Serological Differentiation of Experimentally Induced *Candida dubliniensis* and *Candida albicans* Infections

MARÍA D. MORAGUES, MIREN J. OMAETXEBARRIA, NATALIA ELGUEZABAL, JOSEBA BIKANDI, GUILLERMO QUINDOÑ, DAVID C. COLEMAN, AND JOSÉ PONTÓN

Departamento de Enfermería 1 and Departamento de Inmunología, Microbiología y Parasitología, Facultad de Medicina y Odontología, Universidad del País Vasco, E-48080 Bilbao, Vizcaya, Spain, and Microbiology Research Unit, Department of Oral Surgery and Oral Pathology, School of Dental Science, Trinity College, University of Dublin, Dublin 2, Republic of Ireland

Received 2 April 2001/Returned for modification 2 May 2001/Accepted 3 June 2001

Using a rabbit model of systemic infection, we show that it is possible to differentiate infections caused by *Candida dubliniensis* and other Candida species by detecting the antibody response mounted by the infected animals. These results confirm our previous observation in a patient with *C. dubliniensis* candidemia and suggest that detection of *C. dubliniensis*-specific antibodies is useful in the diagnosis of invasive candidiasis caused by this yeast.

*Candida dubliniensis* has been predominantly recovered from the oral cavities of human immunodeficiency virus-infected individuals (6, 10, 11). However, in recent years, *C. dubliniensis* has been increasingly recovered from individuals not infected with human immunodeficiency virus (6, 7, 12), including individuals with invasive candidiasis (4, 4a, 5, 9). At present, the identification of systemic infections caused by *C. dubliniensis* relies on the isolation of the yeast from clinical specimens followed by identification by conventional methods used in the clinical microbiology laboratory. We have previously shown the existence of antigenic differences between *C. dubliniensis* and its close relative *Candida albicans* (3), and we have shown that it was possible to differentiate candidemias caused by the two organisms due to the specific antibody response detected in a *C. dubliniensis*-infected patient (9). In the present study, a rabbit model of systemic infection was developed to confirm the existence of differences in the antibody response which can be used in the serodiagnosis of infections caused by *C. dubliniensis*.

Apart from *Candida dubliniensis* CD33 (11), which was from the University of Dublin strain collection, all of the yeast strains used in this study were obtained from the National Collection of Pathogenic Fungi (Bristol, United Kingdom). Yeast strains were routinely grown in medium 199 (Sigma Chemical Co., St. Louis, Mo.) as previously described (2), and their cell walls were extracted in the presence of dithiothreitol (Sigma) as reported previously (8). New Zealand White rabbits were intravenously inoculated with 2 × 10⁶ blastospores as described previously (2). In some experiments, a group of rabbits were immunized subcutaneously with two immunoreactive antigens from *C. dubliniensis* with molecular masses of 34 kDa or between 160 and 170 kDa. For the initial immunization, antigens were dissolved in 0.5 ml of complete Freund’s adjuvant (Sigma) and injected subcutaneously into two rows of five sites equidistantly spaced along the rabbit’s back. Subsequent immunizations were performed every week with the antigens in incomplete Freund’s adjuvant. Sera were adsorbed with *C. albicans* NCPF 3153 blastospores using previously described methods (8). Indirect immunofluorescence and Western blotting assays were performed as previously described (2). To obtain the immunoreactive bands of 34 kDa or 160 to 170 kDa from the cell wall of *C. dubliniensis*, a dithiothreitol extract (300 μg of protein) was separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and electroblotted onto a nitrocellulose membrane as described previously (2). Bands of 34 kDa and 160 to 170 kDa were excised, dissolved in 0.5 ml of dimethyl sulfoxide (Sigma), and diluted with 0.5 ml of complete or incomplete Freund’s adjuvant (Sigma).

It was not possible to discriminate between rabbit systemic infections caused by *C. dubliniensis* NCPF 3949 and *C. albicans* NCPF 3153 on the basis of the detection of antibodies directed to the cell wall surface by indirect immunofluorescence. However, the antibody response against antigens extracted from the cell wall was more informative. Adsorbed sera from rabbits infected with *C. dubliniensis* NCPF 3949 reacted predominantly with four antigens in the *C. dubliniensis* NCPF 3949 extract with molecular masses of 160 to 170, 45, 34, and 29 kDa and with two antigens in the *C. albicans* NCPF 3153 extract with molecular masses of 45 and 29 kDa (Fig. 1). Similar results were observed with cell wall extracts from *C. dubliniensis* CD33. Adsorbed sera from rabbits infected with *C. albicans* NCPF 3153 reacted predominantly with five antigens in the *C. dubliniensis* extract with molecular masses of 70, 45, 32, 30, and 28 kDa and with five antigens in the *C. albicans* extract with molecular masses of >200, 70, 45, 44, and 28 kDa (Fig. 1).

Since the antibody response against *C. dubliniensis* cell wall antigens permitted discrimination between infections caused by *C. dubliniensis* from those caused by *C. albicans*, it was of interest to investigate whether infections caused by other *Candida* species could also be differentiated. Antisera from rabbits infected with *Candida krusei* NCPF 3100 recognized two antigens with molecular masses of 57 and 45 kDa, while sera from
rabbits infected with Candida parapsilosis NCPF 3104 reacted with an antigen with a molecular mass of 39 kDa (Fig. 2). The antisera from the rabbits infected with Candida guilliermondii NCPF 3099 or Candida glabrata NCPF 3203 reacted with polydispersed components of molecular masses between 130 and 150 kDa (Fig. 2). The sera from rabbits infected with Candida tropicalis NCPF 3111 stained polydispersed components of molecular masses between 130 and 160 kDa and several antigens with molecular masses between 38 and 88 kDa (Fig. 2).

The results of the studies described above led to the identification of two antigens with molecular masses of 160 to 170 kDa which are potentially specific for C. dubliniensis. To further characterize these antigens, rabbits were immunized separately with antigen extracts and the resulting antisera were used to stain cell wall extracts from several Candida species. The serum directed against the 160- to 170-kDa antigen reacted with this antigen and with two bands of 65 and 34 kDa in the C. dubliniensis extract (Fig. 3, lane C1). This antiserum also stained faint bands of >200, 65, and 60 kDa in the C. albicans extract (Fig. 3, lane C2). The serum directed against the 34-kDa antigen reacted with several bands (73, 65, 45, 44, 34, and 32 kDa) present in extracts from both C. dubliniensis and C. albicans (Fig. 3, B lanes). However, this antiserum also showed a faint reactivity with the 160- to 170-kDa region in the C. dubliniensis extract and with a band of >200 kDa in the C. albicans extract. The antiserum directed against both the 160- to 170-kDa antigen and the 34-kDa antigen showed a positive reaction by indirect immunofluorescence with C. dubliniensis blastospores but not with C. albicans blastospores (data not shown).

As C. dubliniensis can be recovered from cases of invasive infection, it is clear that routine diagnostic laboratories should be in a position to rapidly and accurately identify this species. Due to its close similarity with C. albicans, identification of C. dubliniensis infections may be problematic in most clinical mycology laboratories, and currently, identification is made following isolation of the fungus in culture. However, recovery from blood may be difficult, since the sensitivity of blood culture methods such as lysis centrifugation varies from 28 to 3000.
78%, depending on the number of deep-tissue sites infected by Candida (1). In this regard, antibody detection may help to identify some cases of C. dubliniensis invasive infections, providing a specific response can be demonstrated (9).

In the present study, we have shown that it is possible to differentiate between experimentally induced infections caused by C. dubliniensis and C. albicans and between infections caused by C. dubliniensis and other clinically significant Candida species on the basis of the antibody response mounted by the infected animals. Rabbits infected with C. dubliniensis induced a specific antibody response directed against two cell wall components of 160 to 170 and 34 kDa not found in animals infected with C. albicans or with any of the other clinically relevant Candida species studied. These components seem to be antigenically related and located on the cell wall surface. The results presented in this study confirm and extend our previous observation of a patient with C. dubliniensis candidemia, who exhibited an antibody response against an antigen of 160 to 170 kDa which was not observed in patients with C. albicans candidemia (9). If the antibody responses detected in rabbits infected by C. tropicalis, C. glabrata, C. krusei, C. parapsilosis, or C. guilliermondii could be extrapolated to humans, it is unlikely that patients with infections caused by these Candida species would produce an antibody response similar to that observed in the patient infected by C. dubliniensis.

In conclusion, we present evidence for the existence of a specific antibody response in rabbits infected with C. dubliniensis which is similar to that observed in a patient with C. dubliniensis candidemia. We believe that detection of antibodies specific for C. dubliniensis antigens may have a clinical application in the diagnosis of invasive candidiasis caused by this yeast, especially in patients with negative blood cultures.

This investigation was supported in part by grants UPV 093.327-G01/98 from the Universidad del País Vasco and PM99-0033 from the Spanish Ministerio de Ciencia y Tecnología.

REFERENCES