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A Monoclonal Antibody Specific to Surface Antigen on *Candida krusei*

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A monoclonal antibody (MAb; MAb 6B3) which reacts specifically with a cell wall antigen found in all strains or isolates of *Candida krusei* was developed. MAb 6B3 was extensively tested by immunofluorescence assay for cross-reaction with many *Candida*, *Cryptococcus*, *Saccharomyces*, *Trichosporon*, and *Rhodotorula* species and was found to react only with the species *C. krusei*. The specific epitope is expressed on the surface of fungal cells and appears to reside on a protein moiety. Taking into account the increasing importance of fluconazole-resistant strains in nosocomial fungal infections, the very high degree of specificity of this MAb for *C. krusei* could be useful for the routine detection of *C. krusei* in culture or in tissue samples.

Among the members of the genus *Candida*, *Candida albicans*, *Candida tropicalis*, *Candida glabrata*, and *Candida parapsilosis* formerly represented more than 80% of the usual clinical *Candida* isolates, while *Candida krusei* was only sporadically isolated (11) and was regarded as a commensal organism (13, 24). During the past decade, *C. krusei* has been recognized as a true pathogen, particularly in immunocompromised patients. The resistance of this organism to fluconazole and the systematic use of this drug may explain the significant increase in the numbers of *C. krusei* infections, (9, 20, 31). In contrast to *C. albicans*, only a few articles concerning the potential virulence of *C. krusei* have been published (10, 12, 14, 15, 21, 22, 25, 26). Likewise, relatively few studies have been conducted to characterize antigens of *C. krusei*, and no monoclonal antibody (MAb) specific to this yeast has yet been developed. The present study concerns the description of a MAb (MAb 6B3) specific for the species *C. krusei.*

C. krusei ATCC 44507 (American Type Culture Collection) was used throughout this work unless otherwise indicated. Clinical isolates of *C. krusei*, *Candida* spp., and other fungi including *Rhodotorula*, *Saccharomyces*, and *Cryptococcus* were obtained from the Mycology Laboratory of the Medical Schools in the French cities of Angers, Grenoble, and Lyon. Each isolate was identified by using the ID 32C system (bioMérieux, Marcy l'Étoile-France). Among the isolates of *C. krusei* tested, 43 were typed by restriction endonuclease analysis and hybridization with the CkF1,2 DNA probe as described previously (1, 2).

Cultures were maintained on a Sabouraud glucose agar (SGA) slant (bioMérieux) at 22°C, and blastoconidia were prepared by growing the cells on this medium for 48 h at 37°C. In some experiments, the influences of growth in different media and at different temperatures (22 and 37°C) on the surface expression of the antigen reacting with MAb 6B3 were investigated. Seven isolates of *C. krusei* and one isolate each of *C. albicans*, *C. tropicalis*, or *C. glabrata* were cultured for 48 h in the following five media: SGA, SGA with chloramphenicol, SGA with gentamicin, 5% sheep blood Columbia agar, and chocolate agar (bioMérieux).

Cell antigens were extracted by the following four methods: (i) 10^9 blastoconidia were incubated at 37°C with shaking in 1.5 ml of 50 mM EDTA–0.35 M 2-mercaptoethanol (2ME; pH 9; Sigma Chemical Co., St. Louis, Mo.) for 30 min; (ii) 10^9 blastoconidia were digested with 1 ml of lyticase (1,000 U/ml; *Arthrobacter luteus*; ICN Pharmaceuticals France, Orsay, France) containing 1 mM phenylmethylsulfonyl fluoride (Sigma) for 1 h and 30 min at 37°C with shaking; (iii) 10^9 cells were incubated at 37°C in 1.5 ml of 2% (wt/vol) sodium dodecyl sulfate (SDS; Sigma) (5, 18) for 30 min; and (iv) extraction by dithiothreitol (DTT) (Sigma) was performed as described by Smail and Jones (27). Solubilized antigenic components were recovered by centrifugation at 12,000 × *g* for 15 min, and they were then dialyzed against distilled water and lyophilized.

Enzyme-linked immunosorbent assays (ELISAs) were performed in triplicate in a microtitration plate (Falcon; Becton Dickinson, Lincoln Park, N.J.). Each well was coated with 100 μl of *C. krusei* extract at 10 or 100 μg of protein/ml in phosphate-buffered saline (PBS), and the plates were incubated for 2 h at 37°C or overnight at 4°C. After washing with PBS, the plates were blocked by adding 200 μl of PBS containing 1% bovine serum albumin (fraction V; Sigma). After washing with PBS with 0.05% Tween 20 (PBST), assays were performed by successively incubating the wells with the MAb for 1 h at 37°C, with a 1/2,000 dilution of a commercially available goat anti-mouse immunoglobulin G (IgG) peroxidase conjugate in PBST (Caltag Laboratories, South San Francisco, Calif.) for another hour and then the substrate solution containing *ortho*-phenylendiamine dihydrochloride (Sigma) and hydrogen peroxide in phosphate citrate buffer (0.15 M; pH 5.0) for 30 min. All working volumes were 100 μl. The enzyme reaction was

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stopped by the addition of 50 μl of 1 N H_2SO_4 and optical densities were read with a Titertek Multiscan instrument at 492 nm (Flow Laboratories, Inc., McLean, Va.). The absorbance values given are the averages of three absorbance values.

SDS-polyacrylamide gel electrophoresis (PAGE) was performed by the method of Laemmli (16). Briefly, the extracts being analyzed were dissolved in buffer containing 62.5 mM Tris-HCl and 2% (wt/vol) SDS. After boiling for 2 min or incubation at 56°C for 30 or 60 min, 40 μl of each extract containing 20 or 60 μg of protein was placed on a 5 to 15% gradient polyacrylamide slab gel. Electrophoresis was performed in a gel electrophoresis apparatus (GE-2/4; Pharmacia, Uppsala, Sweden). Subsequently, the gels either were stained with Coomassie blue or were electrophoretically transferred to polyvinylidene difluoride sheets (Immobilon; Millipore Corp., Bedford, Mass.) as described previously (28). After the transfer, the membranes were blocked in 10% nonfat dry milk in PBST, washed in PBS, incubated with 1:20 or 1:100 dilutions of the MAb, washed, and incubated with peroxidase-labelled, affinity-purified goat anti-mouse IgG (Caltag Laboratories). After the washings the sheets were placed in substrate solution (3,3′-diaminobenzidine [Sigma] and hydrogen peroxide) for 30 min.

Indirect immunofluorescence assays (IFAs) were carried out as described previously (19). A suspension of 10^6 blastoconidia/ml in PBS containing NaN_3 (1‰) was prepared. Ten microliters of this suspension was placed in the wells on microscope slides (Polylabo, Strasbourg, France), and the suspension was allowed to dry at 30°C for 1 h. Twenty microliters of hybridoma culture supernatant undiluted or diluted in PBS was dropped over the cells. The slides were placed in a moist chamber at 37°C for 1 h and were then washed twice with PBS. Twenty microliters of fluorescein-conjugated goat anti-mouse immunoglobulin (Caltag Laboratories) was added to the slides at a dilution of 1:100 in PBS, and the slides were then reincubated at 37°C for 30 min and then washed twice with PBS. The preparations were in PBS containing 90% glycerol when they were mounted onto the slides. The preparations were examined with a Nikon microscope equipped with a reflected-light source of fluorescence. The same procedure was used for immunofluorescence studies of yeast cells grown under various conditions and for blastoconidia that were treated with different reagents.

The antigens were characterized by heat, enzymatic, and chemical agent treatments. The heat stabilities of the *C. krusei* antigens recognized by the MAb were tested by heating 10^7 blastoconidia of *C. krusei* in 1 ml of PBS at 56°C for 30 or 60 min and at 100°C for 2 or 5 min. The effects of lyticase (2,000 U/ml; Sigma) and four proteases, pronase E (2.5 mg/ml; Merck, Darmstadt, Germany), proteinase K (16 μg/ml; Merck), trypsin (2.5 mg/ml; Sigma), and α-chymotrypsin (25 μg/ml; Merck), were tested by incubating 10^7 blastoconidia for 30 min at 37°C with shaking in 1 ml of enzymatic reagent in PBS. Control cells were incubated with PBS alone. Periodate oxidation was performed for 1 h at room temperature in the dark with 10^7 blastoconidia and 1 ml of 20 mM of sodium periodate in 20 mM aceto-acetate buffer (pH 5). After washing in this buffer, the blastoconidia were incubated for 30 min with 1 ml of 1% (wt/vol) glycine to block the aldehyde groups generated by the periodate treatment and to prevent nonspecific reactions of the antibodies. The cells were then washed in PBS. Control cells were incubated with acetate buffer alone. Treatment with EDTA-2ME, DTT, or SDS was carried out as described above. After incubation with enzymes or chemical agents, the blastoconidia were washed in PBS and were fixed to the wells of a microscope slide. The antigenic activity of the treated blastoconidia was measured by IFA as described above. All experiments were performed in triplicate.

TABLE 1. IFAs of MAb 6B3 with different *Candida* species and other fungal genera

Organisms	No. of strains tested	No. of strains that reacted with MAb 6B3
Candida krusei	73	73
Candida albicans	45	0
Candida glabrata	37	0
Candida tropicalis	18	0
Candida kefyr	8	0
Candida parapsilosis	10	0
Candida guilliermondii	4	0
Candida norvegensis	6	0
Candida lusitaniae	2	0
Candida lipolytica	2	0
Candida famata	2	0
Candida lambica	2	0
Candida inconspicua	3	0
Rhodotorula spp.	3	0
Cryptococcus neoformans	6	0
Saccharomyces cerevisiae	10	0
Trichosporon spp.	8	0

Preparation of the MAb was carried out by immunization of BALB/c mice (Iffa Credo, l'Arbresle, France) with three subcutaneous injections, at 2-week intervals, of 10^6 formalin-killed blastoconidia emulsified in 100 μl of Freund's complete adjuvant (Sigma) for the first injection and in Freund's incomplete adjuvant (Sigma) for the following two booster injections. A final booster injection of 10^5 formalin-killed blastoconidia in 100 μl of 0.15 M NaCl was given intravenously 3 days before the mouse was killed. Hybridoma cells were produced by the method described by Dippold et al. (3). Splenocytes were fused with mouse plasmocytoma X63/Ag.63 in the presence of 0.4% polyethylene glycol 1500 (Merck). The fusion products were diluted in 200 ml of selective medium containing 100 μg of hypoxanthine, 15 μM thymidine, and 0.4 μM aminopterin (HAT medium; Sigma), and the mixture was plated out at 100 μl per well in 20 96-well trays. Ten days after fusion, aliquots of medium from wells with growing hybridomas were screened by IFA for the production of antibodies directed to blastoconidia of *C. krusei*. Positive hybrids were immediately subcloned twice by limiting dilution in 96-well plates and were stored in liquid nitrogen. MAbs were obtained from confluent hybridoma cultures in a nonselective medium.

Two fusions were carried out, and 593 hybridoma cell lines were produced. Supernatants from 10 of these cell lines produced antibodies that recognized *C. krusei* by IFA. Among them, only one of the cell lines (6B3), preliminarily tested by IFA against blastoconidia from other *Candida* species, indicated that it was specific for *C. krusei*. This hybridoma secreted an IgG and was selected for further studies. To investigate the strain and species specificities of MAb 6B3, 73 isolates or strains of *C. krusei*, 138 isolates of other *Candida* species, and 27 isolates of other fungal genera were cultured on SGA for 48 h at 37°C and were examined by IFA for surface antigen expression. The antigen recognized by MAb 6B3 was not detectable in genera other than *Candida* (Table 1). In the genus *Candida*, no binding to the other species of yeast tested other than *C. krusei* was

TABLE 2. Quantitative IFA titers of MAb 6B3 in culture supernatant tested with treated *C. krusei* ATCC 44507 blastoconidia

Treatment	IFA titer
None	1:200
56°C, 30 min	1:200
56°C, 60 min	1:200
100°C, 2 min	—[a]
100°C, 5 min	—
Lyticase	1:200
Pronase E	1:6
Proteinase K	1:200
Trypsin	1:12
α-Chymotrypsin	1:200
Sodium periodate	1:200
EDTA-2ME	1:100
DTT	1:100
SDS	1:100

[a] —, negative reaction at a 1:3 dilution of culture supernatant.

noticed under the conditions supporting cell development. All isolates and reference strains of *C. krusei* were positive by IFA with MAb 6B3. Both blastoconidia and pseudohyphae (when present) reacted with the MAb. The results of control experiments performed without MAb 6B3 were found to be negative. According to these results, MAb 6B3 recognizes a specific *C. krusei* epitope that is expressed on the surfaces of the blastoconidia of *C. krusei.*

IFA values were not affected by heating the blastoconidia of *C. krusei* for 30 or 60 min at 56°C. However, no immunofluorescent reactivity was obtained after heating at 100°C. Pronase E and trypsin treatment of *C. krusei* reduced strongly the level of fixation of MAb 6B3. Preparations treated with other enzymes such as proteinase K, α-chymotrypsin, or lyticase retained the same activities as those treated with buffer. Exposure of *C. krusei* blastoconidia to sodium periodate, EDTA-2ME, DTT, or SDS did not affect or weakly reduce the level of fixation of MAb 6B3 (Table 2).

Soluble extracts made with DTT, EDTA-2ME, SDS, or lyticase and tested with MAb 6B3 by ELISA showed no positive signal. These extracts were separated by SDS-PAGE and detected immunologically by using MAb 6B3 to identify the apparent molecular mass of the antigenic component. None of these extracts gave a positive reaction, whatever the temperature (100, 56, or 37°C) or the time (2, 30, or 60 min) of sample treatment before electrophoresis. Although the antigen was expressed on the surface of *C. krusei*, it was not identified in the soluble extracts tested by ELISA or Western blotting. These results suggest two hypotheses: (i) MAb 6B3 recognizes a conformational or a native protein epitope which is present on intact cells and which could be denatured following chemical or enzymatic extraction or (ii) the antigen recognized by MAb 6B3 may not be released in the extracts made with EDTA-2ME, lyticase, DTT, or SDS. The hypothesis that antigen is resistant to solubilization is strengthened by the results obtained by IFA: MAb 6B3 still stains the blastoconidial surface after treatments with the various chemicals or lyticase.

The variability of antigen expression was examined by observing the surface reactivities of cells grown under various conditions. Incubation with MAb 6B3 revealed that antigen expression was detectable on the blastoconidia of *C. krusei* whatever medium or temperature (22 or 37°C) was used. However, the reactivity observed by IFA was low when the blastoconidia were obtained on blood agar (Table 3).

Tsuchiya et al. (29, 30) used polyclonal antibodies (PAbs) to study the antigenic patterns of the more medically important yeasts by cross-absorption experiments. For *C. krusei*, by using PAb, Fukazawa et al. (7, 8) and Tsuchiya et al. (30) determined three main antigenic factors: factors 1, 5, and 11. Factor 11 was identified as *C. krusei* specific. The antigen recognized by MAb 6B3 is different from factor 11, which presents a mannan moiety. Several lines of evidence indicate that the epitope recognized by MAb 6B3 presents a protein moiety. First, it was susceptible to treatment with heat at 100°C. Second, treatment with some proteolytic enzymes (pronase, trypsin) resulted in a strong reduction of fluorescent staining. Finally, treatment of *C. krusei* blastoconidia by sodium periodate did not affect recognition by the MAb.

In conclusion, we provide here the first report of the development of a MAb which identifies a *C. krusei*-specific antigen. Its use in the latex test described by Freydiere et al. (6) is useful for the rapid and convenient identification of *C. krusei* colonies. A similar test, Bichrolatex Albicans (Fumouze Diagnostics, Asnière, France), which uses a MAb (MAb LIB 3H8) specific for *C. albicans*, was developed for the rapid identification of *C. albicans* colonies (4, 17, 23). In addition, MAb 6B3 could also be used for the identification of *C. krusei* in tissue samples or in normally sterile fluids.

TABLE 3. Quantitative IFA titers of MAb 6B3 against isolates of *C. krusei* and other *Candida* spp cultured in SGA, SGA with chloramphenicol, SGA with gentamicin, 5% sheep blood Columbia agar, or chocolate agar

Organism	IFA titer in the following medium at the indicated temp:									
	SGA		SGA with chloramphenicol		SGA with gentamicin		SBA sheep blood Columbia agar		Chocolate agar	
	22°C	37°C	22°C	37°C	22°C	37°C	22°C	37°C	22°C	37°C
C. krusei 1	1:100	1:100	1:100	1:100	1:100	1:100	1:25	1:50	1:50	1:50
C. krusei 2	1:200	1:200	1:200	1:200	1:200	1:200	1:25	1:25	1:25	1:25
C. krusei 3	1:200	1:200	1:200	1:200	1:200	1:200	1:25	1:25	1:12	1:12
C. krusei 4	1:100	1:100	1:100	1:100	1:100	1:100	1:12	1:12	1:12	1:12
C. krusei 5	1:200	1:200	1:100	1:200	1:200	1:200	1:25	1:25	1:25	1:25
C. krusei 6	1:100	1:100	1:100	1:100	1:100	1:100	1:12	1:12	1:12	1:12
C. krusei 7	1:100	1:100	1:100	1:100	1:100	1:100	1:12	1:25	1:12	1:12
C. albicans	—[a]	—	—	—	—	—	—	—	—	—
C. tropicalis	—	—	—	—	—	—	—	—	—	—
C. glabrata	—	—	—	—	—	—	—	—	—	—

[a] —, negative reaction at a 1:3 dilution of culture supernatant.

REFERENCES

1. **Carlotti, A., A. Couble, J. Domingo, K. Miroy, and J. Villard.** 1996. Species-specific identification of *Candida krusei* by hybridization with the CkF1,2 DNA probe. J. Clin. Microbiol. **34:**1726–1731.
2. **Carlotti, A., R. Grillot, A. Couble, and J. Villard.** 1994. Typing of *Candida krusei* clinical isolates by restriction endonuclease analysis and hybridization with CkF1,2 DNA probe. J. Clin. Microbiol. **32:**1691–1699.
3. **Dippold, W. G., K. O. Lloyd, L. T. C. Li, H. Ikeda, H. F. Oettgen, and L. J. Old.** 1980. Cell surface antigens of human malignant melanoma; definition of six antigenic systems with mouse monoclonal antibodies. Proc. Natl. Acad. Sci. USA **77:**6114–6118.
4. **Dromer, F., and B. Dupont.** 1996. The increasing problem of fungal infections in the immunocompromised host. J. Mycol. Med. **6:**1–6.
5. **Elorza, M., A. Marcilla, and R. Sentandreu.** 1988. Wall mannoproteins of the yeast and mycelial cells of *Candida albicans*: nature of the glycosidic bonds and polydispersity of their mannan moieties. J. Gen. Microbiol. **134:** 2393–2403.
6. **Freydiere, A. M., L. Buchaille, R. Guinet, and Y. Gille.** 1997. Evaluation of latex reagents for rapid identification of *Candida albicans* and *C. krusei* colonies. J. Clin. Microbiol. **35:**877–880.
7. **Fukazawa, Y., T. Shinoda, A. Nishikawa, K. Kagaya, and Y. Yonezawa.** 1976. Serological and immunological studies of the serotypes of *Candida albicans*, p. 213. *In* K. Iwata (ed.), Yeasts and yeast-like microorganisms in medical science. University of Tokyo Press, Tokyo, Japan.
8. **Fukazawa, Y., T. Shinoda, and T. Tsuchiya.** 1968. Response and specificity of antibodies for *Candida albicans*. J. Bacteriol. **95:**754.
9. **Horn, R., B. Wang, T. Kiehn, and D. Armstrong.** 1985. Fungemia in a cancer hospital: changing frequency, earlier onset and results of therapy. Rev. Infect. Dis. **7:**646–655.
10. **Howlett, J. A.** 1975. The infection of rat tongue mucosa in vitro with five species of *Candida*. J. Med. Microbiol. **9:**309–316.
11. **Hurley, R., J. De Louvois, and A. Mulhall.** 1987. Yeasts as human and animal pathogens, p. 207–281. *In* A. H. Rose and J. S. Harrison (ed.), The yeasts, 2nd ed, vol. 1. Academic Press, London, United Kingdom.
12. **Hurley, R., and V. C. Stanley.** 1969. Cytopathic effects of pathogenic and non-pathogenic species of *Candida* on cultured mouse epithelial cells: relation of the growth rate and morphology of the fungi. J. Med. Microbiol. **2:**63–74.
13. **Hurley, R., and H. I. Winner.** 1966. Pathogenicity in the genus *Candida*. Dermatol. Int. **5:**151–153.
14. **King, R., R. Lee, and A. Morris.** 1980. Adherence of *Candida albicans* and other *Candida* spp. to mucosal epithelial cells. Infect. Immun. **27:**667–674.
15. **Klotz, S. A., D. J. Drutz, and J. E. Zajic.** 1985. Factors governing adherence of *Candida* species to plastic surfaces. Infect. Immun. **50:**97–101.
16. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature **227:**680–685.
17. **Laurent, F., P. Cahen, and P. Honderlick.** 1996. Utilization of the Bichrolatex albicans® Fumouze for rapid identification of *Candida albicans* in blood culture bottles. J. Mycol. Med. **6:**19–21.
18. **Marcilla, A., S. Mormeneo, M. V. Elorza, J. J. Manclus, and R. Sentandreu.** 1993. Wall formation by *Candida albicans* yeast cells—synthesis, secretion and incorporation of two types of mannoproteins. J. Gen. Microbiol. **139:** 2985–2993.
19. **Marot-Leblond, A., R. Robert, J. M. Senet, and T. Palmer.** 1995. Purification of a *Candida albicans* germ tube specific antigen. FEMS Immunol. Med. Microbiol. **12:**127–136.
20. **Merz, W. G., J. E. Karp, D. Schron, and R. Saral.** 1986. Increased incidence of fungemia caused by *Candida krusei*. J. Clin. Microbiol. **24:**581–584.
21. **Miyake, Y., Y. Fuyita, S. Minagi, and H. Suginaka.** 1986. Surface hydrophobicity and adherence of *Candida albicans* to acrylic surfaces. Microbios **46:**7–14.
22. **Ray, T. L., K. B. Digre, and C. D. Payne.** 1984. Adherence of *Candida* spp to human epidermal corneocytes and buccal mucosal cells: correlation with cutaneous pathogenicity. J. Invest. Dermatol. **83:**37–41.
23. **Robert, R., R. Sentandreu, C. Bernard, and J.-M. Senet.** 1994. Evaluation du réactif Bichrolatex albicans pour l'identification rapide de colonies de *Candida albicans*. J. Mycol. Med. **4:**226–229.
24. **Samaranayake, Y. H., and L. P. Samaranayake.** 1994. *Candida krusei*: biology, epidemiology, pathogenicity and clinical manifestations of an emerging pathogen. J. Med. Microbiol. **41:**295–310.
25. **Samaranayake, Y. H., P. C. Wu, L. P. Samaranayake, M. So, and K. Y. Yuen.** 1994. Adhesion and colonisation of *Candida krusei* on host surfaces. J. Med. Microbiol. **41:**250–258.
26. **Segal, E., O. Lehrman, and D. Dayan.** 1988. Adherence in vitro of various *Candida* species to acrylic surfaces. Oral Surg. Oral Med. Oral Pathol. **66:**670–673.
27. **Smail, E. H., and J. M. Jones.** 1984. Demonstration and solubilization of antigens expressed primarily on the surfaces of *Candida albicans* germ tubes. Infect. Immun. **45:**74–81.
28. **Towbin, H., T. Staehelin, and J. Gordon.** 1979. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. Proc. Natl. Acad. Sci. USA **76:**4350–4354.
29. **Tsuchiya, T., Y. Fukazawa, and S. Kawakita.** 1965. Significance of serological studies on yeasts. Mycopathol. Mycol. Appl. **26:**1.
30. **Tsuchiya, T., M. Taguchi, Y. Fukasawa, and T. Shinoda.** 1984. Serological characterization of yeasts as an aid in identification and classification. Methods Microbiol. **16:**75.
31. **Wingard, J. R., W. G. Merz, M. G. Rinaldi, T. R. Johnson, J. E. Karp, and R. Saral.** 1991. Increase in *Candida krusei* infection among patients with bone marrow transplantation and neutropenia treated prophylactically with fluconazole. N. Engl. J. Med. **325:**1274–1277.