Interaction between Cryptococcus neoformans and alveolar macrophages

N. T. GROSS,*†‡ K. NESSA,*† P. CAMNER,† M. CHINCHILLA§ & C. JARSTRAND*
*Division of Clinical Bacteriology, Huddinge University Hospital, Huddinge, Sweden; †Division of Inhalation Toxicology, Institute of Environmental Medicine, Karolinska Institute, Stockholm, Sweden; ‡Division of Microbiology and Immunology, Faculty of Microbiology, University of Costa Rica, San José, Costa Rica; and §Centro de Investigaciones en Enfermedades Tropicales (CIET), Department of Parasitology, Faculty of Microbiology, University of Costa Rica, San José, Costa Rica

Phagocytosis, oxidative metabolism and phagolysosomal pH of rat alveolar macrophages (AM) were studied at different points of time after challenge with Cryptococcus neoformans. Phagocytosis was evaluated using a fluorescent quenching technique which distinguishes between attached and ingested organisms. The nitroblue tetrazolium (NBT) test was used as an indirect measurement of the oxidative metabolism of the phagocytes. The pH of the phagolysosomes was measured using a cytofluorometric technique. Both the attachment and ingestion of serum opsonized C. neoformans by AM were slow during the first hours of incubation, but were considerable after 24 h. The oxidative metabolism of AM challenged with the yeast was insignificant during the first hour, but reached high levels after 24 h. Most phagolysosomes in AM with ingested cryptococci had a pH < 5.5. Our results indicate that these AM defence mechanisms, although poor during the first hours after exposure to the yeast, are of significance after 24 h. Thus, in the immunocompetent host the AM should prevent the dissemination of C. neoformans from the lungs.

Keywords: alveolar macrophages, Cryptococcus neoformans, oxidative metabolism, phagocytosis, phagolysosomal pH

Introduction

Cryptococcosis is a mycosis caused by the encapsulated yeast Cryptococcus neoformans. This yeast is one of the most common life-threatening fungal pathogens that infects patients with AIDS. The prevalence of cryptococcosis in AIDS patients is 3–6% in Europe, 6–10% in USA and 10–30% in some tropical countries, particularly in Central Africa [1]. In spite of the ubiquity of C. neoformans [2], cryptococcosis is rarely seen in immunocompetent persons [3]. The alveolar macrophage which provides the initial host defence against inhaled micro-organisms [4]. However, its role in the defence against C. neoformans remains incompletely elucidated. In vitro phagocytosis of the yeast by rat alveolar macrophages (AM) has been examined, but the observation times have generally been limited to only a few hours [5,6].

Also the methodology employed did not distinguish between yeast cells which are only attached from those that are ingested. Furthermore, the respiratory burst of the macrophage has previously been evaluated only during the first hours of contact with C. neoformans, a stage in which the phagocytic response to the yeast appears to be poor [7,8]. Finally, studies on phagolysosomal pH of AM with ingested cryptococci have not been performed.

The aim of this work was to study the in vitro interaction between C. neoformans and rat AM during extended incubation times. As C. neoformans reproduces slowly, in comparison with encapsulated bacteria, a cellular defence which is activated late can still be efficient. The attachment and ingestion phases of phagocytosis were studied separately. The activation of the oxidative metabolism of the AM upon exposure to this yeast was examined in relation to time. Finally, we were concerned about the pH of the phagolysosomes with ingested cryptococci as we previously had found [9] that Candida albicans can
be located within open phagolysosomes with probably high pH, which means inefficient killing of the organism and leakage of the phagolysosomal content extracellularly. Considering the large size of the encapsulated C. neoformans we suspected that a large number of this organism would be found in open phagolysosomes.

Materials and methods

Yeast strains and silica particles

The encapsulated strain C. neoformans var. neoformans (serotype D, ATCC 24067), used in these studies was maintained in distilled water at 4 °C. The strain was grown for 48 h at 30 °C in Sabouraud glucose broth pelleted by centrifugation for 10 min at 300 g, and diluted with 10 mM sodium phosphate buffer, pH 7-6. Portions were heat-killed by incubation in a 80 °C water bath for 1 h. The organisms were centrifuged for 10 min at 300 g, resuspended in 10 mM sodium phosphate buffer and kept at -20 °C until used. Total diameter of the organism and cell size were determined with photographs using a micrometer scale. Thirty yeast cells were measured. The cell size was 6.1 ± 1.1 μm (± SD) and the width of the capsule was 0.7 ± 0.3 μm.

A strain of C. albicans (ATCC 10231) was used in all assays as a control organism. Maintenance and harvesting of cultures followed the same procedure as that of the cryptococcal strain. The size measured using a light microscope was 3.8 ± 0.4 μm (± SD). Experiments with live C. albicans were not included in the present study as previous experiments have shown no significant differences between heat-killed and live cells [9].

Particles of inert silica with a diameter of 5.0 ± 0.5 μm (mean ± SD), coated with aminopropyl groups (Spherisorb S3 NH, Phase Separations Ltd, Queensferry, Clwyd, UK) were used as control particles.

Preparation of FITC-labelled yeast and silica particles

Heat-killed C. neoformans or C. albicans suspended in 10 mM sodium phosphate buffer, pH 7.6, were pelleted by centrifugation for 10 min at 25 °C and suspended with Ringer acetate solution (pH 6.5) to a concentration of 10⁶ ml⁻¹. Finally the suspensions were divided into 0.5 ml aliquots and stored at -20 °C. For experiments with live C. neoformans the same procedure was performed. The viability of labelled organisms was assessed by plating serial 10-fold dilutions of the organisms onto Sabouraud glucose agar and counting CFU after a 24-h incubation at 37 °C.

Opsonin

Fresh isologous Sprague-Dawley rat serum was obtained by cardiac puncture and kept at -70 °C until used. All three particles were treated with 18% fresh rat serum (FRS) for 1 h at 37 °C. C. neoformans and C. albicans were for selected experiments treated with 18% heat-inactivated rat serum (HIRS), which was obtained by heating at 56 °C for 30 min.

Alveolar macrophages

Adult male Sprague-Dawley rats weighing between 200 and 250 g were used throughout the studies as the source of alveolar macrophages (AM). Rats were killed by an overdose of sodium pentobarbital solution (60 mg ml⁻¹). The lungs and trachea were removed from the thoracic cavity and the AM was obtained by a lavage technique previously described [9]. The procedure yielded 8 × 10⁶ to 15 × 10⁶ cells per rat, approximately 90% of which were macrophages as determined from typical macrophage morphology by light microscopy.

Phagocytosis assay

To distinguish between internalized and attached yeast cells or silica particles, a modification [10] of a method described by Hed [11] was used. Phagocytosis assays using FITC labelled C. neoformans, C. albicans and silica particles were done in duplicates and incubations were performed for 1, 2 or 3 h by a slide technique described in detail in earlier reports [9,10]. After addition of Trypan blue, that does not enter the macrophage, the numbers of attached (blue) and the ingested particles (FITC stained) per macrophage were determined with a fluorescence microscope (Carl Zeiss Instruments, Inc., Austria). In each sample 100 consecutive macrophages were scored.

The small volumes used in the slide technique might lead to desiccation of the medium during extended incubation times. Therefore a Petri dish method, providing adequate environmental conditions for the AM, was used in the 24 h experiments. A suspension of AM (2 ml, 10⁶ cells ml⁻¹) was seeded on round glass coverslips (22 mm, Chance propper Ltd, Warley, England) in Petri dishes (35 × 10 mm, Nunclon, Denmark) and incubated...
for 1 h. The AM were then lavaged and incubated for 24 h with 2 ml of fresh HEPES medium containing $10 \times 10^6$ live or heat-killed *C. neoformans* cells per ml. Then, the coverslips were removed and Trypan blue was added to the preparation for 30 s. The rest of the procedure was done exactly as performed by the slide technique [9]. The 24 h incubation did not affect the viability of the AM as determined by Trypan blue exclusions. These two methods to perform phagocytosis were compared using an incubation time of 3 h whereby no significant differences were found (0.32 ± 0.04 attached and 0.11 ± 0.03 ingested yeast per AM, mean ± SD, n = 4, by the slide technique compared to 0.35 ± 0.02 and 0.14 ± 0.02, respectively, by the Petri dish method).

In order to separate attachment and the ingestion, two parameters were constructed. One, the *accumulated attachment* defined as the sum of the numbers of attached and ingested particles per AM after 1 h, was used as measure of the attachment process. The other, the *ingested fraction* defined as the number of ingested particles per AM after 1 h, divided by the accumulated attachment, was used as a measure of the ingestion process.

**Nitroblue tetrazolium reduction assay**

The oxidative metabolism of rat AM was studied by measuring the reduction of yellow nitroblue tetrazolium (NBT) to dark blue formazan. This reaction is caused by superoxide anions produced by the phagocytes [12]. The experiments were performed as described previously in detail [9]. Briefly, AM in suspension (2 ml, $10^6$ cells ml$^{-1}$) were allowed to adhere to plastic Petri dishes ($35 \times 10$ mm; Nunclon, Denmark) by incubation at $37^\circ$C for 1 h. In one set of experiments, the yeast cells or silica particles were then incubated with NBT for 1 h. The reaction was stopped by adding 0.5% HCl. Produced formazan was dissolved in dimethysulphoxide (DMSO, Merck, Darmstadt, Germany) and the optical density of the solution was measured in a spectrophotometer (Shimadzu, UV-116A) at a 572 nm wavelength.

In another set of experiments, the yeast or silica particles were incubated with the AM for 2 or 23 h. After this time interval, the medium together with the particles which had not been phagocytosed was removed and NBT was added for 1 h.

A suspension of AM without yeast cells or silica particles was incubated together with NBT. It served as a control in these experiments.

**Measurements of phagolysosomal pH**

FITC-labelled particles (2 ml, $1 \times 10^6$ ml$^{-1}$) were incubated with AM (2 ml, $1 \times 10^6$ cells ml$^{-1}$) for 3 and 24 h. Then the fluorescent emission of the FITC-labelled organisms was measured by a modification [13] of the method of Okhuma & Poole [14] which is based on the principle that the fluorescence spectrum is dependent on the pH of the environment. The FITC-labelled particles were used as a probe of pH. The intensity of the fluorescence from a particle in individual phagolysosomes was then measured at two excitation wavelengths, 452 and 488 nm, using a microscope cytofluorometer. One hundred phagolysosomes were scored. The data were converted from fluorescent emission ratios to pH by using a calibration curve constructed from measurements of various pH-buffered suspensions of each fluorescent probe [13].

**Statistics**

Statistical significance was determined by using the paired $t$-test. All comparisons were two sided, and a $P$ value of $< 0.05$ was considered significant.

**Results**

**Phagocytosis studies of live *C. neoformans* with and without opsonins**

Phagocytosis of live, non-opsonized *C. neoformans* was essentially negative after a 24 h incubation (0.08 ± 0.02 attached and 0.00 ± 0.00 ingested yeast cells per AM, mean ± SD, n = 3). Preincubation of yeasts with HIRS had no effect in either binding or ingestion (0.13 ± 0.02 attached and 0.00 ± 0.00 ingested yeast cell per AM, mean ± SD, n = 3). Opsonization with FRS was required for phagocytosis of the cryptococcal strain (Table 1).

**Comparison of live and heat-killed FRS-opsonized *C. neoformans***

Experiments were performed with both live and heat-killed cryptococcal cells, whereby no major differences were found as to phagocytosis and phagolysosomal pH tests (Table 1). A slightly higher uptake of cryptococci was observed after 24 h probably due to a higher number of microorganisms available ($2.0 \times 10^7$ yeast cells ml$^{-1}$ initial inoculum vs $2.2 \times 10^7$ ± $0.5 \times 10^6$, mean ± SD, n = 3, after 24 h in Hepes medium alone). Live *C. neoformans* ($1.0 \times 10^7$ yeast cells ml$^{-1}$) reduce NBT ($0.14 \pm 0.01$, optical density) without the presence of AM. Heat-killed *C. neoformans* did not reduce the dye on its own, thus the rest of the experiments were done with heat-killed yeasts only as results with live yeasts and AM together would be difficult to interpret.

**In vitro phagocytosis studies**

For *C. neoformans*, both the attachment and ingestion processes are significantly reduced compared to silica and
Table 1 Tests with C. neoformans, heat-killed or alive, and opsonized with fresh rat serum

<table>
<thead>
<tr>
<th>Test</th>
<th>C. neoformans Heat-killed</th>
<th>Live</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of attached yeasts per AM after 1 h</td>
<td>0.17 ± 0.03</td>
<td>0.19 ± 0.01</td>
</tr>
<tr>
<td>No. of ingested yeasts per AM after 1 h</td>
<td>0.02 ± 0.01</td>
<td>0.03 ± 0.01</td>
</tr>
<tr>
<td>No. of attached yeasts per AM after 3 h</td>
<td>0.37 ± 0.06</td>
<td>0.41 ± 0.08</td>
</tr>
<tr>
<td>No. of ingested yeasts per AM after 3 h</td>
<td>0.10 ± 0.02</td>
<td>0.12 ± 0.01</td>
</tr>
<tr>
<td>No. of attached yeasts per AM after 24 h</td>
<td>1.01 ± 0.06</td>
<td>1.15 ± 0.07</td>
</tr>
<tr>
<td>No. of ingested yeasts per AM after 24 h</td>
<td>0.84 ± 0.10</td>
<td>0.97 ± 0.08</td>
</tr>
</tbody>
</table>

Phagolysosomal pH

| Phagolysosomes with pH < 5.5 (%) after 3 h | 76 ± 4 | 73 ± 5 |
| Phagolysosomes with pH ≥ 5.5 and < 6.5 (%) after 3 h | 14 ± 2 | 15 ± 3 |
| Phagolysosomes with pH ≥ 6.5 (%) after 3 h | 10 ± 2 | 12 ± 2 |
| Phagolysosomes, mean pH after 3 h         | 5.3 ± 0.05 | 5.4 ± 0.1 |

Table 2 Phagocytosis of C. neoformans compared with C. albicans and silica particles after incubation with AM for 1 h

<table>
<thead>
<tr>
<th>Test</th>
<th>C. albicans</th>
<th>C. neoformans*</th>
<th>C. neoformans†</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of attached particles per AM</td>
<td>1.89 ± 0.25</td>
<td>0.17 ± 0.06*</td>
<td>0.04 ± 0.03§</td>
</tr>
<tr>
<td>No. of ingested particles per AM</td>
<td>1.21 ± 0.57</td>
<td>0.03 ± 0.02†</td>
<td>0.01 ± 0.01§</td>
</tr>
<tr>
<td>Accumulated attachment</td>
<td>3.10 ± 0.49</td>
<td>0.20 ± 0.06‡</td>
<td>0.05 ± 0.04§</td>
</tr>
<tr>
<td>Ingested fraction</td>
<td>0.37 ± 0.14</td>
<td>0.14 ± 0.07‡</td>
<td>0.22 ± 0.22</td>
</tr>
</tbody>
</table>

*Particles opsonized with 18% fresh rat serum.
†Particles opsonized with 18% heat inactivated rat serum.
‡P < 0.01 compared with silica.
§P < 0.01 compared with C. neoformans treated with fresh rat serum.

Each value represents the mean ± SD, n = 7.

C. albicans (P < 0.01). The attachment process is significantly slower with HIRS than with FRS opsonized C. neoformans (P < 0.01). For C. albicans, the ingestion process is significantly increased compared to silica (P < 0.01) but the attachment process is not significantly different (Table 2).

After 2, 3 and 24 h, we observed a time dependent increase in both attachment and ingestion by AM of FRS opsonized cryptococci, with a higher number of attached than ingested yeast per macrophage (Table 3). Both the numbers of attached and ingested FRS opsonized C. neoformans were significantly higher than corresponding numbers with either non-opsonized or HIRS treated yeast cells (Table 3). As for C. albicans, after a 3 h incubation, the numbers of attached but not ingested yeast cells were significantly higher when the yeast was opsonized with FRS compared with HIRS (1.26 ± 0.13 vs 1.05 ± 0.05 attached yeast per AM and 2.90 ± 0.22 vs 2.79 ± 0.12, ingested yeast per AM, mean ± SD).

NBT reduction studies

The results of the NBT test are given in Table 4. During the first hour of phagocytosis we did not find any significant difference in NBT reduction with either FRS opsonized or HIRS treated cryptococci in comparison with the control of unstimulated macrophages. The values of AM stimulated by silica particles were also in the levels of those of the control, FRS opsonized C. albicans, however, induced a significant increase in NBT reduction in AM.

In the 3 h experiments, FRS-opsonized C. neoformans elicited a significant increase in NBT reduction compared with control. No significant differences were observed between HIRS treated C. neoformans and the control. We found a significantly increased NBT reduction by AM stimulated with C. albicans opsonized with either FRS or HIRS compared with control; however, significantly higher values were observed when C. albicans was opsonized with FRS compared with HIRS.
Resistance of the rat to cryptococcosis is similar to that of other species. The phagocytosis, oxidative metabolism and phagolysosomal pH of AM stimulated with yeast and silica was found to be with a pH value of 5.5. After 3 h a considerable fraction with pH > 6.5 was observed for both yeast cells. A small fraction was also found after 24 h phagocytosis. Such a fraction was seen with control silica particles (Table 5). The mean pH values of AM phagolysosomes with C. neoformans, C. albicans and silica particles were similar. The mean pH value was lower for all three particles after 24 h incubation.

**Discussion**

The phagocytosis, oxidative metabolism and phagolysosomal pH of AM stimulated with C. neoformans were studied. The rat model was chosen because the natural resistance of the rat to cryptococcosis is similar to that observed in humans [15], and also the rat AM are of easy accessibility.

Concerning the phagocytic activity, we found that the attachment and ingestion rates for cryptococci were initially markedly slow compared with corresponding rates for silica and C. albicans. However, although slow in the beginning, these processes were steadily proceeding and after 24 h a considerable number of cryptococcal cells were attached and ingested. The initially low uptake of C. neoformans by the phagocytes is most probably due to the cryptococcal capsule as shown by previous studies [16,17].

Our study shows that in contrast to the statements in two recent review articles [18,19] neither specific anticapsular antibody [18] nor exogenous cytokine [19] is needed for a noticeable amount of complement opsonized cryptococci to be internalized by the alveolar macrophage. A prerequisite, however, is that a sufficient duration of time is allowed for the process to take place.

Some authors have found binding [20] or uptake [6] of unopsonized cryptococci by alveolar macrophages, but even with our long incubation time we found basically no uptake unless the cryptococci were opsonized with fresh serum as a source of complement. The same result has also been found by others [8,21,22]. We have no explanation for these contradictory results as to the need of complement for the uptake of cryptococci by AM, but want to stress that there is evidence available for the presence of components of active complement in the respiratory tract [23,24].

As to the NBT reduction, reflecting the oxidative metabolism of the AM, we observed that the amount of formazan produced by the AM during 1 h with cryptococci treated with either HIRS or FRS was in the level of that produced by the AM alone. The small number of C. neoformans bound to the cell membrane of the AM after 1 h explains these findings which are consistent with previous observations [7,8]. Because a higher number of the FRS opsonized yeast was associated to the AM cell membrane after 24 h, NBT studies were performed after this incubation time. The results demonstrate rather high levels of formazan produced by AM with C. neoformans after 24 h, findings which suggest that a considerable number of cell membrane bound cryptococci is required for the AM to generate an increased NBT reduction.

It is of note that the NBT values of the control AM after 3 and 24 h were lower than those seen after 1 h. This difference might be due to a decrease in metabolic activity of the cultured AM and/or loss of AM during the exchange of medium in the 3 and 24 h experiments.

Complement opsonized particles are attached to phagocytes via the C3 receptors, which generally when activated promote phagocytosis but not respiratory burst with...
Table 4 Amount of formazan formed from NBT reduction by superoxide anions during 1 h and 3 and 24 h after onset of phagocytosis

<table>
<thead>
<tr>
<th>Opsonin</th>
<th>Optical density (arbitrary units)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 h</td>
</tr>
<tr>
<td>Control (only AM)</td>
<td>0.19 ± 0.10</td>
</tr>
<tr>
<td>Silica and AM FRS</td>
<td>0.19 ± 0.11</td>
</tr>
<tr>
<td>C. albicans and AM FRS</td>
<td>0.35 ± 0.11*</td>
</tr>
<tr>
<td>C. albicans and AM HIRS</td>
<td>ND</td>
</tr>
<tr>
<td>C. neoformans and AM FRS</td>
<td>0.19 ± 0.11</td>
</tr>
<tr>
<td>C. neoformans and AM HIRS</td>
<td>0.20 ± 0.13</td>
</tr>
<tr>
<td>C. neoformans and AM none</td>
<td>ND</td>
</tr>
</tbody>
</table>

FRS, fresh rat serum; HIRS, heat inactivated rat serum; ND, not determined.
*P < 0.05 compared with control.
†P < 0.05 compared with HIRS treated C. albicans.
‡P < 0.05 compared with HIRS treated C. neoformans.
§Results were obtained from a different group of rats. Control AM had a value of 0.09 ± 0.01 (mean ± SD, n = 4).
Each value represents the mean ± SD, n = 4.

Table 5 Distribution of pH and mean pH values of AM vesicles with heat-killed C. neoformans, C. albicans and silica particles, 3 and 24 h after onset of phagocytosis

<table>
<thead>
<tr>
<th>Incubation</th>
<th>Particle</th>
<th>% vesicles with</th>
<th>% vesicles with</th>
<th>% vesicles with</th>
<th>Mean pH</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>pH &lt; 5.5</td>
<td>pH ≥5.5 and &lt; 6.5</td>
<td>pH ≥6.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>After 3 h</td>
<td>C. neoformans</td>
<td>77.0 ± 2.6</td>
<td>13.5 ± 1.9</td>
<td>9.5 ± 1.9*</td>
<td>54 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>C. albicans</td>
<td>76.5 ± 3.4</td>
<td>14.5 ± 1.9</td>
<td>9.0 ± 2.6*</td>
<td>54 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>Silica</td>
<td>83.4 ± 3.4</td>
<td>16.5 ± 3.4</td>
<td>0.0 ± 0.0</td>
<td>5.2 ± 0.1</td>
</tr>
<tr>
<td>After 24 h</td>
<td>C. neoformans</td>
<td>89.0 ± 3.5</td>
<td>9.5 ± 2.5</td>
<td>1.5 ± 1.9</td>
<td>5.0 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>C. albicans</td>
<td>87.0 ± 2.6</td>
<td>12.0 ± 2.8</td>
<td>1.5 ± 1.9</td>
<td>5.0 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>Silica</td>
<td>91.0 ± 2.6</td>
<td>9.0 ± 2.6</td>
<td>0.0 ± 0.0</td>
<td>5.0 ± 0.0</td>
</tr>
</tbody>
</table>

*P < 0.01 compared with silica.
Data are given as mean ± SD, n = 4.

superoxide production [25]. This phenomenon has earlier been observed as in human monocytes and neutrophils with red blood cells [26], bacteria [27] and C. albicans (Jarstrand, unpublished results) as target cells. It is therefore remarkable that C. neoformans opsonized with complement in this study elicited an increased superoxide production by AM. As previous investigators have found similar observations for AM, with Pneumocystis carinii [28] and as we also found that complement opsonized C. albicans elicited an increased NBT reduction, we suggest that alveolar macrophages in contrast to other phagocytes might have some characteristics which make them prone to react with a respiratory burst when challenged with complement opsonized particles.

In this study, it was demonstrated that most phagolysosomes containing C. neoformans had a pH < 5.5. Comparable values were obtained with silica particles and C. albicans, results which agree well with data shown in our previous studies [9]. Our earlier electron microscopic studies with C. albicans have shown open channels between macrophage cell surface and the yeast containing phagolysosome. Such open vesicles probably have a high pH, which means an impaired killing and disintegration of the organism. In the present study we found after incubation times of 3 and 24 h that 9.5 and 1.5%, respectively, of the cryptococcus containing vesicles had a pH of > 6.5. It is of note that none of the phagolysosomes containing silica particles had a pH > 6.5 even after 3 h. Thus, the size of the particle is of no importance for the formation of open phagolysosomes. However, the existence of open passages between the phagolysosomes with ingested cryptococci and the AM cell membrane which may raise the vacuolar pH cannot be ruled out. The vesicles with higher pH could also be phagocytic vesicles with C. neoformans that have not yet fused with the lysosomes.
In conclusion, the current work indicates that several anticytotoxic mechanisms by alveolar macrophages are poor during the first hour of exposure to the yeast. However, these mechanisms improve with time and after 24 h are of significance. A considerable fraction of the yeast cells is attached and ingested. There is a markedly increased oxidative metabolism, which is of importance as some strains of C. neoformans are susceptible to reactive metabolites [7,29]. Finally, most of the yeast cells are located within phagolysosomes with acid pH. Thus, a prolonged contact between the phagocyte and this organism evokes an efficient defence by the AM in the immunocompetent host. In the immunocompromised host, however, an impaired function of the AM might contribute to the development of cryptococcosis both in the lungs and in other organs.

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