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Activation and Binding of Opsonic Fragments of C3 on Encapsulated *Cryptococcus neoformans* by Using an Alternative Complement Pathway Reconstituted from Six Isolated Proteins

THOMAS R. KOZEL,* MICHAEL A. WILSON, GAIL S. T. PFROMMER, AND ANNETTE M. SCHLAGETER

Department of Microbiology and Cell and Molecular Biology Program, University of Nevada-Reno, Reno, Nevada 89557

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Encapsulated *Cryptococcus neoformans* yeast cells are potent activators of the complement system. We examined the interaction of the yeast cells with an alternative complement pathway reconstituted from isolated factor D, factor B, factor H, factor I, C3, and properdin. Incubation of encapsulated cryptococci with the reconstituted pathway led to activation and binding of C3 fragments to the yeast cells that was quantitatively and qualitatively identical to that observed with normal human serum. Incubation with either normal serum or a mixture of isolated proteins led to binding of 4×10^7 to 5×10^7 C3 molecules to the yeast cells. The kinetics for activation and binding of C3 were identical, with maximum binding observed after a 20-min incubation. Immunoglobulin G was not needed for optimal activation kinetics. C3 fragments eluted from the yeast cells by treatment with hydroxylamine and subsequent analysis by sodium dodecyl sulfate-polyacrylamide gel electrophoresis demonstrated the presence primarily of iC3b on yeast cells incubated with either normal serum or the reconstituted pathway. Ultrastructural examination of the opsonized yeast cells showed that the cryptococcal capsule was the site for binding of C3 activated from normal serum or the reconstituted pathway, with a dense accumulation of C3 at the periphery of the capsule. Thus, incubation of encapsulated cryptococci in the reconstituted pathway led to deposition of opsonic complement fragments at a site that was appropriate for interaction with phagocyte receptors. Cryptococci opsonized with the reconstituted pathway showed a markedly enhanced interaction with cultured human monocytes compared with unopsonized yeast cells, indicating that the alternative pathway alone is opsonic for yeast cells. However, the results indicate that additional serum factors are needed for optimal opsonization of yeast cells because a 35% reduction in the number of cryptococci bound to macrophages was observed with cryptococci opsonized with the reconstituted pathway compared with that observed when yeast cells were opsonized with normal serum.

Incubation of encapsulated *Cryptococcus neoformans* in normal human serum leads to deposition of large numbers of C3 fragments on the yeast cells. Quantitative studies have shown that the number of bound C3 fragments was approximately 10^7 molecules per yeast cell. Ultrastructural studies showed that the C3 fragments are bound within and at the surface of the capsule (12, 14). Activation and binding of C3 is markedly inhibited by EDTA but not by EGTA [ethylene glycol-bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid], suggesting that the alternative complement pathway is the primary mechanism for complement activation by the cryptococcal capsule. However, it has also been suggested that early components of the classical complement pathway may be essential for optimal opsonization of the yeast cells (5).

A role for immunoglobulin G (IgG) in opsonization of the yeast cells was demonstrated by Diamond et al. (5), who found that the opsonic potential of normal human serum was lost when the serum was adsorbed with encapsulated cryptococci at 0°C. The opsonic activity was restored by the addition of IgG isolated from normal human serum. These studies identified a critical role for antibody in normal human serum in opsonization of the yeast cells, but they did not distinguish between the potential role of IgG as an initiator of alternative pathway activation and its possible function as an opsonic ligand.

In the present study we used purified factor D, factor B, factor H, factor I, C3, and properdin to assess the role of these alternative pathway proteins in activation and binding of C3 to the cryptococcal capsule and in opsonization of the yeast cells. The objectives of our study were (i) to determine whether incubation of the yeast cells in a mixture of the six isolated alternative pathway proteins leads to activation and deposition of C3 fragments on the yeast cells, (ii) to compare the ultrastructural location and molecular forms of C3 bound to the yeast cells after incubation in normal human serum or a mixture of alternative pathway proteins, and (iii) to assess the opsonic potential of the alternative pathway proteins. Our results showed that incubation of encapsulated cryptococci with the purified alternative pathway proteins leads to deposition of C3 onto the yeast cells in a manner that is qualitatively and quantitatively identical to activation and binding from normal serum. The alternative pathway proteins effected significant opsonization of the yeast cells, but the opsonic potential of the isolated proteins was significantly less than that of normal serum, suggesting the need for accessory factors in opsonization of encapsulated cryptococci.

MATERIALS AND METHODS

Yeast cells. *C. neoformans* 388 is an encapsulated strain of serotype A that was provided by K. J. Kwon-Chung. The yeast cells were cultured on a liquid synthetic medium (2) on a gyratory shaker at 100 rpm for 72 h at 37°C. Cells used in most experiments were killed with formaldehyde before use (14). Cells used for electron microscopy were collected by centrifugation, washed two times with 0.2 M phosphate

* Corresponding author.

buffer (pH 7.2), and killed by fixation with paraformaldehyde-glutaraldehyde (14).

Serum and isolation of purified proteins. Peripheral blood was collected from 10 volunteers after their informed consent was obtained. The sera were pooled, and portions were stored at −70°C. This pool was used as the source of normal human serum.

Complement proteins were isolated from frozen human plasma. C3 was isolated as described previously (14, 26). Factor I was isolated by use of sodium sulfate precipitation to remove plasminogen (4), ion-exchange chromatography on DEAE-Sephacel (4), affinity chromatography on a column of Sepharose CL-4B coupled to C3b (18), removal of IgG with an anti-IgG affinity column, and molecular sieve chromatography on Sephacryl S-200. Factor H was isolated by differential precipitation with polyethylene glycol 4000 (9), plasminogen depletion on lysine–Sepharose CL-4B (9), ion-exchange chromatography on DEAE-Sephacel (9), molecular sieve chromatography on Sephacryl S-300, and chromatography on Urogel-hydroxyapatite (24). Factor B was isolated by differential precipitation with polyethylene glycol 4000, plasminogen depletion on lysine–Sepharose CL-4B, and ion-exchange chromatography on DEAE-Sephacel as described previously (9), followed by ion-exchange chromatography on CM Sepharose CL-6B (11) and molecular sieve chromatography on Sephacryl S-300. Properdin was isolated by ion-exchange chromatography on QAE Sephadex A-50 (17) and Bio-Rex 70 (25), followed by removal of contaminating IgG by chromatography on protein A–Sepharose CL-4B and an anti-human IgG–Sepharose CL-4B affinity column. Factor D was purified as described previously (15).

Purity of the isolated proteins was assessed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) under reducing conditions with 7% polyacrylamide gels. Then 5 μg (C3, properdin, factor B, and factor H) or 10 μg (factors D and I) of protein was applied to the gel. All proteins were assessed by double immunodiffusion for contamination with IgG. The isolated proteins were examined at five times the physiological concentrations with antiserum specific for human immunoglobulins (goat affinity-purified anti-human immunoglobulin; Southern Biotechnology Associates, Inc., Birmingham, Ala.; catalog no. 2010-01).

C3b was prepared by treatment of C3 with trypsin as described previously (26). iC3b was prepared by treating ^{125}I-labeled C3b (100 μg) with 1 mM phenylmethylsulfonyl fluoride, followed by incubation with factor H (5 μg) and factor I (5 μg) for 60 min at 37°C.

C3 and C3b were labeled with ^{125}I by the Iodogen (Pierce Chemical Co., Rockford, Ill.) procedure (8). Radiolabeled proteins were separated from free iodine by filtration through Sephadex G-25. Typically, 1.0 mg of C3 was labeled to a specific activity of 4×10^5 cpm/μg, and 0.5 mg of C3b was labeled to a specific activity of 1×10^5 cpm/μg. Radiolabeled C3 remained functionally active for at least 7 days but was used within 4 days.

Preparation of mixture of alternative pathway proteins. A mixture of alternative pathway proteins in GVB (sodium Veronal [5 mM]-buffered saline [142 mM] [pH 7.3] containing 0.1% gelatin, 1 mM $MgCl_2$, and 0.15 mM $CaCl_2$) was used at 40% of physiologic concentration. Normal serum concentrations of 1,200 μg of C3 per ml, 200 μg of factor B per ml, 470 μg of factor H per ml, 34 μg of factor I per ml, 20 μg of properdin per ml, and 2 μg of factor D per ml were the basis for these calculations (20, 21). C3 was preincubated with factors H and I for 30 min at 37°C to eliminate any contaminating C3b. The additional three components were added and used immediately.

Assay for C3 fragments bound to yeast cells. Activation and binding of C3 to cryptococci was done with a reaction mixture consisting of 2×10^5 cryptococci, 40 μl of human serum or a mixture of alternative pathway components equivalent to 40 μl of serum, ^{125}I-labeled C3 sufficient to provide a mixture of labeled and unlabeled C3 with a final specific activity of 10,000 cpm/μg of C3, and GVB to bring the reaction volume to 100 μl. The mixture was incubated for 5 to 80 min at 37°C. After incubation, the tubes were immediately placed on ice, and EDTA was added to a final concentration of 13 mM. The cells were washed five times with phosphate-buffered saline (PBS) containing 0.1% SDS, and the amount of bound radioactivity was determined. Tubes containing heat (56°C for 30 min)-inactivated serum in place of normal human serum were used as controls for nonspecific binding. Specific binding was determined by subtracting nonspecific binding from total binding. The number of C3 molecules bound to each yeast cell was calculated from the specific activity of the radiolabeled C3, assuming the molecular weight of C3 to be 187,500 (26).

Analysis of bound C3 fragments by SDS-PAGE. Cryptococci (2×10^6 yeast cells) were incubated for 40 min at 37°C with 1 ml of 40% serum or 1 ml of alternative pathway proteins at concentrations equivalent to 40% serum. ^{125}I-labeled C3 was included in the reaction mixture at a specific activity of 50,000 cpm/μg of C3. After incubation, the cells were immediately separated from the reaction mixture by centrifugation and then washed three times with PBS containing 0.1% SDS. The bound C3 fragments were eluted from the cells by incubation with 125 μl of 1 M hydroxylamine in 0.2 M $NaHCO_3$ (pH 10). The cells were removed by centrifugation, the supernatant fluid containing the eluted fragments was collected, and phenylmethylsulfonyl fluoride was added to a final concentration of 1 mM. The eluted C3 fragments were analyzed under reducing conditions by SDS-PAGE with 7% acrylamide gels.

Radiolabeled C3, C3b, and iC3b were used as standards for the SDS-PAGE analysis. To ensure that the hydroxylamine treatment itself had not altered the molecular form of the C3, these proteins were diluted with 12.5 volumes of 1 M hydroxylamine in 0.2 M $NaHCO_3$ (pH 10) and analyzed under reducing conditions in the same manner as the C3 fragments eluted from the yeast cells.

Ultrastructural localization of C3 fragments. Cryptococci were incubated with normal human serum or with the purified alternative pathway proteins as described above. The cells were fixed, incubated sequentially with peroxidase-labeled monoclonal anti-C3c (Genzyme Corp., Boston, Mass.) and peroxidase-labeled anti-mouse IgG (Bio-Rad Laboratories, Richmond, Calif.), stained, dehydrated with dimethylformamide, and embedded in Epon-araldite as described (14). Thin sections were cut with a diamond knife and placed on Formvar-coated copper grids. The grids were stained with uranyl acetate and lead citrate. Electron microscopy was done with a Philips CM10 electron microscope, and photographs were taken with Kodak 4489 film.

Phagocytosis. Cryptococci were opsonized as described above with 40% normal human serum or with purified alternative pathway proteins at 40% of normal serum concentrations. All opsonized cryptococci were washed once with RPMI 1640 and suspended in RPMI 1640 at 4×10^6 yeast cells per ml.

Cultured human macrophages were used for all phagocytosis experiments. Monocytes were isolated from 40 ml of

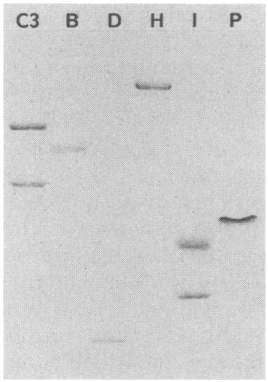

FIG. 1. SDS-PAGE of reduced samples of C3 (C3), factor B (B), factor D (D), factor H (H), factor I (I), and properdin (P). The gel was stained with Coomassie blue dye R250.

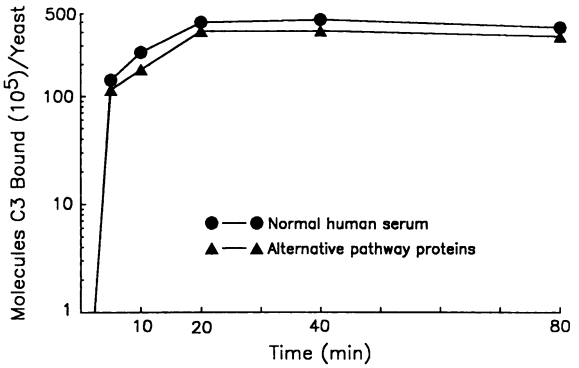

FIG. 2. Kinetics of deposition of ^{125}I-labeled C3 on encapsulated cryptococci incubated with 40% normal human serum or the alternative complement pathway reconstituted at 40% of the physiological concentration of the six isolated proteins.

peripheral blood by using Sepracell-MN (Sepratech Corp., Okalhoma City, Okla.) according to the directions of the manufacturer. The isolated monocytes were suspended at 10^6 cells per ml in RPMI 1640 containing 12% normal human serum and cultured for 7 to 26 days in 50 ml of Teflon-coated Erlenmeyer flasks at 37°C in 5% CO_2 as described previously (27).

Phagocytosis experiments were done in Terasaki plates by a modification of the procedure of Wright (27). Briefly, Terasaki plates were pretreated with PBS containing 1 mg of human albumin per ml. The cultured monocytes were collected by centrifugation and suspended in RPMI 1640 at 1×10^5 to 2×10^5 cells per ml. The plates were washed with PBS, and 10 μl of the macrophage suspension was added to each well. The macrophages were incubated for 1 h at 37°C to facilitate adherence to the plates. The plates were rinsed with PBS, and 10 μl of opsonized cryptococci (4×10^6 cryptococci per ml) was added to each well. The plates were incubated for 60 min at 37°C and rinsed with PBS to remove nonadherent cryptococci. The cells were examined microscopically to determine the number of cryptococci bound to each yeast cell. Twenty-five macrophages were examined in each well. Six wells were examined for each treatment group. The results represent the mean ± the standard error of the mean for seven separate experiments. Data are reported as the number of yeast cells bound to 100 macrophages.

RESULTS

Purity of the isolated complement proteins. All isolated alternative pathway proteins were examined under reducing conditions by SDS-PAGE. The results (Fig. 1) showed bands consistent with the reported molecular weights of each protein or the subunits of each protein. Assay of the isolated proteins by double immunodiffusion against antiserum specific for human IgG showed the absence of detectable contamination when the proteins were examined at five times the physiological concentrations.

Activation of the alternative complement pathway by encapsulated cryptococci. Encapsulated cryptococci were incubated for various time intervals with 40% normal human serum or the alternative pathway proteins at 40% of their physiological concentrations. Deposition of C3 on the yeast cells was assessed by incorporation of ^{125}I-labeled C3 into the reaction mixtures. Incubation of the yeast cells in normal serum led to maximal deposition of approximately 4×10^7 to 5×10^7 molecules of C3 per yeast cell after an incubation period of 20 min (Fig. 2). Incubation of the yeast cells with the alternative pathway proteins similarly produced maximal deposition of 4×10^7 to 5×10^7 molecules of C3 per yeast cell. Identical kinetics for activation and binding of C3 fragments were observed with yeast cells incubated with either normal serum or the isolated alternative pathway proteins.

The fact that identical activation and binding curves were produced by normal serum and the isolated proteins provided strong evidence that IgG was not necessary for activation and binding of C3 fragments onto the yeast cells. The addition of IgG to the alternative pathway proteins at 40% of the physiological concentration did not increase the rate of C3 binding (data not shown), providing further evidence that IgG was not necessary for alternative pathway-mediated activation and binding of C3 to the yeast cells.

Ultrastructural localization of C3 fragments in the cryptococcal capsule. The location of C3 fragments bound to the yeast cells was determined by transmission electron microscopy. Cryptococci were incubated with 40% human serum or with C3, properdin, factor B, factor D, factor H, and factor I at 40% of their physiological concentrations. The location of the bound C3 fragments was determined by incubation of the treated yeast cells with a monoclonal antibody specific for C3c followed by incubation with peroxidase-labeled anti-mouse IgG. Incubation with the isolated proteins produced deposition of C3 fragments on and within the capsule, with particularly dense binding at the periphery (Fig. 3A). This result was similar to the pattern of C3 binding when the yeast cell was opsonized with normal human serum (Fig. 3B). Two additional patterns of binding were observed regardless of whether the yeast cells were incubated with normal serum or the isolated proteins. The first pattern (Fig. 3B) showed binding of C3 extending throughout the capsule to the cell wall. The second pattern (Fig. 3A) showed binding primarily in the outer half of the capsule, with little or no binding at the cell wall. Mixtures of both binding patterns were seen in yeast cells opsonized with either normal serum or the isolated proteins. Binding of C3 was not observed when the yeast cells were incubated in heat-inactivated serum (Fig. 3C).

Identification of C3 fragments bound to the capsule. The previous experiments demonstrated that incubation of cryptococci with alternative pathway proteins led to binding of C3 fragments to the capsule, but the molecular form of the C3 was not identified. The molecular form of the bound C3

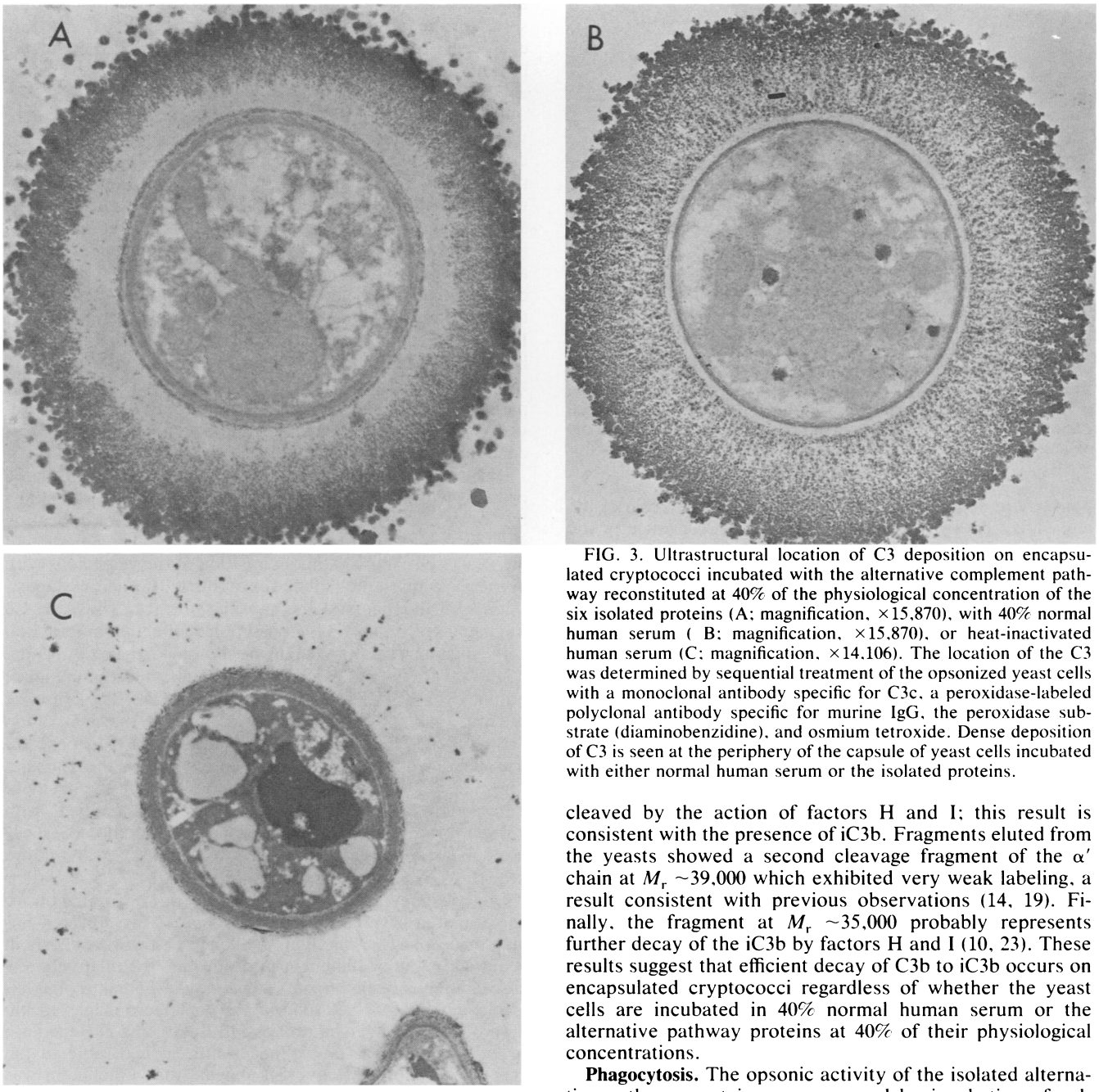

FIG. 3. Ultrastructural location of C3 deposition on encapsulated cryptococci incubated with the alternative complement pathway reconstituted at 40% of the physiological concentration of the six isolated proteins (A; magnification, ×15,870), with 40% normal human serum (B; magnification, ×15,870), or heat-inactivated human serum (C; magnification, ×14,106). The location of the C3 was determined by sequential treatment of the opsonized yeast cells with a monoclonal antibody specific for C3c, a peroxidase-labeled polyclonal antibody specific for murine IgG, the peroxidase substrate (diaminobenzidine), and osmium tetroxide. Dense deposition of C3 is seen at the periphery of the capsule of yeast cells incubated with either normal human serum or the isolated proteins.

was determined by use of hydroxylamine to elute C3 from yeast cells that had been incubated in 40% normal human serum or alternative pathway proteins at concentrations equivalent to 40% human serum. Previous studies have demonstrated that 95 to 100% of the C3 is eluted by such treatment (13). The eluted C3 was analyzed by SDS-PAGE under reducing conditions. Radiolabeled C3, C3b, and iC3b were included to identify protein fragments representative of each molecular form.

The results (Fig. 4) suggest that there was little or no C3b bound to yeasts incubated with either normal serum or the isolated proteins. The absence of the α'-(M_r, ~102,000) chain indicated the relative absence of C3b. The presence of a chain at M_r ~58,000 indicates that the α' chain had been cleaved by the action of factors H and I; this result is consistent with the presence of iC3b. Fragments eluted from the yeasts showed a second cleavage fragment of the α' chain at M_r ~39,000 which exhibited very weak labeling, a result consistent with previous observations (14, 19). Finally, the fragment at M_r ~35,000 probably represents further decay of the iC3b by factors H and I (10, 23). These results suggest that efficient decay of C3b to iC3b occurs on encapsulated cryptococci regardless of whether the yeast cells are incubated in 40% normal human serum or the alternative pathway proteins at 40% of their physiological concentrations.

Phagocytosis. The opsonic activity of the isolated alternative pathway proteins was assessed by incubation of cultured mononuclear phagocytes with cryptococci that were opsonized as described above with 40% normal human serum or alternative pathway proteins at concentrations equivalent to 40% human serum. The results (Fig. 5) showed substantial opsonization of the yeast cells by normal serum. Heat-inactivated serum was unable to facilitate binding of the yeast cells to the cultured macrophages. The isolated alternative pathway proteins effected a significant opsonization of encapsulated cryptococci; however, the number of yeast cells associated with each macrophage was lower than that observed with yeast cells opsonized with normal serum.

DISCUSSION

Previous studies by ourselves (13) and others (3, 5) have demonstrated the critical role of the alternative complement

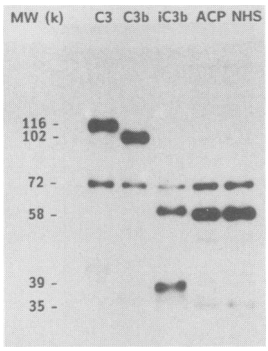

FIG. 4. Autoradiogram of SDS-PAGE analysis of ^{125}I-labeled C3 released by hydroxylamine-SDS treatment of encapsulated cryptococci opsonized by incubation with 40% normal human serum or the six alternative complement proteins reconstituted at 40% of the physiological concentrations. Lanes 1, 2, and 3 contain, respectively, ^{125}I-labeled C3, C3b, and iC3b. Lane 4 contains eluate from cryptococci incubated with the six alternative pathway proteins. Lane 5 contains eluate from cryptococci incubated with 40% normal serum.

pathway in opsonization of *C. neoformans*. The role of the alternative pathway was identified by the resistance of the opsonic potential of serum to chelation with EGTA containing 10 mM $MgCl_2$. EGTA chelates calcium, which is necessary for activation of the classical pathway (7, 22). The ability to activate and bind C3 to the capsule in the presence of magnesium-EGTA provided indirect evidence for involvement of the alternative pathway. Further evidence for involvement of the alternative pathway was the abrogation of the opsonic potential of human serum by heating at 50°C (3, 5) or adsorption with zymosan to remove properdin (5). This accumulated evidence is convincing, but it is indirect and does not exclude the possibility that complement activation by the cryptococcal capsule may involve IgG or early components of the classical pathway (5). The present report provides direct evidence that encapsulated cryptococci can activate the alternative pathway, leading to deposition of opsonic complement fragments in the cryptococcal capsule.

Activation and binding of C3 fragments to the yeast cells with the six isolated alternative pathway proteins was quantitatively identical to that observed with normal serum with regard to both the kinetics of C3 deposition and the maximal amount of bound C3. The total number of molecules bound to each yeast cell approached a similar maximum of approximately 4×10^7 to 5×10^7 regardless of whether normal serum or the isolated proteins were used. Similarly, the rate of C3 deposition was identical with serum and the isolated proteins. Substantial deposition was observed after 5 min, and maximal deposition occurred after 20 min.

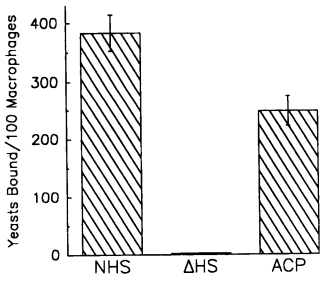

FIG. 5. Opsonization of encapsulated cryptococci by incubation with normal human serum (NHS), heat inactivated human serum (ΔHS), or isolated proteins of the alternative complement pathway (ACP). Opsonization of the yeast cells is expressed as the number of cryptococci associated (attached or ingested) with 100 macrophages. Data are shown as the mean ± the standard errors of the means of five separate experiments.

The fact that the isolated proteins effected efficient activation in the absence of either IgG or components of the classical pathway provides strong evidence that neither IgG nor early classical pathway proteins play a role in complement activation by the cryptococcal capsule. These results are similar to results reported by Bjornson et al. (1) on activation of the alternative pathway by *Bacteroides fragilis* and *Bacteroides thetaiotamicron*. Incubation of *Bacteroides* cells in mixtures of the six alternative pathway proteins led to activation and binding of C3 onto the bacteria. Neither IgG nor classical pathway proteins were required. It should be emphasized that microorganisms differ with regard to their requirements for accessory proteins in alternative pathway activation. For example, alternative pathway activation by type III, group B streptococci requires the presence of type-specific antibody (6).

Activation and binding of the C3 fragments by the isolated proteins was qualitatively identical to activation and binding by normal serum with regard to the ultrastructural location and the molecular form of C3. Ultrastructural examination of the yeast cells showed that incubation of the yeast cells with normal serum or the isolated proteins led to heavy deposition of C3 fragments at the capsular surface. Thus, the C3 fragments were spatially located for effective interaction with phagocyte complement receptors. Analysis of the bound C3 fragments by SDS-PAGE indicated the presence primarily of iC3b, with little or no bound C3b. The presence of large amounts of iC3b provides evidence that incubation with isolated alternative proteins is sufficient to deposit C3 fragments with potent opsonic activity onto the capsule. These results further indicate that regulation of the decay of C3b bound to the cryptococcal capsule is identical with normal serum and the isolated proteins. Thus, no accessory proteins beyond those provided by factors H and I are needed to effect decay of the C3b.

Our phagocytosis studies focused on opsonization of encapsulated cryptococci for phagocytosis by cultured human macrophages. The high incidence of cryptococcosis in acquired immunodeficiency syndrome and the relatively few cases in neutropenic patients suggest that the macrophage is a relevant effector cell. The ability of macrophages to kill cryptococci has been controversial. Levitz and DiBenedetto recently found that macrophages are, indeed, able to kill cryptococci (16). Moreover, it appears that attachment was sufficient for macrophage-mediated killing of cryptococci. This latter observation is particularly relevant to our studies, because our analysis of opsonization of the yeast cells did not differentiate between cryptococci that were attached or ingested.

Our phagocytosis studies indicate that the reconstituted alternative pathway has substantial opsonic potential. Incubation of the yeast cells in the reconstituted pathway markedly enhanced the binding of cryptococci to the macrophages. There is one previous report in which cells of *Escherichia coli* were opsonized by incubation with isolated proteins of the alternative pathway (23). In contrast, in studies of *Bacteroides* spp., Bjornson et al. (1) found that the isolated alternative pathway proteins effectively deposited opsonic fragments of C3 onto the bacterium, but treatment of the *Bacteroides* cells in this manner did not promote

adherence, uptake, or killing of the bacteria by human neutrophils.

Our results indicate that deposition of iC3b onto the cryptococcal capsule is not sufficient for optimal opsonization of the yeast cells. Cryptococci incubated with normal serum or the six isolated proteins did not differ with regard to the amount, location, or molecular form of the C3 fragments bound to the yeast cells. However, a consistent pattern was observed in which yeast cells incubated with the six alternative pathway proteins showed approximately 35% less binding to macrophages than did yeast cells incubated with normal serum. These results implicate the presence of an additional opsonic factor in normal serum. The identity of this opsonin is not known; however, fibronectin, C-reactive protein, and IgG are possible candidates. An IgG antibody in normal serum is a particularly attractive possibility, because Diamond et al. (5) have reported that the opsonic activity of normal serum is markedly depleted by adsorption of normal serum with encapsulated cryptococci at 0°C. The opsonic activity of the adsorbed serum was restored by the addition of purified IgG. Current efforts in our laboratory are directed toward identification of the additional serum component(s) required for effective opsonization of encapsulated cryptococci.

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