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Intracellular Fate of *Cryptococcus neoformans*

J. R. TACKER, F. FARHI, AND G. S. BULMER

Department of Microbiology and Immunology, University of Oklahoma College of Medicine, Oklahoma City, Oklahoma 73190

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Human peripheral leukocytes were found to engulf and kill cells of *Cryptococcus neoformans*. Fewer encapsulated than nonencapsulated cells met this fate, since cryptococcal capsular polysaccharide inhibited phagocytosis. During 10 to 12 hr of incubation of nonencapsulated cells in human serum, sufficient polysaccharide was produced to inhibit phagocytosis by 50%. The polysaccharide inhibitor was found in the sera of four patients with cryptococcosis, but not on the surfaces of their leukocytes. Additional experiments indicated that serum is not essential for effective phagocytosis. However, normal human serum contains anticryptococcal activity which is not inhibited by capsular material. Preliminary findings indicate that the phagocytic index of a patient with cryptococcosis may be correlated with the severity of his disease.

A microorganism engulfed during phagocytosis may be killed or it may survive and use the phagocyte as a vehicle of transport. In the case of pathogenic bacteria, most are destroyed within leukocytes. Few reports have been published about the intracellular fate of pathogenic fungi, however.

Louria and Brayton (12) reported that *Candida* cells were rapidly phagocytized by human polymorphonuclear leukocytes (PMN), but they remained viable after 4 hr of incubation. Lehrer and Cline (10) maintained that neutrophils kill engulfed cells of *C. albicans* with the lysosomal enzyme myeloperoxidase (MPO). The potential importance of MPO as an antimicrobial system has been stressed by numerous investigators in recent years (e.g., in reference 15), although *Aspergillus fumigatus* is not killed after ingestion by neutrophils (11). *Histoplasma capsulatum* cells have been found to actually multiply inside human, mouse, and guinea pig histiocytes (8) and engage in protein synthesis after being phagocytized by mouse histiocytes (9).

Previous work attests to the effectiveness of human peripheral leukocytes in phagocytizing cells of *Cryptococcus neoformans* (2), although the engulfing process is markedly and specifically inhibited by small amounts of cryptococcal capsular material (3). Such inhibition of cellular defense mechanisms—be it permanent, temporary, or briefly transient—may be of key importance in initiating human cryptococcal infection if, as indicated previously (7), only a few hours are required to produce the antiphagocytic capsular material after the nonencapsulated infectious particle enters the pulmonary milieu. This proposal presupposes, hoewever, that phagocytosis of *C. neoformans* by human cells results in their death. The present study was designed to determine whether this is, indeed, the case.

MATERIALS AND METHODS

Information on the following procedures has been published previously: source and maintenance of cultures (4), preparation of low-*p*H (LpH) and high-*p*H media for culturing *C. neoformans* in a nonencapsulated and normally encapsulated state, respectively (7); phagocytosis protocol (2); and isolation of cryptococcal capsular material (ACIA) (3, 7).

Determination of the fate of phagocytized yeast cells. Procedures were similar to those previouly reported for in vitro phagocytosis experiments (2), with the following modifications. Nonencapsulated cells of *C. neoformans*, strain CIA, cultured on LpH agar medium were suspended in unpooled human serum specimens from six normal males and six normal females, in a concentration of approximately 2.0×10^6 cells per ml, as determined with a standard hemocytometer. The individual cryptococcus-serum suspensions were dispensed, 0.5 ml per tube, into three sets of siliconized test tubes (100 by 15 mm). To each tube in set 1, 0.5 ml of a freshly collected homologous leukocyte suspension was added (3×10^6 to 4×10^6 leukocytes per ml), making the ratio of leukocytes to yeast cells in "fate" experiments about 2:1. In set 2, 0.5 ml of sterile 0.85% NaCl was added instead of the leukocyte suspension. In set 3, 0.5 ml of leukocyte suspension was added, followed by 0.5 ml of 0.5% sodium deoxycholate (DOC) to disrupt the leukocytes.

Tubes from sets 1, 2, and 3 were incubated at 37 C on a rotor (Scientific Industries, Inc.; 25 rev/min). At 2- and 4-hr intervals, 0.5 ml of 0.5% DOC was added to sets 1 and 2, and viable plate counts, using Sabouraud dextrose agar medium, were made from all three sets to enumerate the number of viable cells of *C. neoformans*. (Preliminary tests indicated that the

DOC rapidly disrupted all leukocytes and that a final concentration of up to 1.0% DOC had no adverse effect on the viability of *C. neoformans*.) Controls consisted of incubating similar concentrations of *C. neoformans* in 1.0 ml of Sabouraud dextrose broth and 1.0 ml of serum (heated for 45 min at 62 C) under similar conditions. In all instances, the percentage of viable cells was determinated by dividing the number of viable cells, at a given time period, by the number of viable cells (determined by viable counts) in the original suspension.

To determine the effect of serum-free leukocytes on yeast cells, the following experiment was performed. Eighty milligrams of sterile bovine fibrinogen, fraction I, was added to a sterile tube containing 20 ml of heparinized human venous blood. The tube was inverted twice. Equal volumes of this mixture were dispensed into two sterile centrifuge tubes which were then incubated in a 37 C water bath for 40 min. The resulting suspension of leukocytes in supernatant plasma was transferred by capillary pipette to fresh, sterile centrifuge tubes which were spun at 1,000 rev/min for 10 min. The supernatant fluid was discarded, and the packed white cells were suspended and washed three times in Hanks' balanced salt solution. Finally, the leukocytes were suspended in Eagle medium. This suspension was used in the "fate" experiments described above.

During the course of the research, the opportunity arose to determine the percentage of phagocytosis of *C. neoformans* cells from four patients with cryptococcosis, who were being treated at the University of Oklahoma Medical Center. (In each case, cultures of cerebrospinal fluid were positive.) Experiments were performed to determine whether there might be a connection between severity of disease and phagocytic events.

Assays for the production of capsular material in serum. The synthesis of capsular material by nonencapsulated cells of *C. neoformans* incubated in human serum was assayed as follows. First, 0.1 ml of a saline suspension containing 6×10^6 to 7×10^6 LpH medium-grown yeast cells was dispensed into siliconized tubes (100 by 15 mm) containing 0.9 ml of human serum. After 0, 2, 6, 11, and 24 hr of incubation at 37 C, 1.0 ml of autologous leukocyte suspension was added to each tube. Phagocytosis experiments were performed, and the percentage of phagocytosis was determined as described previously. Control tubes contained 1.0 ml of serum with 6×10^6 to 7×10^6 nonencapsulated cells, 1.0 ml of autologous leukocytes, and 0.85 mg of capsular material ACIA per ml.

Specificity of capsular material. It was of interest to explore why capsular material is so specific for *C. neoformans* cells. Cells of *C. neoformans* (strain CIA, cultured for 48 hr on LpH medium) were treated in three ways. (i) An aqueous suspension was autoclaved at 121 C for 15 min. (ii) A heavy suspension was stirred in acetone at 25 C for 8 hr and washed in acetone (repeated six times). (iii) Cells suspended in 0.6% Formalin were stirred for 4 hr at 25 C. After each treatment, cells were washed in sterile saline five times, and their nonviable state was verified by culturing. Capsular material ACIA was then added back to each of these samples to determine whether phagocytic activity was inhibited. If capsular material had been bound to any of these preparations, inhibition of phagocytosis would have occurred.

Location of factor responsible for the inhibition of phagocytosis. Experiments were then designed to determine whether the inhibition of phagocytosis of cells of *C. neoformans* incubated in serum was due to soluble or adhered polysaccharide. In all instances, leukocytes and serum were derived from the blood of a single donor. Nonencapsulated cells of *C. neoformans* (strain CIA, cultured on LpH agar medium) were suspended in 80 ml of human serum in a concentration of 7.5×10^7 cells/ml. The suspension was distributed among four tubes. These tubes, and one containing 8.0 ml of uninoculated serum (hereafter referred to as fresh, or F, serum), were incubated in a rotor at 37 C for 24 hr. The contents of the four tubes containing cells of *C. neoformans* were then pooled and centrifuged at $200 \times g$ for 30 min. (The sediment is hereafter referred to as old, or O, cells and the supernatant fraction as old, or, O serum.) Four ml of O serum was passed through a 0.45-μm membrane filter (Millipore Corp.) before use in the chemical and immunological studies described below. The remaining O serum was used in phagocytosis studies. To each of five siliconized tubes (100 by 15 mm), 1.0 ml of either F serum (three tubes) or O serum (two tubes) was added. To one tube of O serum, 0.1 ml of saline-yeast suspension was added. To one tube of O serum, 0.1 ml of saline-yeast suspension was added, which contained 6×10^6 to 7×10^6 cells of freshly harvested nonencapsulated cells (F cells), cultured for 48 to 72 hr on LpH agar medium, per ml. To one tube of F serum, 0.1 ml of saline which contained 6×10^6 to 7×10^6 O cells was added.

Controls consisted of 0.1 ml of O cells (same concentration used above) suspended in 1.0 ml of O serum, 0.1 ml of F cells in 1.0 ml of F serum, and 0.1 ml of F cells in 1.0 ml of F serum containing 0.85 mg of capsular material ACIA per ml. Autologous leukocytes suspended in 1.0 ml of plasma were added to each control tube. Phagocytosis experiments were performed as previously described.

Immunological and chemical detection of capsular polysaccharide in serum. Hyperimmune serum was prepared by immunizing rabbits with zeolite-treated antigens of *C. neoformans* CIA. (This material was obtained from Dr. R. F. Devlin, Department of Microbiology, University of Oklahoma Medical Center; R. F. Devlin, Ph.D. thesis, Univ. of Oklahoma Medical Center, Oklahoma City, 1969.) The agglutination titer of the serum, pooled from several rabbits, was greater than 1:640 when turbidity of the antigen was 2 McFarland units. To 5.0 ml of the rabbit anticryptococcal serum, 4.0 mg of capsule material ACIA was added from *C. neoformans* CIA. The mixture was incubated at 37 C for 1 hr. After refrigeration at 4 C overnight, the precipitate was removed by centrifugation at $350 \times g$ for 10 min. Because additional antibody was detected in the supernatant fluid when this material was used in a ring test (5), the absorption was carried out twice more with 2.5 and 0.5 mg of antigen, respectively. Thereafter, the ring test indicated a very slight antigen excess. To determine the presence of capsular polysaccharide in O serum, 0.1 ml of O serum

was layered over 1.0 ml of either absorbed or nonabsorbed serum in small test tubes. The tubes were incubated at 37 C for 60 min, and the presence of a precipitin band was recorded arbitrarily as +++ (strong band), ++, +, ±, or − (no band).

In another experiment, a solution was prepared consisting of 20 ml of a 1:10 dilution of O serum (prepared as described above), 4.0 ml of 2% $ZnSO_4 \cdot 7H_2O$, and 4.0 ml of 1.8% $Ba(OH)_2 \cdot 8H_2O$. It was mixed well and centrifuged at 350 × *g* for 10 min at 4 C. The supernatant fluid was dialyzed at 4 C against three changes of tap water and three changes of distilled water, after which it was lyophilized. The resulting material was suspended in 1.0 ml of distilled water. The presence of hexuronic acid was measured by the carbazole test (6). The standard curve for the carbazole test was plotted from readings taken on a Beckman DU spectrophotometer at 535 nm of seven samples. These consisted of the following amounts of capsular material ACIA suspended in 1.0 ml of serum: 2.5, 2.0, 1.5, 1.0, 0.5, 0.25, and 0.00 mg (blank).

RESULTS

Fate of phagocytized yeast cells. Of the nonencapsulated cells of *C. neoformans* ingested by human PMN, 19% remained viable after 4 hr of incubation at 37 C (Fig. 1). After the same incubation in Sabouraud broth or in serum which had been heated at 62 C for 30 min, 80 to 90% of the cells remained viable. Thus, the combination of human PMN leukocytes and serum kills cells of *C. neoformans* ($P < 0.05$). When cells of

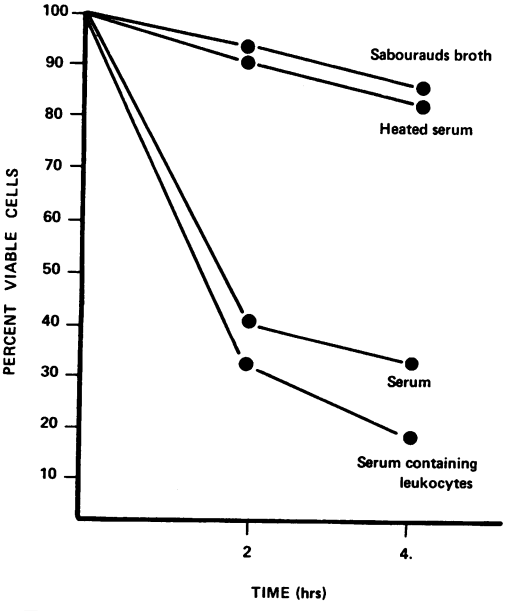

FIG. 1. *Viability of C. neoformans during incubation in Sabouraud broth, human serum (heated and unheated), and serum containing leukocytes from six normal males and six normal females.*

C. neoformans were incubated in serum alone for 4 hr at 37 C, or in serum containing deoxycholate-disrupted leukocytes, 34% of the yeast cells survived. Therefore, disrupted leukocytes with serum had an effect on the viability of nonencapsulated yeast cells similar to that of serum alone. The significant decrease ($P < 0.05$) in the number of viable cells of *C. neoformans* in the absence of intact or disrupted leukocytes indicates that normal human serum has anticryptococcal activity during a 2- to 4-hr incubation period. Since little decrease in viability occurred when the cells were incubated in heated serum, the anticryptococcal activity appears to be heat-labile. The difference between the lethal effect of intact leukocytes in serum, and in serum alone or with disrupted leukocytes (i.e., 19 versus 34% survival), is significant ($P < 0.05$). Thus, phagocytized cells of *C. neoformans* are killed by human peripheral leukocytes. The sex of the donor had no apparent effect on the ability of leukocytes or serum to kill *C. neoformans* cells. In these experiments, clumping of yeast cells did not appear to be important, based on phase-contrast microscope observations of samples periodically removed from the experimental system.

When serum was excluded from the system, and both yeast cells and leukocytes were incubated in Eagle medium, 90 to 100% of the leukocytes were viable after 4 hr of incubation at 37 C; however, only 30% of the yeast cells were viable. Thus, leukocytes effectively killed *C. neoformans* in the apparent absence of serum.

The ability of cryptococcal capsular polysaccharide to inhibit phagocytosis of *C. neoformans* was verified experimentally by using the serum and leukocytes from normal subjects. When cryptococcal capsular material ACIA in final concentration of 0.85 mg/ml was added to a suspension of serum, leukocytes, and nonencapsulated cells of *C. neoformans*, no killing of yeast cells could be attributed to phagocytosis. However, even in the presence of cryptococcal capsular material, the anticryptococcal activity in serum was evident.

When the leukocytes and sera from the four patients with varying stages of cryptococcosis were studied, the percentage of phagocytosis taking place after incubation with *C. neoformans* cells was appreciably lower than that observed with the cells and sera of healthy subjects (Table 1). However, these patients' leukocytes manifested normal phagocytosis of *C. neoformans* when mixed with the serum from a healthy individual. This was not true when patients' sera were tested with leukocytes from healthy subjects.

Specificity of capsular material. A previous study demonstrated that the antiphagocytic effect

of capsular material was highly specific for *C. neoformans* (3). An explanation is apparent from the results of an experiment in which percent phagocytosis was determined for treated and nontreated cells of *C. neoformans* with and without the addition of capsular material (Table 2). The extent of phagocytosis for untreated (viable) cells was 78%; with the addition of capsular material, it was only 26%. Capsular material did not inhibit phagocytosis of the treated cells.

Location and nature of factor responsible for the inhibition of phagocytosis. Figure 2 depicts the results of a series of experiments in which nonencapsulated cells of *C. neoformans* were incubated in human serum at 37 C. At various intervals, cells were removed and added to typical phagocytosis experiments which included fresh serum and homologous leukocytes. After 4 hr, the percent phagocytosis was determined. Thus, in these experiments inhibition of phagocytosis reflected the amount of capsule produced by cells of *C. neoformans* in human sera (2).

TABLE 1. *Phagocytosis of Cryptococcus neoformans with various combinations of leukocytes and serum from normal individuals and cryptococcal patients*

Patient	Phagocytosis[a] (%)		
	P.L. + P.S.	P.L. + N.S.	N.L. + P.S.
FJ	36	72	42
WK	20	77	32
HA	16	74	33
DI	52	—[b]	—

[a] Abbreviations: P = patient, L = leukocytes, S = serum, and N = normal. For each patient, a phagocytic control experiment was performed with the serum and leukocytes from a healthy subject. The average percent phagocytosis (for the four healthy subjects) was 75%.

[b] Subject unavailable.

TABLE 2. *Phagocytosis of treated nonencapsulated cells of C. neoformans with and without the addition of cryptococcal capsular material*

Treatment	Percent phagocytosis	
	Without capsule	With capsular material added[a]
Heat-killed	75	63
Acetone-killed	87	84
Formalin-killed	91	90
None (viable cells)	78	26

[a] Amount added = 850 μg/ml.

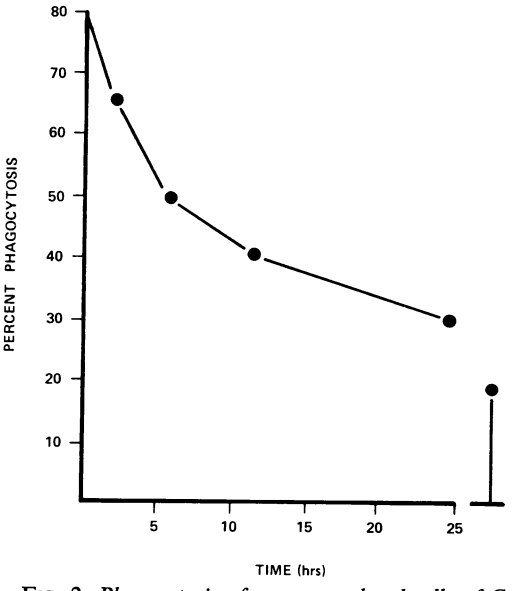

FIG. 2. *Phagocytosis of nonencapsulated cells of C. neoformans after incubation with human serum for varying time periods. Bar on right (control) represents the percent phagocytosis of nonencapsulated cells when 850 μg of capsular material (ACIA) per ml was added to the system.*

As the incubation time in serum increased, phagocytosis decreased. Incubation for 10 to 12 hr was required to reduce phagocytosis by 50%. After 25 hr of incubation, only 30% phagocytosis occurred. The data indicated that when nonencapsulated cells of *C. neoformans* are incubated in human sera for 12 hr, enough capsular material is synthesized to inhibit phagocytosis by 50%. To determine the location of the phagocytic-inhibiting factor, experiments were performed with combinations of treated cells and serum (Table 3). As observed previously, 79% phagocytosis occurred with untreated cells (F) and serum (F); with the addition of capsular material, it decreased to 28%. However, serum in which cells of *C. neoformans* were incubated for 24 hr (O serum) also decreased phagocytosis; this was not true when fresh serum (F) was used. These results confirmed the findings seen with cryptococcal patients, namely, that the phagocytic-inhibitory factor was present in the serum.

Immunological and chemical detection of capsular polysaccharide in serum. Since capsular polysaccharide is antiphagocytic (3), attempts were made to detect it immunologically and chemically in serum which had been incubated with cells of *C. neoformans* for 24 hr (O serum). The presence of capsule was measured with nonabsorbed and absorbed rabbit anticryptococcal

TABLE 3. *Phagocytosis with treated cells of C. neoformans and human serum in differing combinations*

Cells of *C. neoformans*[a]		Serum[b]		Cryptococcal capsular material (ACIA)[c]	Percent phagocytosis
O	F	O	F		
+	−	+	−	−	31
+	−	−	+	−	78
−	+	+	−	−	30
−	+	−	+	−	79
−	+	−	+	+	28

[a] O cells of *C. neoformans* were incubated in serum for 24 hr at 37 C; F cells of *C. neoformans* were freshly harvested (i.e., untreated).

[b] O serum: the serum remaining when O cells were removed after 24 hr at 37 C. F serum was freshly collected, untreated.

[c] Amount added = 850 μg/ml.

antibody. Although the sera reacted very strongly with nonabsorbed rabbit serum, there was very little, if any, reaction with absorbed serum (Table 4). The presence of capsular polysaccharide was also assayed chemically by the carbazole test, in which the presence of hexuronic acid, and to some extent xylose, was measured. As indicated in Table 4, 0.58 to 0.66 mg of capsular material per ml could be detected in the serum. The control represents serum not incubated previously with cells of *C. neoformans*.

DISCUSSION

Phagocytosis has been considered a potential defense mechanism against *C. neoformans* although it has never actually been demonstrated that killing by human peripheral leukocytes follows engulfment of this yeast (7). It is now evident that human peripheral leukocytes can kill phagocytized cells of *C. neoformans*. It was surprising to find that there was no statistical difference in killing between serum containing disrupted leukocytes and serum devoid of them. Apparently, the disruption procedure (DOC) did not release MPO or the assay methods did not detect it. It is well known that human peripheral leukocytes contain this antimicrobial agent (14), and studies currently in progress (*unpublished data*) in this laboratory indicate that MPO is a potent killing agent of *C. neoformans* and therefore probably played a major role in the killing of engulfed cells of *C. neoformans* in this study. However, since it is dangerous to contrast one system with another, or even one cell type from different sources, it is also dangerous to speculate on the roles of phagocytosis and MPO in cells other than neutrophils (e.g., the alveolar macrophages which airborne particles of *C. neoformans* would encounter in the lung milieu).

Human serum does not appear to be necessary for phagocytic killing; however, there is always the possibility that not all of the serum components were removed in the washing process in our experiments.

Human serum contains anticryptococcal activity. For several years it has been known that sera from man, various animals, and birds contain this activity (1, 15); however, many of the reports present conflicting data on the nature of this material. Additionally, since incubation periods of several days are required to demonstrate lethal effect, it has been difficult to relate it to in vivo situations. These data show that it is a potent killing agent during 2 to 4 hr of incubation in vitro, is heat-labile, and is not inhibited by cryptococcal capsular material. Thus, during the disease state, it may function as a potent defense mechanism.

A previous report suggested that the infectious particle of *C. neoformans* was nonencapsulated in soil and that capsular production was initiated when the nonencapsulated cells were incubated with human lung tissue (7). Since cryptococcal capsular material is antiphagocytic in microgram amounts (3), it seemed desirable to examine its synthesis in human serum, as well as to learn whether this material is present in the serum of cryptotoccal patients, why it is so specific for *C. neoformans* (3), and how it inhibits the phagocytic (and thereby killing) process. The experiments described in this report at least partially answer these questions.

When nonencapsulated cells of *C. neoformans* were incubated in human serum for 10 to 12 hr, sufficient capsular material was produced to inhibit phagocytosis by 50%. After 25 hr of incubation, phagocytosis dropped to less than 30%. The time required for capsular synthesis in

TABLE 4. *Immunological and chemical evidence for cryptococcal polysaccharide in human sera*

Experimental subject	Precipitation ring test		
	Nonabsorbed anticrypto-coccal serum	Absorbed anticrypto-coccal serum	Polysaccharide in serum (mg/ml)
1	+++[a]	±	0.60
2	+++	±	0.66
3	++	−	0.58
4	+++	+	0.63
Control	−	−	0.00

[a] See text for explanation of symbols.

serum was much longer than previously observed with lung tissue, probably due to the killing of many yeast cells by the anticryptococcal activity of serum. When patients' sera were added to normal human leukocytes, the percent phagocytosis decreased. Since the converse was not true, the phagocytic inhibitor would appear to reside in the serum. The presence of such an inhibitor in patients' sera, which our findings indicate to be cryptococcal capsular material, is probably important in the pathogenesis of the disease. It may, by depressing phagocytosis, indicate to the physician a prognosis for the patient. Although we studied phagocytosis in only four patients, a correlation appeared to exist between percent phagocytosis and prognosis (i.e., the disease was fatal to the three patients who had a phagocytic capability of less than 36%). The only patient who survived had 52% phagocytosis. Although the findings from such a small sample cannot necessarily be considered valid, they suggest an area for further study.

For several years, cryptococcal capsular material has been known to be remarkably inert in the fluids of body tissues (13). Since this material is antiphagocytic, an attempt to remove it from patients with cryptococcosis would seem worthwhile. By reducing the amount of capsular material, the phagocytes might be rendered more effective or the possibility of reinfection decreased after the disease is under control.

Some insight has been gained into why cryptococcal capsular material is specific for cells of *C. neoformans*. Capsular material did not inhibit the phagocytosis of killed, nonencapsulated cells, regardless of the type of treatment. Thus, either the nonencapsulated cells must be viable or, perhaps, the treatment used on these cells altered cell wall sites responsible for the attachment of the antiphagocytic polysaccharide. If the latter is true, these sites may be constructed so as to receive only the specific capsular material produced by a given strain. Alternatively, these sites may merely represent microtributaries through which capsular material is released through the cell wall, and the demonstrated specificity may represent an affinity between capsular materials which are quantitatively and qualitatively homogeneous.

All of these factors make it apparent that, if cellular defense is an important defense mechanism against *C. neoformans*, it must function effectively within a few hours after the nonencapsulated infectious particle enters a potential host. If this response is depressed or even delayed, by whatever mechanism, the organism will synthesize the antiphagocytic capsular material, and an infection may ensue.

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LITERATURE CITED

1. Baum, G. L., and D. Artis. 1963. Characterization of the growth inhibition factor for *Cryptococcus neoformans* (G.I. Fe) in human serum. Amer. J. Med. Sci. 246:53–57.
2. Bulmer, G. S., and M. D. Sans. 1967. *Cryptococcus neoformans*. II. Phagocytosis by human leukocytes. J. Bacteriol. 94:1480–1483.
3. Bulmer, G. S., and M. D. Sans. 1968. *Cryptococcus neoformans*. III. Inhibition of phagocytosis. J. Bacteriol. 95:5–8.
4. Bulmer, G. S., M. D. Sans, and C. M. Gunn. 1967. *Cryptococcus neoformans*. I. Nonencapsulated mutants. J. Bacteriol. 94:1475–1479.
5. Campbell, D. H., J. S. Garvey, N. E. Cremer, and D. H. Sussdorf. 1964. Methods in immunology. W. A. Benjamin, Inc., New York.
6. Dische, Z. 1950. A modification of the carbazole reaction cf hexuronic acids for the study of polyuronides. J. Biol. Chem. 183:489–494.
7. Farhi, F., G. S. Bulmer, and J. R. Tacker. 1970. *Cryptococcus neoformans*. IV. The not-so-encapsulated yeast. Infect. Immunity 1:526–531.
8. Howard, D. H. 1965. Intracellular growth of *Histoplasma capsulatum*. J. Bacteriol. 89:518–523.
9. Howard, D. H., and V. Otto. 1969. Protein synthesis by phagocytized yeast cells of *Histoplasma capsulatum*. Sabouraudia 7:186–194.
10. Lehrer, R. I., and M. J. Cline. 1969. Interaction of *Candida albicans* with human leukocytes and serum. J. Bacteriol. 98:996–1004.
11. Lehrer, R. I., and R. G. Jan. 1970. Interaction of *Aspergillus fumigatus* spores with human leukocytes and serum. Infect. Immunity 1:345–350.
12. Louria, D. B., and R. G. Brayton. 1964. Behavior of *Candida* cells within leukocytes. Proc. Soc. Exp. Biol. Med. 115:93–98.
13. Neill, J. M., U. Y. Sugg, and D. W. McCauley. 1951. Serologically reactive material in spinal fluid, blood and urine from a human case of cryptococcosis (torulosis). Proc. Soc. Exp. Biol. Med. 77:775–778.
14. Sbarra, A. J., A. A. Jacobs, R. R. Strauss, B. B. Paul, and G. W. Mitchell. 1971. The biochemical and antimicrobial activities of phagocytizing cells. Amer. J. Clin. Nutr. 24:272–281.
15. Szilagyi, G., F. Reiss, and J. C. Smith. 1966. The anticryptococcal factor of blood serum: a preliminary report. J. Invest. Dermatol. 46:306–308.