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Activated neutrophils exhibit enhanced phagocytosis of *Cryptococcus neoformans* opsonized with normal human serum

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SUMMARY

We studied the effect of agents that activate neutrophils on phagocytosis of *C. neoformans*. The amount of CR3 on the surface of neutrophils was used as a marker for neutrophil activation. Surface CR3 was estimated by flow cytometry using phycoerythrin-labelled anti-CR3 (anti-Leu-15) monoclonal antibody. Phagocytosis was determined by incubation of neutrophils with cryptococci that had been preincubated with normal human serum. We found that treatment of neutrophils with (i) the chemotactic peptide N-formyl-methionyl-leucyl-phenylalanine, (ii) zymosan activated serum, (iii) supernatant fluid from a mixed leucocyte culture, or (iv) supernatant fluid from human leucocytes cultured with phytohaemagglutinin produced a dose-dependent increase in CR3 density. These agents also markedly enhanced phagocytosis of opsonized cryptococci in a parallel dose-dependent fashion. These results indicate that phagocytosis of cryptococci opsonized with normal human serum is markedly enhanced by treatment of neutrophils with reagents that stimulate neutrophils. Our results demonstrate that neutrophils activated in an appropriate manner are capable of efficient phagocytosis of encapsulated cryptococci. This potential phagocyte activity may account in part for the high natural resistance to cryptococcosis.

Keywords phagocytosis *Cryptococcus neoformans* neutrophil activation opsonization capsule

INTRODUCTION

Cryptococcosis is a fungal infection produced by the yeast *Cryptococcus neoformans*. The disease occurs most frequently in patients who are compromised by diabetes mellitus, corticosteroid therapy or lymphoreticular malignancy (Diamond & Bennett, 1974). More recently, cryptococcosis has emerged as a leading cause of death in patients with acquired immune deficiency syndrome (Kovacs *et al.*, 1985). The yeast is ubiquitous, suggesting that natural resistance to disease is high. The reasons for this are unknown. Incubation of encapsulated cryptococci in normal human serum leads to the deposition iC3b (Kozel, Highison & Stratton, 1984; Kozel & Pfrommer, 1986) and IgG (Kozel *et al.*, 1984; McGaw & Kozel, 1979) within and at the surface of the capsule. Despite the presence of these potential opsonic ligands, phagocytosis of the yeast is limited (Bulmer & Sans, 1967; Davies *et al.*, 1982; Diamond *et al.*, 1974; Kozel *et al.*, 1984). However, once phagocytized, the yeast is rapidly killed by neutrophils and monocytes (Diamond, Root & Bennett, 1972). Thus, a key

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factor in resistance to cryptococcosis is the ability of phagocytic cells to overcome the antiphagocytic action of the cryptococcal capsule.

It has become evident that human neutrophils are activated by a variety of stimuli. The characteristics of activated neutrophils include: enhanced expression of complement receptors CR1 and CR3 (Berger *et al.*, 1984; Fearon & Collins, 1983), increased capacity of complement receptors to promote binding and phagocytosis of C3b- and iC3b-coated erythrocytes (Kay, Glass & Salter, 1979; Wright & Meyer, 1986), increased cell locomotion (Kay *et al.*, 1979), increased expression of the neutrophil-associated proteins p90 and p170 (Tetteroo *et al.*, 1986), increased expression of receptors for formyl-methionyl-leucyl-phenylalanine (f-mlp; Fearon & Collins, 1983), changes in cell shape (Hoffstein, Friedman & Weissmann, 1982), and increased adhesiveness (Hoffstein *et al.*, 1982). Stimuli that induce one or more of these correlates of activation include the chemotactic peptide f-mlp (Fearon & Collins, 1983; Berger *et al.*, 1984), phorbol myristate acetate (PMA; Wright & Meyer, 1986; Tetteroo *et al.*, 1986), incubation of purified neutrophils at 37°C (Fearon & Collins, 1983), mixed lymphokines obtained from human leucocytes cultured with phytohaemagglutinin (PHA; Kay *et al.*, 1979), and C5a des Arg (Fearon & Collins, 1983).

The ability of neutrophils to acquire multiple new properties on activation suggested that activated neutrophils might exhibit increased phagocytosis of encapsulated cryptococci. As a consequence, we examined the ability of several potential activators to influence phagocytosis of cryptococci that were opsonized with normal human serum. Expression of neutrophil CR3 was used as a marker for neutrophil activation (Berger *et al.*, 1984). The results showed that activated neutrophils had a markedly enhanced ability to phagocytize cryptococci, and the increased phagocytic activity closely paralleled the level of neutrophil activation.

MATERIALS AND METHODS

Yeast cells. A *C. neoformans* isolate of serotype D (ATCC 24067; American Type Culture Collection, Rockville, MD) was used throughout this study. Yeast cells were grown in a yeast extract dialysate medium (Huppert & Bailey, 1963), killed with formalin (Kozel & Mastroianni, 1976), and stored at 4°C as a suspension in sterile saline (0·15 M NaCl).

Preparation of neutrophil activators. The synthetic chemoattractant N-formyl-methionyl-leucyl-phenylalanine (f-mlp; Sigma Chemical Co., St Louis, MO) was prepared as a 10^{-3} M stock solution in dimethyl sulphoxide and stored at −70°C. Zymosan was boiled in 0·15 M NaCl for 30 min and washed once with 0·15 M NaCl before use. Zymosan-activated serum was prepared by incubating normal human serum with 0·5 mg zymosan per millilitre serum for 2 h at 37°C. Zymosan was removed from the activated serum by centrifugation and filtration through a 0·44 μm Millipore filter. The activated serum was stored at −70°C.

Supernatant fluid was obtained from a mixed leucocyte culture (MLC). Twenty millilitres of peripheral blood was collected from each of five donors and was treated with 10 U/ml heparin to prevent coagulation. The blood was diluted with an equal volume of heparinized (10 U/ml) Hanks balanced salt solution (HBSS; Gibco Laboratories, Grand Island, NY), and the mononuclear cells were isolated by centrifugation through a Ficoll-Hypaque gradient (Böyum, 1968). The isolated mononuclear cells were washed twice with HBSS and resuspended at a final concentration of 2×10^6 cells/ml in RPMI 1640 (Gibco Laboratories) containing 1 mM sodium pyruvate, 50 U penicillin/ml, 50 μg streptomycin/ml, 5×10^{-5} M 2-mercaptoethanol, and 5% fetal calf serum (complete RPMI). After incubation for 48 h at 37°C in 5% CO_2, the cells were removed by centrifugation, the supernatant fluid was sterilized by filtration through a 0·22 μm Millipore membrane, and the MLC supernatant was stored at −70°C. Complete RPMI 1640 medium containing pyruvate, penicillin, streptomycin, 2-mercaptoethanol, and fetal calf serum was used as a negative control.

Phytohaemagglutinin (PHA; Burroughs Wellcome Co., Research Triangle Park, NC) was also used to stimulate release of lymphokines by lymphocytes. A mixed leucocyte culture was prepared as described above, with the exception that PHA was added to the culture medium at a final concentration of 1·0 μg/ml. The leucocytes were incubated with PHA for 24 h at 37°C in 5% CO_2. The PHA-stimulated supernatant was collected and stored as described above for the MLC supernatant. Complete RPMI medium containing PHA at 1·0 μg/ml served as a negative control.

Neutrophil isolation and treatment. Peripheral blood was drawn from normal donors, anticoagulated with heparin (10 U/ml), and the neutrophils were isolated on discontinuous Ficoll-Hypaque density gradients (English & Andersen, 1974). Isolated neutrophils were washed twice with HBSS containing 0·5% BSA (HBSS-BSA), and resuspended at 5×10^5 cells/ml in HBSS-BSA. Unless otherwise indicated, isolated neutrophils were kept at 0°C before use. Neutrophils were treated with activating agents by incubating $2{\cdot}5 \times 10^6$ neutrophils with the indicated concentration of activating agent in a final volume of 2·5 ml for 30 min at 37°C. The cells were immediately placed on ice, washed once with HBSS-BSA, and resuspended in HBSS-BSA at 5×10^5 cells/ml.

Phagocytosis. Cryptococci opsonized with normal human serum were prepared by incubating 5×10^6 yeasts (50 μl in sterile saline) with 500 μl fresh frozen human serum and 1·95 ml HBSS for 30 min at 37°C. The opsonized cryptococci were collected by centrifugation, washed once with HBSS, and resuspended in 2·5 ml HBSS-BSA.

Treated or untreated neutrophils (0·5 ml HBSS-BSA containing $2{\cdot}5 \times 10^5$ cells) were mixed with an equal volume of HBSS-BSA containing 1×10^6 opsonized cryptococci. The cells were then tumbled on a rotator for 1 h at 37°C. Slides were prepared with a cytocentrifuge; the cells were fixed with methanol, stained with Giemsa stain, and examined microscopically. The percentage of neutrophils with ingested yeasts as well as the number of ingested yeasts per neutrophil were determined. The large size of the yeast permitted a clear distinction between attached and ingested yeasts. The data are reported as the phagocytic index which is the number of ingested yeasts per 100 neutrophils. Two hundred neutrophils were examined on each slide. Four replications were done for each experiment. All data reported are the average of three separate experiments ± the standard error of the mean (s.e.m.).

Quantification of CR3 density on neutrophils by flow cytometry. CR3 density was determined by staining neutrophils with phycoerythrin-labelled anti-Leu-15 (Becton-Dickinson, Mountain View, CA), an antibody reactive with an epitope on human CR3 (Ross, Cain & Lachmann, 1985), followed by flow cytometry. Isolated neutrophils (8×10^5 cells) were collected by centrifugation and resuspended in 50 μl PBS containing 1% fetal calf serum and 0·1% sodium azide (staining buffer). Phycoerythrin-labelled anti-Leu-15 (20 μl) was added, and the cells were incubated for 30 min at 0°C. As a negative control for non-specific Fc-mediated binding, neutrophils were incubated with a similar amount of phycoerythrin-labelled mouse IgG_{2a}. This monoclonal antibody (Becton Dickinson, cat. no. 9053) is of the same heavy chain isotype as the anti-Leu-15, but it has no specificity for human neutrophil antigens. Unstained neutrophils incubated with staining buffer alone served as controls to establish a baseline for measurement of fluorescence. After incubation with the labelled monoclonal antibody, 1·0 ml of staining buffer was added, and the cells were collected by centrifugation. The cells were washed once with 1·0 ml staining buffer, resuspended in 1 ml of the same buffer, and held at 0°C until examination by flow cytometry.

Flow cytometry was performed with a FACS Analyzer (Becton Dickinson) equipped with the standard phycoerythrin–fluorescein filter pack, Coulter volume sensor and right angle light scatter detector. The FACS Analyzer was standardized with 7·8 μm diameter FITC-beads (Becton Dickinson). Since the neutrophils contained contaminating platelets and erythrocytes, volume and right angle light scatter gates were set to collect data only from neutrophils (10,000 per assay). All data were collected using three decade logarithmic amplification and converted to their linear equivalent. Unstained neutrophils displayed the same amount of autofluorescence for all cells in the same experiment regardless of treatment. Stained neutrophils that were kept at 0°C were assigned a relative fluorescence value of 1·0 to reflect the amount of CR3 on unstimulated neutrophils (Berger *et al.*, 1984; Fearon & Collins, 1983). All other fluorescence data in each experiment were normalized to this value. Samples are compared by the arithmetic means of these normalized data. Data are reported as the mean of three independent experiments. Relative volume (rv) of a given group of cells was converted to relative surface area (rsa) using the equation $(rv)^{2/3} = (rsa)$.

RESULTS

Our initial experiments were done to determine the ability of neutrophils treated by incubation with or without f-mlp at 37°C to engulf cryptococci that were opsonized by incubation with either

Table 1. Effect of incubation at 37°C and treatment with f-mlp on the expression of CR3 and the phagocytic activity of neutrophils isolated from human blood

PMN treatment	Phagocytic index* Yeast treatment† 56°C NHS	Phagocytic index* Yeast treatment† NHS	Anti-CR3 relative fluorescence‡
0°C	0·2±0·1	53±16	1·0
37°C	1·0±1·0	53±15	5·4±2·1
37°C+f-mlp (10^{-6} M)	1·3±1·2	114±28	22·4±6·4

* Phagocytosis data are reported as the mean numbers of ingested yeasts/100 neutrophils (phagocytic index). Data are reported as the means of three separate experiments±s.e. Each experiment contained four replications

† Encapsulated cryptococci were preincubated for 30 min at 37°C with normal human serum (NHS) or normal human serum that had been heat inactivated for 30 min at 56°C (56° NHS).

‡ Fluorescence data are reported on a linear scale. Data shown are the means of three separate experiments±s.d.

Table 2. Increased phagocytic activity and CR3 density following incubation of neutrophils with various concentrations of f-mlp

PMN treatment*	Phagocytic index†	Anti-CR3 relative fluorescence‡
37°C, HBSS alone	58±10	3·8±2·3
37°C, f-mlp, 10^{-11} M	48±2	4·4±1·8
37°C, f-mlp, 10^{-10} M	49±3	4·7±1·9
37°C, f-mlp, 10^{-9} M	85±3	9·4±3·1
37°C, f-mlp, 10^{-8} M	118±3	18·8±8·2
37°C, f-mlp, 10^{-7} M	112±6	20·3±8·7
37°C, f-mlp, 10^{-6} M	111±3	18·1±7·7

* Neutrophils were incubated with HBSS or HBSS containing f-mlp for 30 min at 37°C.

† Phagocytosis data are reported as the mean number of ingested yeasts/100 neutrophils (phagocytic index). All yeasts were opsonized with normal human serum. Data are reported as the means of three separate experiments±s.e. Each experiment contained four replications.

‡ Fluorescence data are reported on a linear scale. Data shown are the means of three separate experiments±s.d.

normal or heat inactivated human serum. Heat inactivated serum was used as a control to verify that opsonization of the yeast was due to heat-labile opsonins (Kozel *et al.*, 1984; Kozel & Pfrommer, 1986). A parallel assay was done to determine the level of CR3 expression on the neutrophils. CR3 expression was used as a marker for neutrophil activation (Berger *et al.*, 1984; Fearon & Collins, 1983; O'Shea, *et al.*, 1985; Wright & Meyer, 1986). The results (Table 1) showed that neutrophils incubated at 37°C and labelled with anti-CR3 showed a 5-fold increase in fluorescence compared with the fluorescence observed on neutrophils held at 0°C. Neutrophils

Table 3. Effect of zymosan-treated serum on neutrophil phagocytosis and surface CR3 density

PMN treatment*	Phagocytic index†	Anti-CR3 relative fluorescence‡
37°C, HBSS alone	40±2	4·4±1·4
37°C, 0·2% treated serum	81±6	9·8±1·8
37°C, 1·0% treated serum	96±9	14·4±2·2
37°C, 5·0% treated serum	120±14	16·1±2·4

* Neutrophils were incubated with the activating agent for 30 min at 37°C.

† Phagocytosis data are reported as the mean numbers of ingested yeasts/100 neutrophils (phagocytic index). All yeasts were opsonized with normal human serum. Data are reported as the means of three separate experiments±s.e. Each experiment contained four replications.

‡ Fluorescence data are reported on a linear scale. Data shown are the means of three separate experiments±s.d.

incubated with 10^{-6} M f-mlp demonstrated a 22-fold increase in labelling with anti-CR3. The phagocytic activity of neutrophils preincubated at 37°C was equivalent to the activity of neutrophils that were kept at 0°C before the phagocytosis assays (Table 1). Presumably, the 60 min incubation used for the phagocytosis assay was sufficient to permit activation of neutrophils that had been kept previously at 0°C. Neutrophils that were activated with f-mlp had a markedly enhanced phagocytosis of cryptococci opsonized with normal serum. Cryptococci opsonized with heat-inactivated serum were not ingested efficiently by any group of neutrophils, regardless of the manner of neutrophil activation.

We compared the dose of f-mlp that was required to activate neutrophils as shown by increased CR3 density with the dose of f-mlp required to enhance phagocytosis of cryptococci opsonized with human serum. Neutrophils were incubated for 30 min at 37°C with HBSS-BSA or HBSS-BSA

Table 4. Effect of supernatant fluid from mixed leucocyte culture (MLC) on neutrophil phagocytosis and surface CR3 density

PMN treatment	Phagocytic index*	Anti-CR3 relative fluorescence†
37°C, HBSS alone	40±3	4·4±1·4
37°C, 1·0% control medium‡	43±12	4·6±1·5
37°C, 5·0% control medium	42±9	5·3±1·6
37°C, 20% control medium	65±6	7·5±2·5
37°C, 1·0% MLC supernatant§	62±3	9·6±2·4
37°C, 5·0% MLC supernatant	79±4	11·5±2·0
37°C, 20% MLC supernatant	100±8	14·0±2·4

* Phagocytosis data are reported as the mean numbers of ingested yeasts/100 neutrophils (phagocytic index). All yeasts were opsonized with normal human serum. Data are reported as the means of three separate experiments±s.e. Each experiment contained four replications.

† Fluorescence data are reported on a linear scale. Data shown are the means of three separate experiments±s.d.

‡ Complete RPMI medium.

§ Supernatant medium from a 48 h mixed leucocyte culture.

Table 5. Effect of supernatant fluid from phytohaemagglutinin (PHA) stimulated leucocytes on neutrophil phagocytosis and surface CR3 density

PMN treatment	Phagocytic index*	Anti-CR3 relative fluorescence†
37°C, HBSS	40±3	4·4±1·4
37°C, 1·0% control medium‡	38±8	4·1±1·1
37°C, 5·0% control medium	42±8	4·4±1·1
37°C, 20% control medium	51±6	5·5±2·5
37°C, 1·0% PHA supernatant§	75±12	7·5±3·6
37°C, 5·0% PHA supernatant	96±9	12·2±4·8
37°C, 20% PHA supernatant	130±22	17·9±2·6

* Phagocytosis data are reported as the mean numbers of ingested yeasts/100 neutrophils (phagocytic index). All yeasts were opsonized with normal human serum. Data are reported as the means of three separate experiments ± s.e. Each experiment contained four replications.

† Fluorescence data are reported on a linear scale. Data shown are the means of three separate experiments ± s.d.

‡ RPMI medium containing 1 μg PHA/ml.

§ Supernatant medium from leucocytes cultured for 24 h with PHA (1 μg/ml).

containing various concentrations of f-mlp that ranged from 10^{-6} to 10^{-11} M. The results (Table 2) showed that a dose-dependent enhancement of phagocytosis occurred at the same concentrations of f-mlp required to stimulate expression of neutrophil CR3.

The increase in labelling with phycoerythrin-labelled anti-Leu-15 could reflect changes in non-specific binding via the neutrophil Fc receptor. To exclude this possibility, we incubated neutrophils with a phycoerythrin-labelled irrelevant monoclonal antibody of the same heavy chain isotype as the anti-Leu 15. Neutrophils that were held at 0°C, incubated at 37°C, incubated at 37°C with 10^{-9} M f-mlp, or incubated at 37°C with 10^{-7} M f-mlp showed relative staining with the irrelevant antibody of 1·0, 0·98, 0·98, and 1·03, respectively. Thus, the increased labelling of the stimulated neutrophils could not be attributed to an alteration in non-specific binding mediated by Fc receptors. Furthermore, the increased labelling appeared to reflect an increase in CR3 density since the relative volume of neutrophils treated by incubation at 37°C alone or with 10^{-6} M f-mlp increased only by a factor of approximately 1·1 (data not shown).

Fearon & Collins (1983) showed that neutrophils were activated by incubation with C5a desArg. Therefore, we examined the ability of zymosan-activated serum to influence the ability of neutrophils to engulf cryptococci opsonized with normal human serum. Neutrophils incubated with zymosan-activated serum in final concentrations ranging from 0·2–5% exhibited a dose-dependent increase in expression of CR3 (Table 3). These stimulated neutrophils displayed a concomitant enhancement of phagocytosis of cryptococci that were opsonized with normal human serum.

Kay *et al.* (1979) found that neutrophils were activated by incubation with supernatant fluid from cultured neutrophils. This prompted us to examine the activating properties of lymphokine-containing supernatants from MLC or PHA-stimulated lymphocytes. Both the MLC and the PHA-stimulated supernatants activated neutrophils as shown by increased expression of neutrophil CR3 over a dose range of 1–20% supernatant fluid (Tables 4 and 5). These treated neutrophils exhibited increased phagocytosis of cryptococci over the same dose range. The results of control experiments (Tables 4 and 5) showed that neither the culture medium alone nor culture medium containing PHA could account for the enhanced expression of CR3 and increased phagocytic activity of neutrophils.

DISCUSSION

Our results showed that neutrophils activated by a variety of stimuli display enhanced phagocytosis of cryptococci that were opsonized with normal human serum. Increased expression of membrane CR3 was used as an indicator of neutrophil activation (Berger *et al.*, 1984). Expression of CR3 and phagocytosis of cryptococci were concomitantly enhanced by all stimuli that were examined, e.g. incubation at 37°C, incubation with f-mlp, incubation with zymosan-activated serum, and incubation with mixed lymphokines. These same stimuli have been shown by other investigators to induce one or more of the parameters characteristic of activated neutrophils (Berger *et al.*, 1984; Fearon & Collins, 1983; Kay *et al.*, 1979). In addition, there was a close correlation between the dose of the stimulant required to stimulate CR3 expression and enhanced phagocytosis of the yeast. This parallel dose-response effect was observed with all stimulants that were examined. Taken together, these data provide strong evidence that activation of neutrophils leads to enhanced phagocytosis of cryptococci that are opsonized with normal human serum.

It is clear that the enhanced phagocytosis is dependent upon opsonic fragments of the complement cascade. Cryptococci that were incubated with heat-inactivated serum were not engulfed by neutrophils, regardless of the level of activation (Table 1). Several investigators have shown that incubation of encapsulated cryptococci in normal human serum leads to deposition of C3 fragments on the yeast (Diamond *et al.*, 1974; Goren & Warren, 1968). We used immunoperoxidase staining and electron microscopy to determine that the C3 fragments bound to the capsule are located primarily at and near the surface of the capsule (Kozel *et al.*, 1984). Little or no C3 was found deep within the capsule or at the cell wall. In subsequent studies, encapsulated cryptococci were incubated with human serum supplemented with ^{125}I-labelled C3. The bound C3 was eluted with hydroxylamine, and the released fragments of C3 were identified on the basis of molecular weight as iC3b (Kozel & Pfrommer, 1986). Thus, potentially opsonic C3 fragments are spatially located in a manner that would permit interaction with phagocyte complement receptors.

The presence of large amounts of opsonically active fragments of C3 at the surface of the capsule and the enhanced expression of complement receptors on activated neutrophils suggest a causal relationship between the quantitative increase in expression of CR1 and/or CR3 and phagocytosis of the yeast. However, other alternatives are also possible. Enhanced phagocytosis may be due to qualitative changes in the complement receptors. For example, Wright & Meyer (1986) found that treatment of neutrophils with PMA induced not only a quantitative increase in the amount of surface CR3 but also a qualitative change in the ability of the neutrophils to bind and ingest erythrocytes coated with iC3b. Enhanced phagocytosis of opsonized cryptococci may also be due to a general increase in the phagocytic activity of activated neutrophils or to an increase in the activity of Fc receptors. This latter alternative is less likely because neutrophil activation does not appear to be associated with increased Fc receptor expression (Berger *et al.*, 1984). Indeed, a decrease in Fc receptor activity has been reported to follow activation with PMA (Wright & Meyer, 1986).

Our results do not indicate the basal level of phagocytosis that would be exhibited by a freshly isolated and unstimulated neutrophil because the incubation of neutrophils at 37°C for a phagocytosis experiment would also activate the neutrophils (Berger *et al.*, 1984; Fearon & Collins, 1983). Therefore, phagocytosis of cryptococci by neutrophils kept at 0°C before the phagocytosis experiment (Table 1) is probably due to phagocytosis by neutrophils that have been activated as a consequence of the phagocytosis experiment itself. It is possible, even likely, that unstimulated neutrophils would exhibit a very limited phagocytosis of cryptococci opsonized with normal human serum.

Our results suggest a reconsideration of the role of the cryptococcal capsule in phagocytosis of this yeast. Unlike most encapsulated microorganisms, the cryptococcal capsule focuses opsonic complement fragments at the surface of the yeast. Thus, an appropriate ligand is available for interaction with phagocyte complement receptors. A crucial event appears to be the availability and activity of these receptors. It is evident that resting neutrophils express a limited number of receptors for C3b and iC3b (Berger *et al.*, 1984; Fearon & Collins, 1984). This number increases to a moderate extent when the phagocytes are isolated and incubated at 37°C. This increase in

expression appears to be due to a translocation of CR3 from an existing intracellular pool to the neutrophil membrane because the increased expression of neutrophil CR1 and CR3 is complete within minutes and is not blocked by inhibitors of protein synthesis (Berger *et al.*, 1984). In addition to the quantitative increase in CR3 expression, there is a qualitative change in the ability of CR3 to bind surface-bound ligands and to promote phagocytosis (Wright & Meyer, 1986). The consistent reports (Bulmer & Sans, 1967; Davies *et al.*, 1982; Diamond *et al.*, 1974; Kozel *et al.*, 1984) of reproducible but low efficiency phagocytosis of the yeast by neutrophils are probably due to CR3 receptors that are translocated during the phagocytosis experiments. It is apparent that the full potential of neutrophils for phagocytosis of the yeast has not been reported because the neutrophils were not stimulated in a manner that would permit full expression of this potential.

Our results also have importance for the pathogenesis of cryptococcosis. Cryptococcosis is an opportunistic infection that occurs infrequently. Our results suggest that phagocytes are capable of efficient phagocytosis of the yeast if the phagocytes are stimulated in an appropriate manner. This may account in part for the high natural resistance to cryptococcosis. These results further suggest that patients with an inability to stimulate expression of phagocyte CR3 will be at increased risk for cryptococcosis. Little is known about the *in vivo* factors that influence neutrophil activation, but a study of these factors will doubtless contribute to our understanding of why certain patients are at increased risk for cryptococcosis.

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