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Accelerated Decay of C3b to iC3b When C3b Is Bound to the *Cryptococcus neoformans* Capsule

GAIL S. T. PFROMMER, SARAH M. DICKENS, MICHAEL A. WILSON, BRYAN J. YOUNG, AND THOMAS R. KOZEL*

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

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Incubation of encapsulated and nonencapsulated *Cryptococcus neoformans* in normal human serum (NHS) leads to activation and binding of potentially opsonic fragments of complement component C3 to the yeast cells. Analysis of the molecular forms of C3 after incubation of encapsulated cryptococci in NHS showed that the percentage of bound C3 occurring as iC3b approached 100% after 8 min. The percentage of bound C3 occurring as iC3b on nonencapsulated cryptococci never exceeded 70%, even after 60 min of incubation in NHS. Conversion of C3b to iC3b was assessed further by incubating C3b-coated cryptococci for various times with a mixture of complement factors H and I at 40% of their respective physiological concentrations. Most, if not all, of the C3b on encapsulated cryptococci was converted to iC3b at a single fast rate. Conversion of C3b to iC3b on nonencapsulated cryptococci did not follow a single rate constant and appeared to have a fast and a slow component. Studies of the requirements for factors H and I in cleavage of C3b to iC3b showed steep dose-response curves for both factors in the case of encapsulated cryptococci and shallow curves with C3b bound to nonencapsulated cryptococci. Taken together, our results indicate that C3b molecules bound to encapsulated cryptococci have a uniformly high susceptibility to conversion to iC3b by factors H and I. In contrast, a significant portion of the C3b bound to nonencapsulated cryptococci is very resistant to conversion to iC3b by factors H and I.

Incubation of encapsulated *Cryptococcus neoformans* in normal human serum (NHS) leads to the deposition of 10^7 to 10^8 C3 fragments onto the average yeast cell (9, 22). Activation occurs entirely via the alternative complement pathway (10, 11, 21). The capsule itself is the site for binding of the C3 fragments (7, 9), and the bound C3 is almost entirely in the form of iC3b (8, 11).

The characteristics of activation and binding of C3 to nonencapsulated cryptococci differ in several important respects from the features observed with encapsulated cells. First, fewer C3 molecules bind to nonencapsulated cryptococci, approximately 2×10^6 per yeast cell (10). Second, initiation of the complement system by nonencapsulated cryptococci involves antibody-dependent activation of the classical pathway (10, 21). This produces a kinetic profile for the early accumulation of C3 fragments on nonencapsulated cryptococci that is much more rapid than occurs with encapsulated cells (10). Finally, the kinetic pattern for activation and binding of C3 fragments to encapsulated cryptococci is characterized by an abrupt termination in amplification that occurs after approximately 15 min when the cryptococci are incubated with NHS. In contrast, nonencapsulated cryptococci and zymosan continue to accumulate C3 fragments over incubation periods of 60 min or longer. Continued amplification such as that observed with nonencapsulated cryptococci and zymosan requires the presence of C3b, which is an essential component of the alternative pathway C3 convertase. The abrupt termination in amplification of C3 on the surface of encapsulated cryptococci may reflect a premature conversion of capsule-bound C3b to iC3b.

The almost exclusive presence of iC3b on the surface of encapsulated cryptococci coupled with the apparently premature termination of amplification suggests that there may be more rapid conversion of C3b to iC3b on encapsulated cells than occurs when C3b is bound to nonencapsulated cryptococci or zymosan. Conversion of C3b to iC3b occurs when the plasma serine protease factor I cleaves the α' chain of C3b into two fragments with molecular weights of approximately 67,000 and 40,000. Both fragments remain bound to the C3 β chain via disulfide bonds (18). Cleavage of C3b by factor I requires that the complement regulatory protein factor H be bound to the C3b (17). Thus, formation of iC3b is dependent on interaction between particle-bound C3b and factor H.

There are several mechanisms that could account for increased conversion of C3b to iC3b on encapsulated cryptococci. First, factor H could act more effectively in the microenvironment of the cryptococcal capsule. Second, the capsule could provide an environment that directly increases the efficiency of factor I-mediated cleavage of the C3b α' chain. Finally, the capsule could act as a cofactor for enzymatic degradation of C3b to iC3b by factor I. For example, it has been shown that C3b on sheep erythrocytes is inactivated by factor I in the absence of factor H (2, 3, 19).

Our study examined requirements for factors H and I in decay of C3b on encapsulated and nonencapsulated cryptococci. The goals were (i) to assess the time course for decay of yeast-bound C3b brought about by purified factors H and I and (ii) to determine whether cleavage of C3b bound to encapsulated and nonencapsulated cryptococci had different requirements for factors H and I. Our results showed that cleavage of C3b to iC3b by purified factors H and I occurred more rapidly and more precipitously on encapsulated than nonencapsulated cryptococci. The slope of dose-response curves showed that C3b bound to nonencapsulated cryptococci was much more heterogeneous than C3b bound to

* Corresponding author.

encapsulated cryptococci with regard to requirements for factors H and I.

MATERIALS AND METHODS

Yeast cells. *C. neoformans* 388, a serotype A strain provided by K. J. Kwon-Chung, was the encapsulated isolate (capsular width, ≈4.5 μm) used throughout the study (22). The nonencapsulated isolate was *C. neoformans* 602 (6). Both strains exhibited melanin production characteristic of *C. neoformans* (1) and produced a reaction on CGB agar characteristic of *C. neoformans* var. *neoformans* (12). The yeast cells were grown, killed with formaldehyde, and prepared for use as described previously (21). Each strain displayed the kinetics for activation and binding of C3 fragments that were expected, respectively, of encapsulated and nonencapsulated cryptococci.

Buffers, serum, and serum components. The following buffers were used: VBS (sodium Veronal [5 mM]-buffered saline [142 mM] [pH 7.3]), VBS^{2+} (VBS containing 0.15 mM $CaCl_2$ and 1 mM $MgCl_2$), GVB (VBS containing 0.1% gelatin), GVB-Ni (GVB containing 1.0 mM $NiCl_2$), GVBE (GVB containing 20 mM EDTA), and GVB^{2+} (VBS^{2+} containing 0.1% gelatin).

Peripheral blood was collected from 7 to 14 volunteers after their informed consent was obtained. The sera were pooled and stored at −80°C. This pool was used as the source of NHS. C3, factor B, factor D, factor H, and factor I were isolated from frozen human plasma as described previously (11). C3 was labeled with ^{125}I by the Iodogen (Pierce Chemical Co., Rockford, Ill.) procedure (4). Typically, 1 mg of C3 was labeled to a specific activity of 4×10^5 cpm/μg.

Deposition of C3b on cryptococcal cells. Trypsin-generated metastable C3b was used for deposition of initial C3b onto encapsulated and nonencapsulated cryptococci. Briefly, 2.5×10^7 yeast cells were resuspended in 125 μl of GVB-Ni containing 500 μg of a cocktail of C3 and ^{125}I-C3 that were mixed in proportions calculated to produce a specific activity of 100,000 cpm/μg. The tube was incubated for 2 min in a 37°C water bath, and 10 μg of TPCK-treated trypsin (Sigma Chemical Co., St. Louis, Mo.; catalog no. T-8642) in 10 μl of VBS^{2+} was added and incubated for 1 min at 37°C. The reaction was stopped by addition of 1 ml of GVB containing 250 μg of soybean trypsin inhibitor (Sigma; T-9003) and immediately placed in an ice bath. The cells were washed three times with GVB containing soybean trypsin inhibitor and one time with GVB-Ni.

Nickel-stabilized C3 convertase was used for amplification of the bound C3b. Yeast cells initially coated with trypsin-generated C3b were resuspended in 500 μl of GVB-Ni containing 45 μg of factor B and 0.5 μg of factor D and incubated for 2 min at 37°C. The yeast cells were washed two times with ice-cold GVB-Ni and resuspended in 1 ml of GVB-Ni, and 200 μl of GVB-Ni containing 800 μg of a cocktail of C3 and ^{125}I-C3 with a specific activity of 100,000 cpm/μg was added and incubated for 20 min at 37°C. The cells were washed two times with GVB-Ni, and the number of C3 molecules bound per cell was determined.

Amplification of the bound C3 was repeated two times. After the third amplification cycle, the cells were resuspended in 1 ml of GVBE and refrigerated overnight. Near maximal levels of bound C3b were achieved after two amplification cycles, and no further binding of C3b was produced by more than three amplification cycles. Analysis of the binding using ^{125}I-C3 showed that the three amplification cycles typically led to binding of 10^7 C3b molecules per encapsulated yeast cell and 2×10^6 to 5×10^6 C3b molecules per nonencapsulated yeast cell. This level of binding is similar to levels observed when these strains are incubated for 30 min in 40% NHS. Elution of the C3b with methylamine and analysis by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) showed the presence of only C3b, with no detectable intact C3 or iC3b.

Treatment of yeast-bound C3b with factors H and I. Cryptococci (2.5×10^6) coated with radiolabeled C3b were incubated with purified factors H and I in 1 ml of GVB^{2+}. The incubation times and concentrations of factors H and I varied with the experimental protocol. All experiments using factors H and I were based on the assumption that the concentrations of these factors in NHS were 560 and 34 μg/ml, respectively (16).

Analysis of bound C3 fragments. Bound C3 was eluted from the yeast cells by incubating the cells (2.5×10^6) for 90 min at 37°C with 100 μl of 25 mM methylamine in 0.2 M $NaHCO_3$ (pH 11) containing 1% SDS. After incubation, phenylmethylsulfonyl fluoride was added to a final concentration of 1 mM, the cells were removed by centrifugation, and the supernatant fluid containing the eluted C3 fragments was collected. The cells were washed one time with 50 μl of the methylamine solution, and the supernatant fluid from the wash was pooled with the original eluate. Treatment with methylamine released approximately 93% of the C3 fragments bound to encapsulated cryptococci and approximately 88% of the C3 bound to nonencapsulated cryptococci. Phenylmethylsulfonyl fluoride was added again (1 mM), and the samples were dialyzed against 0.05 M Tris (pH 6.8). The molecular form of eluted and reduced C3 was determined by SDS-PAGE and autoradiography. Radiolabeled C3, C3b, and iC3b were used as standards. Conversion from C3b to iC3b was determined by densitometer scanning of autoradiograms of the SDS-PAGE gels and was calculated as the loss of the α′ band of C3b. Lane-to-lane variation in sample size was corrected by normalizing the density of each band as a percentage of the sum of the densities of the C3 α′ chain (molecular weight, 106,000), the C3 β chain, and the 68,000-molecular-weight cleavage fragment that is characteristic of iC3b. Data are reported as the percentages of the total C3 fragments that are in the form of iC3b, using C3 or C3b as a standard.

Sigmoid curves showing conversion to iC3b were analyzed with a four-parameter logistic equation (GraphPAD InPlot version 3.0; GraphPAD Software, San Diego, Calif.). A 50% effective dose for dose-response curves, a Hill coefficient or slope factor for each curve, and 95% confidence intervals (CI) for each parameter were calculated from the four-parameter logistic equation.

RESULTS

Effect of incubation time in NHS on the molecular form of C3. An initial experiment was done to determine the molecular form of C3 bound to encapsulated or nonencapsulated cryptococci incubated for various times in NHS. The bound fragments were eluted with methylamine, reduced, and analyzed by SDS-PAGE. Similar amounts of C3 were loaded into each lane to facilitate comparisons of the molecular forms of C3 at each time point. Conversion of C3b to iC3b was assessed as the loss of the α′ band relative to the α band in purified C3. Data from individual lanes were normalized on the basis of the total density of all C3 cleavage fragments within a lane. It was assumed in this calculation that the

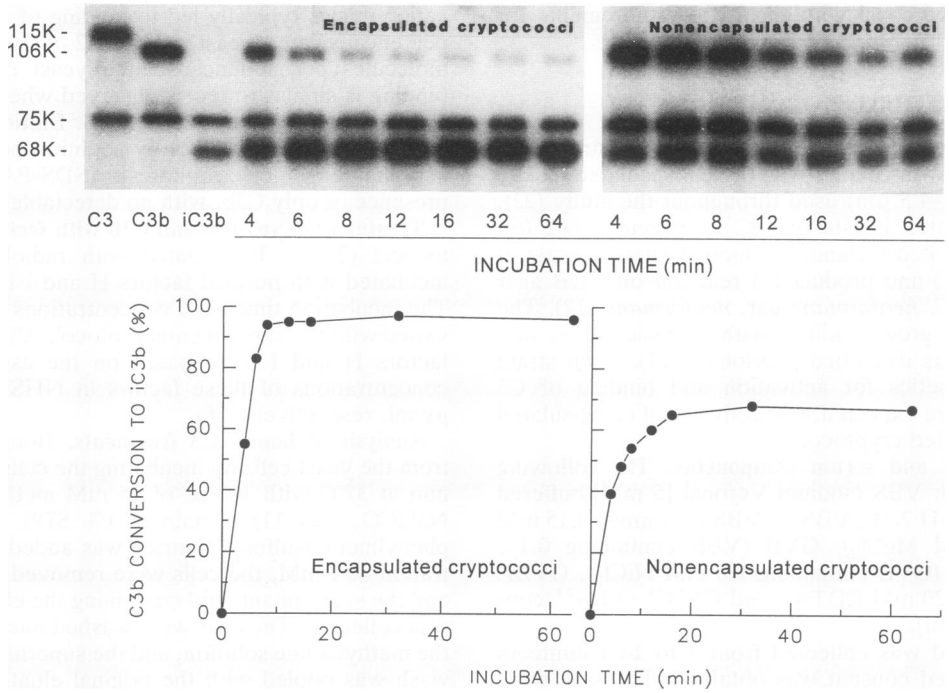

FIG. 1. Molecular form of C3 fragments on encapsulated and nonencapsulated cryptococci that were incubated for various times in NHS. The C3 fragments were eluted from the yeast cells and analyzed by SDS-PAGE and autoradiography.

contribution of C3a to the radioactivity of the α chain was negligible. The characteristic SDS-PAGE autoradiogram patterns of reduced C3, C3b, and iC3b are shown in Fig. 1. The results (Fig. 1) showed that greater than 80% of the C3 fragments bound to encapsulated cryptococci were in the form of iC3b after a 6-min incubation with NHS. The percentage of C3 occurring as iC3b approached 100% after 8 min. In contrast, less than 50% of the C3 bound to nonencapsulated cryptococci was in the form of iC3b after a 6-min incubation in NHS, and the total amount occurring as iC3b never exceeded 70%, even after 60 min of incubation.

Time course for cleavage of C3b by factors H and I. Previously reported kinetic studies showed that amplification of the number of bound C3 molecules on the encapsulated strain 388 was complete after approximately 15 min, whereas C3 fragments continued to accumulate on nonencapsulated strain 602 throughout a 60-min incubation (10). This finding indicates that the apparent molecular form of the C3 shown in Fig. 1 was a composite of newly deposited C3b and iC3b formed by decay of C3b deposited at earlier times. The occurrence of such simultaneous processes did not allow for an accurate comparison of the rate of decay of C3b on the yeast cells. As a consequence, an experiment was done in which encapsulated and nonencapsulated cryptococci were coated with C3b by use of purified C3, factor B, and factor D as described in Materials and Methods. An absence of factors H and I in this amplification system ensured that all C3 bound to the yeast cells was in the form of C3b. Yeast cells with bound C3b were incubated for various times with a mixture of purified factors H and I at 40% of their physiological concentrations. The bound fragments were eluted with methylamine and analyzed by SDS-PAGE under reducing conditions. The results (Fig. 2) showed that the rate of conversion of C3b to iC3b was much more rapid on encapsulated cryptococci than on nonencapsulated yeast cells. Purified factors H and I converted approximately 95% of the capsule-bound C3b to iC3b after only 4 min. Incubation of C3b-coated nonencapsulated cryptococci with purified factors H and I resulted in conversion of only 70% of the C3b to iC3b after 4 min, and the amount of iC3b never exceeded 90%, even after a 64-min incubation.

The data in Fig. 2 were consistent with pseudo-first-order rate kinetics (Fig. 3). For the analysis, the natural log of the fraction of C3b remaining was expressed as a function of time. At least 90% of the C3b on encapsulated cryptococci was converted to iC3b as a single fast first-order rate process. In contrast, conversion of C3b on nonencapsulated cryptococci appeared to occur by at least two first-order rate processes. The fast component had a rate similar to that observed with encapsulated cells. A second reaction occurred at a much slower rate. Extrapolation of the slow component to zero time indicates that 30 to 40% of the C3b on nonencapsulated cryptococci is converted at the slow rate.

Concentrations of factors H and I needed for conversion of C3b to iC3b. Conversion of C3b to iC3b occurs when the complement regulatory protein factor H binds to C3b and facilitates cleavage of the α′ chain by the serine protease factor I. Rapid cleavage of C3b to iC3b on encapsulated cryptococci could represent increased binding by and/or efficiency of factor H, increased activity of factor I, or both. As a consequence, an experiment was done in which encapsulated and nonencapsulated cryptococci were coated with C3b and incubated for 30 min at 37°C with factor I at 40% of its physiological concentration and variable amounts of factor H. A second experiment held the concentration of factor H at 40% of its physiological concentration while the concentration of factor I was varied.

Incubation of C3b-coated encapsulated cryptococci with a constant amount of factor I and a variable amount of factor H showed an acute dose-response curve in the range of

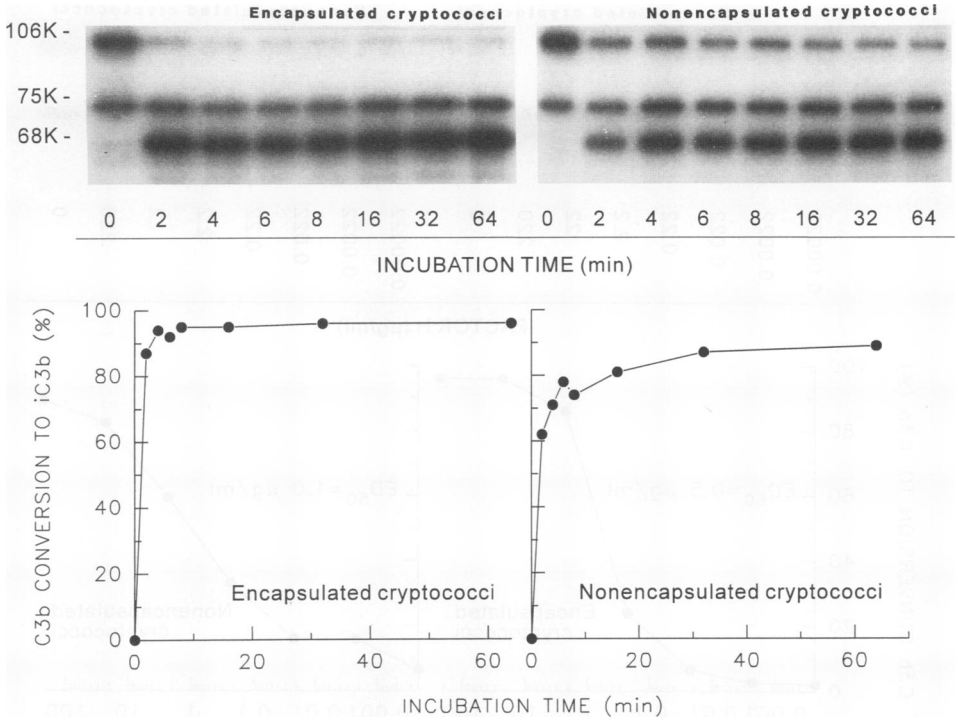

FIG. 2. Conversion of C3b to iC3b after incubation of C3b-coated encapsulated and nonencapsulated cryptococci for various times with factors H and I at 40% of their physiological concentrations. The C3 fragments were eluted from the yeast cells and analyzed by SDS-PAGE and autoradiography. Autoradiograms were scanned by densitometry. Conversion of C3b to iC3b shown in the graphs was calculated as the loss of the α′ band (106K) in each lane relative to the α′ band of C3b eluted from yeast cells that were not treated with factors H and I.

concentrations in which reactions with factor H were rate limiting for C3b conversion (Fig. 4). Analysis of the curve by using a four-parameter logistic equation showed that 50% conversion of C3b required 0.5 μg of factor H per ml (95% CI = 0.41 to 0.72 μg/ml). This corresponds to 0.09% of the physiological concentration of factor H. A plot of log factor H concentration versus percent conversion had a Hill slope of 1.2 (95% CI = 0.92 to 1.5 μg/ml). Incubation of C3b-coated nonencapsulated cryptococci with an excess of factor I and variable amounts of factor H produced a much shallower dose-response curve with a Hill slope of 0.44 (95% CI = 0.37 to 0.52 μg/ml). Conversion of 50% of the C3b required 1.0 μg of factor H per ml (95% CI = 0.69 to 1.4 μg/ml). The shallow slope of the curve found with nonencapsulated cryptococci suggests that C3b bound to nonencapsulated cryptococci displays considerably more heterogeneity in requirements for factor H than does C3b bound to encapsulated cryptococci.

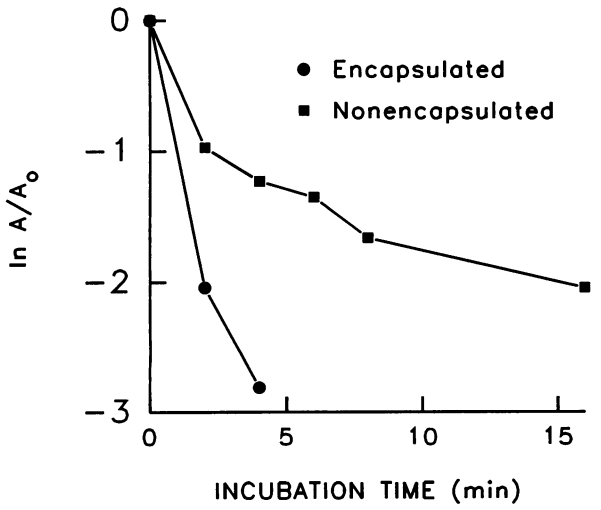

FIG. 3. First-order rate plot for conversion of C3b to iC3b after incubation of C3b-coated encapsulated and nonencapsulated cryptococci for various times with factors H and I at 40% of their physiological concentrations.

Incubation of C3b-coated encapsulated cryptococci with a constant amount of factor H and a variable amount of factor I showed that 50% conversion of C3b occurred at 0.03 μg of factor I per ml (95% CI = 0.02 to 0.05 μg/ml) in the range of concentrations in which reactions with factor I were rate limiting for C3b conversion (Fig. 5). This corresponds to 0.09% of the physiological concentration of factor I. A plot of log factor I concentration versus percent conversion had a Hill slope of 0.95 (95% CI = 0.57 to 1.3 μg/ml). As when factor H concentrations were varied, incubation of C3b-coated nonencapsulated cryptococci with an excess of factor H and variable amounts of factor I produced a curve that was much shallower (Hill slope = 0.27; 95% CI = 0.21 to 0.33 μg/ml) than the curve observed with encapsulated cells. Fifty percent conversion of C3b occurred at 0.16 μg of factor I per ml (95% CI = 0.08 to 0.32 μg/ml).

DISCUSSION

Studies of C3 fragments bound to zymosan found that most of the bound C3 could be released by treatment with nucleophiles such as hydroxylamine or methylamine (13).

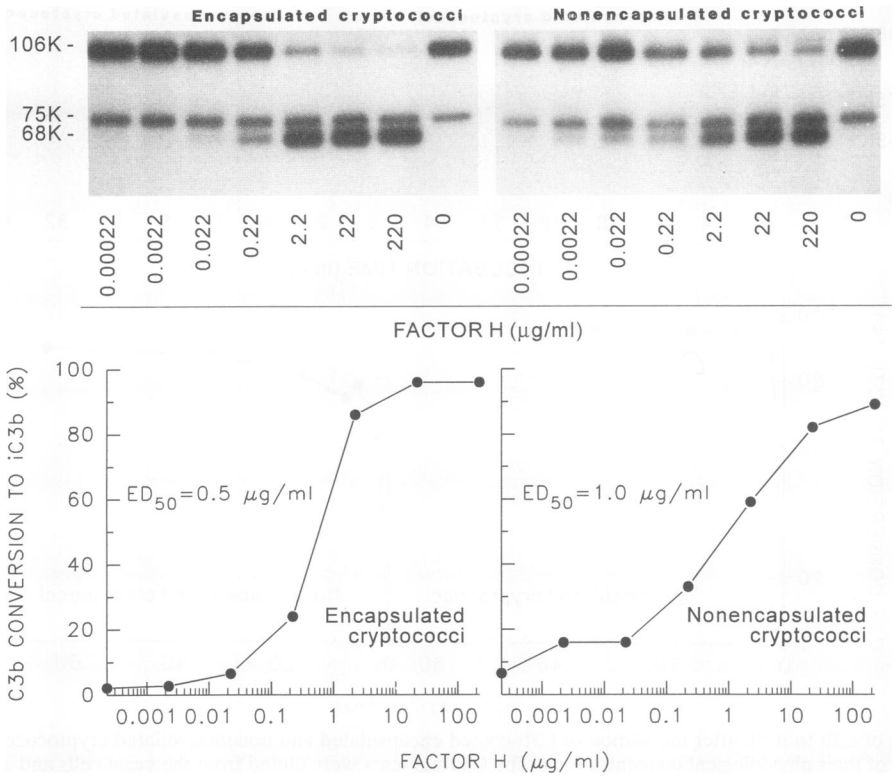

FIG. 4. Requirement for factor H in conversion of C3b to iC3b on encapsulated and nonencapsulated cryptococci. C3b-coated cells were incubated for 30 min with a mixture of factor I at 40% of its physiological concentration and various amounts of factor H. The C3 fragments were eluted from the yeast cells and analyzed by SDS-PAGE and autoradiography. Autoradiograms were scanned by densitometry. Conversion of C3b to iC3b shown in the graphs was calculated as the loss of the α′ band (106K) in each lane relative to the α′ band of C3b eluted from yeast cells that were either untreated (time zero) or treated with factor I in the absence of factor H. Identical results were produced by either manner of calculation. ED_{50}, 50% effective dose.

Release by treatment with hydroxylamine indicated that the C3 fragments were bound by ester bonds (13, 14). Analysis of the released C3 fragments by SDS-PAGE showed that the C3 fragments consisted of both C3b and iC3b (13). We have

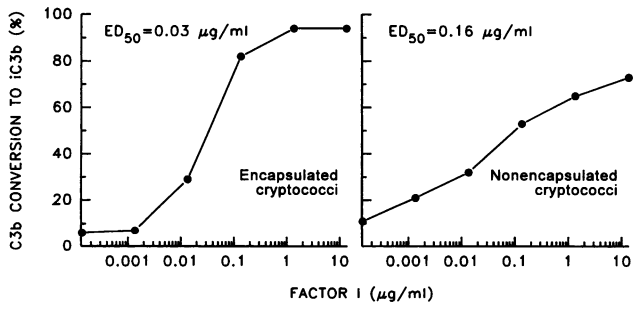

FIG. 5. Requirement for factor I in conversion of C3b to iC3b on encapsulated and nonencapsulated cryptococci. C3b-coated cells were incubated for 30 min with a mixture of factor H at 40% of its physiological concentration and various amounts of factor I. The C3 fragments were eluted from the yeast cells and analyzed by SDS-PAGE and autoradiography. Autoradiograms (not shown) were scanned by densitometry, and conversion of C3b to iC3b as shown in the graphs was calculated as the loss of the α′ band in each lane relative to the α′ band of C3b eluted from yeast cells that were either untreated (time zero) or treated with factor H in the absence of factor I. Identical results were produced by either manner of calculation. ED_{50}, 50% effective dose.

done similar experiments in an effort to analyze the C3 fragments bound to encapsulated and nonencapsulated cryptococci after incubation in NHS. Results shown in Fig. 1 demonstrate that C3 fragments bound to encapsulated cryptococci are almost entirely in the form of iC3b. This result is consistent with previous studies of complement fragments bound to encapsulated cryptococci (8, 9, 20). However, previous studies examined C3 fragments only after incubation of yeast cells in NHS for 30 min. Results shown in Fig. 1 indicate that an incubation time as short as 8 min produces C3 on encapsulated cryptococci that is >90% iC3b. In contrast, the iC3b on nonencapsulated cryptococci never exceeded 70%, even after extended incubation times. The pattern of C3 fragments found on nonencapsulated cryptococci is similar to the pattern of C3 found on zymosan (13). These results suggest an extremely efficient mechanism for conversion of C3b to iC3b on encapsulated cryptococci relative to the conversion that occurs on nonencapsulated cryptococci or zymosan.

Incubation of C3b-coated encapsulated or nonencapsulated cryptococci with purified factors H and I at 40% of their physiological concentrations provided a more accurate assessment of the sensitivity of C3b to conversion to iC3b. The results showed a much more rapid conversion to iC3b when the C3b was bound to encapsulated cryptococci. Analysis of the conversion of C3b to iC3b as a first-order rate plot provided insight as to a possible mechanism for the apparent resistance of C3b on nonencapsulated cryptococci to breakdown by factors H and I. Most, if not all, of the C3b

on encapsulated cryptococci was converted with a single fast rate constant. The rate of conversion on nonencapsulated cryptococci did not follow a single exponential process and appeared to have a fast and a slow component. The rapid phase of conversion was similar to that observed with C3b on encapsulated cryptococci. However, it appeared that 30 to 40% of the C3b bound to nonencapsulated cryptococci was converted at a much slower rate.

Kinetic analysis of cleavage of C3b to iC3b on encapsulated and nonencapsulated cryptococci suggested that there was a variation in the efficiency with which factor H, factor I, or both could influence conversion of C3b to iC3b on nonencapsulated cryptococci. This was confirmed by dose-response studies of the concentrations of factors H and I needed for cleavage of C3b on the two cell types. Dose-response curves for both factor H and factor I were quite steep when cleavage of C3b to iC3b was assessed on encapsulated cryptococci. This indicated that C3b molecules on encapsulated cryptococci were uniform in their requirements for these proteins. Dose-response curves for factors H and I in conversion of C3b to iC3b on nonencapsulated cryptococci showed much shallower slopes than did the curves obtained for encapsulated cryptococci. This indicates that C3b molecules bound to nonencapsulated cryptococci were quite heterogeneous in their requirements for factors H and I. This was particularly evident when the concentration of factor I was varied. Complete cleavage of C3b was never observed, even at the highest concentration of factor I. This suggests that a fraction of the C3b bound to nonencapsulated cryptococci is highly resistant to the action of factors H and I, a result that is consistent with the kinetic data, which showed that 30 to 40% of the C3b on nonencapsulated cryptococci was cleaved at a lower rate than the remainder of the C3b. The results also showed that approximately five times more factor I was required for conversion of 50% of the C3b on nonencapsulated cryptococci than was required for conversion of C3b on encapsulated cryptococci. This is also consistent with the presence of a resistant fraction of C3b on nonencapsulated cells.

There are at least two possible mechanisms for the slower rate of conversion of C3b to iC3b on nonencapsulated cryptococci. In one case, the differences could be due to changes in rate within the tertiary complex of C3b, factor H, and factor I. In the other case, differences in the rate of conversion of C3b to iC3b could be due to differences in the rate constants of reaction steps involving the combination of C3b with factors H and I. The shallow dose-response curves for both factors H and I and the inability of either H or I to saturate the reaction converting C3b to iC3b on nonencapsulated cryptococci suggest that the latter mechanism distinguishes encapsulated from nonencapsulated cells. In this case, the heterogeneity in conversion of C3b to iC3b would arise from the heterogeneity of combination of C3b with H and I, such that even at high concentrations of H and I, the formation of the C3b-H-I complex remains the limiting step at some sites.

Our data which show heterogeneity in the ability of factor H to facilitate cleavage of C3b on nonencapsulated cryptococci are consistent with a study by Horstmann et al. (5) which found considerable heterogeneity in the affinity of factor H for C3b bound to human erythrocytes, rabbit erythrocytes, and zymosan. Since rabbit erythrocytes and zymosan are activators of the human alternative pathway while human erythrocytes are not, heterogeneity in binding of factor H is not a feature which distinguishes activators from nonactivators. One possible explanation for the uniformity with which factor H binds to C3b in the cryptococcal capsule is the homogeneous nature of the capsule itself. This uniform structure, coupled with the three-dimensional matrix of the capsule, may provide an identical binding environment for all C3b molecules. In contrast, the complexity of the cryptococcal cell wall and the two-dimensional nature of the surface of the cell wall may restrict access or provide a variable background environment for interaction of factors H and I with C3b.

Results of this study provide an explanation for the predominance of iC3b on the surface of encapsulated cryptococci. The results also explain additional characteristics of complement activation by encapsulated cryptococci. Previous studies of the kinetics of C3 activation and binding to encapsulated strain 388 showed that rapid accumulation of C3 fragments on strain 388 is abruptly terminated after incubation in NHS for approximately 15 min. In contrast, nonencapsulated cryptococci continue to accumulate C3 fragments after incubation times as long as 60 min. Amplification can proceed only if C3b is available at the amplification site for formation of C3 convertase. The heterogeneity in susceptibility of C3b to inactivation by factors H and I means that a significant portion of the C3b will be resistant to cleavage. As a consequence, C3b will remain to support further amplification. The uniformly susceptible C3b on encapsulated cryptococci means that all available C3b will be converted within a short time to iC3b, and there can be no further amplification. Finally, our results may explain a previous study which described the relative ease with which activation and binding of C3 fragments from NHS to the cryptococcal capsule is disrupted by incorporation of additional factor H into the reaction mixture. A 200% increase in the concentration of factor H in 40% NHS completely blocked activation and binding of C3 fragments to encapsulated cryptococci. A similar increase in factor H retarded but did not block activation and binding of C3 fragments to nonencapsulated cryptococci. This result is consistent with a model in which all C3b molecules have a uniformly high sensitivity to the action of factors H and I, whereas a portion of the C3b molecules bound to nonencapsulated cryptococci are relatively resistant to the action of factors H and I.

Our results predict that both C3b and iC3b will be available at the surface of encapsulated and nonencapsulated cryptococci for interaction with phagocyte complement receptors. However, the rapid conversion of C3b to iC3b on encapsulated cryptococci suggests that iC3b may be a more important opsonic ligand for encapsulated cryptococci. In contrast, the relative resistance of C3b to conversion on nonencapsulated cryptococci suggests that C3b may be a more important ligand in the case of nonencapsulated cryptococci. These predictions are in good agreement with studies by Levitz and Tabuni (15) which showed that phagocytosis of encapsulated and nonencapsulated cryptococci that were opsonized with NHS involved multiple complement receptors. However, phagocytosis of NHS-opsonized encapsulated cryptococci was blocked more effectively by treatment of phagocytes with antibodies specific for complement receptor 3, a receptor with specificity for iC3b, than by treatment with antibodies specific for complement receptor 1, a receptor with specificity for C3b. Conversely, phagocytosis of NHS-opsonized nonencapsulated cryptococci was blocked more effectively by treatment of phagocytes with antibodies specific for complement receptor 1 than by treatment with antibodies specific for complement receptor 3.

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