

Identification of C3d Receptors on *Candida albicans*

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Pseudohyphal but not yeast forms of *Candida albicans* possess both iC3b and C3d receptors, as determined by rosetting with erythrocytes carrying iC3b (EAC3bi) or C3d (EAC3d). Rosetting with EAC3d was markedly reduced when pseudohyphae were heat killed or treated with trypsin or pronase but was not inhibited by several saccharides or aminosaccharides, including α -methyl-D-mannoside, or by pretreatment of pseudohyphae with concanavalin A. However, mannoproteins obtained by concanavalin A affinity chromatography of whole pseudohyphal extracts inhibited the attachment of EAC3d to *C. albicans*, whereas soluble (nonmannosylated) proteins were less active. Thus, although the C3d receptors appeared to be glycosylated, the oligosaccharide component of the receptor was apparently not involved in the recognition of C3d. To isolate these receptors, whole-cell extracts were separated by DEAE-Trisacryl chromatography. Fractions that inhibited rosetting were pooled and affinity purified by C3d-Thiol-Sepharose chromatography. The eluate from this affinity column inhibited attachment of *C. albicans* to EAC3d. Monoclonal antibodies to *C. albicans* were prepared, and three of these antibodies blocked rosetting. Western blotting (immunoblotting) with these antibodies indicated the presence of 62- and 70-kilodalton receptors for C3d in the extracts purified by C3d affinity chromatography and separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis.

Human peripheral blood cells possess several membrane glycoproteins (CR1, CR2, CR3, and CR4) that bind cleavage products of the third component of complement. CR1 and CR3 preferentially bind C3b and iC3b, respectively (reviewed in reference 1), whereas CR2 and CR4 recognize C3dg/C3d (5, 8, 16, 19). Receptors with these specificities are not found exclusively on mammalian cells. Recently, Heidenreich and Dierich (6) demonstrated that *Candida albicans* and *C. stellatoidea* rosette with erythrocytes coated with iC3b (EAC3bi) and C3d (EAC3d), whereas this property was not associated with less pathogenic *Candida* spp., such as *C. tropicalis*, *C. parapsilosis*, and *C. krusei*. These investigators suggested that a lectinlike interaction occurred between these C3 fragments and *C. albicans*, since D-mannose and D-glucose partially inhibited rosetting. The presence of *Candida* receptors for iC3b and C3d has been substantiated by Edwards et al. (3), who have, in addition, examined the ability of a series of monoclonal and polyclonal antibodies against CR1, CR2, and CR3 to bind to *C. albicans* and inhibit rosetting with EAC3bi and EAC3d. Of these antibodies, only one monoclonal antibody against CR3 (Mo-1) bound significantly to *C. albicans*. However, at high concentrations, one monoclonal antibody (HB-5), in the presence of a second enhancing antibody and a polyclonal antibody against CR2, blocked rosetting.

Although considerable emphasis has been placed on defining the biological consequences of the interaction of mammalian cell receptors with C3b and its further cleavage products, the functional significance of the receptors for C3 fragments on *Candida* spp. remains unknown. To facilitate investigations concerning the potential pathogenic role of these receptors on *Candida* spp. and to compare them with receptors of similar specificity on human cells, studies were initiated to identify the C3d receptor(s) on *C. albicans*.

MATERIALS AND METHODS

Preparation of cells. *C. albicans* 4918 was used throughout these studies and has been described previously (7). Yeast forms were obtained from brain heart infusion agar plates which had been incubated overnight at 37°C. Cells were collected in phosphate-buffered saline (PBS) containing 0.02 M PO₄ and 0.15 M NaCl (pH 7.2), washed twice in PBS, and suspended in a Veronal-sucrose buffer containing 1 mM MgCl₂, 0.15 mM CaCl₂, and 0.1% gelatin (GVB-GVBS; μ = 0.065 [14]).

For rosetting assays, the pseudohyphal phase of *C. albicans* was induced in RPMI 1640 (GIBCO Laboratories, Grand Island, N.Y.) in the absence of serum. Yeast cells from an overnight culture grown at 37°C on brain heart infusion agar were washed in PBS, and cells at a density of $2 \times 10^7/0.1$ ml were added to 10 ml of RPMI 1640 in sterile screw-cap test tubes. For most experiments, the hyphae were collected after 18 h (37°C), washed in PBS, and suspended in GVB-GVBS. For other experiments, cells were harvested at earlier time intervals as indicated. The final concentration of pseudohyphal particles used in the rosetting assays was $2 \times 10^6/0.1$ ml of GVB-GVBS.

Preparation of erythrocyte-complement intermediates. Functionally purified human complement components (C1, C2, C3, and C4) were obtained from Diamedix Corp., Miami, Fla. Optimally sensitized sheep erythrocytes were suspended in GVB-GVBS, and 1 ml of the suspension (5×10^8 erythrocytes) was incubated with C1 (5,000 U) at 30°C for 30 min. Subsequently, C4 (1,000 U), C2 (1,000 U), and C3 (1,000 U) were added in sequence, and the cells were washed with GVB-GVBS between each step. Incubations were done at 37°C for 30 min with C4, for 8 min with C2, and for 1 h with C3. Portions of erythrocyte intermediates (designated E, EA, EAC1, EAC14, EAC142, and EAC1423b) were examined for rosetting with pseudohyphae. EAC3b was

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converted to EAC3bi by incubation at 37°C for 1 h with 50 μ l of human C3 inactivator (Factor I; Diamedix) and 5 μ l of Factor H; Factor H was diluted 1:300 (vol/vol) in GVB-GVBS. Factor H was kindly provided by Michael M. Frank (National Institute of Allergy and Infectious Diseases, Bethesda, Md.). Conversion of EAC3bi to EAC3d was accomplished by trypsin (20 μ g) digestion for 1 h at 37°C. The reaction was terminated by soybean trypsin inhibitor (40 μ g).

Rosetting assay. Portions of the erythrocyte intermediates (1.4×10^8 in 0.1 ml) were incubated with 0.1 ml of yeast or pseudohyphal particles (2×10^6) for 30 min at 37°C and overnight at 4°C. Samples were then removed, and the percentage of yeast cells or pseudohyphae with adhering erythrocytes was determined. Results were evaluated with light microscopy (magnification, $\times 1,000$). At least 100 pseudohyphal or yeast forms were counted in each incubation mixture. Adherence was defined as the binding of at least four erythrocytes. To measure rosette inhibition, EAC3d was incubated for 30 min at 37°C with potential inhibitors before the addition of pseudohyphae. Saccharides used as inhibitors were obtained from Sigma Chemical Co., St. Louis, Mo., and included lactose, glucose, fucose, α -methyl-D-MANNOSIDE, sialic acid, galactosamine, glucosamine, rhamnose, mannosamine, galactose, and *N*-acetylglucosamine (all at 50 mM). In other experiments, pseudohyphae were treated with 0.6 to 3.3 mg of trypsin or pronase (Sigma) per ml or 2.5 to 25 μ g of concanavalin A (ConA; Sigma) per ml at 37°C for 1 h or were heated for 15 min at 100°C.

Preparation of pseudohyphal extracts. Yeast cells, grown on brain heart infusion agar overnight at 37°C, were inoculated into Phytone peptone broth (BBL Microbiology Systems, Cockeysville, Md.; 500 ml per 2-liter flask) for conversion to the pseudohyphal form (final concentration, 10^6 yeast cells per ml). Cultures were grown for 48 h at 37°C, collected by centrifugation, washed and suspended in PBS containing 1 mM phenylmethylsulfonyl fluoride (PMSF; Sigma), and homogenized with glass beads at high speed on a Vortex mixer (The Vortex Manufacturing Co., Cleveland, Ohio) with intermittent cooling in an ice bath. This procedure released cell wall components. Subsequently, the cell homogenate was centrifuged at $10,000 \times g$ for 20 min, at $25,000 \times g$ for 20 min, and finally at $100,000 \times g$ for 1 h. The clear supernatant was then membrane filtered and lyophilized. For DEAE chromatography, the supernatant was precipitated with ammonium sulfate (70%) and dialyzed before lyophilization.

Generation of monoclonal antibodies. CBYJ mice were injected with pseudohyphal cell walls purified according to the procedure of Reiss et al. (15). The mice received a total of 100 mg (dry weight) of cell walls. A total of 50 mg in Freund complete adjuvant was given intraperitoneally on day 0, and 21 days later, 50 mg in saline was administered intraperitoneally. Three days after injection 2 (day 24), serum was obtained and assayed for antibody by agglutination of *C. albicans* pseudohyphae. Spleen cells from the mice with positive antibody titers were fused with NS-1-G-P₂ myeloma cells as described by Margulies et al. (13). Cell culture supernatants were screened for *C. albicans*-specific monoclonal antibodies by an enzyme-linked immunosorbent assay. Tissue culture plates (no. 3596; Costar, Cambridge, Mass.) were coated with pseudohyphae or solubilized cell wall proteins prepared according to the procedure of Chaffin and Stocco (2). Positive hybrids were subcloned twice by limiting dilution and expanded, and the supernatants were retested for antibody production by enzyme-linked immuno-

sorbent assay. Ascitic fluid was obtained according to the method of Luka et al. (12). Isotypes were determined by immunodiffusion with class-specific antisera (Litton Bionetics, Kensington, Md.).

ConA fractionation. The lyophilized extracts were subjected to affinity chromatography on ConA (ConA-Sepharose; Pharmacia, Uppsala, Sweden) by the procedure of Ellsworth et al. (4). A 200-mg sample of lyophilized material dissolved in PBS containing 1 mM PMSF was separated on a column (0.7 by 18 cm) at 4°C at a flow rate of 8 ml/h. A pool of the unbound soluble protein and a pool of the mannoprotein complexes eluted with α -methyl-D-mannoside were dialyzed against PBS or distilled H₂O containing PMSF and lyophilized.

DEAE-Trisacryl chromatography. Pseudohyphal extracts were fractionated on DEAE-Trisacryl M (LKB, Bromma, Sweden) by elution with a stepwise gradient of NaCl in 0.05 M Tris hydrochloride (pH 7.8) containing 0.02% sodium azide and 1 mM PMSF. The NaCl concentrations chosen were 0.04, 0.10, 0.20, 0.28, and 1.0 M. A glass column (2.5 by 14 cm) was packed with 50 ml of beads and equilibrated with 0.05 M Tris hydrochloride (pH 7.8). Lyophilized pseudohyphal whole-cell extract (150 mg) was dissolved in the starting buffer, filtered (Millex filter; 0.22- μ m pore size; Millipore Corp., Bedford, Mass.), and applied to the column. Fractions (3 ml) were collected at a flow rate of 36 ml/h, and the A_{280} was determined. Approximately 10 tubes were collected for each NaCl step. Fractions were assayed for inhibition of rosetting, as previously described, by incubating 0.1 ml of the fractions with EAC3d before addition of the pseudohyphal particles. Those fractions which showed inhibitory activity were pooled, dialyzed against distilled H₂O, concentrated (CX10; Millipore), and lyophilized.

C3d-Thiol-Sepharose affinity chromatography. C3d-Thiol-Sepharose was prepared according to the method of Iida et al. (8). One milligram of purified C3 (Cytotech, Richmond, Calif.) was incubated with 20 μ g of tolylsulfonyl phenylalanine chloromethyl ketone trypsin (Sigma) for 5 min at 37°C in the presence of 0.25 g of Thiol-Sepharose beads (Pharmacia) in 0.1 M Tris hydrochloride (pH 8.0) containing 0.1 M NaCl. PMSF (1 mM) was added to stop the reaction. The beads were washed with the Tris buffer and incubated twice in succession with 30 μ g of porcine elastase at 37°C for 5 h. The reaction was terminated by the addition of the porcine elastase inhibitor elastatinal (Sigma). The coupling of C3d to the Sepharose was confirmed by treating a portion of beads with 0.05 M L-cysteine in 0.01 M Tris buffer (pH 8.0) and analyzing this eluate by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) with 10% polyacrylamide. Cysteine treatment released a single protein of 35 kilodaltons (kDa). C3d affinity chromatography of a pool of inhibitory fractions from DEAE-Trisacryl was performed as follows. A total of 1 ml (approximately 1.0 mg of protein) was added to 2.5 ml of C3d-Thiol-Sepharose beads which had been placed in a sterile screw-cap glass test tube. The mixture was incubated overnight at 4°C with gentle mixing and then packed into a column (1 by 3 cm) to give a bed volume of approximately 1.5 ml. Nonbinding material was collected (approximately 2.5 ml), and the column was subsequently washed with 0.065 M sodium borate (pH 8.0) containing 0.05 M NaCl and then with Tris hydrochloride (pH 7.8) containing 0.2 M NaCl. The beads were then eluted with 0.5 ml of 0.01 M acetate buffer (pH 4.1) containing 0.5 M NaCl. Unbound and bound fractions were pooled, dialyzed exhaustively against PBS, and lyophilized.

SDS-PAGE and western blotting (immunoblotting). SDS-

PAGE was performed under reducing conditions essentially according to the method of Laemmli (9) with a Mini Protean II (Bio-Rad Laboratories, Richmond, Calif.) apparatus. Samples were separated on 10% acrylamide gels at 200 V for 40 min. After completion of electrophoresis, the gels were removed and stained with either Coomassie brilliant blue R-250 (Bio-Rad) or silver (Bio-Rad) or the SDS-PAGE-separated proteins were electrophoretically transferred to 0.45- μ m-pore-size nitrocellulose paper (Bio-Rad). Electrophoretic transfer was accomplished with constant power at 100 V for 90 min at 10°C in Tris glycine buffer (pH 8.3) with 20% methanol (Trans Blot Cell; Bio-Rad). The transfers were blocked with 5% bovine serum albumin in Tris-buffered saline (TBS) (20 mM Tris hydrochloride, 0.2 M NaCl [pH 7.5]) for 1 h at 37°C, washed twice for 5 min with fresh TBS containing 0.05% Tween-20, and incubated with monoclonal antibodies (ascites diluted 1:100 [vol/vol]) in Tween 20-TBS containing 1% gelatin for 2 h at room temperature or at 4°C overnight. After subsequent washing (twice for 10 min in Tween 20-TBS), bands were detected by protein A-gold complemented by the gold enhancement procedure (Bio-Rad) according to the instructions of the manufacturer. Some nitrocellulose paper transfers were parallel blotted by the method of Luka et al. (12) and incubated with monoclonal antibodies as described above. The bands were detected with alkaline phosphatase-conjugated anti-mouse immunoglobulin G (1:1,000 [vol/vol]; Sigma). Other nitrocellulose paper transfers were stained directly with 0.1% amido black (Sigma) in 45% methanol-10% glacial acetic acid in distilled water, washed in glacial acetic acid-methanol-water, and dried.

Total protein was estimated according to the method of Lowry et al. (11), with bovine serum albumin as a standard.

RESULTS

Rosetting with E, EA, or EAC intermediates. Few yeast forms of *C. albicans* rosette with any complement intermediate, whereas pseudohyphae rosette with EAC3bi and EAC3d but not E, EA, EAC1, EAC142, or EAC1423b (Table 1). The interactions of pseudohyphae with EAC3d and EAC1423b can be seen in Fig. 1. Rosetting with EAC3bi was similar to that observed with EAC3d (data not shown). These patterns are similar to those published previously (3,

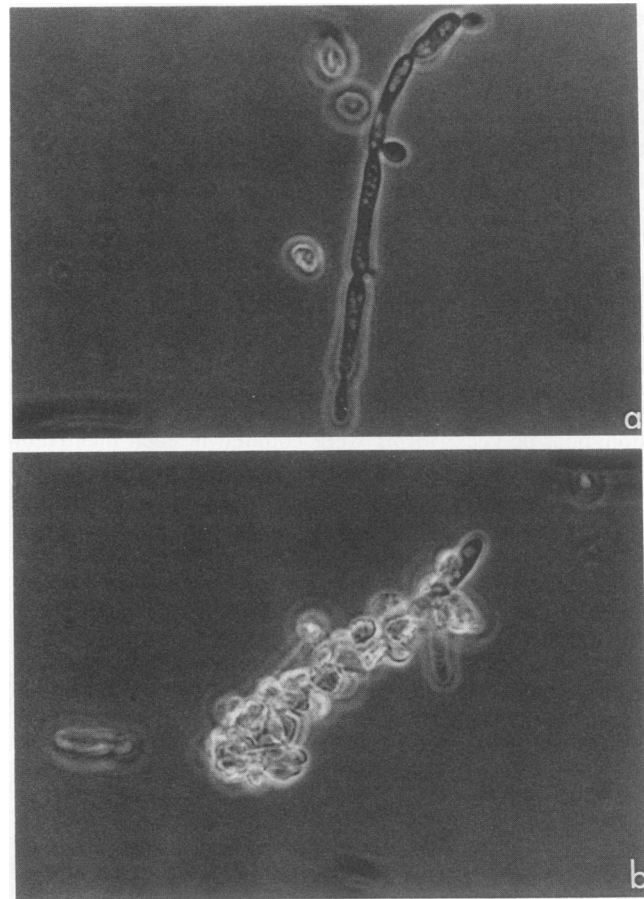


FIG. 1. Incubation of *C. albicans* pseudohyphae with EAC3b (a) or EAC3d (b). Note that the rosetting occurred only in panel b.

TABLE 1. Rosetting of *C. albicans* yeast cells or pseudohyphae with E, EA, or EAC intermediates

Complement intermediate	% Yeast cells or pseudohyphae with rosettes ^a	
	Yeast	Pseudohyphae
E	3.7 \pm 1.6	3.2 \pm 1.5
EA	1.0 \pm 0.7	4.1 \pm 1.0
EAC1	0.9 \pm 0.7	3.1 \pm 1.0
EAC142	0.9 \pm 0.5	3.0 \pm 2.0
EAC1423b	5.0 \pm 1.0	5.5 \pm 1.7
EAC1423bi	3.0 \pm 1.0	75.0 \pm 5.4
EAC1423d	4.0 \pm 1.0	51.8 \pm 3.7

^a Percent rosetting was determined by counting 100 yeast or pseudohyphal particles. Data are expressed as the mean \pm standard deviation for samples assayed in triplicate.

6) in that extensive coating of the pseudohyphae by EAC3bi and EAC3d occurred. In further exploring the interaction of *Candida* spp. with C3 cleavage products, we have chosen to focus our attention on the C3d receptor.

Since this receptor was observed primarily on pseudohyphae, its expression during the conversion of yeast forms to the pseudohyphal forms was examined. Yeast cells were germinated in RPMI 1640, and at 0, 1, 2, 3, 4, and 18 h, the percentages of yeast forms converting to pseudohyphae, as well as the percentages of rosetting particles, were determined. A direct correlation can be seen in Fig. 2 between the number of yeast cells which converted to pseudohyphal forms and the percentage of rosette-positive pseudohyphae. Yeast cells which did not convert to pseudohyphae at any of the time periods rarely formed rosettes, and the number of rosette-forming yeast cells was no greater with EAC3d than with E.

Effects of heat, enzymes, ConA, and saccharides on rosetting of EAC3d by pseudohyphae. Rosetting of EAC3d was completely abolished by heating pseudohyphae at 100°C for 15 min and was considerably reduced by pretreatment of the *Candida* spp. with trypsin or pronase (Table 2). The incubation of pseudohyphae with ConA over a concentration range of 2.5 to 25 μ g before the addition of EAC3d did not inhibit rosetting. At final concentrations of 50 mM, lactose, glucose, fucose, α -methylmannoside, sialic acid, galactosamine, glu-

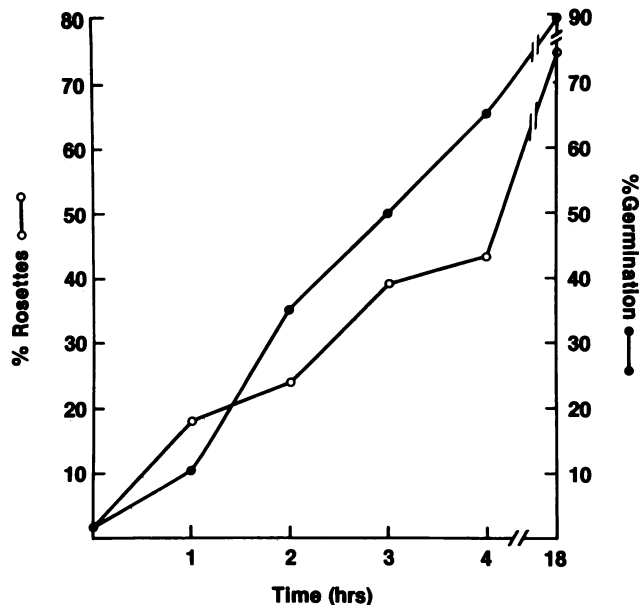


FIG. 2. Expression of C3d receptor activity on *C. albicans* and conversion of yeast cells to pseudohyphae as functions of time. Yeast cells were incubated in RPMI 1640. At 0, 1, 2, 3, 4, and 18 h, samples were removed, washed in GVB-GVBS, and incubated with EAC3d, and percent rosetting activity, as well as percent cells which had germinated, was determined. Data are expressed as the mean of triplicate samples.

cosamine, rhamnose, mannosamine, galactose, and *N*-acetylglucosamine were not inhibitory (data not shown).

Inhibition of rosetting of EAC3d by monoclonal antibodies. Four monoclonal antibodies prepared against *C. albicans* were screened for their ability to block EAC3d rosetting by pseudohyphae. These monoclonal antibodies were of the immunoglobulin G1 subclass. Three of the four antibodies (CA-A, CA-C, and CA-D) blocked rosetting significantly at dilutions of up to and including 1:256 (Table 3) when incubated with pseudohyphae before the addition of EAC3d, whereas the other one, CA-B, was inactive.

Comparison of mannoproteins and nonmannosylated proteins as inhibitors of EAC3d rosetting. ConA-Sepharose-bound mannoproteins and nonmannosylated proteins were assayed for their ability to block rosetting of EAC3d by

TABLE 2. Effect of pretreatment of *C. albicans* pseudohyphae with heat, enzymes, and ConA on EAC3d receptor activity

Treatment and concn	% Rosetting ^a
Untreated (GVB-GVBS).....	88.3 ± 2.1
Heat killed ^b	2.1 ± 1.0
Trypsin	
3.3 mg/ml	25.6 ± 1.7
0.6 mg/ml	20.0 ± 2.7
Pronase (3.3 mg/ml)	18.4 ± 5.1
ConA (2.5 µg/ml) ^c	83.8 ± 2.7

^a Data are expressed as the mean ± standard deviation for samples assayed in triplicate and are representative of three experiments.

^b Heat killing was done at 100°C for 15 min.

^c Identical results were observed over a concentration range from 2.5 to 25 µg/ml.

TABLE 3. Effect of anti-*C. albicans* monoclonal antibodies on rosetting of EAC3d by pseudohyphae

Monoclonal antibody ^a	% Rosetting	
	Expt 1	Expt 2
Control (GVB-GVBS)	85.5	88.9
CA-A	12	10.6
CA-B	83.9	88.9
CA-C	10.6	8.1
CA-D	6.1	13.6

^a Ascites were diluted 1:256 (vol/vol).

pseudohyphae. The results strongly suggest that C3d receptors are mannoproteins (Fig. 3). At a protein concentration of 0.6 µg/0.1 ml, mannoproteins from pseudohyphal whole-cell extracts inhibited rosetting by 50%, whereas 50% inhibition was not observed at concentrations of nonmannosylated proteins as high as 50 µg/0.1 ml.

Fractionation of pseudohyphal extracts by DEAE-Trisacryl and C3d-Thiol-Sepharose chromatography. Pseudohyphal whole-cell extracts were chromatographed on DEAE-Trisacryl, and four protein peaks were obtained by using a stepwise NaCl gradient. Only fractions that were eluted with 0.20 M NaCl (peak III) contained components that inhibited rosetting of EAC3d by pseudohyphae (Fig. 4). These fractions were pooled, lyophilized, and designated as pool III. Multiple bands were detected after separation of both the starting material and pool III by SDS-PAGE (Fig. 5, lanes 1 and 2).

Pool III was then affinity purified by C3d-Thiol-Sepharose chromatography. The unbound material and the low-pH

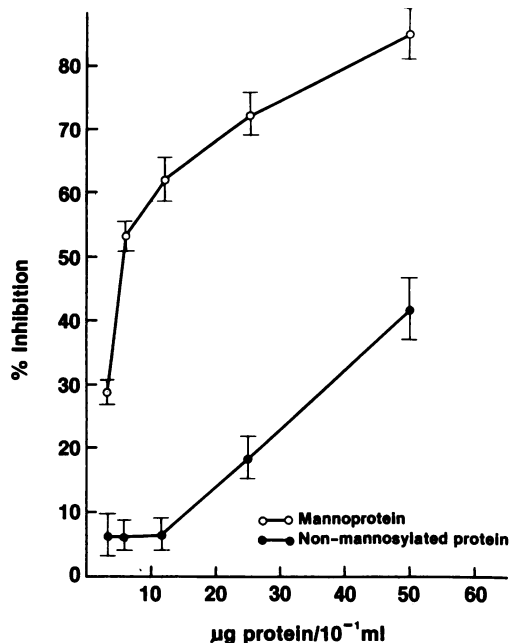


FIG. 3. Effect of *Candida* mannoproteins (or mannan) and nonmannosylated proteins on the rosetting of EAC3d by pseudohyphae. Whole-cell extracts were affinity chromatographed on ConA-Sepharose. Unbound and bound fractions were preincubated with EAC3d (30 min at 37°C), at which time pseudohyphae were added, and the percent rosetting was determined. Controls consisted of EAC3d incubated with pseudohyphae and GVB-GVBS only. Data represent an average of three experiments.

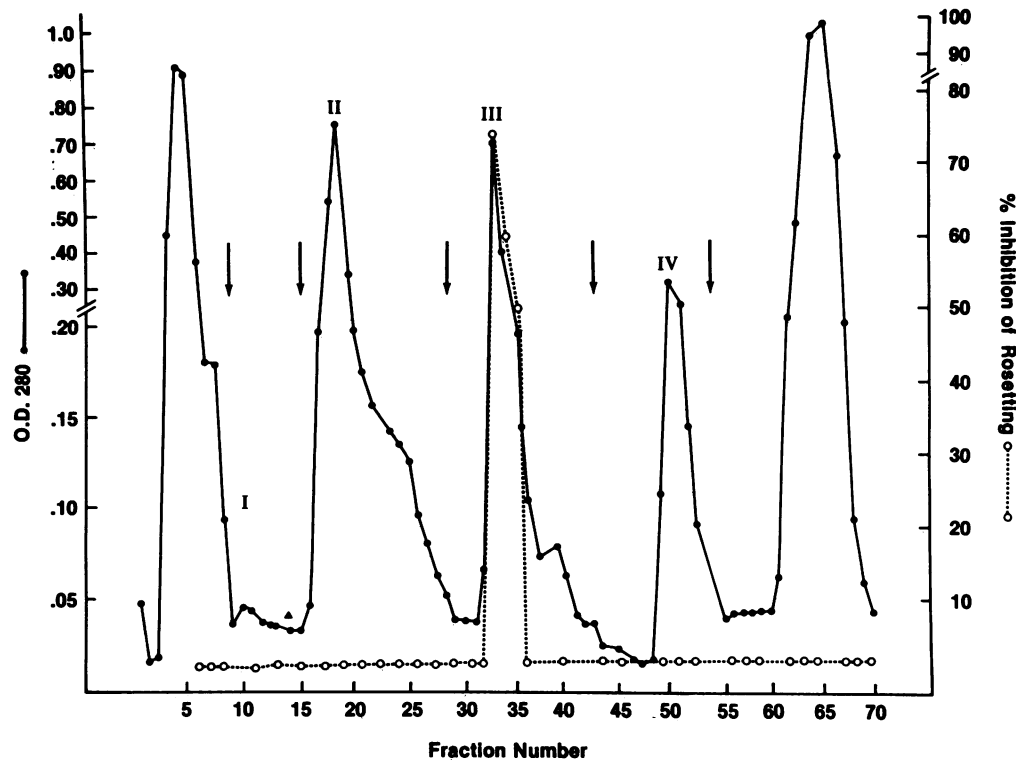


FIG. 4. DEAE-Trisacryl step gradient chromatography of a whole-cell extract of *C. albicans*. Extract (150 mg) was applied and eluted with Tris buffer containing the following concentrations of NaCl (arrows): peak I, 0.04 M; peak II, 0.10 M; peak III, 0.20 M; peak IV, 0.28 M; and the column was cleared of protein with 1.0 M NaCl. The optical density at 280 nm of each fraction was determined, and alternate fractions were assayed for inhibition of rosetting. Peak III contained components which inhibited rosetting. (Similar data were obtained from four column separations).

eluate were dialyzed and assayed for inhibition of rosetting. The low-pH eluate inhibited rosetting by 50% at a concentration of 0.6 μ g of protein per 0.1 ml. Components that did not bind to the column possessed minimal inhibitory activity at concentrations of up to 50 μ g of protein per ml (data not shown). The low-pH eluates were separated by SDS-PAGE. Silver staining of the gel revealed a band of approximately 70 kDa (Fig. 5, lane 3). C3d-binding proteins with molecular sizes of approximately 62 and 70 kDa were detected by Western blotting with CA-A, one of the monoclonal antibodies that inhibited rosetting of *C. albicans* with EAC3d (Fig. 5, lane 4). Neither these nor any other bands reacted with Coomassie blue or amido black on the nitrocellulose paper transfers or the original gels.

DISCUSSION

Two proteins of approximately 62 and 70 kDa that bind the C3d fragment of C3 have been identified in extracts of *C. albicans* pseudohyphae. Purification of these C3d receptors was achieved by sequential chromatography of whole-mycelium extracts on DEAE-Trisacryl and C3d-Thiol-Sepharose. These receptors were detected throughout the purification procedures by inhibition of rosetting of *C. albicans* with EAC3d and identified by Western blotting with an anti-*Candida* monoclonal antibody (CA-A) that blocked rosetting. This monoclonal antibody consistently recognized bands of both 62 and 70 kDa, providing evidence of structural similarity between the components in these bands in

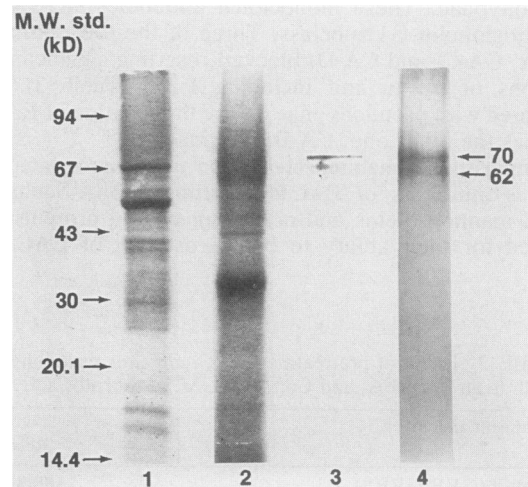


FIG. 5. SDS-PAGE of *C. albicans* extracts before and after chromatography on DEAE-Trisacryl and C3d-Thiol-Sepharose. The gels shown in lanes 1 and 2 were stained with Coomassie brilliant blue R-250, and that in lane 3 was stained with silver. Lane 1, Pseudohyphal extract; lane 2, DEAE-Trisacryl pool III; lane 3, low-pH eluate from C3d-Thiol-Sepharose; lane 4, immunoblot of low-pH eluate from C3d-Thiol-Sepharose detected by monoclonal antibody CA-A and protein A-gold. A similar blotting pattern was observed with alkaline phosphatase staining. The molecular size standards are indicated in lane 1, and the sizes (in kilodaltons) of the bands in lane 4 are also indicated.

that at least one epitope was shared. The same two bands were found when the monoclonal antibody was used to purify the putative C3d receptors from *Candida* extracts (R. Calderone, manuscript in preparation). It is conceivable that the lower-molecular-size material was a degradation product even though a protease inhibitor was included in all extraction and purification procedures. Alternatively, the finding of two bands may reflect slightly different receptors or different degrees of glycosylation of the same protein. The present studies have focused entirely on the characterization of the C3d receptor. This and other investigations (3, 6) have clearly demonstrated that *Candida* spp. also rosette with EAC3bi. Whether this activity represents interaction with these or different receptors has not been explored.

The presence of a receptor for C3d on *C. albicans* and *C. stellatoidea* was initially described by Heidenreich and Dierich (6) and later confirmed by Edwards et al. (3). In the former study, this receptor and a receptor for iC3b were observed on both yeast and hyphal forms, whereas they were detected only on hyphal forms by Edwards et al. (3). In the present study, C3d receptor expression was found primarily on pseudohyphal forms and conversion of yeast cells to pseudohyphae correlated with an increase in receptor expression.

These receptors, which are heat and protease sensitive, are probably mannosylated, since a ConA affinity-purified fraction from whole-cell extracts of pseudohyphae was approximately 10-fold more active as an inhibitor of rosetting with EAC3d than was the fraction that did not bind to ConA. Further support for the association of mannose with the C3d receptors has recently been obtained by the finding that ConA detects both the 62- and 70-kDa bands on nitrocellulose transfers of monoclonal antibody CA-A affinity-purified receptors separated by SDS-PAGE (unpublished observations). However, this saccharide does not appear to be involved in the recognition of C3d, since pretreatment of the pseudohyphae with ConA had no effect on rosetting and α -D-methylmannoside failed to inhibit this activity. These findings are in contrast to the previously described partial inhibition of rosetting (30%) by mannose (6).

Different approaches are being used to characterize the C3 receptors on *Candida* spp. and to compare them with mammalian receptors of similar specificity. Edwards et al. (3) have used monoclonal antibodies against human C3 receptors as potential inhibitors of rosetting of *Candida* spp. with erythrocytes coated with C3 fragments. Some similarity to CR2 was suggested by their findings that HB-5, a monoclonal anti-CR2 antibody, and anti-GP140, a polyclonal antibody against CR2, blocked rosetting with EAC3bi and EAC3d. This effect was only observed at high antibody concentrations, and inhibition by the monoclonal antibody required the addition of an enhancing antibody. Binding of these antibodies to *Candida* spp. was not detectable, indicating very low affinity interactions. Our approach, which has been to directly examine the C3d binding proteins on *Candida* spp., indicates at least one similarity between CR2 and the *Candida* receptor in that both are glycosylated and the carbohydrate moiety is not apparently involved in receptor recognition (18). The molecular sizes of the *Candida* receptors (62 and 70 kDa) are significantly lower than that of CR2 (140 kDa). They are, however, similar to the molecular size of a cleavage product of CR2 isolated from the culture medium of Raji lymphoblastoid cells (10). The possible relationship of the *Candida* receptor to CR4, the neutrophil and platelet C3dg/C3d receptor (5, 16, 17), has not been explored but will be of interest as further information con-

cerning the physicochemical properties of the mammalian receptor becomes available.

The finding of C3 receptors exclusively on the more pathogenic *Candida* spp. is highly suggestive of their involvement in disease processes. However, the mechanisms by which these receptors may participate in pathogenesis have not been defined. As suggested previously (6), the C3d receptors may facilitate the formation of aggregates of the organism which are not readily phagocytosed or they may mediate adhesion to certain mammalian cells. It is anticipated that characterization of these receptors will provide a basis for future investigations concerning their functional significance.

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