INFECTION AND IMMUNITY, May 1991, p. 1832–1838
0019-9567/91/051832-07$02.00/0
Copyright © 1991, American Society for Microbiology
Vol. 59, No. 5

Evidence for Expression of the C3d Receptor of *Candida albicans* In Vitro and In Vivo Obtained by Immunofluorescence and Immunoelectron Microscopy

TOSHIO KANBE,[1] REN-KAI LI,[1] ELSA WADSWORTH,[2] RICHARD A. CALDERONE,[2] AND JIM E. CUTLER[1]*

Department of Microbiology, Montana State University, Bozeman, Montana 59717,[1] and Department of Microbiology, School of Medicine and Dentistry, Georgetown University, Washington, D.C. 20007[2]

Received 28 December 1990/Accepted 8 February 1991

The complement conversion product C3d binds to a receptor on the cell surface of *Candida albicans*. While the function of this receptor is still uncertain, we investigated whether it is expressed during a murine infection. Rabbit antiserum raised against purified receptor was used in conjunction with immunofluorescence microscopy and immunocolloidal gold electron microscopy to examine kidney tissue and peritoneal lavages from infected mice for receptor expression by *C. albicans* in vivo. Specificity of the antiserum was indicated by reactivity with purified receptor (55 to 60 kDa) and with a protein of similar molecular mass from whole hyphal extracts in Western blots (immunoblots). In vitro analysis by immunofluorescence microscopy showed that the antiserum reacted with both yeast and pseudohyphal forms of the organism, but reactivity was strongest with pseudohyphae. Immunocolloidal gold electron microscopy of fungal cells from peritoneal lavages revealed intense staining of mother cells of germinative forms, germ tubes, and pseudohyphae. Staining of the mother cells was heaviest at the innermost layers of the cell wall but only scant on the cell surface. In contrast, staining was observed throughout the cell walls of germ tubes and pseudohyphae. In kidney, expression of the C3d receptor was found primarily on the cell walls of hyphae and pseudohyphae, although some staining was observed in the cytoplasm. These data support that the C3d receptor of *C. albicans* is expressed in vivo.

Candida albicans may be a harmless member of the normal flora of mucosal surfaces, but in immunocompromised individuals the organism is a major cause of infectious disease. Investigations are beginning to unravel some of the complexities of this fungus as it relates to interactions with the host. For example, the cell surface antigenic characteristics are dynamic and influenced by a variety of environmental conditions and developmental stages of the fungus (1–3, 8, 22, 25). Biological activities of cell surface moieties are becoming identified, and several may serve as receptor molecules for a variety of host humoral components, such as fragments of complement component C3, fibronectin, laminin, and others (6, 7, 10, 11, 13, 21, 25). Although definitive evidence is lacking, some of these receptors may have pathogenetic implications (11, 20). Clearly, more information is needed, including evidence that such molecules are expressed in vivo.

Information has been provided that *C. albicans* expresses a receptor for the mammalian complement fragment C3d (6, 10, 13). Expression of the C3d receptor was characterized by documenting rosette formation of C3d-coated erythrocytes (EAC3d) with cells of *C. albicans*. This method is able to detect relatively high expression levels of the receptor, but detection is confined to the cell surface of the fungus and the observations are limited to in vitro grown cells. To understand the role, if any, of the C3d receptor in pathogenesis of candidiasis, in vivo expression must be documented.

In this study, we examined expression of the C3d receptor in yeast and filamentous forms of *C. albicans* in vitro and in vivo by immunofluorescence microscopy (IFM) and immunoelectron microscopy (IEM). We found that the receptor appears to be produced by both forms of the fungus under in vitro and in vivo conditions.

* Corresponding author.

MATERIALS AND METHODS

Preparation of anti-C3d receptor antiserum. Rabbits were immunized against 300 μg of the C3d receptor purified as described previously (23). The receptor was solubilized in saline, emulsified in complete Freund adjuvant, and injected subcutaneously into each of three rabbits. Three weeks after the primary immunization, each animal was boosted with a subcutaneous injection of 150 μg of antigen emulsified in incomplete adjuvant. The resulting antiserum was used successfully specifically to detect *C. albicans* proteins which exhibit receptor activity (23). The antiserum was diluted as needed in phosphate-buffered saline (PBS; 0.02 M, pH 7.4) supplemented with 3% bovine serum albumin (BSA; fraction V; Sigma Chemical Co., St. Louis, Mo.). This buffer is designated PBS-BSA.

SDS-PAGE and Western blot (immunoblot). Hyphae of *C. albicans* were induced in germination medium of Lee et al. (16) in the following manner. Yeast cells from a 24-h culture grown on Sabouraud agar slants (25°C) were suspended in saline, washed twice with saline, and inoculated into germination medium at a final concentration of 10^6 cells per ml. Cultures were incubated for 24 h (25°C), collected by centrifugation, washed, and inoculated into the germination medium at 37°C at a density of 5×10^7 cells per ml (final concentration). Hyphae were collected at 20 h, washed with saline, and homogenized with a Braun homogenizer as described previously (6, 18). The homogenate was centrifuged and the supernatant collected (18) was used as a source of antigen. Purification of C3d receptor from *C. albicans* hyphae was described previously (18, 23).

Sodium dodecyl sulfate-polyacrylamide gel electrophore-

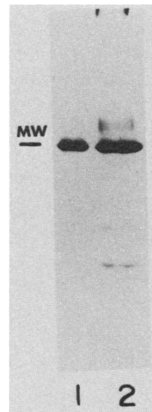

FIG. 1. Western blot of purified C3d receptor (lane 1) and a whole hyphal cell extract (lane 2) reacted with specific, hyperimmune antiserum and detected by protein A-alkaline phosphatase. The molecular weight (MW) marker indicates a protein of approximately 55 to 60 kDa. A minor protein of 67 to 68 kDa and faint reactivity with two bands in the 20-kDa range were also observed in the whole-cell extract (lane 2).

sis (SDS-PAGE) was done by using 10% polyacrylamide under reducing conditions (18). A total of 10 μg of antigen protein (whole-cell homogenate or purified receptor) was loaded per well. Proteins were transferred and blotted with the antiserum described above (1:250 dilution) and washed (18). Bound antibody was detected with a protein A-alkaline phosphatase assay (Cappel Labs, West Chester, Pa.).

In vitro experiment. *C. albicans* strain 1, used in previous studies and which has many characteristics in common with other strains (5, 17), was induced to pseudohyphal forms as described previously (6). Briefly, yeast cells were grown on brain heart infusion (BHI) agar in petri dishes at 37°C for 24 h and harvested by washing the plate with PBS (0.02 M sodium phosphate buffer, 0.15 N sodium chloride, pH 7.4). Cells were washed three times in PBS, and transferred to RPMI 1640 tissue culture medium (Sigma). The final concentration of the suspension in RPMI 1640 medium was 2×10^6 cells per ml; by 18 h of incubation at 37°C, >90% of the cells developed pseudohyphae as confirmed by light microscopy and appropriate criteria (12). The cells were fixed for IFM and IEM as described below.

In vivo experiment. In vivo expression of the C3d receptor on *C. albicans* was investigated in a mouse model used in previous work (4). Yeasts were grown in YEPD (0.3% yeast extract, 1% peptone, and 2% dextrose) for 24 h at 37°C with rotary shaking at 180 rpm (New Brunswick Scientific, New Brunswick, N.J.). In this growth medium, C3d receptor expression was essentially identical to expression of cells grown on brain heart infusion agar (data not shown). Cells were harvested by centrifugation, washed three times in PBS, and kept in an ice bath for up to 30 min before injection into mice. Five- to eight-week-old male BALB/cByJ mice were inoculated with *C. albicans* either intraperitoneally (i.p.) or intravenously. Yeasts were injected intravenously in doses of 5×10^4, 10^5, or 5×10^5 cells per 0.1 ml. Mice were sacrificed 6 days after the injection, and kidneys with macroscopic cortical abscesses were fixed for periodic acid-Schiff/hematoxylin (PASH) stain and for IFM and IEM as described below. For i.p. inoculation, similar methods were used as described before (4). Yeast cells were suspended in PBS, and 2×10^9 cells in 0.2 ml were injected into the peritoneal cavities of mice. Five hours after injection, fungal cells were collected from the peritoneal cavity by saline lavage. The collected cells were immediately processed for IFM and IEM as described below.

Tissue processing for PASH stain. Kidneys were embedded in O.C.T. compound (Miles Inc., Elkart, Ind.) immediately after removal from mice and rapidly frozen on dry ice-acetone for cryosectioning (Reichert-Jung FGIGOCUT 2800N cryostat). Sections (approximately 8 μm thick) were collected on glass slides, air dried for 30 min, fixed in cold (−20°C) acetone for 5 min, and air dried. PASH staining was done as described previously (9).

Indirect IFM. *C. albicans* cells obtained from disparate cultural methods were studied by IFM. Samples included cells grown on brain heart infusion agar plates for 24 h (in vitro yeast phase) or in RPMI 1640 (in vitro pseudohyphae) and cells from the mouse peritoneal cavity 5 h after injection (in vivo yeasts, pseudohyphae, and germ tubes). Cells were fixed with periodic acid (0.01 M)-lysine (0.075 M)-paraformaldehyde (2%) in 0.037 M phosphate (PLP) (19) at 4°C overnight, washed four times in PBS, and incubated with 50 μl of 100-fold-diluted anti-*C. albicans* C3d receptor rabbit polyclonal serum for 90 min at 22 to 24°C. Samples were washed in PBS-BSA buffer and treated for 90 min with affinity-purified fluorescein isothiocyanate-conjugated anti-rabbit immunoglobulin G (diluted as suggested by the manufacturer; Sigma). After three washings in PBS, the cells were placed onto a glass slide, a drop of Aqua-mount (Lehner Laboratories, Pittsburgh, Pa.) was added and mixed, and the slide was covered with a cover slip.

Kidneys from infected mice were also examined for in vivo expression of the C3d receptor by *C. albicans*. The infected organ was cut into approximately three equal longitudinal portions with a razor blade, fixed in PLP overnight at 4 to 8°C, and infiltrated at the same temperature with an increment of sucrose (Sigma) in PBS at 10, 15, and 20% (wt/vol) for 12 h each, respectively. Fixed samples were then embedded in O.C.T. compound and frozen by dry ice-acetone for cryosectioning. Sections were mounted on glass slides, postfixed with acetone at −20°C for 5 min, air dried, and stained for IFM as described above. In some experiments, fresh (unfixed) infected kidney was cryosectioned for IFM. Samples were examined with a fluorescence microscope (Nikon Episcopic fluorescence microscope, type 104, equipped with a high-pressure mercury lamp [100W/2]) and photographed with Kodak Tmax 400 or Tri-X pan black-and-white film.

IEM. Freeze substitution was used for fixation of in vitro grown yeasts and pseudohyphae and in vivo cells grown in the mouse peritoneal cavity. The technique was similar to that recently reported (15, 17) with the exception that osmium tetroxide was not used in the substitution fluid. Briefly, copper grids (0.3-mm mesh; Sheet Mesh, Nissin EM Co., Ltd., Tokyo, Japan) were cleaned in 0.1 N HCl, rinsed in deionized water, and air dried. Each grid was thinly layered with *C. albicans* cells by touching one side of the grid to cells freshly collected on a Whatman no. 1 filter paper. Immediately following layering, each grid was dipped into a mixture of liquid propane and ethane precooled by liquid nitrogen to approximately −190°C. The frozen samples were transferred to a substitution fluid composed of acetone and methanol (1:1; J. T. Baker Chemical Co., Phillipsburg, N.J.) at −80°C for 72 h. Samples were incrementally brought up to room temperature (4 h at −20°C, 1 h at 4°C, and then 22 to 24°C), washed three times in anhy-

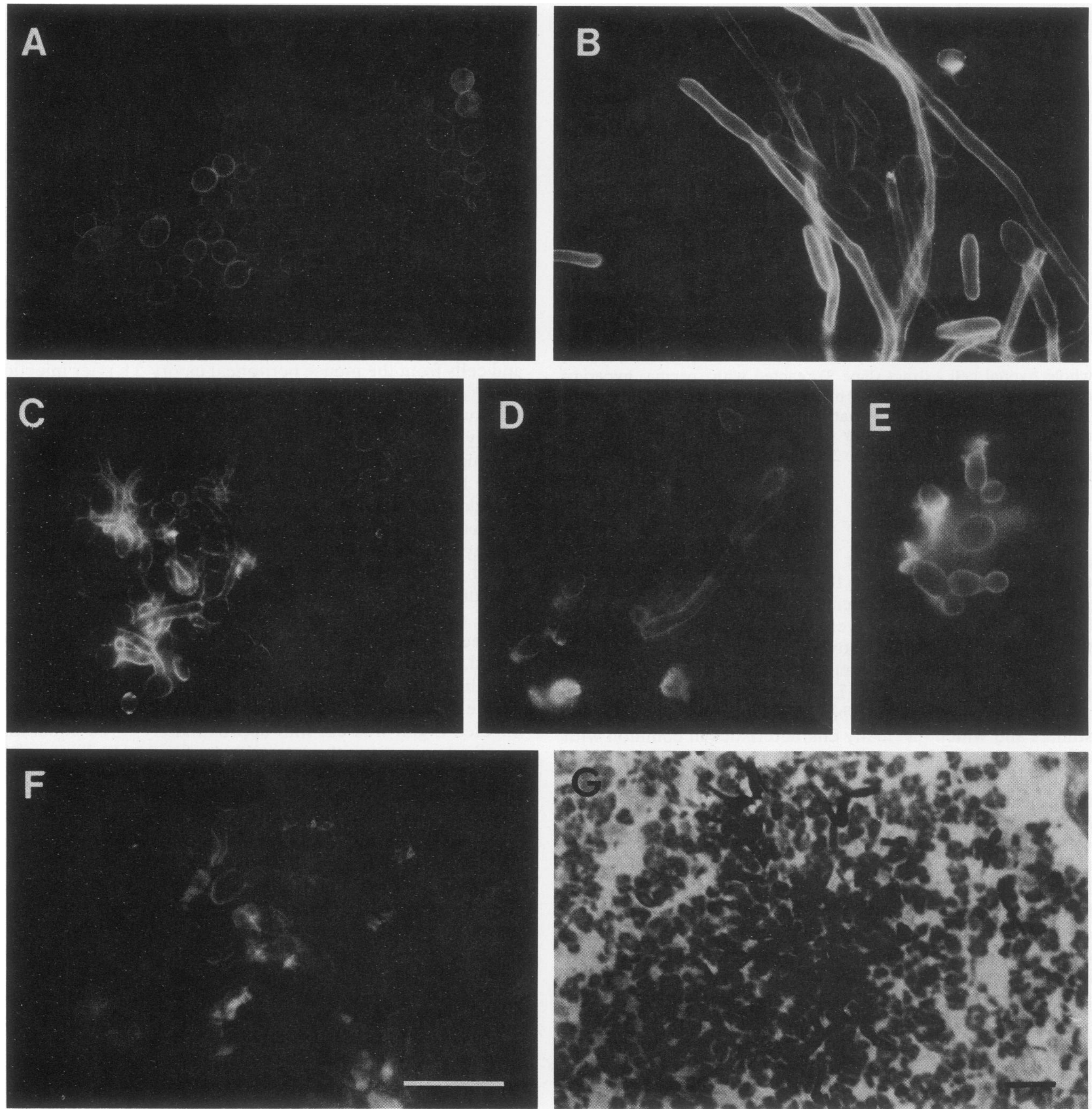

FIG. 2. IFM of C3d receptor expression by *C. albicans* in vitro and in vivo. (A) Yeast cells grown on BHI agar at 37°C generally showed faint fluorescence, but intensity of fluorescence was heterogeneous. (B) Yeast and pseudohyphae grown in RPMI 1640 for 18 h at 37°C with shaking. Pseudohyphae generally showed higher fluorescence intensity than yeast forms, but some pseudohyphae were only weakly fluorescent. (C) In vivo expression of C3d receptor on germinative forms obtained from the peritoneal cavities of mice 5 h after injection of yeast form cells. (D) Pseudohyphae and (E) yeasts in kidney which was fixed in PLP before freezing and sectioning. (F) Pseudohyphae in kidney not fixed prior to freezing and sectioning. Note that fluorescence of *C. albicans* in kidney is generally of a similar intensity as that of yeast cells in vitro. (G) PASH-stained section of the kidney lesion studied above revealing predominance of hyphal and pseudohyphal elements.

drous acetone, and finally infiltrated and embedded in Epon 812 resin for thin sectioning.

Fixation of kidneys was done by using more traditional chemical fixation procedures. The kidneys from mice infected intravenously with *C. albicans* were removed, sliced into small pieces with a razor blade, and placed into cold PLP. After overnight fixation, the presence of fungal cells in tissue pieces was confirmed at the light microscopic level by examination of thin tissue slices produced by cutting with a razor blade. Fixed samples were washed in PBS followed by

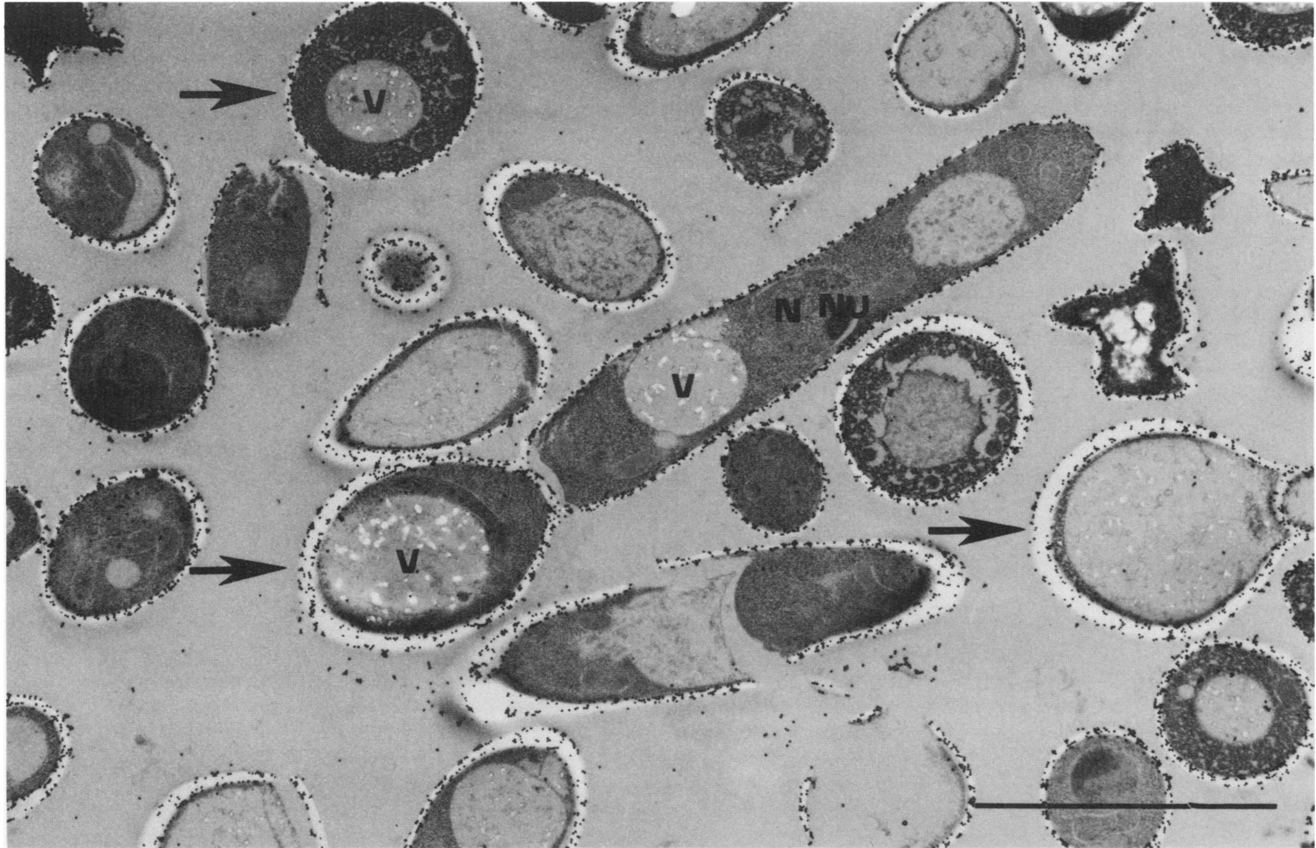

FIG. 3. IEM of C3d receptor expression by *C. albicans* in vitro. *C. albicans* pseudohyphae were fixed by freeze substitution, embedded, ultra-thin sectioned, reacted with rabbit anti-C3d, and developed with anti-rabbit immunoglobulin G–30-nm colloidal gold. Essentially identical results were obtained with protein A–15-nm gold conjugate as the developer (not shown). Note the presence of the C3d receptor, as evidenced by the gold particles, in the cell walls of yeast and pseudohyphal forms. Yeasts and mother cells (arrows) of germinative forms had a high density of the receptor near the inner and outer surfaces of the cell wall. Cell walls of hyphal forms were generally thin but appeared to have heavy expression of the receptor. N, Nucleus; Nu, nucleolus; V, vacuole. Bar, 5 μm.

deionized water, dehydrated through a graded series of acetone, flat embedded in Epon 812 resin (Ted Pella, Inc., Tustin, Calif.), and allowed to polymerize for 24 h at 65°C. The embedded materials were checked under a light microscope for the presence of *C. albicans* in the kidney tissue. The flat block was trimmed, placed on top of a resin block, and fixed in position by addition of a drop of Epon which was then allowed to polymerize.

Ultrathin sections (Reichert-Jung OmU2 Ultracut microtome) were collected on nickel grids (300 mesh), bleached with 1% hydrogen peroxide for 20 min at 21 to 23°C, washed in PBS, and blocked with PBS-BSA before being treated at 4°C overnight with a 1:400 dilution of the antibody specific for the C3d receptor. The sections were washed, blocked again, and treated with either a 1:30 dilution of secondary antibody-conjugated gold particles (30-nm colloidal gold-conjugated goat anti-rabbit immunoglobulin G, heavy and light chains; Amersham International plc, Amersham, United Kingdom) or a 1:20 dilution of protein A-conjugated gold particles (AuroProbe EM protein A G15; Amersham) for 2 h at 21 to 23°C. After washing in PBS, sections were fixed in 3% glutaraldehyde in PBS for 30 min and stained with uranyl acetate and lead citrate. Control grids were reacted with preimmune rabbit serum instead of the primary antibody. Samples were viewed with a Zeiss EM10C/CA microscope at 65 kV.

RESULTS

Specificity of the antiserum for the C3d receptor. Hyperimmune serum used in this study reacted in a Western blot with the purified C3d receptor of *C. albicans* (Fig. 1, lane 1). To examine the specificity of the antiserum, a Western blot was also done with a whole, hyphal cell extract prepared by Braun homogenization (Fig. 1, lane 2). We observed a major band at 55 to 60 kDa, a minor reaction with a 67- to 68-kDa band, and slight reactivity with two bands in the 20-kDa range (standards not shown).

C3d expression as evidenced by IFM. Surface expression of the C3d receptor on *C. albicans* was investigated by IFM (Fig. 2A to F). Yeast forms grown on BHI agar showed faint fluorescence; however, some cells had a higher intensity of fluorescence than others (Fig. 2A). Pseudohyphae induced in RPMI 1640 medium appeared to express a much higher density of the C3d receptor (Fig. 2B) than the majority of yeasts, although heterogeneity in the intensity of fluorescence on pseudohyphae was also observed (Fig. 2B). Control cells which were reacted with preimmune serum, instead

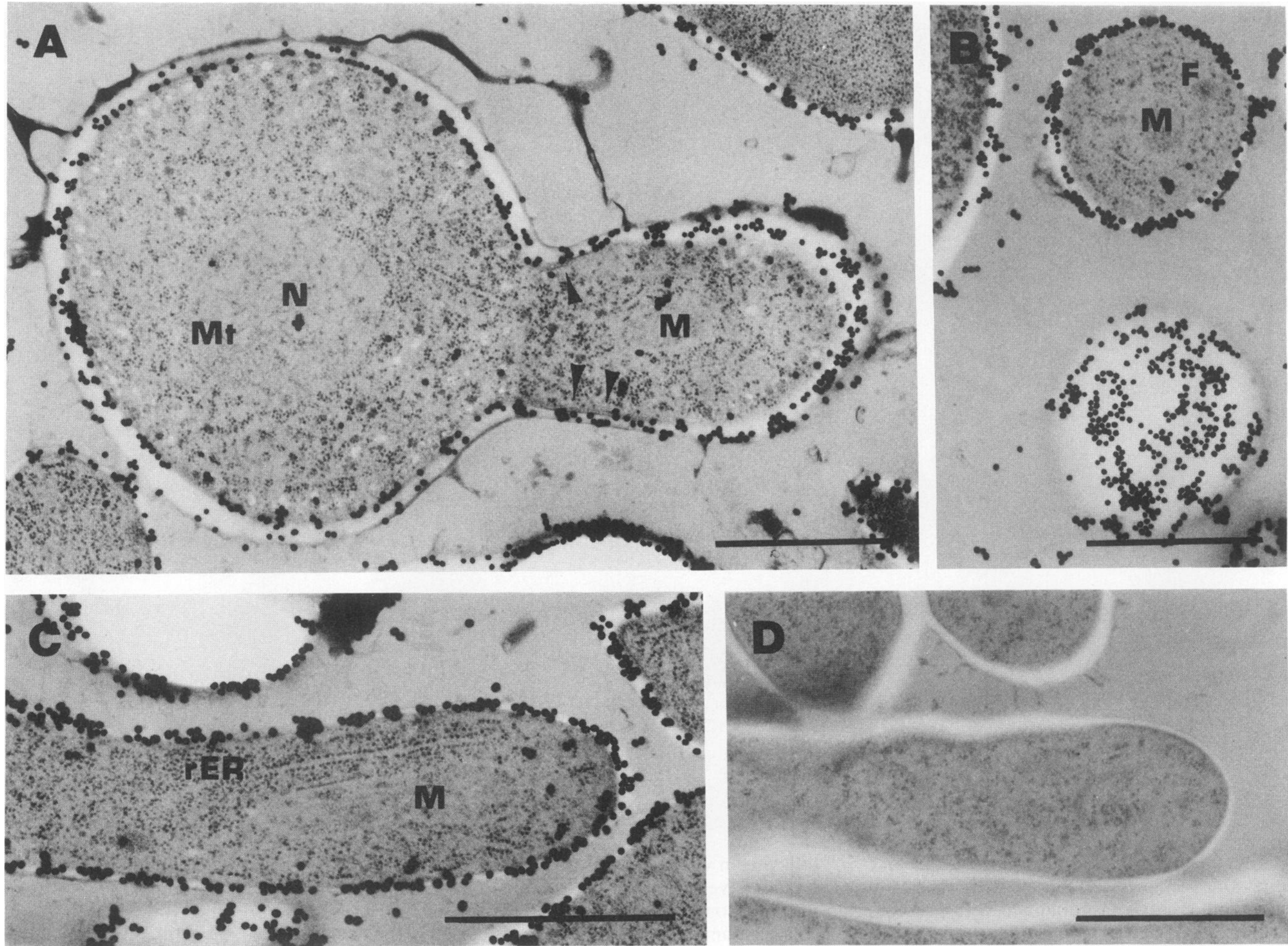

FIG. 4. IEM of C3d receptor expression by *C. albicans* grown in the mouse peritoneal cavity. Yeast forms, 2×10^8, of *C. albicans* were injected into the peritoneal cavities of mice, retrieved by lavage 5 h later, and prepared for IEM by freeze substitution. (A) C3d receptor was especially dense on the mother cells of germ tubes. Cross section of a filamentous structure was seen in the transition zone between the mother cell and the germ tube (arrowheads). (B) Mitochondrion (M) and filasome (F) denote probable subapical region of a hyphal tip. Note the high density of the C3d receptor throughout the cell wall. (C) Longitudinal section of hyphal cell showing receptor expression throughout the cell wall. (D) Control section reacted with preimmune serum instead of anti-C3d. N, Nucleus; Mt, microtubule; rER, rough endoplasmic reticulum; F, filasome; M, mitochondrion. Bar, 1 μm.

of the specific hyperimmune antiserum, showed no fluorescence (data not shown). These data indicate that all yeast cells produce at least a small amount of C3d receptor on their surfaces.

Most of the *C. albicans* yeast cells either germinated or produced pseudohyphae in the mouse peritoneal cavity (Fig. 2C). The general pattern of distribution of C3d receptor expression on these cells appeared similar to that observed above for yeast and pseudohyphal forms which developed in vitro. Detection of expression of C3d receptor of *C. albicans* in mouse kidney tissue differed from in vitro or in vivo i.p. grown cells in that fluorescence intensity of pseudohyphae and hyphae of *C. albicans* was no higher than that on the mother yeast cells. Actually, intensity of fluorescence of the filaments was more like that of in vitro grown yeast cells (cf. Fig. 2A with D). This was not due to fixation of samples with PLP (Fig. 2D and E) because low fluorescence intensity was also observed with unfixed samples (Fig. 2F). Sections stained with PASH showed that hyphae and pseudohyphae were the dominant forms present in the kidney lesions (Fig. 2G).

C3d expression as evidenced by IEM. Cells grown in vitro as either yeast forms or pseudohyphae were fixed by freeze substitution. Results shown were obtained from sections developed with goat anti-rabbit immunoglobulin G coupled to 30-nm gold particles (Fig. 3), but essentially identical results occurred when protein A–15-nm gold conjugate was used for development (not shown). Receptor expression appeared on the surface and subsurface areas of the cell wall, with little or no cytoplasmic organellar association. In yeasts, mother cells of pseudohyphae, and pseudohyphal filaments, gold particles were found associated with the cell wall. Furthermore, in yeasts and mother cells which displayed thick cell walls, gold deposition was essentially equally heavy near the outer and inner surfaces of the cell walls (Fig. 3, arrows).

Cells collected from the peritoneal cavities of mice were also fixed by freeze substitution. Most of the antigen ex-

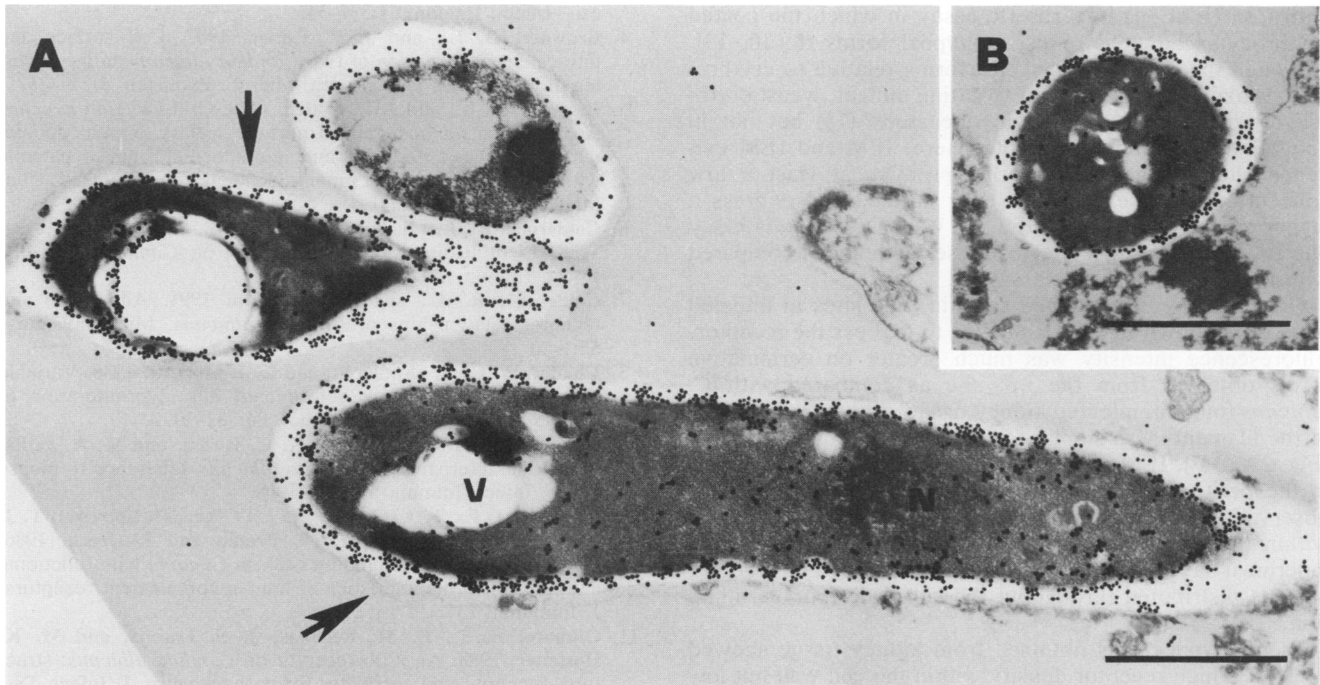

FIG. 5. IEM of C3d receptor expression by *C. albicans* in mouse kidney. Mice were infected intravenously with *C. albicans*, kidneys were removed 6 days later, and microabscesses in the cortex were removed, fixed in PLP at 4°C, sectioned, and immunostained. (A) Longitudinal section of a hyphal cell. Note localization of the receptor in cell wall and cytoplasm. (B) Cross section of hyphal cell in which C3d receptor expression is localized in the cell wall. Membranelike structure surrounding hyphal cell is indicated by arrows. N, Nucleus; V, vacuole. Bar, 1 μm.

pressed on mother cells was located deep in their relatively thick cell walls (Fig. 4A). The thin cell walls of germ tubes appeared to express the C3d receptor heavily (Fig. 4C). The presence of a filamentous structure at the transition region from the mother cell to hypha (Fig. 4A, arrowheads) and nonconstriction of the emerging cell are evidence of a germ tube (24). Cross sections of hyphae (Fig. 4B) showed dense expression of C3d receptor throughout the cell wall. The presence of a mitochondrion (M) and filasome (F) is suggestive of the subapical region of germ tubes (15). A section of hypha cut longitudinally showed the presence of a mitochondrion (M), rough endoplasmic reticulum (rER), and heavy expression of the C3d receptor through the cell wall (Fig. 4C). Negative control cells which were reacted with preimmune rabbit serum instead of the primary antibody showed no nonspecific binding (Fig. 4D).

Expression of the C3d receptor on *C. albicans* in mouse kidney fixed by PLP was also investigated by IEM. Hyphae and pseudohyphae were found predominantly in the infected tissue (Fig. 2G). Expression of the C3d receptor in these cells was primarily on the cell wall, but was also detectable in the cytoplasm (Fig. 5A and B). Membranelike structures around the pseudohyphae were observed (Fig. 5A, arrows). The results were generally the same as indicated above for in vitro and i.p. developed pseudohyphal cells, except that less surface expression of the C3d receptor was observed on pseudohyphae found in kidney tissue. Possible reasons for this observation are discussed below.

DISCUSSION

Detection of the C3d receptor was done by use of a polyclonal antiserum as applied to IFM and IEM techniques. Although the CA-A (18) receptor-specific monoclonal antibody is available, it adheres nonspecifically to embedding resin used in electron microscopy, which limits its use in postembedment staining of sectioned material (our unpublished findings). The polyclonal antiserum worked well, however, and is specific for the receptor as evidenced by its detection of the same bands on Western blots as CA-A (23) and ability to block rosetting function of the receptor (unpublished data). The antiserum also appears not to detect a determinant shared by unrelated molecules in the cell wall for the following two reasons. First, if the receptor determinant is associated with other cellular components, multiple bands from the whole cell extract would be expected to react with the antiserum instead of a single major band (Fig. 1). The minor band detected at about 67 to 68 kDa has also been found to have C3d-binding activity (6), and the two faint bands in the 20-kDa range are of doubtful significance at the dilution of antiserum used in the studies. Second, the relative fluorescence intensity in the IFM studies showed much greater surface expression for pseudohyphal cells over yeast forms (Fig. 2A and B). This correlates with detection of C3d receptors on pseudohyphae by the rosette assay as reported earlier (6) and receptor expression by in vitro grown yeast cells reported by others (see below). Despite these arguments, we cannot state that the antiserum necessarily detects functional receptor molecules at all levels within the cell wall. In fact, determining whether the receptor molecule has binding function deep within the cell wall or is a prereceptor at this site may yield important information on receptor development and biogenesis of the cell wall of *C. albicans*.

Existence of a C3d-binding molecule on *C. albicans* was observed by investigators who used erythrocytes coated

with C3d (EAC3d) in a rosette assay in which the coated erythrocytes adhered to pseudohyphal forms (6, 10, 13). Although the small size of yeast forms relative to erythrocytes makes interpretation of rosetting difficult, yeast erythrocyte rosettes were noted in one study (13) but not in another (6). In studies presented here, IFM and IEM evidence showed that yeast forms produce at least a low concentration of receptor on their surface and express a higher amount deep within the cell wall. Detection is likely due to increased sensitivity of these methods as compared with the EAC3d rosetting assay.

C. albicans cells obtained from in vivo sites in infected mice were shown by IFM and IEM to express the receptor. Fluorescence intensity was much greater on germinative forms obtained from the i.p. site as compared with *C. albicans* found in infected kidney tissue, where the intensity of the filaments was similar to that of in vitro grown yeasts (Fig. 2A to F). IEM studies on *C. albicans* which developed in the peritoneal cavity yielded results similar to in vitro observations. Dense receptor expression was detected within the cell wall of mother cells and especially at the innermost layers of the cell wall. However, the receptor became distributed throughout the cell wall upon germination.

Pseudohypal forms obtained from kidney tissue showed by IEM a high receptor density within the cell wall but low density on the cell surface. Although it is tempting to compare these findings with results on fungal cells obtained from the peritoneal cavity, the more conventional fixation (PLP) was used to prepare kidney tissue rather than freeze substitution for i.p. developed cells. PLP fixation is done at relatively warm temperatures and is more prone to artifactual changes compared with freeze substitution, which also may account for the apparent cytoplasmic association of the receptor in cells obtained from kidney tissue. Unfortunately, the thickness of kidney tissue precludes use of freeze substitution for fixation of these specimens (14; our unpublished observations). *C. albicans* pseudohyphae in kidney tissue also had a membranous covering (Fig. 5) which we speculate is host derived and could be responsible for blocking antibody reactivity at the cell surface. An alternative explanation, that reduced expression of receptor occurs in kidney as compared with developing cells in the peritoneal cavity, seems unlikely because the receptor is heavily produced within deeper cell wall layers (Fig. 5). Whether the membranous material is due to C3d deposition and/or a multitude of other host proteins that can bind to *C. albicans* is under investigation.

ACKNOWLEDGMENTS

This work was supported by Public Health Service grants AI 25738 (R.A.C.) and AI 24912 (J.E.C.) from the National Institutes of Health.

REFERENCES

1. **Brawner, D. L., and J. E. Cutler.** 1984. Variability in expression of a cell surface determinant on *Candida albicans* as evidenced by an agglutinating monoclonal antibody. Infect. Immun. **43:** 966–972.
2. **Brawner, D. L., and J. E. Cutler.** 1986. Ultrastructural and biochemical studies of two dynamically expressed cell surface determinants on *Candida albicans*. Infect. Immun. **51**:327–336.
3. **Brawner, D. L., and J. E. Cutler.** 1986. Variability in expression of cell surface antigens of *Candida albicans* during morphogenesis. Infect. Immun. **51**:337–343.
4. **Brawner, D. L., and J. E. Cutler.** 1987. Cell surface and intracellular expression of two *Candida albicans* antigens during *in vitro* and *in vivo* growth. Microb. Pathogen. **2**:249–257.
5. **Brawner, D. L., and J. E. Cutler.** 1989. Oral *Candida albicans* isolates from nonhospitalized normal carriers, immunocompetent hospitalized patients, and immunocompromised patients with or without acquired immunodeficiency syndrome. J. Clin. Microbiol. **27**:1335–1341.
6. **Calderone, R., L. Linehan, E. Wadsworth, and A. L. Sandberg.** 1988. Identification of C3d receptors on *Candida albicans*. Infect. Immun. **56**:252–258.
7. **Calderone, R. A., and P. C. Braun.** 1991. Adherence and receptor relationships of *Candida albicans*. Microbiol. Rev. **55**:1–20.
8. **Chaffin, W. L., J. Skudlarek, and K. J. Morrow.** 1988. Variable expression of a surface determinant during proliferation of *Candida albicans*. Infect. Immun. **56**:302–309.
9. **Cutler, J. E., D. L. Brawner, K. C. Hazen, and M. A. Jutila.** 1990. Characteristics of *Candida albicans* adherence to mouse tissue. Infect. Immun. **58**:1902–1908.
10. **Edwards, J. E., T. A. Gaither, J. J. O'Shea, D. Rotrosen, T. J. Lawley, S. A. Wright, 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.
11. **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.
12. **Hazen, K. C., and J. E. Cutler.** 1979. Autoregulation of germ tube formation by *Candida albicans*. Infect. Immun. **24**:661–666.
13. **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.
14. **Hoch, H.** 1986. Freeze-substitution of fungi, p. 183–212. *In* H. C. Aldrich and W. J. Todd (ed.), Ultrastructure techniques for microorganisms. Plenum Press, New York.
15. **Kanbe, T., I. Kobayashi, and K. Tanaka.** 1989. Dynamics of cytoplasmic organelles in the cell cycle of the fission yeast *Schizosaccharomyces pombe*: three-dimensional reconstruction from serial sections. J. Cell Sci. **94**:647–656.
16. **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.
17. **Li, R. K., and J. E. Cutler.** 1991. J. Gen. Microbiol., in press.
18. **Linehan, L., E. Wadsworth, and R. Calderone.** 1988. *Candida albicans* C3d receptor, isolated by using a monoclonal antibody. Infect. Immun. **56**:1981–1986.
19. **McLean, I. W., and P. K. Nakane.** 1974. Periodate-lysine-paraformaldehyde fixative. A new fixative for immunoelectron microscopy. J. Histochem. Cytochem. **22**:1077–1083.
20. **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.
21. **Page, S., and F. C. Odds.** 1988. Binding of plasma proteins of *Candida* species *in vitro*. J. Gen. Microbiol. **134**:2693–2702.
22. **Poulain, D., V. Hopwood, and A. Vernes.** 1985. Antigenic variability of *Candida albicans*. Crit. Rev. Microbiol. **12**:223–270.
23. **Saxena, A., and R. A. Calderone.** 1990. Purification and characterization of the extracellular C3d-binding protein of *Candida albicans*. Infect. Immun. **58**:309–314.
24. **Soll, D. R., and L. H. Mitchell.** 1983. Filament ring formation in the dimorphic yeast *Candida albicans*. J. Cell Biol. **96**:486–493.
25. **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.