INFECTION AND IMMUNITY, June 1993, p. 2662–2669
0019-9567/93/062662-08$02.00/0
Copyright © 1993, American Society for Microbiology
Vol. 61, No. 6

Isolation of Avirulent Clones of *Candida albicans* with Reduced Ability To Recognize the CR2 Ligand C3d

STEPHAN FRANZKE,[1] RICHARD A. CALDERONE,[2]* AND KLAUS SCHALLER[1,3]

Institut für Mikrobiologie, Universität Hannover, Hannover,[1] and Institut für Chemotherapie, Bayer AG, Wuppertal,[3] Germany, and Department of Microbiology, Georgetown University, Washington, D.C. 20007[2]

Received 14 January 1993/Accepted 12 March 1993

Four clones of the yeast *Candida albicans*, isolated on the basis of their tolerance to clotrimazole, were compared with their parental strain in terms of growth, morphology, virulence, and cell surface complement receptor activity. In a newly described synthetic medium, these clones, designated C1, C2, N, and P, produced germ tubes or pseudohyphae, but no true hyphae, in a pattern which was specific for each strain. The growth of each clone at 37°C, under conditions which favor the filamentous growth form of the organism, was equal to that of the parental strain (H12). The pathogenicity of each clone was tested in an intravenous mouse model. None of the mice infected with the tolerant clones but all of the mice infected with H12 developed severe renal candidiasis after infection with 1.4×10^6 to 2.0×10^6 CFU/ml. The number of CFU of each clone from the mouse kidney was reduced about 3 or 4 orders of magnitude in comparison with the wild type. As a correlate, we measured the complement receptor activity (CR2 and CR3) of each clone. The C3 ligands, iC3b and C3d, were conjugated to sheep erythrocytes (E) sensitized with antibody (A) to the erythrocytes (EA). We found that all tolerant clones showed reduced recognition of C3d-bearing sheep erythrocytes (EAC3d) in rosetting assays. Clone P showed more than an 80% reduction in rosetting of EAC3d in comparison with H12 cells. In contrast, recognition of iC3b (EAiC3b) by each of the clones was similar to that by H12 cells. When dithiothreitol extracts of clone P and H12 were compared by immunoblot, both quantitative and qualitative differences in reactivities were observed with antibodies specific for the *Candida* C3d receptor and with antiserum from a patient with chronic mucocutaneous candidiasis.

Several virulence factors have been proposed for the pathogenic yeast *Candida albicans*. Conversion of blastoconidia to the hyphal morphology may be essential for deep-tissue penetration (7, 30). In association with this morphological transition, secretory protease (1, 22, 26, 29) may also play an important role in tissue invasion. Adhesion of the organism to host cells, an essential early part of the infectious process, is mediated by cell surface mannoproteins (3, 8, 20). The *Candida*-host cell recognition system appears to be complex; mannoproteins of the organism recognize either fucosyl or glucosamine glycosides of epithelial cells (8, 37) or arginine-glycine-aspartic acid-containing peptides (RGD) of endothelial cells or their extracellular matrix proteins (21).

Strain specificity in *C. albicans* has been observed in regard to adherence to epithelial cells; some strains recognize *N*-acetylglucosamine instead of fucose residues (3, 8). The *Candida* mannoprotein which is involved in the adherence of the organism to epithelial cells thus possesses lectin-like properties.

The adhesin(s) which recognizes RGD sequences is similar to the complement receptors (CR) of mammalian cells (18, 28). In this regard, *C. albicans* expresses CR2- and CR3-like proteins which recognize the C3 conversion products iC3b and C3d (3, 5, 9, 14, 15, 17, 25). Adherence to endothelial cells may be mediated by the CR3-like protein of this organism in that antibody to the mammalian CR3 as well as RGD-containing ligands blocked adherence of the organism to cultured endothelial cells (15).

The purification and characterization of the CR-like proteins have been pursued. A mannoprotein which recognizes C3d has been purified, by using both monoclonal antibody and C3d-ligand affinity chromatography, from hyphae (5, 25) and from culture media of hyphal cultures (34). Recent data indicate that blastoconidia, although not expressing the protein on their cell surface, possess an epitope at the level of the plasma membrane which reacts with a monospecific antibody to the *Candida* C3d-binding protein (or CR2) (19). Immunoelectron microscopy (19) has been used to show that the *Candida* CR2 is expressed during experimental disease; also, lymphocytes from infected animals undergo blastogenesis when incubated in vitro with the purified CR2 (12), indicating that the CR2 is expressed in vivo. The *Candida* CR2 has a molecular mass of 60 kDa and reacts weakly in Western immunoblot assay with antibody to the mammalian CR2.

A protein of approximately 130 kDa has been shown to react with antibody to the mammalian CR3 (10). Despite the apparent differences in molecular mass, it is uncertain whether a single protein or different proteins recognize iC3b and C3d. Proteins of 60 and 68 kDa from the organism recognize a variety of ligands including laminin, C3d, and fibrinogen (38), similar to the mammalian CR3, which recognizes RGD-containing ligands as well as C3d.

An understanding of the role of these adhesins in virulence can be achieved in part through the use of cell surface mutants which are defective in their ability to express the adhesins. In this regard, a cell surface mutant of *C. albicans* which has reduced CR3-like activity (32) and is relatively avirulent in animal models of endocarditis and vaginitis has been reported (4, 24). However, studies with additional strains are needed before correlates can be made in regard to the role of the CR2 and CR3 of *C. albicans* in adherence and virulence. In this report we describe the isolation and some of the phenotypic properties of clotrimazole-tolerant clones

* Corresponding author.

of *C. albicans* with emphasis on their CR-like activity and virulence.

MATERIALS AND METHODS

Strain. *C. albicans* H12 was isolated from the urine and stool of a patient with systemic candidiasis.

Media and culture methods. For the isolation of clones, yeast nitrogen base (YNB; Difco Laboratories, Detroit, Mich.) plus 1.6% glucose (YNBG) was used. To produce solid medium, 1% agar (agar no. 1; Unipath, Basingstoke, England) was added. The medium (MTM) used for converting blastoconidia to hyphae combines previously described transformation-inducing factors (2, 23, 27) including 1 g of *N*-acetylglucosamine (Sigma Chemie GmbH, Deisenhofen, Germany), 1 g of glucose (E. Merck, Darmstadt, Germany), 5 ml of nonessential amino acids, 5 ml of BME amino acids without glutamine, 10 ml of glutamine (200 mM), and 1,000 ml of Dulbecco's phosphate-buffered saline (the last four were obtained from Biochrom KG, Berlin, Germany). For germination of blastoconidia, clones were preincubated on YNBG agar slants at 27°C overnight in shake culture. Kidney isolates were plated on *Candida* B plates (Biologische Arbeitsgemeinschaft, Lich, Germany).

Selection of mutants. We incubated 10^6 blastoconidia of *C. albicans* H12 per ml in YNBG plus 32 or 128 μg of clotrimazole per ml at 37°C overnight. Subsequently, cells were diluted (10^0, 10^{-1}, 10^{-2}, and 10^{-3}), plated on YNBG, and then incubated at 37°C for 4 days. Individual colonies were isolated and subcultured several times on YNBG plates. Germination of clotrimazole-tolerant clones and H12 was evaluated by using MTM. Clones (C1, C2, P, and N) which underwent morphogenic conversion from blastoconidia but did not form true hyphae were chosen for further study.

Animal model. The virulence of wild-type strain (H12) and the clotrimazole-tolerant clones C1, C2, N, and P was tested in a systemic mouse model. Male B6D2F1 mice (Winkelman GmbH & Co. KG, Borchen, Germany), weighing 20 g each, were infected intravenously (via the tail vein) with a 0.2-ml suspension of *C. albicans* containing 3×10^6, 1×10^7, or 3×10^7 blastoconidia per ml in YNB plus 2% glucose at 37°C. The state of health of the mice (10 mice per treatment group) was evaluated every day over a period of 21 days.

Kidneys from infected mice (five mice per treatment group) were isolated under sterile conditions, pooled, and, after the addition of deionized H_2O to a final volume of 10 ml, homogenized with a Potter S homogenizer (B. Braun, Melsungen, Germany). Dilutions of each homogenate were replated on *Candida* B plates. The number of CFU of each clone was determined after a 2-day incubation at 37°C.

Assay for iC3b and C3d receptors. Each clone was incubated for 24 h in MTM at 37°C to induce its filamentous phase (see above), washed three times in Veronal-sucrose buffer containing 0.1% gelatin, and resuspended in the same buffer for rosetting assays, all by previously published methods (5, 25). Sheep erythrocytes (EA), coated with iC3b (EAiC3b) or C3d (EAC3d), were incubated at 37°C with H12 or one of the clones (final volume, 100 μl). The percent rosetting caused by each clone and H12 was determined by counting 100 particles (hyphae or pseudohyphae) incubated with either EAiC3b or EAC3d. A particle was counted as receptor positive when more than eight EA bound per organism (25). All experiments were repeated at least twice.

Preparation of DTT extracts. After conversion at 37°C in MTM as described above, hyphae and pseudohyphae were collected by filtration (Whatman no. 1 paper; Whatman Inc., Clifton, N.J.), washed extensively, and extracted with dithiothreitol (DTT; Sigma Chemical Co., St. Louis, Mo.) as described previously (36). The extracts were dialyzed extensively for 48 h against distilled water and concentrated to about 0.5 ml with Centriprep-10 concentrators (Amicon, Beverly, Mass.). To inhibit protease activity, a cocktail of 1 mM EDTA, 1 μM leupeptin, and 0.2 mM AEBSF was added. Protein concentration was determined with the Coomassie protein assay reagent (Pierce, Rockford, Ill.).

SDS-PAGE and immunoblotting. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE; reducing conditions) and immunoblotting were performed by established procedures (6, 25, 32, 34) with a Minigel system (Bio-Rad Laboratories, Richmond, Calif.) and 10% polyacrylamide. Each lane was loaded with a total of 1 μg of protein. Either rabbit immunoglobulin G or a monoclonal antibody (CA-A) to the *C. albicans* CR2 (25, 34) was used as the primary antibody at a 1:1,500 dilution. Also, a serum sample from a patient with chronic mucocutaneous candidiasis was used in immunoblotting at a 1:200 dilution (32). Either anti-rabbit peroxidase or protein G-peroxidase (Bio-Rad) in a 1:1,500 dilution was used a secondary antibody. SDS-PAGE-separated proteins transferred to nitrocellulose were stained with Aurodye (Amersham Corp., Arlington Heights, Ill.).

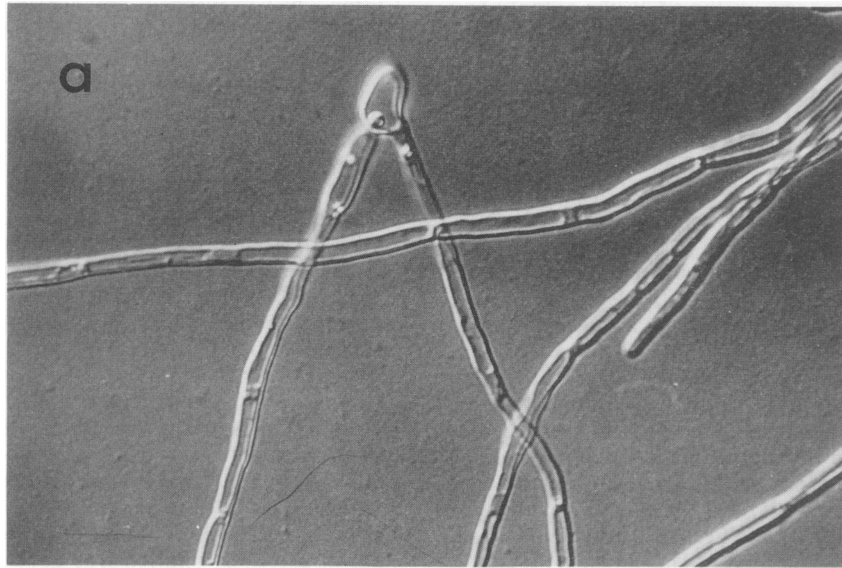
RESULTS

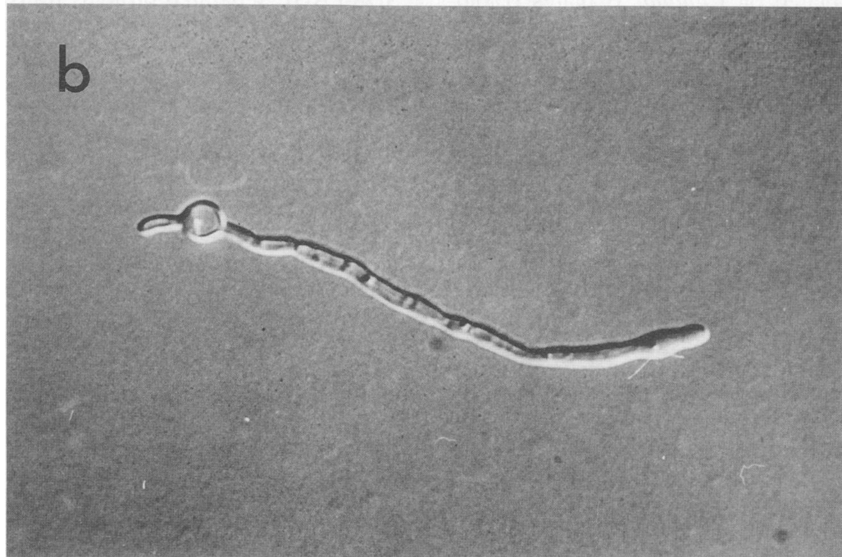
Selection of clones. Wild-type *C. albicans* (H12) was incubated in YNBG plus 32 or 128 μg of clotrimazole per ml at 37°C overnight. The cell suspension was diluted and transferred to YNBG agar plates. Approximately 90% of the cells died after treatment at each concentration of clotrimazole. Colonies, which first appeared at about 4 to 5 days, were selected for further study if they resembled the colony morphology of the parent strain. Following subculture onto YNBG plates, a total of 116 colonies from cultures treated with the lower concentration of clotrimazole (two separate experiments) and 150 colonies from cultures treated with the high concentration of clotrimazole (one experiment) were isolated.

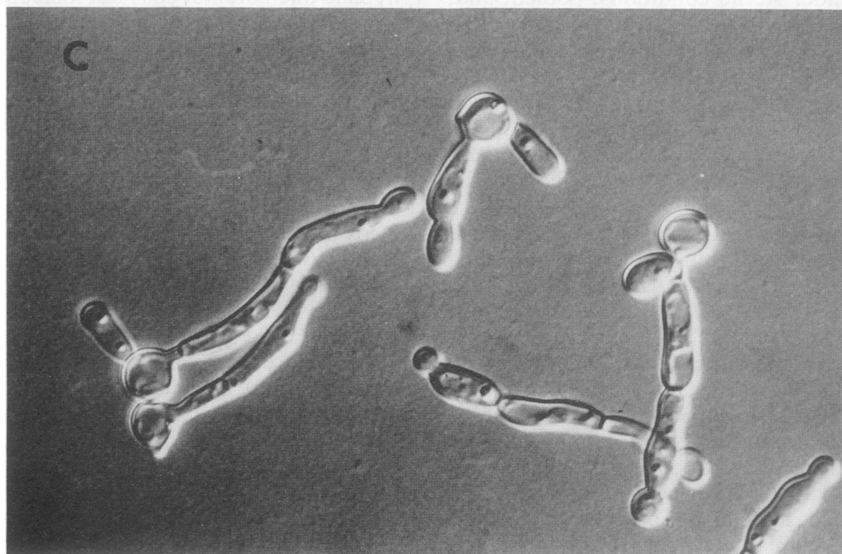
Approximately 60 clotrimazole-tolerant clones were tested in MTM for their ability to form hyphae like the wild-type H12. A total of six clones which did not form true hyphae like parental cells but instead formed pseudohyphae and germ tubes from blastoconidia were chosen for further study. Of these six clones, two reverted to the H12 type of true hyphae upon isolation from infected animals and remained virulent following passage in vitro. The other four clones (C1, C2, N, and P) were investigated further. C1, C2, and N were selected from low-dose clotrimazole treatment, whereas clone P was derived from incubation at the higher concentration of clotrimazole.

Morphology of the wild type and clones in MTM. Blastoconidia of H12 and clones C1, C2, N, and P were cultured on YNBG slants for 2 days at 27°C. When transferred to MTM at 37°C, H12 began to form germ tubes within 2 h. Short hyphae and large mycelial aggregates were formed after 7 and 24 h, respectively. Clones C1, C2, N, and P differed from H12 in their pattern of conversion. C1 formed predominantly germ tubes but did not form hyphae even after 48 h at 37°C. Clones C2, N, and P converted to different percentages of pseudohyphae and germ tubes. All clones and H12 grew in MTM at 24°C as blastoconidia. The morphology of H12 and the clotrimazole-tolerant clones is shown in Fig. 1.

The growth of each clone was determined in either serum


a


b


c

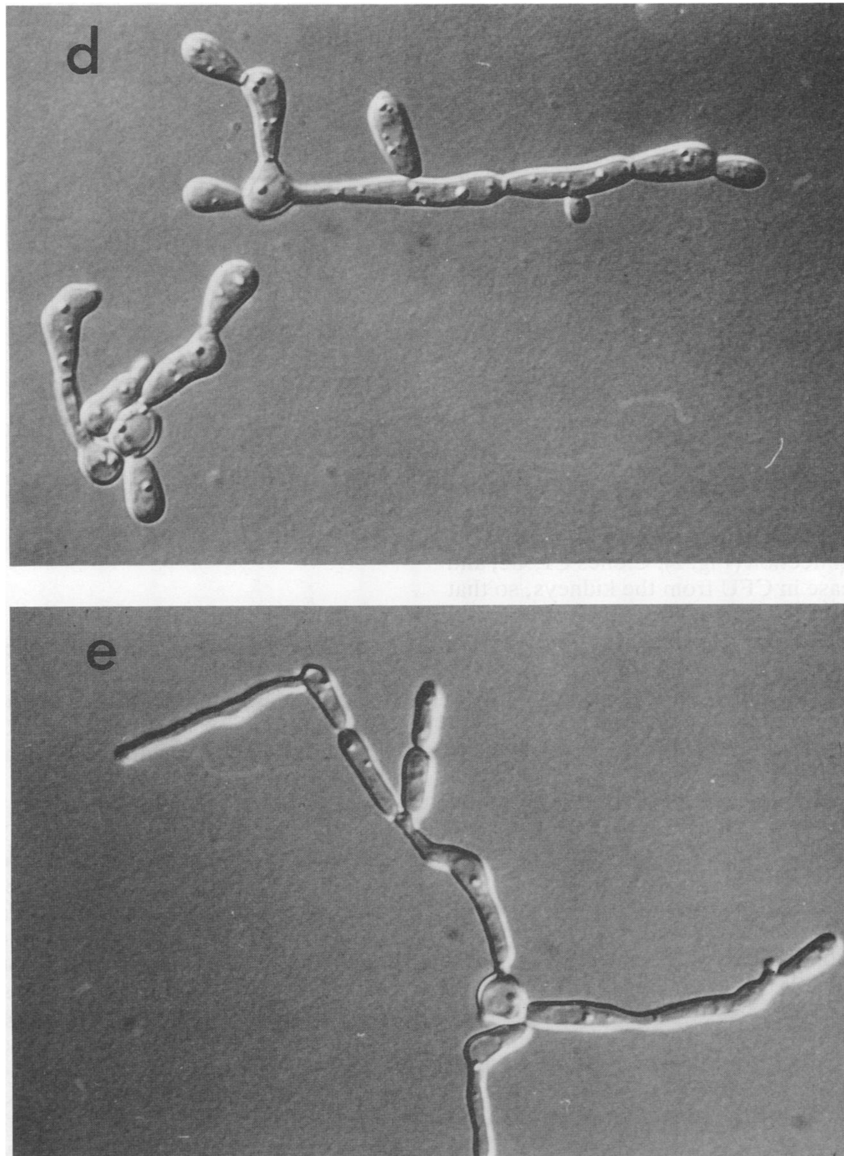


FIG. 1. Microscopic view of *C. albicans* H12 and clotrimazole-tolerant clones after overnight incubation in MTM at 37°C. (a) H12; (b) C1; (c) C2; (d) N; (e) P. Magnification, ×1,062.5.

or MTM at 37°C for 24 to 72 h, conditions which induced morphogenic conversion. Growth was measured as either total-cell protein per culture or viable units (CFU per milliliter of medium). In both media and with either parameter, the growth of each clone was similar if not identical to that of H12 cells (data not shown).

Virulence of the wild type and clones. The virulence of *C. albicans* C1, C2, N, and P in comparison with H12 was tested in a mouse model by intravenous infection of male B6D2F1 mice with several doses of the organism. Of 10 animals infected with H12 at the lowest infection dose (0.4×10^6 CFU per mouse), 7 died within 21 days postinfection. After infection with 1.4×10^6 CFU per mouse, all mice died overnight. Clones C1 and C2 showed no virulence in this model even at the highest dose (4.4×10^6 CFU per mouse). Similarly, clone P did not cause mortality at any of the doses. Mice infected with clone N all died within 6 days when infected with 6.6×10^6 CFU per mouse, but at lower doses clone N did not cause death in any treatment group. The data obtained for each clone from one of three experiments are shown in Table 1. Similar data were obtained for the other two experiments.

Colonization of mouse kidneys. Since the kidney is one of the main target organs in mice infected intravenously with *Candida* species (4, 35), we determined the number of organisms (CFU per kidney) from the kidneys of animals infected with H12, C1, C2, N, and P. The number of organisms was determined at 10 min, 4 and 7 h, and 1, 2, 4, 7, 11, 14, and 21 days after infection. The number of *Candida* organisms isolated from the kidneys of animals infected with H12 and each clone at 10 min postinfection was nearly the same (around 10^4 CFU per kidney), data which probably represent trapping of the organism by the kidneys. Subsequently (within the first 7 h), a decrease in the CFU per

TABLE 1. Pathogenicity of *C. albicans* H12, C1, C2, N, and P in an intravenous systemic murine model

Clone	No. dead/no. infected with infection dose (CFU/mouse) of[a]:		
	0.6×10^6	2×10^6	6×10^6
H12	7/10	10/10[b]	10/10[c]
C1	0/10	0/10	0/10
C2	0/10	0/10	0/10
N	0/10	0/10	10/10[d]
P	0/10	0/10	0/10

[a] The numerator represents the number of mice dead at 21 days postinfection, except as indicated below.
[b] All mice dead 5 days postinfection.
[c] All mice dead 1 day postinfection.
[d] All mice dead 6 days postinfection.

kidney was observed for all strains; however, with H12 cells only, an increase in this value was observed within the first 2 days following infection, reaching a maximum of 10^7 CFU per kidney 12 days postinfection (Fig. 2). Clones C1, C2, and P showed a rapid decrease in CFU from the kidneys, so that the total number of organisms isolated from the kidneys was reduced by more than 3 orders of magnitude in 10 days compared with the number of H12 organisms (Fig. 2). Clone N also showed a decrease in CFU within the first 2 days. Then the number of CFU of clone N increased over time but never reached the levels of the H12 isolate. At 21 days and 6 weeks postinfection, 2×10^2 (Fig. 2) and less than 1×10^2 (data not shown) CFU per kidney, respectively, were recovered from animals infected with clone N. These data indicate that on the basis of mortality and tissue load of the organism, differences in virulence were observed among these clones.

Ability of *C. albicans* to cause rosetting of EAiC3b and EAC3d. The ability of filamentous forms of both H12 and the clotrimazole-tolerant clones (N, P, C1, and C2) to cause rosetting of EAiC3b and EAC3d was studied. The results of the rosetting experiments are shown in Fig. 3. All data are compared with results for H12 cells, which are expressed as 100% rosetting. Rosetting with H12 (expressed as the percentage of hyphae with adhering EA) was 94.3% ± 4.5% for EAiC3b and 77.0% ± 4.3% for EAC3d. Clones C1, C2, and

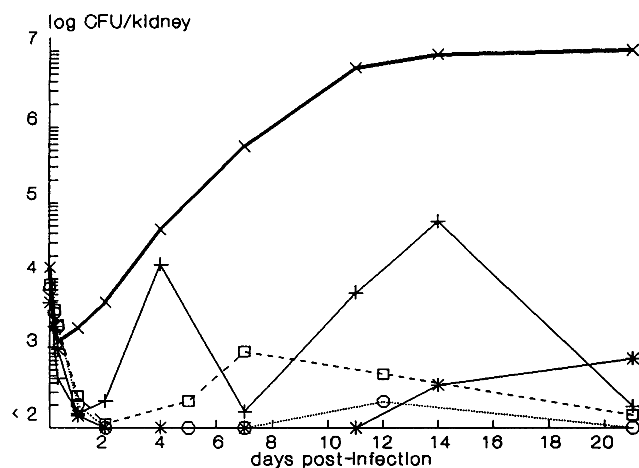


FIG. 2. Quantitation (CFU per kidney) of *C. albicans* H12 (×), C1 (□), C2 (○), N (+), and P (*) from the kidneys of infected mice. The infection dose was 2×10^5 CFU per mouse.

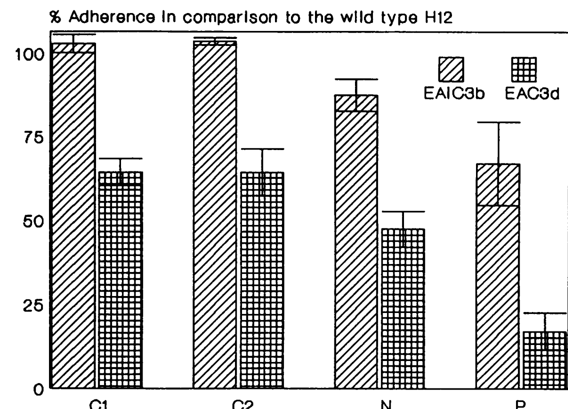


FIG. 3. Ability of *C. albicans* clones C1, C2, N, and P to cause rosetting of EAiC3b and EAC3d. The wild type (H12) is indicated as causing 100% rosetting. Rosetting (percentage of hyphae with EAiC3b or EAC3d) caused by H12 is 94% ± 2.3% and 77% ± 3.2% for EAiC3b and EAC3d, respectively. The cells were grown in MTM at 37°C overnight.

N did not show a significant reduction in their ability to recognize EAiC3b, whereas strain P showed a 33% reduction of binding in comparison with the H12 cells. The ability of all clones to cause rosetting of EAC3d was lower than that of H12 cells. The percentage of rosetting with clones C1 and C2 was 49.3% ± 1.7% and 49.3% ± 4.1%, respectively, whereas that for mutant N was 36.7% ± 5.3%. A drastic reduction in the rosetting of EAC3d with clone P was observed: in comparison with H12, rosetting of EAC3d with clone P was reduced by more than 80% (13.3% ± 4.6%).

Although clone P appeared to lack a functional C3d-binding protein by the rosetting assay, we also sought to determine whether a functional protein could be found in extracts of the organism. For these experiments, either EAiC3b or EAC3d was preincubated with DTT extracts of H12 or P (50 μl; 30 μg of protein per ml). Subsequently, H12 hyphae were added to the mixture, and the rosetting was determined. With both extracts, we found a reduction in EAC3d rosetting with H12 hyphae. The reduction with the extract from H12 cells (51% ± 4%) was significantly higher than with the extract from P cells (40% ± 4%; data not shown). Rosetting of EAiC3b by H12 cells was inhibited equally by extracts of either H12 or P cells.

Immunoblotting. DTT extracts were separated by SDS-PAGE, transferred to nitrocellulose paper, and blotted with a monospecific rabbit immunoglobulin G to the *C. albicans* C3d-binding protein. This antibody was chosen for study since we found that it blocked the rosetting of EAC3d when preincubated with hyphae of H12 (51% inhibition of rosetting; data not shown). In addition, in Western blot analyses of both extracts we used both a monoclonal antibody to the *Candida* CR2 (CA-A) and a serum sample from a patient with chronic mucocutaneous candidiasis. Both the CA-A and patient serum blocked rosetting by wild-type *C. albicans* (25, 32). Western blots of extracts from H12 and P are shown in Fig. 4. Mutant P was selected for study because of its low level of rosetting of EAC3d. Proteins of 94, 68, 60, 50, and 31 kDa showed strong reactivity to the monospecific antibody (Fig. 4A). Differences between the extracts were observed. With the extract from clone P, the 94-, 60-, and 50-kDa bands showed less intensity while the 31-kDa protein reacted to a greater extent with the antibody (Fig. 4A, compare lanes 1

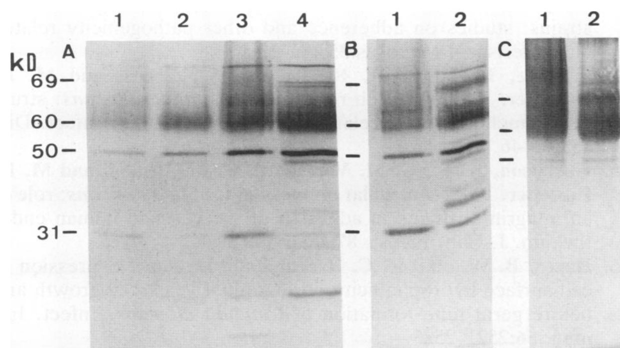


FIG. 4. Immunoblots of DTT extracts from wild-type H12 and mutant P. (A) Monospecific anti-*Candida* CR2 antibody. Lanes 1 and 3 contain P, and lanes 2 and 4 contain H12; lanes 1 and 2 represent a 1:5 dilution of antigen used in lanes 3 and 4. (B) Human antiserum from a patient with chronic mucocutanous *Candida* mycoses; lane 1 contains P, and lane 2 contains H12. (C) Monoclonal anti-*Candida* CR2 antibody (CA-A); lane 1 contains P, and lane 2 contains H12.

and 3 with lanes 2 and 4). A doublet of 69 and 68 kDa was visible only in the H12 extract (Fig. 4A, compare lanes 2 and 4 with lanes 1 and 3). Western blots with the human antiserum also showed significant differences between H12 and P (Fig. 4B). Proteins of 68, 37 and 35 kDa were observed only from H12 extracts, whereas proteins of 60 and 50 kDa were quantitatively more reactive from H12 extracts. The 31-kDa protein appeared to be more reactive from P extracts, similar to that observed with the monospecific serum (Fig. 4A, lanes 1 and 3). In Fig. 4C, reactions of the DTT extracts with CA-A are shown. A 68-kDa protein was observed only from H12 cells. The 60-kDa protein from H12 cells may be more reactive.

DISCUSSION

Several approaches have been used to isolate cell surface mutants of *C. albicans* (4, 39). Calderone et al. (4) isolated cerulenin-resistant, spontaneous mutants of *C. albicans*, one of which (m-10) was impaired in its ability to adhere to a variety of host cells in vitro, was avirulent in endocarditis and vaginitis models (4, 24), and had significantly reduced levels of iC3b binding (31). On the basis of this rather limited analysis of strains with impaired iC3b binding, one might suggest that this protein is essential for virulence. More recently, Whelan et al. (39) isolated several cell surface mutants of *C. albicans* by nonagglutination with a hyperimmune rabbit antiserum against wild-type cells. Each of four mutants tested caused reduced levels of rosetting of either EAiC3b or EAC3d (39). Correlations with CR activity and virulence were not possible because virulence studies were not completed, although one of the mutants was as virulent as the parent in a rabbit model of systemic disease (39).

More recently, Ghannoum has reported that polyene- and azole-resistant strains of *C. albicans* did not adhere as readily to human buccal epithelial cells as wild-type cells did (13). The virulence of the nonadhering strains was not determined.

Odds et al. found that azole antibiotics could inhibit the conversion from blastoconidia to hyphae in *C. albicans* and used this property of azoles to study the role of hyphal forms of the organism in virulence (31). In the present study, we were able to isolate clones which could grow in the presence of high concentrations of azole and were still able to convert from blastoconidia to pseudohyphae or form germ tubes following incubation with clotrimazole overnight. Interestingly, these clones are not more resistant to clotrimazole than are H12 cells, since MIC studies showed that the growth of H12 and clones was inhibited in different media at concentrations of approximately 1 μg of clotrimazole per ml (11).

The growth of each clone under conditions which favor morphogenic conversion was similar to that of H12 cells. Although each clone grew to the same extent as H12, the pattern of growth was different from that of H12 and was clone specific. Clone P, for example, produced abundant pseudohyphae and germ tubes but did not produce true hyphae like H12. The association of hyphal formation with virulence in *C. albicans* is unclear (7, 33, 35). Shepherd isolated nitrosoguanidine-derived mutants which formed only blastoconidia or hyphae (35). He compared their virulence in an intravenously infected mouse model and concluded that each mutant could invade the host. In other experiments, Ryley and Ryley compared the virulence of the same strains with that of strain MM2002, which produced blastoconidia but only a few hyphae in vitro (33). They found that strain MM2002 was just as virulent for mice as its wild-type, germinative strain was, and they concluded that hyphae are not required for the initiation of lesion formation in the kidneys.

In *C. albicans*, surface mannoproteins (6), adhesins (3), and hydrophobicity proteins (16) are differentially expressed depending on the morphological form. Since our clones differed in their growth form at 37°C in MTM, we measured the CR activity of each clone by using EAiC3b and EAC3d. Since an association of CR3 activity with adherence of the organism to endothelial cells (15) and virulence in animal models (4, 24) has been observed, we reasoned that our clones may have reduced CR activity.

Each of the clones caused reduced levels of rosetting of EAC3d but wild-type levels of rosetting of EAiC3b. The extent of the reduction in rosetting of EAC3d was strain specific. Of the four clotrimazole-tolerant clones tested, clone P caused the least rosetting of EAC3d, and for this reason it was selected for immunoblot studies. DTT extracts of both H12 and P were prepared, electrophoresed, and transferred to nitrocellulose. Using a monospecific antibody and a monoclonal antibody to *C. albicans* CR2, we observed a quantitative loss in reactivity of both the 50- and 60-kDa proteins from strain P compared with H12. In comparison, the 31-kDa protein, visualized only with the monospecific antibody, was observed in the DTT extract of strain P. Immunoblotting with a patient serum sample revealed several differences in protein profiles. Proteins of 68, 48, 45, and 40 kDa were observed only in H12 cells. Quantitative changes in the 60- and 50-kDa proteins were also seen, as described above for other antibodies. The reduced activity with the 60-kDa protein is similar to that reported with other cell surface mutants (39).

Since each of the clotrimazole-tolerant strains is avirulent in a murine model of systemic candidiasis, there is a suggestion that C3d recognition is associated with the ability of the organism to cause infection by intravenous inoculation. Interestingly, *C. albicans* m-10, which has reduced levels of iC3b binding but normal levels of C3d binding and is avirulent in endocarditis and murine vaginitis models (4, 24), is virulent when injected intravenously in mice (unpublished data). Therefore, in this model the presence or absence of an

iC3b-binding protein is irrelevant to virulence. However, decreased expression of the C3d-binding protein is associated with reduced infectivity in the intravenous murine model of systemic *Candida* mycosis. That *C. albicans* could have multiple virulence factors is not surprising when one considers the variety of infections caused by this organism and the selective pressures encountered during its colonization and growth in different tissues.

Although only the morphologic and CR phenotypes have been described, other properties of the four clones have been examined (11a). Clones C1 and C2 have reduced lipase activity and therefore would appear to be different from clones P and N. All clones discussed in this communication produce protease at the same level as do H12 cells. Likewise, each of the clones is killed by human neutrophils to a significantly greater extent than is H12 (25a).

ACKNOWLEDGMENTS

This research was supported in part by Public Health Service grant AI 25738 from the National Institutes of Health (to R.C.) and by grants from the Deutsche Forschungsgemeinschaft (to S.F.) and Bayer AG.

We thank Elsa Wadsworth for her advice on the immunoblotting experiments and H. Diekmann for his very helpful discussions and proofreading.

REFERENCES

1. **Borg, M., and R. Rüchel.** 1988. Expression of extracellular acid protease by proteolytic *Candida* spp. during experimental infection of the oral mucosa. Infect. Immun. **56:**626–631.
2. **Buffo, J., M. A. Herman, and D. R. Soll.** 1984. A characterization of pH-regulated dimorphism in *Candida albicans*. Mycopathologia **85:**21–30.
3. **Calderone, R. A., and P. C. Braun.** 1991. Adherence and receptor relationships of *Candida albicans*. Microbiol. Rev. **55:**1–20.
4. **Calderone, R. A., R. L. Cihlar, D. Lee, K. Hoberg, and W. M. Scheld.** 1985. Yeast adhesion in the pathogenesis of endocarditis due to *Candida albicans*: studies with adherence-negative mutants. J. Infect. Dis. **152:**710–715.
5. **Calderone, R. A., L. Linehan, E. Wadsworth, and A. L. Sandberg.** 1988. Identification of C3d receptors on *Candida albicans*. Infect. Immun. **56:**252–258.
6. **Casanova, M., M. L. Gil, L. Cardeñoso, J. P. Martinez, and R. Sentandreu.** 1989. Identification of wall-specific antigens synthesized during germ tube formation by *Candida albicans*. Infect. Immun. **57:**262–271.
7. **Cutler, J. E.** 1991. Putative virulence factors of *Candida albicans*. Annu. Rev. Microbiol. **45:**187–218.
8. **Douglas, L. J.** 1991. Adhesion of *Candida albicans* to host surfaces. FEMS Symp. **50:**43–48.
9. **Edwards, J. E., T. A. Gaither, J. J. O'Shea, D. Rotrosen, T. J. Lawley, M. M. Frank, and I. Green.** 1986. Expression of specific binding sites on *Candida* with functional and antigenic characteristics of human complement receptors. J. Immunol. **137:**3577–3583.
10. **Eigentler, A., T. F. Schulz, C. Larcher, E. M. Breitwieser, B. Myones, A. L. Petzer, and M. P. Dierich.** 1989. iC3b-binding protein on *Candida albicans*: temperature-dependent expression and relationship to human complement type receptor 3. Infect. Immun. **57:**616–622.
11. **Franzke, S.** 1989. Isolierungund partielle Charakterisierung von apathogenen Mutanten der Hefe *Candida albicans*. M. S. Diplomarbeit. Universität Hannover, Hannover, Germany.

11a. **Franzke, S., R. A. Calderone, and K. Schaller.** Unpublished data.

12. **Fukayama, M., E. Wadsworth, and R. Calderone.** 1992. Expression of the C3d-binding protein (CR2) from *Candida albicans* during experimental candidiasis as measured by lymphoblastogenesis. Infect. Immun. **60:**8–12.
13. **Ghannoum, M. A.** 1992. *Candida albicans* antifungal-resistant strains: studies on adherence and other pathogenicity related characteristics. Mycoses **35:**131–139.
14. **Gilmore, B. J., E. M. Retsinas, J. S. Lorenz, and M. K. Hostetter.** 1988. An iC3b receptor on *Candida albicans*: structure, function and correlates for pathogenicity. J. Infect. Dis. **157:**38–46.
15. **Gustafson, K. S., G. M. Vercellotti, C. M. Bendei, and M. K. Hostetter.** 1991. Molecular mimicry in *Candida albicans*: role of an integrin analogue in adhesion of the yeast to human endothelium. J. Clin. Invest. **87:**1896–1902.
16. **Hazen, B. W., and K. C. Hazen.** 1988. Dynamic expression of cell surface hydrophobicity during initial yeast cell growth and before germ tube formation of *Candida albicans*. Infect. Immun. **56:**2521–2525.
17. **Heidenreich, F., and M. P. Dierich.** 1985. *Candida albicans* and *Candida stellatoidea*, in contrast to other *Candida* species, bind iC3b and C3d but not C3b. Infect. Immun. **50:**598–600.
18. **Hynes, R.** 1989. Integrins: a family of cell surface receptors. Cell **56:**549–554.
19. **Kanbe, T., R.-K. Li, E. Wadsworth, R. A. Calderone, and J. E. Cutler.** 1991. Evidence for the expression of the C3d receptor of *Candida albicans* in vitro and in vivo obtained by immunofluorescence and immunoelectron microscopy. Infect. Immun. **59:**1832–1838.
20. **Kennedy, M. J., R. A. Calderone, J. M. Cutler, T. Kanbe, M. H. Riesselman, R. Robert, J.-M. Senet, V. Annaix, A. Bouali, C. Mahaza, G. Trochin, J.-P. Bouchara, M. Miegeville, A. Marot-Leblond, and E. Segal.** 1992. Molecular basis of *Candida albicans* adherence. J. Med. Vet. Mycol. **30**(Suppl. 1):95–122.
21. **Klotz, S. A.** 1992. Fungal adherence to the vascular compartment: a critical step in the pathogenesis of disseminated candidiasis. Clin. Infect. Dis. **14:**340–347.
22. **Kwon-Chung, K. J., D. Lehmann, C. Good, and P. T. Magee.** 1985. Genetic evidence for role of extracellular proteinase in virulence of *Candida albicans*. Infect. Immun. **49:**571–575.
23. **Lee, K. L., H. R. Buckley, and C. C. Campbell.** 1975. An amino acid liquid synthetic medium for the development of mycelial and yeast forms of *Candida albicans*. Sabouraudia **13:**148–153.
24. **Lehrer, N., E. Segal, R. L. Cihlar, and R. A. Calderone.** 1986. Pathogenesis of vaginal candidiasis: studies with a mutant which has reduced ability to adhere *in vitro*. J. Med. Vet. Mycol. **24:**127–131.
25. **Linehan, L., E. Wadsworth, and R. Calderone.** 1988. *Candida albicans* C3d receptor, isolated by using a monoclonal antibody. Infect. Immun. **56:**1981–1986.

25a. **Lohmann-Matthes, M.** Personal communication.

26. **MacDonald, F., and F. C. Odds.** 1983. Virulence for mice of a proteinase-secreting strain of *Candida albicans* and a proteinase-negative mutant. J. Gen. Microbiol. **129:**431–438.
27. **Mattia, E., G. Carruba, L. Angiolella, and A. Cassone.** 1982. Induction of germ tube formation by *N*-acetyl-D-glucosamine in *Candida albicans*: uptake of inducer and germinative response. J. Bacteriol. **152:**555–562.
28. **Nemerow, G. R., M. Moore, and N. R. Cooper.** 1990. Structure and function of the B-lymphocyte Epstein-Barr virus/C3d receptor. Adv. Cancer Res. **54:**273–300.
29. **Odds, F. C.** 1985. *Candida albicans* proteinase as a virulence factor in the pathogenesis of *Candida* infections. Zentralbl. Bakteriol. Mikrobiol. Hyg. 1 Abt. Orig. A **260:**539–542.
30. **Odds, F. C.** 1985. Morphogenesis in *Candida albicans*. Crit. Rev. Microbiol. **12:**45–93.
31. **Odds, F. C., A. Cocknayne, J. Hayward, and A. B. Abbott.** 1985. Effects of imidazole- and triazole-derivative antifungal compounds on the growth and morphological development of *Candida albicans* hyphae. J. Gen. Microbiol. **131:**2581–2589.
32. **Ollert, M. W., E. Wadsworth, and R. A. Calderone.** 1990. Reduced expression of the functionally active complement receptor for iC3b but not for C3d on an avirulent mutant of *Candida albicans*. Infect. Immun. **58:**909–913.
33. **Ryley, J. F., and N. G. Ryley.** 1990. *Candida albicans*—do mycelia matter? J. Med. Vet. Mycol. **28:**225–239.
34. **Saxena, A., and R. A. Calderone.** 1990. Purification of the

extracellular C3d-binding protein of *Candida albicans*. Infect. Immun. **58**:309–314.

35. **Shepherd, M. G.** 1985. Pathogenicity of morphological and auxotrophic mutants of *Candida albicans* in experimental infections. Infect. Immun. **50**:541–544.
36. **Smail, E. H., and J. M. Jones.** 1984. Demonstration and solubilization of antigens expressed primarily on the surface of *Candida albicans*. Infect. Immun. **45**:74–81.
37. **Tosh, F. D., and L. J. Douglas.** 1992. Characterization of a fucose-binding adhesin of *Candida albicans*. Infect. Immun. **60**:4734–4739.
38. **Tronchin, G., J. P. Bouchara, and R. Robert.** 1989. Dynamic changes of the cell wall surface of *Candida albicans* associated with germination and adherence. Eur. J. Cell Biol. **50**:285–290.
39. **Whelan, W. L., J. M. Delga, E. Wadsworth, T. J. Walsh, K. J. Kwon-Chung, R. Calderone, and P. N. Lipke.** 1990. Isolation and characterization of cell surface mutants of *Candida albicans*. Infect. Immun. **58**:1552–1557.