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Specific and Common Antigenic Determinants of *Candida albicans* Isolates Detected by Monoclonal Antibody

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We isolated a hybridoma cell line which produced monoclonal antibody to one determinant of an exoantigen of *Candida albicans*. The immunoglobulin G antibody product was characterized by using a Western blot technique and was used for a serological analysis of numerous homologous and heterologous yeast isolates. Based on specific immunologic determinants, *C. albicans* strains were identified and clustered into five groups. The monoclonal antibodies were effective reagents for identifying and serotyping our *C. albicans* isolates; they have potential application in the epidemiology of yeast infections.

Monoclonal antibodies can be invaluable tools for studying the properties of large groups of microorganisms, including fungi. The most common use of monoclonal antibodies is in the development of immunodiagnostic tests for detecting infection by specific organisms.

Previous reports of immunoidentification of pathogenic fungi (4) and yeasts (7) performed with polyvalent antisera prompted us to evaluate the potential use of monoclonal antibodies for identification and serotyping of the pathogenic yeast *Candida albicans*. The question which we asked was whether monoclonal antibodies, because of their specificity, could be more useful than polyvalent antisera.

In this paper we describe the production and characterization of a monoclonal antibody to *Candida albicans* soluble antigen for use in the Western blot technique.

MATERIALS AND METHODS

Cultures. The yeast isolates used were from our collection or were kindly furnished by the Division of Mycotic Diseases, Centers for Disease Control, Atlanta, Ga.; Centraalbureau voor Schimmelcultures, Baarn, The Netherlands; and Istituto Superiore di Sanità, Rome, Italy (Table 1).

Reference antigens. All of the yeast culture antigens were prepared by the following procedure. Yeast isolates were cultured on Sabouraud dextrose agar slants at 25°C for 48 h. An 8-ml portion of a Merthiolate-water (1:5,000) solution was added to each culture, and the cultures were allowed to stand overnight at room temperature. After this, a 5-ml portion of each solution was filtered and lyophilized. The product was suspended in 0.1 ml of sterile water (50× concentrated) and adjusted to a final concentration of 2,500 μg of protein per ml, as determined by the method of Lowry et al. (3).

Mouse immunization. *Candida albicans* CDC B385 antigen was the antigen used for mouse immunizations. BALB/c mice were immunized according to the following schedule: 0.1 ml of the reference antigen, containing 2,500 μg of protein per ml and 550 μg of carbohydrate per ml (6), was mixed with 0.1 ml of incomplete Freund adjuvant and injected intraperitoneally once a week for 1 month; an intraperitoneal booster injection (0.1 ml of the soluble reference antigen) was given 3 days before the mice were killed to obtain spleen cells for fusion experiments.

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Cells and media. Cell line NS1, which was kindly provided by Lenore Pereira, Virology Laboratory, Department of Health, Berkeley, Calif., was the myeloma cell line used in this study. The myeloma cell line and the hybrids derived from its fusion with BALB/c mouse spleen cells were grown in RPMI 1640 medium (GIBCO Laboratories, Grand Island, N.Y.) supplemented with 20% fetal bovine serum (lot 12103059; GIBCO).

Hybridization and selection of antibody-producing hybrids. The hybridization procedure, the selection of hybrids which produced the desired monoclonal antibodies, and the check of the monoclonality of the antibodies were carried out as previously described (1, 5). The immunoglobulin class was determined by immunodiffusion with rabbit-specific antisera (Miles Laboratories, Inc., Elkhart, Ind.).

Western blot of yeast proteins. A 5-ml portion of yeast antigens was lyophilized and then added to 150 μl of a solution (150 μg/ml) of molecules having known molecular weights (RNase A [molecular weight, 13,700], chymotrypsinogen [25,000], ovalbumin [43,000]); this preparation was dissolved in 0.5 ml of disruptor buffer, which contained 1 M Tris (pH 7.0), 60% (wt/vol) sucrose, 2% (wt/vol) sodium dodecyl sulfate, 5% (wt/vol) β-mercaptoethanol, and 0.02% saturated bromphenol blue solution as a tracing dye. The solution was boiled for 5 min before loading onto sodium dodecyl sulfate–10% polyacrylamide gels and subsequent electrophoresis (15 mA for 18 h) in a vertical gel system (Bethesda Research Laboratories, Inc., Gaithersburg, Md.). Electrophoretic transfer of yeast peptides to nitrocellulose paper (0.45 μm; Schleicher & Schuell, Inc., Keene, N.H.) was performed by a modification of the Western blot method of Towbin et al. (8). Polyacrylamide gels were equilibrated for 10 to 15 min in transfer buffer (25 mM Tris base, 192 mM glycine, 20% methanol) and then placed on a wet sponge and filter paper (Whatman no. 1), and sheets of nitrocellulose were layered over the gels. A sandwich was composed by adding a second piece of filter paper and sponge. The sandwich was placed between supports into a Trans Blot cell (Bio-Rad Laboratories, Richmond, Calif.) with the nitrocellulose sheet facing the anode. The chamber was filled with the transfer buffer, and 60 V was applied for 90 min at room temperature. After this, the transfer buffer was substituted, and the voltage was applied for another 90 min.

Detection of monoclonal antibodies to polypeptides trans-

TABLE 1. Western blot analysis of yeast isolates with *Candida albicans* monoclonal antibody UCSC 1

Species	Strain[a]	Mol wt (10^3) of bands
Blastoschizomyces capitatus	UCSC 0	19, 27, 33, 45
Candida albicans	CDC B385	12, 20, 35
	CBS 5983	12, 54
	CBS 103	12
	CBS 9931	12, 50
	CDC B612	12, 35
	CDC B1073	12
	UCSC 0	12
	UCSC 10	12
	UCSC 66	12
	UCSC 101	12
Candida quilliermondii	CBS 566	25, 45, 78
Candida krusei	CBS 573	25, 48, 78
Candida parapsilosis	CBS 604	37, 66
Candida pseudotropicalis	CBS 607	25, 45, 70
Candida tropicalis	CBS 94	20, 44, 60
Cryptococcus neoformans	CDC B551	20, 45, 68
Malassezia furfur	ISS F1	13, 24, 45, 80
Malassezia pachy dermatis	ISS P1	21, 25, 100
Rhodotorula glutinis	UCSC 0	21
Saccharomyces cerevisiae	CDC B2210	34, 42, 58, 78, 92
Torulopsis glabrata	CBS 138	17

[a] CDC, Centers for Disease Control, Atlanta, Ga.; UCSC, Università Cattolica del Sacro Cuore, Rome, Italy; CBS, Centraalbureau voor Schimmelcultures, Baarn, The Netherlands; ISS, Istituto Superiore di Sanità, Rome, Italy.

ferred to nitrocellulose. A sheet of nitrocellulose paper to which peptides had been transferred was cut into strips for reaction with monoclonal antibodies. The nitrocellulose strips were incubated for 30 min at room temperature in phosphate-buffered saline (PBS) containing 5% (vol/vol) horse serum. Each nitrocellulose strip was then incubated at 37°C for 30 min in a shaker with 0.2 ml of hybridoma culture fluid diluted 1:10 in PBS containing 5% horse serum. The strips were then washed twice in PBS to remove unbound antibodies. To detect bound monoclonal antibodies, each strip was then incubated in 3 ml of PBS containing 5% horse serum for 30 min at room temperature in a shaker. Horseradish peroxidase-conjugated rabbit anti-mouse immunoglobulin G (Miles Laboratories) was diluted 1:500 in PBS containing 5% horse serum, and 3 ml was added to each strip for 30 min at 37°C in a shaker. After extensive washing in PBS, the strips were developed in 100 ml of water with 1 ml of a 1% solution of 4-chloro-1-naphthol (Sigma Chemical Co., St. Louis, Mo.) in methanol. Hydrogen peroxide was added to a final concentration of 0.01%. As bluish bands appeared, the strips were collected and dried. A few strips from each gel were differentially fixed and stained with 0.1% Coomassie blue for visualizing the molecular weight markers and controlling the efficiency of transfer. Plotting the migration patterns permitted accurate evaluation of the molecular weights of the antigenic determinants in the strips that reacted with the monoclonal antibodies (1).

RESULTS

Nine hybrids producing monoclonal antibodies belonging to the immunoglobulin G class were produced by fusion. When the *Candida albicans* reference antigen was electrophoretically separated in denaturing gels and then immobilized on nitrocellulose strips, we did not detect any diversity in the monoclonal antibodies to yeast proteins. *Candida albicans* monoclonal antibody UCSC 1 was selected for its avidity properties. When the antigens of all of the yeast isolates studied were tested by the Western blot technique, *Candida albicans* monoclonal antibody UCSC 1 displayed a potential to discriminate among various isolates (Fig. 1).

All of the *Candida albicans* strains had a species-specific determinant located at a molecular weight of 12,000. *Candida albicans* monoclonal antibody UCSC 1 was used to characterize five different presumptive serotypes within the species which reacted in different isolates with other antigenic determinants having different molecular weights. Cross-reactions occurred with other yeast species having different molecular weights (Table 1). Interestingly, the antigenic determinant with the closest molecular weight was detected in the *Malassezia furfur* isolate.

DISCUSSION

Traditionally, identification of *Candida albicans* isolates has been based on biochemical and morphological criteria. More recently, serological methods have been used for classification of the *Candida* species and their serotypes (7). However, serological methods may prevent autoagglutination, require combined biological tests, and recognize only the two serotypes (serotypes A and B) generally accepted within the species *Candida albicans* (2).

For this study, we had to make a preliminary choice (on grounds other than questionable immunization procedures) between using intact cells and using soluble antigens as immunogens. Because this study was intended to be directed to immunoidentification of opportunistic yeasts, we used a soluble antigen, namely, a reference exoantigen, since this type of antigen has previously been highly effective for the

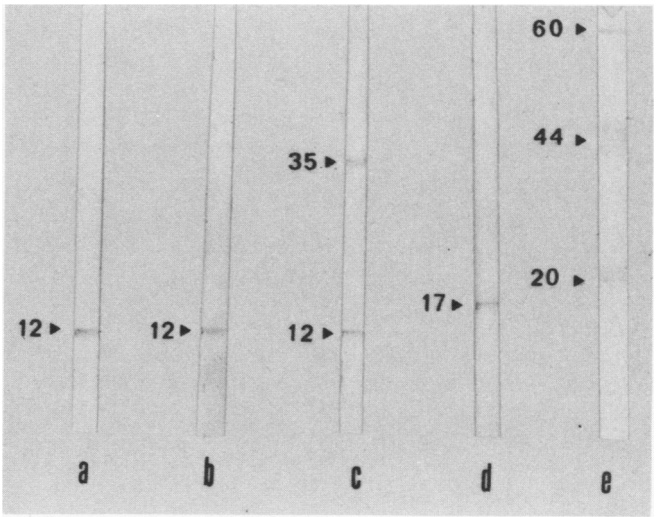

FIG. 1. Western blot analysis of yeast isolates with *Candida albicans* monoclonal antibody UCSC 1. Lane a, *Candida albicans* CDC B1073; lane b, *Candida albicans* UCSC 66; lane c, *Candida albicans* CDC B612; lane d, *Torulopsis glabrata* CBS 138; lane e, *Candida tropicalis* CBS 94 exoantigens. The molecular weights ($\times 10^3$) of bands are indicated to the left of each lane.

rapid and specific identification of mycelial cultures of pathogenic fungi (4).

Using a monoclonal antibody to a soluble antigen of *Candida albicans*, we demonstrated the presence of 12,000-dalton antigenic determinant that was shared by the type strain and by all of the *Candida albicans* isolates tested. No yeast isolate belonging to any other species investigated had an identical antigenic determinant. The similarity in molecular weight between the 12,000-dalton antigenic determinant of *Candida albicans* strains and the 13,000-dalton determinant of the *M. furfur* isolate examined suggests that there is a common function, but does not pose any diagnostic problem; thus, monoclonal antibodies can be readily adapted for identification purposes.

Furthermore, we found that such antigenic determinants may be expressed at different molecular weights in different strains within the species, since they are located on different molecules. Based on specific immunological determinants, the *Candida albicans* strains were clustered in five presumptive serotypes.

Additional experiments will be needed to determine whether the antigenically related proteins having different molecular weights are modifications of a common protein or the polymerized states of a protein which was not separated under the denaturing conditions used in this study.

The data presented in this paper show that monoclonal antibodies are powerful reagents for the identification and serotyping of *Candida albicans* strains. They also have potential application to the epidemiology of yeast infections. We expect that the serotypes are dependent upon the number of selected monoclonal antibodies used for analysis and that additional groupings should emerge as more hybridomas are used.

The poor variability in the responses of monoclonal antibodies to the immunization antigen could be attributed to a dominant immunogenic component. In our institute, work is in progress to achieve partial purification of the exoantigen by using *Candida albicans* monoclonal antibody UCSC 1 as an immunoadsorbent in affinity chromatography. This procedure should permit us to obtain a purified antigen which is depleted of the relative antigenic determinant. The use of such a purified antigen for mouse immunization should permit the production of different monoclonal antibodies.

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