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Karen L. Wozniak
University of Massachusetts Medical School

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Cryptococcus neoformans Enters the Endolysosomal Pathway of Dendritic Cells and Is Killed by Lysosomal Components

Karen L. Wozniak and Stuart M. Levitz*
Department of Medicine, Division of Infectious Diseases, University of Massachusetts Medical School, Worcester, Massachusetts

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Cryptococcus neoformans is an opportunistic fungal pathogen that primarily causes disease in immunocompromised individuals. Dendritic cells (DCs) can phagocyte C. neoformans, present cryptococcal antigen, and kill C. neoformans. However, early events following C. neoformans phagocytosis by DCs are not well defined. We hypothesized that C. neoformans traffics to the endosome and the lysosome following phagocytosis by DCs and is eventually killed in the lysosome. Murine bone marrow-derived DCs (BMDCs) or human monocyte-derived DCs (HDs) were incubated with live, encapsulated C. neoformans yeast cells and opsonizing antibody. Following incubation, DCs were intracellularly stained with antibodies against EEA1 (endosome) and LAMP-1 (late endosome/lysosome). As assessed by confocal microscopy, C. neoformans trafficked to endosomal compartments of DCs within 10 min and to lysosomal compartments within 30 min postincubation. For HDs, the studies were repeated using complement-sufficient autologous plasma for the opsonization of C. neoformans. These data showed results similar to those for antibody opsonization, with C. neoformans localized to endosomes within 20 min and to lysosomes within 60 min postincubation. Additionally, the results of live real-time imaging studies demonstrated that C. neoformans entered lysosomal compartments within 20 min following the initiation of phagocytosis. The results of scanning and transmission electron microscopy demonstrated conventional zipper phagocytosis of C. neoformans by DCs. Finally, lysosomal extracts were purified from BMDCs and incubated with C. neoformans to determine their potential to kill C. neoformans. The extracts killed C. neoformans in a dose-dependent manner. This study shows that C. neoformans enters into endosomal and lysosomal pathways following DC phagocytosis and can be killed by lysosomal components.

* Corresponding author. Mailing address: Department of Medicine, Division of Infectious Diseases, University of Massachusetts Medical School, Worcester, Massachusetts 01605. Phone: (508) 856-1525. Fax: (508) 856-1828. E-mail: Stuart.Levitz@umassmed.edu.
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entry into early endosomes of DCs and DC lysosomal degradation of *C. neoformans* have not been explored. We hypothesized that following phagocytosis by DCs, *C. neoformans* enters the endosomal/lysosomal pathway, where it is killed and degraded for antigen presentation to T cells. Therefore, in the present studies, we determined the intracellular location of *C. neoformans* organisms following phagocytosis by murine DCs and HDCs. Moreover, we examined the capacity of lysosomes isolated from DCs to kill *C. neoformans*.

**MATERIALS AND METHODS**

Reagents. Unless otherwise stated, chemical reagents of the highest quality available were obtained from Sigma-Aldrich Co. (St. Louis, MO), tissue culture media were purchased from Gibco Life Technologies (Rockville, MD), and plasticware was purchased from Fisher Scientific (Pittsburgh, PA). The medium used in murine bone marrow-derived DC (BMDC) experiments was RPMI 1640 supplemented with 10% heat-inactivated fetal bovine serum, 2 mM L-glutamine, 100 U penicillin/ml, 100 µg of streptomycin/ml, and 50 mM 2-mercaptoethanol (complete medium). The medium used in HDC experiments was RPMI 1640 supplemented with 5% heat-inactivated fetal bovine serum, 2 mL/L-glutamine, 100 U penicillin/ml, 100 µg of streptomycin/ml, 50 mM 2-mercaptoethanol, and 10 mM HEPES (HDC medium). All cell culture incubations were performed at 37°C in a humidified environment supplemented with 5% CO2.

**Culture of Cryptococcus.** *Cryptococcus neoformans* serotype A encapsulated strain ATCC 62070 (American Type Culture Collection, Manassas, VA) was cultured for 24 h at 30°C in yeast extract-peptone-dextrose plus 2% glucose. Live *C. neoformans* organisms were washed with sterile phosphate-buffered saline (PBS), counted, and resuspended in sterile PBS to the concentration needed for each experiment.

**Fluorescent labeling of *Cryptococcus*.** Live *C. neoformans* organisms were washed with sterile 0.1 M sodium bicarbonate buffer, pH 8.0 (staining buffer), counted, and resuspended to 5 × 10^6^/mL. *C. neoformans* yeast was incubated with 2 µg/mL Oregon green 488 (Molecular Probes, Eugene, OR) at room temperature in the dark for 1 h. The organisms were then washed three times with sterile PBS, counted, and resuspended in sterile PBS to the concentration needed for each experiment.

**Fluorescent labeling of 3C2 antibody.** Opossum anti-capillary monoclonal 3C2 antibody (gift of Thomas Kozel, University of Nevada, Reno, NV) (50) was diluted in staining buffer to 100 µg/mL and the mixture was incubated at room temperature in the dark for 1 h. The antibody was separated from excess dye by using a Sephadex G-25 column.

BMDCs, C57BL/6 mice were purchased from Jackson Laboratory (Bar Har bor, ME) and were housed under pathogen-free conditions in microisolator cages according to institutionally recommended guidelines at the University of Massachusetts Medical School Department of Animal Medicine. BMDC culture was performed as previously described (22, 30). Briefly, bone marrow was flushed from the femurs and tibiae of C57BL/6 mice. Cells were washed, counted, and plated in complete medium supplemented with 10% fetal-streched supernatant from the J558L cell line (which constitutively produces granulocyte-macrophage colony-stimulating factor) (39). One half of the medium was replaced every three days, and the cells were harvested on day 8 or 9 following plating. The cells were then purified by positive selection using magnetically labeled CD11c antibodies (Miltenyi Biotec, Auburn, CA).

HDCs. Monocyte-derived HDCs were obtained as described previously (44). Briefly, peripheral blood was obtained from healthy volunteers by venipuncture following informed consent, using a protocol approved by the University of Massachusetts Medical School Institutional Review Board. The blood was anti-coagulated with heparin (American Pharmaceutical Partners, Inc., Los Angeles, CA) and diluted 1:1 with Hank’s balanced salt solution (BioWhittaker, Walk ersville, MD). Peripheral blood mononuclear cells (PBMCs) were purified in a Leukosep tube (Greiner Bio-One, Germany) over a Lymphoprep gradient (Accurate Chemical & Scientific Corp., Westbury, NY). The tubes were centrifuged at 1,000 × g for 10 min without the brake. After separation, the autologous diluted plasma was collected and stored at −20°C until use. The PBMC layer was isolated and washed three times with HDC medium. PBMCs (1 × 10^6^/well) were added to a six-well tissue culture plate for 2 h at 37°C to allow monocyte adherence and then gently washed to remove nonadherent cells. HDCs were cultured for 7 days with 50 ng/ml recombinant human interleukin-4 (Peprotech, Rocky Hill, NJ) and 150 ng/ml recombinant human granulocyte-macrophage colony-stimulating factor (sargramostim; Bayer, Wayne, NJ). The cells were then positively selected for CD1c (BDCA-1) expression by using magnetically labeled CD1c antibodies (Miltenyi Biotec).

**DC phagocytosis of *C. neoformans*.** BMDCs or HDCs were harvested, purified, and counted. DCs and *C. neoformans* organisms were incubated at a 2:1 ratio in the presence of 1 µg/mL of Oregon green-stained 3C2 opsonizing antibody for 10, 20, 30, and 60 min at 37°C in 1.7-mL microcentrifuge tubes (Costar, Corning, NY). DC phagocytosis with plasma opsonins, HDCs were incubated with Oregon green-labeled *Cryptococcus* in the presence of autologous human plasma.

**Intracellular staining.** Following incubation of DCs and *C. neoformans* organisms, DCs were fixed with 2% paraformaldehyde in PBS for 10 min at room temperature. After fixation, the cells were washed and permeabilized with 0.1% saponin for 10 min at room temperature. While permeabilized, the cells were intracellularly stained with either anti-EEA1 (ABR Affinity Bioreagents, Golden, CO) or anti-LAMP-1 (mouse; BioSource, San Diego, CA, or human; Santa Cruz Biotechnology, Santa Cruz, CA). For both BMDCs and HDCs, purified anti-EEA1 was the primary antibody and was followed by anti-rabbit immunoglobulin G (IgG) conjugated to Alexa 488 (Molecular Probes). For BMDCs, biotinylated LAMP-1 was used, followed by streptavidin-Alexa 568 (Molecular Probes). For HDCs, purified LAMP-1 was the primary antibody and was followed by anti-rabbit IgG conjugated to Alexa 568 (Molecular Probes). Controls antibodies matched for species and isotype were used for each experiment as follows: for EEA1, rabbit IgG (Sigma); for human LAMP-1, mouse IgG1 (eBioscience); and for mouse LAMP-1, rat IgG2a (eBioscience). Phagocytosis was defined as the detection of the presence of intracellular *C. neoformans* organisms by confocal microscopy. Intracellular staining of live *C. neoformans* yeast cells was confirmed by visualization of both BMDCs and HDCs organisms within the stained compartments.

**Live imaging of phagocytosis.** BMDCs were harvested, purified, and incubated with *C. neoformans* yeast cells at a 2:1 ratio in the presence of 1 µg/mL of Oregon green-stained 3C2 opsonizing antibody for 30 min. Samples were incubated with 2% paraformaldehyde in PBS and washed with 1% bovine serum albumin in PBS. Samples were placed on ice until live imaging by confocal microscopy. For imaging, the sample was placed in a glass-bottomed, 35-mm culture dish (Mat Tck Corp., Ashland, MA) on a stage heated to 37°C. Once the interaction of DCs with *C. neoformans* was observed, confocal images were obtained every 30 s in order to determine whether entry of *C. neoformans* into the stained lysosomal compartment occurred.

**Examination of phagocytosis by electron microscopy.** BMDCs were harvested, purified, and incubated with *C. neoformans* yeast cells at a 2:1 ratio in the presence of 1 µg/mL of 3C2 opsonizing antibody for 10, 20, 30, 40, 50, or 60 min. Following incubation, cells were fixed with 2.5% glutaraldehyde and processed for transmission electron microscopy (TEM) or scanning electron microscopy (SEM). Following sample preparation, cells were examined on a Philips CM10 Transmission Electron Microscope (TEM) in the presence of C. neoformans organisms. For SEM, cells were examined on an FEI Quanta 200 FEG SEM for the uptake of *C. neoformans* by DCs.

**Lysosomal extract purification from DCs.** BMDCs were cultured, harvested, and purified as described above. Crude lysosomal extracts were obtained (S. L. Newman and W. Lemen, unpublished data). Briefly, 1 × 10^5^ lysosomal extraction buffer (Sigma-Aldrich) was added to 2.7 ml per 3 × 10^6^ cells. DCs were homogenized with a PowerGen 700 homogenizer (Fisher Scientific, Pittsburgh, PA) using a 7- by 110-mm homogenizer tip (Fisher Scientific). The homogenizer was passed through the cells 20 to 25 times (which disrupted 75 to 80% of the cells), and then cells were centrifuged for 10 min at 1,000 × g to remove intact cells and cellular debris. The supernatants were collected and centrifuged for 20 min at 20,000 × g to pellet lysosomes. The supernatants were discarded, and the pellet containing the lysosomes was resuspended in 1 mL of 1× extraction buffer. The sample was then sonicated for 20 s at a setting of 40% on a model 500 Sonic Dismembrator (Fisher Scientific). The resultant following sonication was the crude lysosomal extract.

**Lysosomal killing of *C. neoformans*.** *C. neoformans* killing assays were performed as previously described (28, 33, 42). Briefly, following culture of encapsulated *C. neoformans* yeast cells, the organisms were washed three times with sterile PBS and resuspended in 10 mM phosphate buffer with 2% RPMI 1640, pH 5.5 (lysosomal buffer). The fungi were then added to 96-well plates in a volume of 50 µL (2.5 × 10^5^/mL). Lysosomal extracts were added at 10, 25, and 50%, and the wells were filled with lysosomal buffer to a total volume of 100 µL. The plates were then incubated for 24 h at 37°C. Following incubation, the organisms were diluted in sterile PBS and plated on Sabouraud dextrose agar.
plates. The plates were incubated at room temperature for 3 days, and then CFU were counted.

Statistical analysis. For statistical comparisons, we utilized one-way analysis of variance with Tukey’s multiple correction test. P values of <0.05 were considered significant. The data were analyzed by using GraphPad Prism software (GraphPad Software, Inc., San Diego, CA).

RESULTS

Intracellular trafficking of C. neoformans yeast following phagocytosis by DCs. In order to determine the intracellular location of C. neoformans yeast cells following phagocytosis by DCs, BMDCs and HDCs were incubated with C. neoformans. The DCs were then fixed, intracellularly stained with antibodies for EEA1 (early endosomes) or LAMP-1 (late endosomes/lysosomes), and examined by confocal microscopy. The results showed that for both BMDCs and HDCs, more than half of the C. neoformans-containing phagosomes had fused with EEA1-positive compartments by 10 min postincubation (Fig. 1A). By 20 min postincubation, the majority of C. neoformans-containing phagosomes had fused with EEA1-positive compartments, and this remained constant through 60 min postincubation. LAMP-1-positive compartments fused with few C. neoformans-containing phagosomes at early time points, but the majority had fused by 30 min postincubation and continued to be fused through 60 min postincubation (Fig. 1).

Live imaging of C. neoformans phagocytosis by DCs. Although the data from the intracellular staining confirmed the entry of C. neoformans organisms into endosomes and lysosomes, those experiments were timed from the beginning of the incubation, not the beginning of phagocytosis. In order to determine how quickly entry into lysosomes occurred following contact and phagocytosis, live confocal imaging was performed. The results showed that the majority of C. neoformans organisms appeared to enter the lysosomal compartment of
BMDCs (stained with LysoTracker red) within 20 min following uptake (Fig. 2; see Fig. S2 in the supplemental material).

Cryptococcus trafficking following opsonization with complement-sufficient plasma. In natural human infections, anticycrtococcal antibodies may be absent or complexed with shed capsule. In those situations, binding and phagocytosis are dependent upon complement opsonization (22, 26, 51). Phagocytosis by complement- and Ig-opsonized organisms proceeds by different receptors (complement receptors and Fc receptors, respectively) (4). Therefore, we next determined the intracellular fate of C. neoformans in DCs following opsonization with complement-sufficient autologous human plasma. HDCs were incubated with C. neoformans yeast and autologous plasma, and then the DCs were intracellularly stained with EEA1 or LAMP-1 and examined by confocal microscopy (Fig. 3). The results showed that the C. neoformans-containing phagosomes had fused with EEA1-positive compartments by 20 min postincubation and remained fused through 60 min postincubation. The majority of C. neoformans-containing phagosomes had fused with LAMP-1-positive compartments by 30 min postincubation and continued to be fused when observed at 60 min postincubation.

Electron microscopy of internalized C. neoformans organisms in DCs. Phagocytosis of microorganisms by DCs can proceed by conventional (“zipper”) or “coiling” mechanisms (6–8, 14, 40). To determine how DC phagocytosis of C. neoformans proceeded and to confirm that C. neoformans was indeed found within intracellular compartments of the DCs, SEM and TEM were performed following the incubation of BMDCs with C. neoformans yeast (Fig. 4). By 10 min, the earliest time point examined, C. neoformans yeast cells were observed in the process of being internalized by conventional phagocytosis (Fig. 4A to D). Coiling phagocytosis was not observed. In addition, the TEM images demonstrated that fully phagocytosed C. neoformans organisms were surrounded by intact membranes that abutted the organisms’ capsules (Fig. 4D to F).

Fungicidal activity of DC-derived lysosomal extract against C. neoformans. The results of the above studies demonstrated fusion of C. neoformans organisms with DC lysosomes. As the results of previous studies demonstrated that DCs kill C. neoformans (22), we next sought to determine whether lysosomes had direct antifungal activity. Lysosomes were isolated from DCs and disrupted by sonication. This crude lysosomal extract, isolated from $3 \times 10^8$ cells per ml, contained 1,000 to 1,250 mg protein and 6 to 10 units of acid phosphatase activity (a marker of lysosomal activity) per ml. In addition, prior to sonication, the crude lysosomes stained positively for LAMP-1 by confocal microscopy. The extract was incubated with C. neoformans yeast for 24 h, following which the numbers of CFU were determined. The lysosomal extracts had dose-dependent antifungal activity (Fig. 5). The inoculum was almost completely killed following the incubation of C. neoformans with 50% lysosomal extract added.

DISCUSSION

DCs have been shown to be involved in the phagocytosis of C. neoformans both in vivo and in vitro (22, 59). Phagocytosis and the subsequent intracellular events are critical factors determining the fate of the pathogen and the cell-mediated immune response. Previous data from our laboratory showed that pulmonary DCs exposed to C. neoformans yeast in vivo were able to present cryptococcal antigens to antigen-specific T cells ex vivo (59). In addition, BMDCs and HDCs kill complement- and antibody-opsonized C. neoformans organisms in vitro (22). Based on these data, we hypothesized that, following DC phagocytosis, C. neoformans traffics to endosomal and lysosomal compartments, where it is ultimately killed.

To determine where the C. neoformans organisms localized following DC phagocytosis, we incubated DCs with anticytopenic antibody and C. neoformans yeast and examined fusion with the endosome (EEA-1) and late endosome/lysosome (LAMP-1). In human monocyte-derived macrophages, C. neoformans yeast has been shown to localize to LAMP-1-positive compartments and survive this acidic environment (29). In addition, C. neoformans can colocalize with CD63-positive compartments in immature murine DCs (2). In our current confocal microscopy studies, in both murine DCs and HSCs, phagocytosed C. neoformans organisms trafficked to endocytic compartments within 10 to 20 min and to lysosomal compartments within 30 to 60 min following fungal challenge. These findings correlated well with the results of studies of endosomal and lysosomal localization of soluble antigens that demonstrate that endosomal entry occurred between 2.5 and 10 min postuptake and lysosomal entry between 30 and 60 min postuptake (45).

Phagocytosis of organisms by DCs can proceed via different mechanisms, including the conventional zipper-type mecha-
nism and coiling phagocytosis (6–8, 40). In order to examine the mechanisms by which DCs phagocytose \textit{C. neoformans}, we performed TEM and SEM. DC phagocytosis of \textit{C. neoformans} appeared to proceed through the conventional zipper-type phagocytosis mechanism, as evidenced by the presence of symmetrical pseudopods and nonoverlapping pseudopods. No evidence of coiling phagocytosis was observed. In addition, the results of TEM confirmed that phagocytosed \textit{C. neoformans} organisms were indeed inside membrane-bound compartments of DCs.

Vaccination strategies designed to elicit anticapsular antibodies and passive antibody administration represent promising strategies for the prevention and treatment, respectively, of cryptococcosis (reviewed in references 10 and 11). However, the results of studies demonstrating that phagocytosis of \textit{C. neoformans} yeast by HDCs is dependent upon heat-labile serum opsonins strongly suggest a predominant role for complement in phagocytosis (22). As the specific receptors mediating uptake can be critical determinants of subsequent intracellular trafficking events (reviewed in reference 4), it was important to determine whether the fate of \textit{C. neoformans} differed depending upon whether entry was via complement or Fc receptors. We found that \textit{C. neoformans} trafficked into the endosome and then into the lysosome regardless of whether opsonization was with complement-sufficient plasma or antibody. In contrast, recent data from examining the interaction of \textit{C. albicans} with DCs found that the fate of the fungus was linked to the receptor mediating uptake. Thus, entry via dectin-1 resulted in fungal killing by stimulating NADPH oxidase activity, whereas \textit{C. albicans} could escape the oxidative damage by entering DCs through receptors not involved in NADPH oxidase activation, such as the mannose receptor, CD206 (13).

Our studies focused on early time points, and it remains possible that surviving organisms could escape the phagolysosome at later time points. While \textit{C. neoformans} can be killed by DCs (22) and by macrophages (5, 27, 28), under some conditions, \textit{C. neoformans} has also been shown to survive the phagolysosome of macrophages (29). Several mechanisms have

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**FIG. 3.** Localization of \textit{C. neoformans} to early endosomes and lysosomes following serum opsonization and phagocytosis by HDCs. HDCs were incubated with encapsulated \textit{C. neoformans} in the presence of complement-sufficient autologous plasma. Following incubation, HDCs were fixed, permeabilized, and stained with anti-EEA1 antibody (Alexa 568) or anti-LAMP-1 antibody (Alexa 568). (A) Line graph of average percentages of \textit{C. neoformans} organisms inside EEA1- and LAMP-1-positive compartments of HDCs following phagocytosis at 10, 20, 30, and 60 min postincubation. These data are from three independent experiments. Three to four images (including z-stack images) were obtained at each time point. (B) Representative confocal images of \textit{C. neoformans} organisms in EEA1-positive compartments at 20 min postincubation. (C) Representative confocal images of \textit{C. neoformans} organisms in LAMP-1-positive compartments at 30 min postincubation. Scale bar = 11.9 μm. In panels B and C, “Merge” panels at far right show merged images of bright-field, red, and green panels.
been described for macrophages by which *C. neoformans* can escape the phagosome and even the phagocyte. *C. neoformans* can produce phospholipases that cause phagolysosomal membrane permeability, which can lead to the dissemination of the organism (46). In addition, growth, either by capsular expansion or budding, could mechanically disrupt the membrane. Further, recent data have shown that *C. neoformans* can exit from macrophages via extrusion of the phagosome (1). Whether such escape mechanisms exist for *C. neoformans* in DCs is not known.

Mechanisms have been described by which microbial pathogens escape killing and degradation by the lysosome of DCs. For example, *Mycobacterium tuberculosis* can translocate from phagolysosomes to the cytosol of DCs, thus escaping killing and degradation (57). At certain stages of its life cycle, *Leishmania major* resides in DC endocytic compartments where it can escape lysosomal killing by blocking the fusion of lysosomes (24). *Salmonella enterica* serovar Typhimurium is found in DC compartments that lack lysosomal markers, suggesting that this organism pre-

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**FIG. 4.** Results of electron microscopy of *C. neoformans* phagocytosis by DCs. Following culture, BMDCs were purified and incubated for specified times with encapsulated *C. neoformans* cells and opsonizing antibody. DCs were then fixed and examined by SEM or TEM. (A) SEM of two *C. neoformans* yeast cells shown in the process of being phagocytosed by a DC at 10 min postincubation. Arrow points to a pseudopod from the DC attached to the yeast cell. Original magnification, ×19,000. Scale bar = 4 μm. (B) SEM of two *C. neoformans* yeast cells being phagocytosed by a DC at 10 min postincubation. Original magnification, ×16,000. Scale bar = 5 μm. (C) Close-up of the boxed area from panel B demonstrating a *C. neoformans* yeast cell partially covered by the “flap” of a DC pseudopod (arrow). Original magnification, ×50,000. Scale bar = 1 μm. (D) One *C. neoformans* yeast cell is seen inside a membrane-bound compartment of a DC, while another is just beginning to be phagocytosed (arrow) at 50 min postincubation. Original magnification, ×7,100. Scale bar = 2 μm. (E) A *C. neoformans* yeast cell is seen inside a membrane-bound compartment of a DC at 20 min postincubation. Original magnification, ×19,500. Scale bar = 0.5 μm. (F) Close-up of the boxed area from panel E demonstrating a *C. neoformans* cell surrounded by a contiguous endosomal membrane (arrows). Original magnification, ×40,000. Scale bar = 200 nm.

**FIG. 5.** Killing of *C. neoformans* by lysosomal extracts from BMDCs. *C. neoformans* yeast cells were incubated in lysosomal buffer with the indicated concentrations of lysosomal extracts for 24 h at 37°C, following which the numbers of CFU in the wells were determined as described in Materials and Methods. The numbers of CFU in the inoculum are also shown. Data shown are means ± standard errors of the means of the results of four independent experiments, with each condition performed in duplicate. An asterisk indicates a significant difference compared to the results for 0% extract (*P* < 0.0001).
vents phagolysosomal fusion (15). However, based on the results of the studies presented herein, at least in the first hour following phagocytosis, *C. neoformans* yeast localizes to and remains in the endosomal and lysosomal compartments of DCs.

DC killing of fungi can proceed by oxidative and/or nonoxidative mechanisms. Antifungal activity against *C. neoformans* was partially reduced in the presence of respiratory burst inhibitors, suggesting roles for both oxidative and nonoxidative systems (22). However, inhibitors of the respiratory burst did not affect the ability of HDCs to kill phagocyted *C. albicans* or *H. capsulatum* (17, 38). Moreover, the addition of suramin, an inhibitor of phagosome-lysosome fusion, to *Histoplasma*-infected DCs inhibited phagosome-lysosome fusion and DC fungicidal activity (17). Lysosomal killing and degradation by DCs could be important for both pathogen clearance and MHC-restricted antigen presentation to naïve T cells. Therefore, we determined whether lysosomal components from DCs were capable of killing *C. neoformans*. We found that crude lysosomal extracts from DCs had dose-dependent anticyclicidal activity, with nearly complete killing observed when the extracts were diluted 50%. Current studies in our laboratory are focused on determining the antifungal effectors in DC lysates that are responsible for killing *C. neoformans*. For human neutrophils, multiple peptides and proteins, including defensins, have been shown to kill *C. neoformans* (33).

In summary, we have shown that *C. neoformans* traffics into the endosomal and lysosomal compartments of HDCs and murine DCs following phagocytosis. In addition, this trafficking was independent of the method of opsonization, suggesting that multiple mechanisms exist for the entry of phagocyted organisms into the endosomal pathway. Moreover, we have shown that purified lysosomal components of DCs are able to kill *C. neoformans*. Based on the data presented herein, we suggest that the intracellular trafficking and delivery of *C. neoformans* organisms to the lysosome for degradation may be important in the antigen presentation of cryptococcal antigens to T cells. DCs play a key role in the host defense against several pathogenic microorganisms by initiating adaptive cell-mediated immune responses. Understanding the interaction of DCs and their lysosomal components with *C. neoformans* yeast will help in gaining insights into its pathogenesis and may also lead to the development of innovative immunotherapies to help control cryptococcal infection.

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**REFERENCES**


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