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Inhibition of Phagocytosis by Cryptococcal Polysaccharide: Dissociation of the Attachment and Ingestion Phases of Phagocytosis

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The effects of cryptococcal polysaccharide and selected serum factors on (i) the attachment of *Cryptococcus neoformans* to macrophages and (ii) the subsequent ingestion of yeast cells by the macrophages were investigated. Percent attachment was measured after incubation of yeast cells with macrophages at 4 C. Percent engulfment was determined after incubation of yeast cells and macrophages at 37 C. Nonencapsulated yeast cells readily attached to macrophages at the low temperature and were engulfed at a high rate at 37 C, whereas encapsulated yeast cells attached to macrophages at low rates and were engulfed at low rates. Addition of varying doses of purified cryptococcal polysaccharide to nonencapsulated yeast cells inhibited attachment at approximately the same concentration of polysaccharide required for inhibition of engulfment. Nonencapsulated yeast cells that attached to macrophages at 4 C were eluted from the macrophages by addition of purified cryptococcal polysaccharide to the incubation medium. Heat-labile opsonins were not required for attachment of yeast cells to macrophages, but they were necessary for maximal initial rates of phagocytosis. Heat-stable components of serum facilitated attachment of cryptococci, but their most important function appeared to be triggering the ingestion of attached yeast. Specific antiserum had no effect on the attachment and engulfment of nonencapsulated cryptococci, and the antiserum produced only a small enhancement of the engulfment of encapsulated cryptococci.

Cryptococcus neoformans is a yeast highly dependent upon a polysaccharide capsule for its virulence. Macrophages (15), neutrophils (6, 21), and monocytes (6) are able to phagocytize and destroy the yeast, but this process is inhibited by microgram amounts of cryptococcal polysaccharide (3, 6). Since reports have suggested that cell-mediated responses to the organism are largely responsible for control of infection (6, 7, 10), a clear understanding of the mechanism by which the capsular polysaccharide inhibits phagocytosis is needed.

Phagocytosis of particles by leukocytes involves at least two separate steps (16). The first step is attachment of the particle to the surface of a leukocyte. The second step is the uptake or ingestion of the attached particle. The effects of cryptococcal polysaccharide and various serum factors on the overall phagocytic process have been reported (3, 5, 6); however, the effects of cryptococcal polysaccharide on the individual stages of phagocytosis are not known.

Rabinovitch (16) reported that erythrocytes attach to macrophages in vitro at temperatures below 37 C, but ingestion of the cells is substantially inhibited at temperatures below 30 C. If the incubation temperature was raised to 37 C, phagocytosis of erythrocytes proceeded through the ingestion stage. Using this technique, we have studied the effects of cryptococcal polysaccharide and selected serum factors on (i) the attachment of cryptococcal cells to macrophages and (ii) the subsequent ingestion of yeast cells by the macrophages.

MATERIALS AND METHODS

Cryptococcal isolates and soluble polysaccharide. *C. neoformans* strain 602 is a nonencapsulated strain that has been described previously (11). *C. neoformans* strain 613 is a moderately encapsulated isolate, producing a capsule that averages 1.2 μm in width on Littman's CCA medium (14).

Organisms used in all assays were grown for 3 days on slants of potato dextrose agar (Difco) and were washed from the slants with 0.15 M sodium chloride containing formaldehyde at a final concentration of 0.33%. The yeasts were incubated overnight at room temperature in the formalinized saline, washed three times with Hanks balanced salt solution (HBSS; International Scientific Industries), buffered with sodium bicarbonate to pH 7.2, and resuspended in HBSS. Cell counts were determined with a hemocytometer.

The procedure for purification of cryptococcal soluble polysaccharide has been described previously (11). Polysaccharide was stored in a lyophilized state at room temperature and was prepared for use at the desired concentration as a saline solution.

Preparation of macrophages. Peritoneal exudate cells were obtained from unstimulated 7- to 8-week-old Swiss Webster mice (Microbiological Associates), using slight modifications of a procedure described by Cohn and Benson (4). Siliconized tubes and pipettes were used throughout the procedure. Mice were killed by cervical dislocation, and the abdominal skin was reflected. Two milliliters of HBSS containing 10 U of heparin per ml and antibiotics (100 U of penicillin and 100 μg of streptomycin per ml; Grand Island Biological Co.) was injected intraperitoneally. The abdomen was massaged gently, and the fluid was withdrawn with a Pasteur pipette. The cells were sedimented at 400 $\times$ g for 10 min, washed once with HBSS, and counted in a hemocytometer, and the cell concentration was adjusted with HBSS to 5 $\times$ 10^5 macrophages/ml. The procedure yielded approximately 2 $\times$ 10^6 to 3 $\times$ 10^6 leukocytes per mouse, of which approximately 50% were macrophages. Viability was greater than 90% as determined by trypan blue dye exclusion.

Monolayers of macrophages were prepared in four-chamber tissue culture chamber slides (Lab-Tek Products, Division Miles Laboratories, Inc.). One-half milliliter of the peritoneal cell suspension was added to each chamber and incubated at 37 C for 1 h in 5% CO_2. The monolayers of adherent cells were washed once with HBSS; the medium was replaced with medium 199 (International Scientific Industries) containing sodium bicarbonate, antibiotics, and 10% calf serum; and the cultures were reincubated for 24 to 48 h before use. Monolayers prepared in this manner had a viability exceeding 80% and consisted of at least 90% macrophages. No differences in phagocytic activity were noted between 24- and 48-h cultures.

Assays for attachment and ingestion. Culture medium was poured off the macrophage monolayers, and 1 ml of a test yeast suspension was added to each monolayer. The test yeast suspension consisted of (i) cryptococci at a concentration to provide eight yeast cells per macrophage; (ii) unless otherwise indicated, calf serum at a final concentration of 10%; (iii) when required by experimental protocol, 0.25 ml of additional reactants (cryptococcal polysaccharide, immune serum, etc.) in saline; and (iv) enough HBSS to give a final volume of 1 ml.

Attachment assays were done by incubating the monolayers at 4 C for 2 h immediately after addition of yeast suspensions. At this low temperature, engulfment was negligible, amounting to less than 10% of the total percent attachment. Assays for ingestion were done by incubating cryptococci with macrophages at 37 C for 2 h. At the 37 C temperature, attachment in the absence of ingestion occurred infrequently and amounted to less than 10% of the percent ingestion.

After incubation, the medium was aspirated and the monolayers were washed gently in HBSS to remove nonadherent yeast. The monolayers were air dried, fixed in methanol, and stained by the Giemsa procedure.

At least 200 macrophages were examined per monolayer, and the percentage of macrophages with only attached yeast (percent attachment) as well as the percentage of macrophages with ingested yeast (percent phagocytosis) were determined. Results are presented as mean values from at least four replications. Statistical analyses were done by analysis-of-variance techniques.

Serum. Calf serum (Grand Island Biological Co., lot no. C548421 and C951404) was used as the serum source in attachment and ingestion assays. Studies requiring immune serum utilized cryptococcal antiserum prepared in rabbits against whole cells of *C. neoformans* strain 613 (11) or prepared against purified cryptococcal polysaccharide complexed with methylated bovine serum albumin (13). The antiserum prepared against whole cryptococci was previously shown to contain large amounts of specific immunoglobulin M (IgM) (12). Both serum specimens contained specific anticryptococcal IgG as determined by indirect immunofluorescence (9) using fluorescein-conjugated anti-rabbit IgG. The indirect fluorescent-antibody (IFA) technique as described by Goren and Warren (9) was used to determine antibody titers. Concentrations of specific antibody were expressed in terms of IFA units (twice the lowest concentration of anticryptococcal serum required to give strong fluorescence upon addition of optimally titrated fluorescein-conjugated anti-rabbit immunoglobulins). Fluorescein-conjugated IgG fractions of goat anti-rabbit immunoglobulins (IgA + IgG + IgM), goat anti-rabbit IgG (heavy chain), and goat anti-rabbit IgM (heavy chain) were obtained from Cappel Laboratories, Inc. (lot no. 7500, 6152, and 7998, respectively).

RESULTS

Inhibition of attachment by cryptococcal polysaccharide. An initial experiment was done to determine the effect of encapsulation on attachment of *C. neoformans* to macrophages. The nonencapsulated strain 602 and encapsulated strain 613 were added to macrophages and incubated at 4 or 37 C to measure attachment or phagocytosis, respectively. Strain 602 readily attached to macrophages at the lower temperature and was ingested at a high rate when incubated at 37 C (Table 1). Both attachment and phagocytosis of the encapsulated strain were markedly inhibited.

Several investigators have shown that purified cryptococcal polysaccharide can inhibit phagocytosis of nonencapsulated isolates of *C. neoformans*. The results in Fig. 1 suggest that this inhibition of phagocytosis is accounted for by polysaccharide-mediated suppression of the ability of the organism to attach to macrophages. Addition of cryptococcal polysaccharide to strain 602 effectively blocked attachment of the organism to macrophages. Moreover, this

inhibition of attachment required approximately the same concentration of polysaccharide needed for inhibition of phagocytosis.

It was of interest to determine the ability of cryptococcal polysaccharide to block ingestion of yeast cells that had been permitted to attach to macrophages in the absence of polysaccharide. Cells of strain 602 were incubated with macrophages for 1.5 h at 4 C. The monolayers were then washed three times to remove nonadherent yeast cells and incubated at 37 C for 1 h in HBSS containing 100 μg of cryptococcal polysaccharide per ml and 10% calf serum. If cryptococcal polysaccharide were able to block ingestion, yeast cells were expected to remain attached to the macrophages. If the polysaccharide had no effect on engulfment of attached yeast, cryptococci were expected to be found within macrophages. As a negative control, polysaccharide (100 μg/ml) was added to the initial 4 C incubation medium to prevent attachment. As a positive control, cells were allowed to attach at 4 C, and ingestion was allowed to proceed at 37 C in the absence of polysaccharide. Both attachment and engulfment of the yeast were determined after the 37 C incubation. As an additional positive control, cells were fixed and stained after the attachment stage to demonstrate that the yeast had, in fact, attached to macrophages. The results (Table 2) show that yeast cells allowed to attach to macrophages in the absence of polysaccharide were engulfed when the temperature was raised to 37 C. The percent engulfment did not differ significantly from the percent attachment observed with preparations fixed and stained immediately after the attachment stage of phagocytosis (data not shown). Therefore, almost all attached yeast cells can be expected to undergo phagocytosis when the incubation temperature is raised to 37 C. Addition of cryptococcal polysaccharide to the culture medium during the 4 C incubation effectively blocked attachment, and few yeast cells were found to be attached or engulfed after the 37 C incubation. When cryptococcal polysac-

TABLE 1. *Attachment and phagocytosis of encapsulated and nonencapsulated strains of C. neoformans by macrophages.*

Strain	Percent attachment[a]	Percent phagocytosis[b]
602	38.7 ± 11.2	85.8 ± 4.3
613	5.3 ± 3.5	3.8 ± 1.6

[a] Expressed as mean percent ± standard deviation; determined by incubation of yeast and macrophage at 4 C.

[b] Expressed as mean percent ± standard deviation; determined by incubation of yeast and macrophage at 37 C.

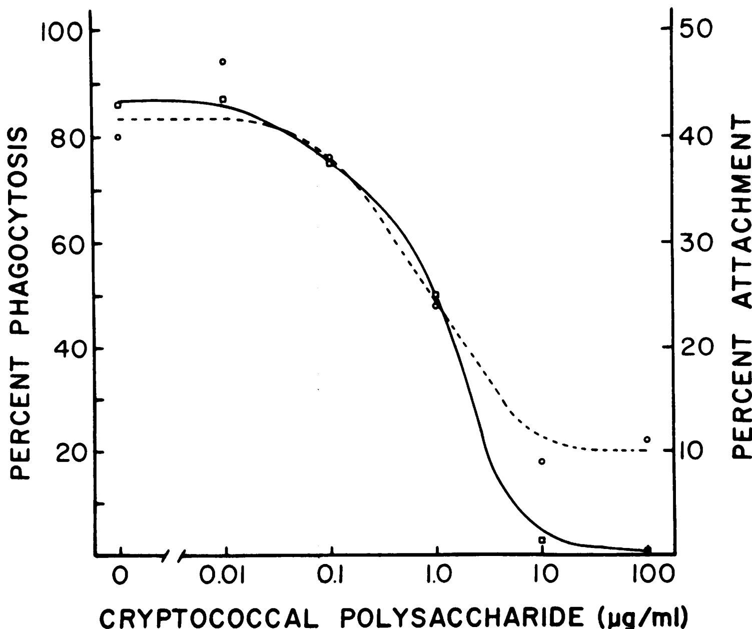

FIG. 1. *Attachment (○) and ingestion (□) of C. neoformans by macrophages in presence of cryptococcal polysaccharide. Attachment was determined after incubation at 4 C. Ingestion was determined after incubation at 37 C.*

TABLE 2. *Elution of attached cryptococci from macrophages by purified cryptococcal polysaccharide.*

Presence of polysaccharide[a] (μg/ml)		Percent attachment[b]	Percent engulfment[b]
4 C incubation	37 C incubation		
None	None	2.0 ± 1.6	44.7 ± 6.8
100	None	6.9 ± 5.0	5.8 ± 4.2
None	100	3.2 ± 2.6	5.9 ± 2.6

[a] Macrophages were incubated with strain 602 for 1.5 h at 4 C with or without added polysaccharide. Cultures were washed three times with HBSS to remove nonadherent yeast cells and then incubated at 37 C in the presence or absence of polysaccharide for 1 h. Percent attachment and percent engulfment were determined after the 37 C incubation.

[b] Expressed as mean ± standard deviation.

charide was added to attached yeast cells and the incubation temperature was raised to 37 C, the yeast cells were not engulfed, nor did they remain attached to the macrophages. Thus, cryptococcal polysaccharide somehow elutes attached yeast cells from the surface of macrophages.

Role of serum factors in attachment of nonencapsulated cryptococci. Incubation of cryptococci with macrophages at 4 and 37 C was used to study attachment and phagocytosis, respectively, at various time intervals over a 2-h incubation period. The effects of incubation in medium containing no serum, normal serum, or serum heated at 56 C for 30 min were noted. Hemolytic complement activity was absent in the heat-inactivated serum. In an effort to minimize presence of cytophilic antibody adherent to the macrophages (15, 22), all monolayers were washed two times with warm (37 C) HBSS, incubated for 30 min at 37 C, and washed once more before use. No further attempt was made to establish the absence of cytophilic antibody. Incubation of strain 602 and macrophages in medium containing heat-inactivated serum produced no significant effect on attachment of the yeast to macrophages when compared with medium containing normal serum (Fig. 2). However, phagocytosis in the presence of heated serum was significantly ($P < 0.01$) impaired. Both attachment and phagocytosis were depressed when the yeast was incubated with macrophages in the absence of serum. The effect on attachment was greatest during the early time intervals, whereas in the absence of serum a marked depression of phagocytosis was observed at all time intervals.

The possible opsonizing activities of specifi-

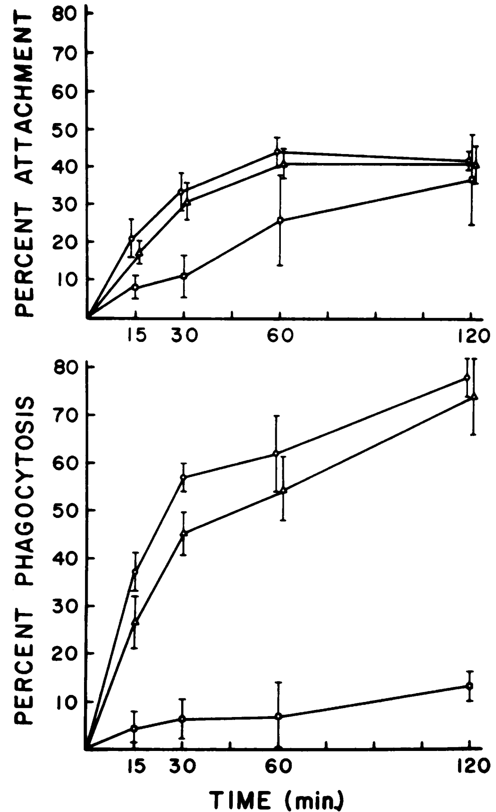

FIG. 2. *Attachment and ingestion of C. neoformans by macrophages when incubated in medium containing normal serum (○), serum heated at 56 C for 30 min (△), or medium containing no serum (□). Attachment was determined after incubation at 4 C. Ingestion was determined after incubation at 37 C.*

cally induced antibody were studied by using antiserum prepared in rabbits against whole encapsulated cryptococci or a methylated bovine serum albumin conjugate of cryptococcal polysaccharide. Immune antisera were incorporated into the incubation medium at a concentration of 2 IFA units of antibody per ml. Neither antiserum preparation had any effect on either the rate of attachment or ingestion of strain 602 (Table 3). Phagocytosis of strain 613 was slightly, but significantly ($P < 0.01$), enhanced in the presence of immune serum.

DISCUSSION

Diamond et al. (5) have demonstrated requirements for IgG, the classical complement pathway, and the alternate complement pathway in phagocytosis of cryptococcus by neutrophils. Our studies show that heat-labile opsonins (HLO) are not an absolute requirement for

TABLE 3. *Effect of immune serum on attachment and phagocytosis by macrophages of encapsulated and nonencapsulated strains of C. neoformans.*

Antiserum[a]	Percent attachment[b]		Percent phagocytosis[c]	
	602	613	602	613
None	51.7 ± 5.2	8.2 ± 2.0	83.4 ± 3.6	6.2 ± 2.5
Anti-whole cell	52.0 ± 8.5	11.4 ± 5.0	88.6 ± 4.8	13.9 ± 6.7
Anti-Polysaccharide-MBSA[d]	56.8 ± 3.5	10.9 ± 5.2	89.0 ± 4.4	21.2 ± 6.4

[a] Antiserum was added in a final concentration of 2 IFA units/ml to the standard attachment and phagocytosis medium described in Materials and Methods.
[b] Expressed as mean ± standard deviation; determined by incubation at 4 C.
[c] Expressed as mean ± standard deviation; determined by incubation at 37 C.
[d] MBSA, Methylated bovine serum albumin.

attachment of the yeast to macrophages. HLO were necessary for maximal initial rates of phagocytosis, however, inasmuch as the absence of HLO depressed the percent phagocytosis at early time intervals, with near-normal rates of phagocytosis attained by completion of the 2-h incubation period. HLO appear to operate in this system primarily by stimulating the process of ingestion rather than through enhancement of yeast-macrophage affinity. This observation is similar to a report by Stossel (20), who used a kinetic analysis of overall reaction rates to suggest that complement components activated via the alternate pathway enhance phagocytosis of albumin-coated paraffin oil particles by stimulating the engulfment process rather than by enhancing cell-particle affinity. The kinetics of ingestion of strain 602 in the absence of HLO also resemble an observation by Diamond et al. (5) that uptake of cryptococci by neutrophils in C4- or C2-deficient serum is delayed at early time intervals, but normal phagocytic indexes are reached by the end of a 2-h incubation period. They also found that phagocytosis by neutrophils was highly dependent on the presence of HLO, presumably the C3 proactivator, at all time intervals studied (5). Mitchell and Friedman (15) have also noted an obligatory requirement for HLO in the phagocytosis of cryptococcus by glycogen-stimulated rat peritoneal macrophages. The decreased dependence of our system on HLO may be due to differences in encapsulation of yeast strains or to our use of peritoneal macrophages from unstimulated animals. Rabinovitch and DeStefano (19) and Bianco et al. (2) have shown that macrophages from stimulated and unstimulated animals differ in their response to the opsonizing effects of complement.

Heat-stable components of serum appear to facilitate the attachment of cryptococci to macrophages, but their most important function may be to trigger the ingestion of attached yeast. Attachment readily occurred in the absence of serum, although heat-stable opsonins were required for maximal rates of attachment, particularly at early time intervals. In the absence of serum, ingestion occurred at a rate only 10 to 15% of normal phagocytic rates at all time intervals. It is likely that the heat-stable opsonin is the naturally occurring IgG described by Diamond et al. (5). Rabinovitch has similarly shown that attachment of aldehyde-treated erythrocytes could occur in the absence of serum factors (16; Fed. Proc. 25:728, 1966), but the ingestion phase was highly dependent on IgG for maximum activity (17, 18).

The role of specifically induced antibody as an opsonin for cryptococcus remains unresolved. Serum known to contain specific IgG and IgM to the encapsulated organism or its soluble polysaccharide had no measurable effect on the rate of attachment or phagocytosis of nonencapsulated cryptococci. A significant increase in the percent phagocytosis of the encapsulated yeast was observed. The effect was greatest using antiserum prepared against a methylated bovine serum albumin conjugate of the soluble polysaccharide. This increased phagocytosis was quite small, however, and amounted to less than 25% of the normal percent phagocytosis observed with nonencapsulated cryptococci. Diamond et al. (6) have observed that serum obtained from individuals who developed positive skin tests after repeated injection of cryptococcin had no effect on rate of ingestion or killing of cryptococci by neutrophils or monocytes. The report did not indicate the level of antibody activity or the class of immunoglobulin involved. In a later report, Diamond et al. (5) demonstrated that the opsonizing activity of normal human serum could be selectively removed by adsorption with cryptococci, and, although immunoglobulins were not demonstrable on the yeast by IFA, the opsonizing activity was restored by purified IgG. It

is likely, therefore, that opsonization of cryptococcus is not mediated by polysaccharide-specific IgM; rather, it is mediated by IgG specific for cryptococcal antigens that may or may not include the capsular polysaccharide. This explanation correlates well with reports that resistance to experimental cryptococcosis in mice does not parallel presence of humoral anticapsular antibody as measured by the agglutination technique (1, 8), a procedure having a relatively high sensitivity for specific IgM.

Numerous investigators (3, 6, 15) have demonstrated the antiphagocytic properties of cryptococcal capsules. It appears from this study that these antiphagocytic properties are due to the ability of the polysaccharide to prevent attachment of encapsulated yeast cells to phagocytic cells. Further, the polysaccharide concentration required to inhibit phagocytosis of nonencapsulated cryptococci corresponds closely with the amount of polysaccharide required for inhibition of attachment. It is evident from Table 2, however, that the action of the polysaccharide extends beyond a simple inhibition of effective contact between the cryptococci and macrophages, since cryptococcal polysaccharide was able to remove attached cryptococci from the surface of macrophages. At least two possible mechanisms may be proposed for such a phenomenon. The present study has shown that serum components greatly facilitate the attachment of cryptococci to macrophages. The work of Diamond et al. (5) suggests that in human serum this component is IgG. It may be possible that cryptococcal polysaccharide competitively inhibits the binding of such opsonic adherence antibodies to either the surface of the yeast or the surface of the macrophage, or both. Alternately, the polysaccharide may bind to the macrophage or yeast at sites other than those occupied by opsonizing antibody. In such a situation, the capsular material might act sterically, inhibiting intimate contact between the yeast cell and the macrophage. Present studies in our laboratory are investigating these possibilities.

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LITERATURE CITED

1. **Abrahams, I.** 1966. Further studies on acquired resistance to murine cryptococcosis: enhancing effect of *Bordetella pertussis*. J. Immunol. 96:525–529.
2. **Bianco, C., F. M. Griffin, Jr., and S. C. Silverstein.** 1975. Studies of the macrophage complement receptor. Alteration of receptor function upon macrophage activation. J. Exp. Med. 141:1278–1290.
3. **Bulmer, G. S., and M. D. Sans.** 1968. *Cryptococcus neoformans*. III. Inhibition of phagocytosis. J. Bacteriol. 95:5–8.
4. **Cohn, Z. A., and B. Benson.** 1965. The differentiation of mononuclear phagocytes. Morphology, cytochemistry, and biochemistry. J. Exp. Med. 121:153–170.
5. **Diamond, R. D., J. E. May, M. A. Kane, M. M. Frank, and J. E. Bennett.** 1974. The role of the classical and alternate complement pathways in host defenses against *Cryptococcus neoformans* infection. J. Immunol. 112:2260–2270.
6. **Diamond, R. D., R. K. Root, and J. E. Bennett.** 1972. Factors influencing killing of *Cryptococcus neoformans* by human leukocytes in vitro. J. Infect. Dis. 125:367–376.
7. **Gadebusch, H. H.** 1972. Mechanism of native and acquired resistance to infection with *Cryptococcus neoformans*, p. 3–12. *In* A. Laskin and H. Lechevalier (ed.), Macrophages and cellular immunity. CRC Press, Cleveland, Ohio.
8. **Goren, M. B.** 1967. Experimental murine cryptococcosis: effect of hyperimmunization to capsular polysaccharide. J. Immunol. 98:914–922.
9. **Goren, M. B., and J. Warren.** 1968.. Immunofluorescence studies of reactions at the cryptococcal capsule. J. Infect. Dis. 118:215–229.
10. **Graybill, J. R., and R. H. Alford.** 1974. Cell-mediated immunity in cryptococcosis. Cell. Immunol. 14:12–21.
11. **Kozel, T. R., and J. Cazin, Jr.** 1971. Nonencapsulated variant of *Cryptococcus neoformans*. I. Virulence studies and characterization of soluble polysaccharide. Infect. Immun. 3:287–294.
12. **Kozel, T. R., and J. Cazin, Jr.** 1972. Immune response to *Cryptococcus neoformans* soluble polysaccharide. I. Serological assay for antigen and antibody. Infect. Immun. 5:35–41.
13. **Kozel, T. R., and J. Cazin, Jr.** 1974. Induction of humoral antibody response by soluble polysaccharide of *Cryptococcus neoformans*. Mycopathol. Mycol. Appl. 54:21–30.
14. **Littman, M. L., and E. Tsubura.** 1959. Effect of degree of encapsulation upon virulence of *Cryptococcus neoformans*. Proc. Soc. Exp. Biol. Med. 101:773–777.
15. **Mitchell, T. G., and L. Friedman.** 1972. In vitro phagocytosis and intracellular fate of variously encapsulated strains of *Cryptococcus neoformans*. Infect. Immun. 5:491–498.
16. **Rabinovitch, M.** 1967. The dissociation of the attachment and ingestion phases of phagocytosis by macrophages. Exp. Cell. Res. 46:19–28.
17. **Rabinovitch, M.** 1967. The role of antibodies in the ingestion of aldehyde-treated erythrocytes attached to macrophages. J. Immunol. 99:232–237.
18. **Rabinovitch, M.** 1967. Studies on the immunoglobulins which stimulate the ingestion of glutaraldehyde-treated red cells attached to macrophages. J. Immunol. 99:1115–1120.
19. **Rabinovitch, M., and M. DeStefano.** 1973. Particle recognition by cultivated macrophages. J. Immunol. 110:695–701.
20. **Stossel, T. P.** 1973. Quantitative studies of phagocytosis. Kinetic effects of cations and heat-labile opsonin. J. Cell. Biol. 58:346–356.
21. **Tacker, J. R., F. Farhi, and G. S. Bulmer.** 1972. Intracellular fate of *Cryptococcus neoformans*. Infect. Immun. 6:162–167.
22. **Tizard, I. R.** 1972. Elution of macrophage-bound immunoglobulins by temperature changes in vitro. Immunology 22:69–73.