the present study. Since our results indicate that epitopes recognized by MoAb RJ5 may be associated with proteins but not with carbohydrates, MoAb RJ5 may thus be used in further characterization of the 41-kD antigen of *C. albicans* and in the molecular

cloning of the gene. It may also be useful in the purification of the 41-kD antigen from the crude extract and be used as a probe in the detection of *Candida* antigens in the sera of patients with invasive infections of *C. albicans*.

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