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In Vivo Complement Activation and Binding of C3 to Encapsulated *Cryptococcus neoformans*

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Tissues from mice infected with *Cryptococcus neoformans* were examined by immunofluorescence to determine the extent of deposition of complement component C3 on encapsulated cryptococci. The relative percentages of cryptococci in each tissue having readily visible C3 were greatest for liver and lung tissues, with the kidney tissue having the next highest percentage and the spleen having the lowest percentage. Binding of C3 fragments to cryptococci in brain tissue was essentially absent.

In vitro studies have shown that encapsulated cells of *Cryptococcus neoformans* are powerful activators of the alternative complement pathway. Incubation of encapsulated cryptococci in normal human serum leads to the activation and binding to the average yeast cell of 1×10^7 to 5×10^7 cleavage fragments of C3 (5). The capsule is the site for activation and binding of C3 in vitro. The cell wall plays no role in activation of the complement system by encapsulated cryptococci (6, 10).

Little is known about the ability of encapsulated cryptococci to activate the complement system in vivo. Marked depletion of serum complement components was noted for three patients with cryptococcal septicemia (7). Similarly, studies with animal models showed that hypocomplementemia occurs during disseminated cryptococcosis (3, 7). Thus, there is indirect evidence that cryptococci activate the complement system in vivo. It is clear, however, that activation of the complement system does not occur in all tissues. For example, little or no C3 was detectable on the surface of cryptococci obtained from the spinal fluid specimens of four patients with cryptococcal meningitis (1).

The objectives of our study were (i) to determine whether encapsulated cryptococci serve as sites for activation and binding of C3 fragments in vivo and (ii) to determine whether there was variation in the extent of C3 deposition on encapsulated cryptococci in different tissues.

BALB/c mice were injected intravenously via the lateral tail vein with 1.2×10^5 cells of *C. neoformans* 104, an isolate of serotype A provided by K. J. Kwon-Chung. The cryptococcal cells had been grown for 72 h at room temperature on Sabouraud dextrose agar. Viability of the inoculum always exceeded 85%. Mice were sacrificed when the animals showed visible signs of the hydrocephalus characteristic of cryptococcal meningoencephalitis. Typically, this occurred 12 to 21 days after infection. Tissues (brain, kidney, liver, lung, and spleen) were removed and snap frozen in liquid nitrogen. Frozen sections were cut, and slides were prepared for examination by immunofluorescence.

Tissue sections were stained for both cryptococcal polysaccharide and murine C3. Slides were first incubated with a 1/100 dilution of fluorescein isothiocyanate (FITC)-conjugated goat $F(Ab')_2$ fragment to mouse C3 (Organon Teknika-Cappel Corp., Durham, N.C.). The slides were then incubated with a 1/10,000 dilution of monoclonal antibody 439 (2 mg/ml), a murine monoclonal antibody specific for cryptococcal polysaccharide (9). Finally, the sections were incubated with a 1/10 dilution of affinity-purified, Texas red-labeled goat anti-mouse immunoglobulin G (IgG) heavy chains (Southern Biotechnology Associates, Inc., Birmingham, Ala.). Control experiments showed that the goat anti-mouse IgG does not bind to the goat anti-mouse C3. All incubations were done for 30 min at room temperature. All antibodies were diluted with PBS (phosphate [10 mM]-buffered saline [127 mM], pH 7.3). Slides were washed three times with PBS after incubation with each antibody. Tissue sections were mounted in a medium of 90% glycerol–0.001% *p*-phenylenediamine in PBS.

Slides were examined with a Leitz Orthoplan microscope equipped for epifluorescence. Binding of FITC-labeled anti-mouse C3 was assessed with a wide-band-blue, high-intensity Leitz H3 filter block cube. Binding of the Texas red-labeled antibody specific for murine IgG was assessed with a fluorescence filter set prepared by Omega Optical, Inc. (Brattleboro, Vt.) for use with Texas red. As a control for the specificity of the fluorescent staining, encapsulated cryptococci were incubated for 30 min at 37°C with 25% mouse serum in Veronal sodium (5 mM)-buffered saline (142 mM) containing 0.15 mM $CaCl_2$ and 1 mM $MgCl_2$ and were stained for the presence of either bound C3 (FITC) or the cryptococcal capsule (Texas red). Yeast cells stained with the FITC-labeled anti-C3 showed intense staining with the appropriate filters for FITC and no staining with the filters for Texas red. Similarly, yeast cells incubated with the polysaccharide-specific monoclonal antibody and the Texas red-labeled anti-mouse IgG showed intense staining with the filter set specific for Texas red and no staining with the Leitz H3 filter block cube.

Cryptococci found in spleen, lung, kidney (results not shown), and liver tissues showed conspicuous binding of C3 fragments (Fig. 1). The percentage of yeast cells that contained readily detectable amounts of C3 varied with the tissue. For six sections of each tissue (two sections from each of three mice) examined, the highest percentages of cryptococci having attached C3 were found in the lung and liver tissues (94% ± 8% and 93% ± 3%, respectively [means ± standard deviations]), followed by the kidney and spleen tissues (66% ± 14% and 41% ± 8%, respectively). Cryptococci found in brain tissue showed no apparent binding of C3 (1% ± 1%) (Fig. 1). The absence of bound C3 on cryptococci

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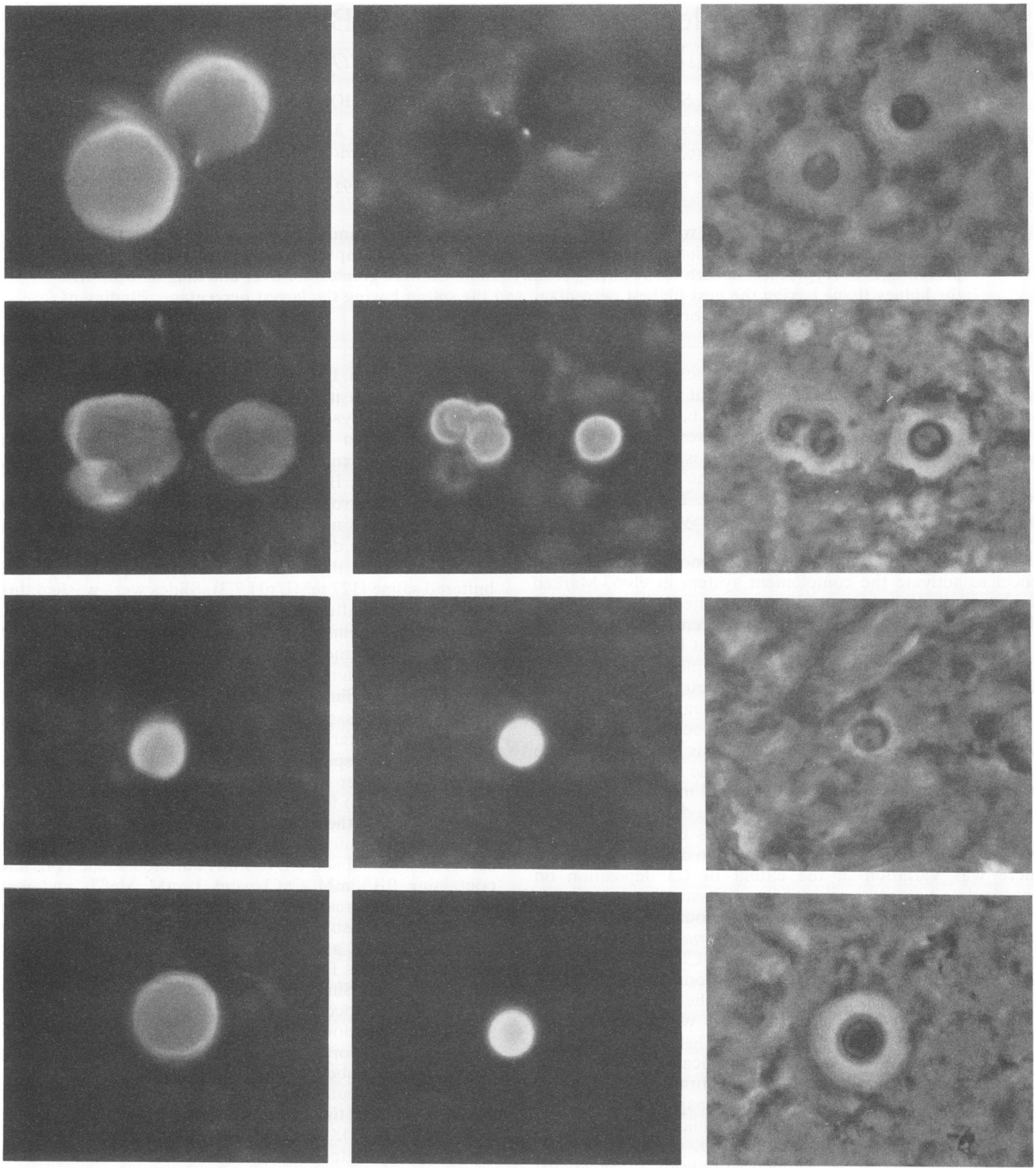

FIG. 1. Tissue sections of brain (first row), liver (second row), lung (third row), and spleen (fourth row) from mice infected intravenously with *C. neoformans*. Mice were sacrificed when they showed signs of the hydrocephalus characteristic of cryptococcal meningoencephalitis. Tissue sections were dually stained, first with FITC-labeled goat anti-mouse C3 and then with a mouse monoclonal antibody specific for cryptococcal polysaccharide followed by Texas red-labeled goat antiserum specific for mouse IgG. Left column, tissue sections examined with the filter set appropriate for Texas red; center column, the same microscopic field examined with the FITC filter set; right column, phase-contrast microscopy of the sections. All sections are shown at the same magnification.

in brain tissue was not due to an absence of yeast cells or a lack of encapsulation because the Texas red stain for presence of the capsule clearly identified encapsulated cryptococci in brain tissue.

Goren and Warren (4) reported that cryptococci found in lung homogenates from infected mice were readily stained with FITC-conjugated antiserum raised against mouse globulins. The specificity of the antiserum was not known with certainty, but those authors speculated that the reactivity might be due to the presence of complement components in the capsule. The results of our study indicate that large amounts of C3 are indeed found on cryptococci in infected lung tissue. The high level of C3 observed on cryptococci in lung tissue may explain, in part, the extraordinary predilection of cryptococci for the lungs of C5-deficient mice. Rhodes found that C5-deficient mice develop an acute, fatal cryptococcal pneumonia after intravenous inoculation of *C. neoformans* whereas C5-sufficient mice do not (8). The absence of C3 on cryptococci found in brain tissue is most likely explained by the absence of one or more components of the alternative pathway at that site. For example, C3 concentrations in human cerebrospinal fluid are only 3.0 μg/ml, compared with 1,170 μg/ml in serum (2). The absence of deposition of C3 onto cryptococci in brain tissue may provide an additional explanation for the apparent niche that particular anatomic location provides for *C. neoformans*.

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REFERENCES

1. **Diamond, R. D., J. E. May, M. A. Kane, M. M. Frank, and J. E. Bennett.** 1974. The role of the classical and alternative complement pathways in host defenses against *Cryptococcus neoformans* infection. J. Immunol. **112**:2260–2270.
2. **Dujardin, B. C. G., P. C. Driedijk, A. F. M. Roijers, and T. A. Out.** 1985. The determination of the complement components C1q, C4 and C3 in serum and cerebrospinal fluid by radioimmunoassay. J. Immunol. Methods **80**:227–237.
3. **Gadebusch, H. H., and A. G. Johnson.** 1966. Natural host resistance to infection with *Cryptococcus neoformans*. IV. The effect of some cationic proteins on the experimental disease. J. Infect. Dis. **116**:551–565.
4. **Goren, M. B., and J. Warren.** 1968. Immunofluorescence studies of reactions at the cryptococcal capsule. J. Infect. Dis. **118**:215–229.
5. **Kozel, T. R., G. S. T. Pfrommer, A. S. Guerlain, B. A. Highison, and G. J. Highison.** 1988. Strain variation in phagocytosis of *Cryptococcus neoformans*: dissociation of susceptibility to phagocytosis from activation and binding of opsonic fragments of C3. Infect. Immun. **56**:2794–2800.
6. **Kozel, T. R., M. A. Wilson, and J. W. Murphy.** 1991. Early events in initiation of alternative complement pathway activation by the capsule of *Cryptococcus neoformans*. Infect. Immun. **59**:3101–3110.
7. **Macher, A. M., J. E. Bennett, J. E. Gadek, and M. M. Frank.** 1978. Complement depletion in cryptococcal sepsis. J. Immunol. **120**:1686–1690.
8. **Rhodes, J. C.** 1985. Contribution of complement component C5 to the pathogenesis of experimental murine cryptococcosis. Sabouraudia **23**:225–234.
9. **Spiropulu, C., R. A. Eppard, E. Otteson, and T. R. Kozel.** 1989. Antigenic variation within serotypes of *Cryptococcus neoformans* detected by monoclonal antibodies specific for the capsular polysaccharide. Infect. Immun. **57**:3240–3242.
10. **Wilson, M. A., and T. R. Kozel.** 1992. Contribution of antibody in normal human serum to early deposition of C3 onto encapsulated and nonencapsulated *Cryptococcus neoformans*. Infect. Immun. **60**:754–761.