Candida albicans Is Phagocytosed, Killed, and Processed for Antigen Presentation by Human Dendritic Cells

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Candida albicans is a component of the normal flora of the alimentary tract and also is found on the muco-cutaneous membranes of the healthy host. Candida is the leading cause of invasive fungal disease in premature infants, diabetics, and surgical patients, and of oropharyngeal disease in AIDS patients. As the induction of cell-mediated immunity to Candida is of critical importance in host defense, we sought to determine whether human dendritic cells (DC) could phagocytose and degrade Candida and subsequently present Candida antigens to T cells. Immature DC obtained by culture of human monocytes in the presence of granulocyte-macrophage colony-stimulating factor and interleukin-4 phagocytosed unopsonized Candida in a time-dependent manner, and phagocytosis was not enhanced by opsonization of Candida in serum. Like macrophages (Mφ), DC recognized Candida by the mannose-fucose receptor. Upon ingestion, DC killed Candida as efficiently as human Mφ, and fungicidal activity was not enhanced by the presence of fresh serum. Although phagocytosis of Candida by DC stimulated the production of superoxide anion, inhibitors of the respiratory burst (or NO production) did not inhibit killing of Candida, even when phagocytosis was blocked by preincubation of DC with cytochalasin D. Further, although apparently only modest phagolysosomal fusion occurred upon DC phagocytosis of Candida, killing of Candida under anaerobic conditions was almost equivalent to killing under aerobic conditions. Finally, DC stimulated Candida-specific lymphocyte proliferation in a concentration-dependent manner after phagocytosis of both viable and heat-killed Candida cells. These data suggest that, in vivo, such interactions between DC and C. albicans may facilitate the induction of cell-mediated immunity.

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For these adaptive immune responses to occur, the DC either would have to take up soluble fungal antigens or phagocytose the fungus themselves. If the latter occurs, it would require not only uptake of the fungus but also killing, degradation, and processing of fungal antigens. Teleologically, it
would seem to be more efficient if the DC ingested the Candida rather than their having to wait for antigens to be shed or regurgitated by other immune cells such as Mø. Recently, we demonstrated that immature human DC were able to phagocytose, kill, and degrade the fungal pathogen Histoplasma capsulatum (24). Furthermore, the DC were able to process H. capsulatum antigens and stimulate T-cell proliferation.

Human and mouse Mø bind and internalize Candida cells via the mannose-fucose receptor (MFR) (37, 47). As human DC and Langerhans cells also express the MFR on their surface (6, 13), we hypothesized that immature DC might phagocytose Candida through the MFR and subsequently kill the organism, process Candida-specific antigens, and stimulate lymphocyte proliferation.

Most recently it was reported that mouse DC phagocytose and kill Candida and that recognition was through the MFR (19). The data presented herein confirm these observations for human DC and also demonstrate two important differences in the interaction of human and mouse DC with Candida. The first is that human and mouse DC apparently kill Candida by different mechanisms. The second difference is that Candida-infected human DC stimulate T-cell proliferation without the requirement for the addition of exogenous IL-2.

**MATERIALS AND METHODS**

Reagents. Nω-nitroarginine methyl ester (LNMMA) was purchased from Calbiochem, La Jolla, Calif. Fluorescein isothiocyanate (FITC), type III ferricytochrome C, Nω-nitroarginine methyl ester (NAME), superoxide dismutase (SOD), catalase, mannosylglycosylation kit (CD), t-flucose, galactose, N-acetyl-t-glucosamine, o-glucosamine, o-methyl-1-nanomannose, mannansylated bovine serum albumin (BSA), and o-mannose were purchased from Sigma, St. Louis, Mo. Diphenylene iodonium bisulfate (DPI) was a gift of Andrew Cross, The Scripps Research Institute, La Jolla, Calif. A 1 mM stock concentration of DPI was prepared in dimethyl sulfoxide (DMSO) and stored at −80°C. DPI was diluted into RPMI 1640 (Gibco-BRL, Gaithersburg, Md.) to the desired concentration just before use. CD was dissolved in DMSO at a concentration of 1 mg/ml, and aliquots were stored at −80°C until use. Cytochrome c (1 mM) was dissolved in Krebs-Ringer phosphate buffer containing 0.2% dextrose and stored at −20°C. All other reagents were prepared in RPMI 1640 and sterilized filtered. Pooled human serum (PHS) prepared from six to eight individual donors was pooled and stored in 200-μl aliquots at −80°C until use.

Preparation of human DC and Mø. Monocytes were isolated by sequential centrifugation (37), washed twice in RPMI 1640 containing 0.05% sodium azide, and prepared in dimethyl sulfoxide (DMSO) for storage. Monocyte preparations were counted by hemacytometer and standardized to 2 × 10⁶ cells/ml. HK cells were prepared in dimethyl sulfoxide (DMSO) and stored at −80°C. The yeast cells were counted on a hemacytometer and standardized to the correct concentration in HBSA.

Reagents. Reagents were purchased from Sigma, St. Louis, Mo., or from other sources as noted. Monolayers were prepared in 6-well tissue culture plates (Corning-Costar, Cambridge, Mass.) at 60% confluence in RPMI 1640 containing 200 mM glutamine, 50 μg of kanamycin/ml, 1% nonessential amino acids (BioWhittaker, Walkersville, Md.), and 1% pyruvate (CellGro, Virginia Beach, Va.). The DC were plated at 5 × 10³ cells/well. Ten microliters of sample then was dispensed into 12- by 75-mm polypropylene tubes for 30 min at 37°C in a water bath with orbital shaking at 150 rpm. At each time point, the cells and yeast cells were centrifuged, the supernatant removed, and the cells were resuspended in 200 μl of RPMI 1640 containing 10% serum and plated in 24-well plates. Ten microliters of sample was added to each well. Ten microliters of HBSA containing one or more yeast cells were added to each well. Yeast cells were counted by hemacytometer and standardized to the correct concentration in HBSA.

Fluorescence-activated cell sorter (FACS) analysis. DC (5 × 10⁶) were incubated with primary monoclonal antibodies (mAbs) at 4°C for 45 min. After two washes in phosphate-buffered saline (PBS) containing 1% BSA (PBS-BSA), the cells were incubated with 1% paraformaldehyde or were incubated with a fluorescein-labeled secondary antibody for an additional 45 min at 4°C. The cells were then washed twice with PBS-BSA and fixed overnight with 1% paraformaldehyde before analysis by flow cytometry on a FACScan fluor flow cytometer (Becton Dickinson, San Jose, Calif.) with standard optics and filter. The acquired data were analyzed with CELLQuest software (Becton Dickinson). Nonspecific antibody binding was blocked by preincubation of DC with 250 μg of human immunoglobulin G (μgG) Sigma/ml) for 30 min at 4°C for the addition of primary mAbs. The following mAbs were used: FITC-labeled CD11a, CD11b, CD11c, CD80, CD18, CD40, and mouse IgG1, IgG2a, and IgM (Ancell, Bayport, Maine); unconjugated CD19, CD13, CD16, and goat anti-rat IgG and IgM (Ancell); CD83 (Serotec, Raleigh, N.C.); CD86 (PharMingen, San Diego, Calif.); HLA-DR (CAL-Tag, South San Francisco, Calif.); CD1a (Bio-source International, Menlo Park, Calif.); and CD56 (Tecoll Diagnostics, Inc., Woburn, Mass.). The DC expressed high levels of CD11a, CD11b, CD11c, HLA-DR, and VLA-5 and moderate levels of CD11a, CD11b, and CD86. DC were negative for Mø, T-cell, B-cell, and NK markers (24).

**Phagocytosis assay.** Phagocytosis of Candida by human DC and Mø was quantitated as described previously for H. capsulatum (52). HK Candida cells were opsonized with 5% PHS at 2 × 10⁷ yeast/ml for 30 min at 37°C. After washing twice in HBSA, opsonized Candida cells were resuspended in HBSA. DC were harvested from 6-well plates, and Mø were harvested from bead bags after 5 to 7 days of culture, washed in HBSA, and standardized to 2 × 10⁶ yeast/ml. DC or Mø (1 × 10⁶) were incubated with or without FITC- or HBSA-labeled HK Candida cells (5 × 10⁴) in a total volume of 1 ml at 37°C in a water bath with orbital shaking at 150 rpm for various periods of time. At the end of the incubation period, trypan blue (1 mg/ml in PBS) was added for 15 min at 25°C to quench the fluorescence of bound but uningested organisms (52). The cells then were washed with HBSA, cytotoxicentrifuged on glass slides, and fixed in 1% parafomaldehyde in 4°C. Coverslips were mounted in 90% glycerol in PBS, and phagocytosis was quantified by phase-contrast and fluorescence microscopy. A total of 100 cells were counted per slide and the number of ingested or bound but uningested yeast cells was determined. Results are expressed as the mean ± standard error of the mean (SEM) of the phagocytic index (PI) (the total number of yeast cells ingested per 100 DC or Mø) as the percent ingestion (the percentage of DC or Mø containing one or more yeast cells).

Binding assays. DC and Mø were harvested after 5 to 7 days of culture, washed twice in HBSA, and resuspended in HBSA with or without HBSA-labeled yeast cells (Amer sham-Burroughs, Arlington Heights, Ill.) and resuspended in HBSA. HK Candida cells were incubated in 12- by 75-mm polypropylene tubes for 30 min at 37°C with various sugars or HBSA only. Fifty microliters of FITC-labeled Candida cells (2 × 10⁷/ml) then were added to each tube and incubated for 20 min at 37°C in a water bath with orbital shaking at 150 rpm. Ten microliters of sample then was mounted on a clean glass slide and coverslipped for immediate quantitation via phase-contrast and fluorescence microscopy. One hundred DC were counted per slide, and the results are expressed as the mean ± SEM of the attachment index (the total number of organisms bound per 100 cells).

For binding assays with Mø, the cells (2.5 × 10⁵) were allowed to adhere for 1 h at 37°C in 5% CO₂, 95% air in the wells of a Terasaki tissue culture plate (Miles Scientific Division, Naperville, Ill.) that previously had been coated with 1% human serum albumin. The cells were washed twice with HBSA and then incubated with various sugars or HBSA for 30 min at 37°C. Five microliters of FITC-labeled HK Candida cells (2 × 10⁶/ml) then was added to each well and the mixture was incubated for 20 min at 37°C. Unbound organisms were removed by washing with HBSA and the monolayers were fixed with 1% paraformaldehyde. Attachment of the yeast cells was quantitated via fluorescence microscopy on an inverted microscope (Diaphot; Nikon Inc. Instrument Group, Melville, N.Y.) by counting 100 Mø per well. Results are expressed as mean ± SEM of the attachment index (52).

Quantitation of DC and Mø fungicidal activity. DC and Mø (5 × 10⁵) were incubated with Candida cells (5 × 10⁴) in the presence or absence of 10% PHS in polypropylene tubes for 1, 2, or 4 h at 37°C in a water bath with orbital shaking at 150 rpm. At each time point, the cells and yeast cells were centrifuged, the
supernatant was aspirated, and the mixtures were resuspended in 1 ml of sterile water. The contents then were vortexed vigorously prior to serial dilution and plating on Sabouraud dextrose agar plates. Inspection of the lysate by light microscopy confirmed that the yeast cells were not clumped. After incubation at 30°C for 48 h, CFU were counted and the percent Candida cells that were killed was calculated by comparison with the CFU obtained from the original inoculum. Some experiments were performed under anaerobic conditions in a Forma Scientific (model 1024; Marietta, Ohio) anaerobic chamber.

Quantitation of PL fusion. DC (1 × 10^6) were incubated for 2 h at 37°C in M199 (Gibco-BRL) containing 10% human serum, 10 μg of gentamicin/ml, and 18-nm diameter colloidal gold beads stabilized with horseradish peroxidase (HRP:Au18) (53). The DC were washed three times with medium and then incubated an additional 2 h at 37°C to ensure that the HRP:Au18 entered the lysosomal compartments. After the second incubation, 1 × 10^6 viable Candida cells were added and phagocytosis was allowed to proceed for 1 h at 37°C. After 1 h the DC were washed three times with ice-cold 0.1 M sodium cacodylate buffer (pH 7.4) and then processed for electron microscopy. DC phagolysosomal fusion (PL fusion) was quantified by counting the number of 18-nm gold particles in phagosomes containing Candida (53). The data are expressed as the mean ± SEM of the number of gold particles per yeast-containing phagosome. H. capsulatum and Saccharomyces cerevisiae also were studied for comparison.

Quantitation of superoxide anion (O_2^-) production. O_2^- generation by DC and Mφ was quantified as the SOD-inhibitable reduction of ferricytochrome c (type III; Sigma) as described previously (69). DC and Mφ (2 × 10^6) were incubated for 1 h at 37°C with opsonized or unopsonized Candida cells (1 × 10^6) in RPMI containing phosphate buffer with 0.2% dextrose containing 80 μM cytochrome c. Control wells contained cytochrome c without Candida (resting cells) or the Candida plus 40 μg of SOD/ml. At the end of the incubation, the supernatants were collected by centrifugation at 4°C. The absorbance of the supernatants at 550 nm was measured in a Spectronic Genesys 5 (Milton Roy, Rochester, N.Y.), and the background absorbance in control tubes containing only buffer and cytochrome c was subtracted. All experiments were performed in triplicate and results were calculated as nanomoles of O_2^- using E_{550 × 2.1 × 10^4} = 1.26. Results are expressed as nanomoles of cytochrome c reduced per 2 × 10^6 cells per hour.

T-cell isolation. At day 6 of the DC culture, blood was obtained from the same donor, and mixed mononuclear cells were isolated on Ficoll-Hypaque gradients (52). The mononuclear cells were standardized to 7.5 × 10^6/ml in RPMI 1640 containing 5% FCS and 10 μg of gentamicin/ml, warmed to 37°C, and passed over a nylon wool column at 37°C. After 1 h of incubation on the column, the first 15 ml of column flowthrough was collected and cultured at 2 × 10^6/ml overnight in a T flask (Corning-Costar). After 24 h of culture, the nonadherent cells were collected, washed in Dulbecco’s PBS (DPBS) containing 2% FCS, and incubated for 1 h on ice with mouse Mabs to human CD56 (Becton Dickinson) and human CD16 (Medarex, Annandale, Calif.) to eliminate any remaining NK cells. The cells then were washed in DPBS containing 2% FCS and incubated for 1 h at 4°C on a rocking shaker (Thermolyne, Dubuque, Iowa) with goat anti-mouse IgG magnetic beads (Per保利 Biosystems, Framingham, Mass.) at 10 beads per cell. Purified T cells were obtained after incubation of the cell-bead suspension on a Dynal MPC-1 magnet (Oslo, Norway) for 10 min. The T cells remaining in suspension were collected and used in the antigen presentation assays as described below. T cells were 98.5% CD3^+ by FACs analysis (24).

Antigen presentation assays. Antigen presentation by DC to T cells was quantified by the incorporation of [3H]thymidine (24). DC were incubated with either HK or viable Candida cells for 1 h at 37°C in a 96-well plate to allow for phagocytosis of the yeast cells. Autologous T cells then were added to each well and the plate was cultured at 37°C for 7 days. In these experiments, the media contained 10% autologous serum rather than PH, in wells containing viable Candida cells, amphotericin B (1.25 μg/ml) was added after the first 48 h of culture to prevent overgrowth of the yeast cells. On day 7 of culture, 1.0 μCi of [3H]thymidine (specific activity, 6.7 Ci/mmol; Dupont-New England Nuclear) in RPMI 1640 was added to each well. After further incubation for 24 h at 37°C, the contents of the wells were harvested onto glass fiber filters and counts per minute were determined in a liquid scintillation counter. The results are expressed as the mean ± SEM of the counts per minute incorporated by T cells in the presence of various amounts of DC and Candida. DC and T cells were greater than 95% viable on all days of culture, as determined by trypan blue dye exclusion.

Statistics. Statistical analysis of the data was performed using Sigma Stat (Jandel Scientific, San Rafael, Calif.). Student’s t-test or the Mann-Whitney rank sum test was used to analyze the data for statistical significance, and results were considered significant at a P level of <0.05.

RESULTS

Phagocytosis and killing of C. albicans by human DC. Previously, we have demonstrated that immature human DC phagocytose and kill H. capsulatum (24), and others have shown that immature human DC can phagocytose some bacteria and protozoa (1, 21, 32, 78). Therefore, we first examined the ability of human DC to phagocytose Candida and compared their phagocytic activity to human monocytoid-derived Mφ. DC and Mφ were incubated in suspension with unopsonized FITC-labeled HK Candida for various periods of time, and phagocytosis was quantified as described in Materials and Methods. Figure 1 shows that DC ingested Candida in a time-dependent manner. Phagocytosis was evident as early as 10 min, and after 1 h almost 60% of DC had ingested at least 1 Candida yeast cell, with an average of over 5 yeast cells per DC. Opsonization of the yeast cells did not enhance DC phagocytosis of Candida (Table 1) nor did it affect the percentage of DC-associated yeast cells that were ingested (61% for unopsonized yeast cells and 58% for opsonized yeast cells).

To determine if ingested Candida cells were killed by DC, DC and Mφ were incubated with viable Candida cells in the presence or absence of 10% PHS for 1, 2, and 4 h at 37°C. The data in Fig. 2 demonstrate that over the 4-h incubation period, both DC and Mφ were equally efficient at killing Candida, and fungicidal activities were equivalent regardless of whether or not PHS was present to opsonize the yeast cells.

Binding of Candida cells to human DC is mediated by the MFR. Our original rationale for these experiments was based on the fact that DC contain high levels of the MFR on their surface (6, 13). As Mφ utilize the MFR to recognize and phagocytose unopsonized Candida cells (37, 47), we hypothesized that human DC MFR might perform the same function. To test this hypothesis, DC and Mφ were preincubated with various sugars and their subsequent capacity to bind FITC-labeled HK Candida cells was quantified. Because Mφ bound Candida poorly in suspension, they were studied under adherent conditions in Terasaki plates. DC or Mφ were preincubated with various sugars for 30 min at 37°C and then incubated with FITC-labeled Candida cells for an additional 20 min. Preincubation of DC or Mφ with mannose, fucose, or mannosylated BSA, but not galactose, significantly inhibited the binding of Candida, strongly suggesting that binding was mediated through the MFR (Table 2).

Mechanism of DC fungicidal activity against C. albicans. As it has long been known that polymorphonuclear cells kill Candida predominantly via the production of toxic oxygen metabolites (16, 77, 81), we next sought to determine if incubation of DC with Candida would stimulate a respiratory burst. Therefore, DC and Mφ were incubated for 1 h with opsonized or unopsonized C. albicans cells, and superoxide anion production was quantified by the SOD-inhibitable reduction of cytochrome c. Figure 3 shows that both unopsonized and opsonized Candida stimulated the release of O_2^- to levels roughly half that produced by Mφ. However, preincubation of DC or Mφ with either SOD (200 μg/ml), catalase (100 μg/ml), or mannitol (0.04 M) did not result in inhibition of fungicidal activity by either cell type (data not shown).

Because lack of inhibition by respiratory burst inhibitors may be caused by an inability of these agents to gain entrance
to the phagosome (36), we next sought to determine if DC would kill Candida in the absence of phagocytosis. The rationale was that if killing took place extracellularly, respiratory burst inhibitors might be more effective in inhibiting killing. Therefore, DC and Mφ were preincubated for 5 min with 2 μg of CD/ml to disrupt actin microfilaments and inhibit phagocytosis, and then killing of Candida was quantified after incubation with the yeast cells for 1 h at 37°C. The data in Table 3 show that both DC and Mφ killed Candida equally well regardless of whether or not the yeast cells were ingested. Visual inspection of aliquots taken from the tubes containing CD confirmed that yeast cells remained extracellular but were still bound to DC and Mφ. Furthermore, in control experiments, CD did not affect the viability of Candida or its ability to germinate. In addition, extracellular killing of C. albicans occurred over a wide range of pHs. Thus, when the extracellular medium was buffered to pH 4.0, 7.2 (standard assay), 8.0, or 10.0, there was no difference in the amount of fungicidal activity exhibited by either DC or Mφ (data not shown).

Having demonstrated that DC and Mφ killed Candida yeast cells even when they remained outside the cell, we then sought to determine if inhibitors of the respiratory burst would inhibit killing of Candida. Preincubation of DC and Mφ with SOD, catalase, and mannitol still did not block killing of Candida by either DC or Mφ. We also attempted to block killing using the NO synthase inhibitors DPI (10 μM) (74), L-NMMA (1 mM) (56), and L-NAME (1 mM) (41), but these agents also were without effect (data not shown). Finally, we tested DPI and SOD in combination (Table 4), but again there was no inhibition of DC or Mφ fungicidal activity.

As the production of toxic oxygen radicals did not appear to be the mechanism of DC or Mφ fungicidal activity, we sought to determine if DC ingesting Candida exhibited significant PL fusion. DC were loaded with HRP:Au 18 and incubated with

![Dendritic cells](image1)

**FIG. 1.** Human DC phagocytose C. albicans in a time-dependent manner. DC or Mφ (2 × 10⁶) were incubated with unopsonized FITC-labeled Candida (1 × 10⁷) for various periods of time. At the end of each time period 1 mg of trypan blue/ml was added to each sample for 15 min to quench the fluorescence of uningested organisms. An aliquot of cells was cytocentrifuged onto glass slides and phagocytosis was quantified by phase and fluorescence microscopy. The data are presented as the percent ingesting (percent DC or Mφ containing at least one ingested organism) and the phagocytic index (the total number of organisms ingested/100 DC or Mφ). The data are the mean ± SEM of four experiments with different donors.

![Macrophages](image2)

**FIG. 2.** Human DC kill C. albicans as well as Mφ. Viable Candida (5 × 10⁴) were incubated with DC or Mφ (5 × 10⁵) in the presence or absence of 5% PHS in suspension for 1, 2, and 4 h at 37°C. At each time point, remaining CFU were quantified as described in Materials and Methods. The data are the mean ± SEM of five experiments.

### Table 1. DC phagocytosis of C. albicans is not enhanced by serum

<table>
<thead>
<tr>
<th>Treatment</th>
<th>% Ingestion</th>
<th>Phagocytic Index</th>
<th>No. of bound but uningested Candida/100 DC</th>
</tr>
</thead>
<tbody>
<tr>
<td>No serum</td>
<td>57 ± 3</td>
<td>314 ± 87</td>
<td>202 ± 58</td>
</tr>
<tr>
<td>5% PHS</td>
<td>60 ± 5</td>
<td>230 ± 70</td>
<td>164 ± 51</td>
</tr>
</tbody>
</table>

a DC were incubated with Candida in the presence or absence of 5% PHS for 1 h at 37°C. Phagocytosis was quantified by phase-contrast and fluorescence microscopy. Data are the mean ± SEM of three experiments performed in duplicate.

b Percentage of DC ingesting 1 or more Candida cells.

c Number of Candida cells ingested per 100 DC.
Candida, H. capsulatum, or S. cerevisiae cells for 1 h at 37°C and then processed for electron microscopy. Figure 4 shows that after 1 h of phagocytosis, DC containing Candida had an average of almost 4 gold particles per phagosome. In contrast, DC that had phagocytosed S. cerevisiae had less than 2 gold particles per phagosome, while DC that had ingested H. capsulatum had about 13 gold particles per phagosome.

Our cautious interpretation of these data is that modest PL fusion occurred upon ingestion of C. albicans by human DC, even though it was only one-third the amount of PL fusion that occurred when DC phagocytosed H. capsulatum. The question that remained unanswered was whether this amount of PL fusion was biologically significant. In other words, was it enough to kill Candida?

To address this issue and rule out a role for toxic oxygen radicals, DC and MΦ fungicidal activities were quantified under anaerobic conditions. DC, MΦ, and Candida cells were equilibrated in the anaerobic chamber for 30 min prior to initiating the killing assay. The DC and MΦ then were incubated with opsonized and unopsonized Candida cells for 1 or 2 h and killing of Candida was quantified as described previously. The data in Table 5 show that both DC and MΦ killed Candida under anaerobic conditions almost as efficiently as under aerobic conditions.

Antigen presentation by DC. As DC phagocytosed and killed Candida, we next tested the ability of DC to process and present Candida antigens to T cells. In preliminary experiments, we determined that the optimal concentration of DC was 10^4/well (data not shown). Next, DC (10^4) were incubated

![Graph showing superoxide anion production by DC and MΦ](https://example.com/graph.png)

**FIG. 3.** C. albicans (Ca) stimulates human DC to produce superoxide anion. DC and MΦ (2 × 10^6) were incubated with opsonized (OP) or unopsonized Candida (1 × 10^5) in the absence or presence of SOD (50 μg/ml), and the production of superoxide anion was quantified by the reduction of cytochrome c. The data are the mean ± SEM of eight experiments.
for 1 h with various concentrations of HK or viable *Candida* cells and then cultured for 1 week with autologous CD3+ T cells. Lymphocyte proliferation over the last 24 h was then quantified by measuring the incorporation of [3H]thymidine. As shown in Fig. 5, T-cell proliferation was stimulated by both viable and HK cells in a concentration-dependent manner.

**DISCUSSION**

DC are the most potent APC of the immune system and are vital for the initiation of primary T-cell-mediated immune responses that are the hallmark of cell-mediated immunity (CMI) (70). As the host defense against *C. albicans* requires

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**TABLE 4. Inhibitors of the respiratory burst and NO production do not block the killing of *Candida* even in the absence of phagocytosis**

<table>
<thead>
<tr>
<th>Target</th>
<th>Inhibitor</th>
<th>% <em>Candida</em> killed by:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>DC</td>
</tr>
<tr>
<td>Candida</td>
<td>None</td>
<td>76 ± 2</td>
</tr>
<tr>
<td>Op Candida</td>
<td>None</td>
<td>75 ± 5</td>
</tr>
<tr>
<td>Candida</td>
<td>SOD</td>
<td>80 ± 5</td>
</tr>
<tr>
<td>Op Candida</td>
<td>SOD</td>
<td>76 ± 3</td>
</tr>
<tr>
<td>Candida</td>
<td>DPI</td>
<td>69 ± 5</td>
</tr>
<tr>
<td>Op Candida</td>
<td>DPI</td>
<td>67 ± 3</td>
</tr>
<tr>
<td>Candida</td>
<td>Both</td>
<td>76 ± 1</td>
</tr>
<tr>
<td>Op Candida</td>
<td>Both</td>
<td>71 ± 2</td>
</tr>
</tbody>
</table>

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<sup>a</sup> DC and Mφ were incubated with unopsonized *Candida* or *Candida* opsonized in 5% PHS (Op Candida) in the presence 2 μg of CD/ml and in the absence or presence of SOD (200 μg/ml), DPI (10 μM), or both SOD and DPI for 1 h at 37°C. Fungicidal activity then was quantified as described in Materials and Methods. Data are the mean ± SEM of four individual experiments.

<sup>b</sup> DC and Mφ were incubated with *Candida* in the absence or presence of 10% PHS (Op Candida) for 1 or 2 h at 37°C under anaerobic conditions. Fungicidal activity then was quantified as described in Materials and Methods. Data are the mean ± SEM of four individual experiments.

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**FIG. 4. *C. albicans* (Ca) stimulates PL fusion in human DC.** DC were labeled with HRP:Au18 and then incubated with *Candida*, *H. capsulatum* (Hc), or *S. cerevisae* (Sc) cells for 1 h at 37°C. After fixation and processing for electron microscopy, the number of gold particles in yeast-containing phagosomes was quantified. The data are presented as the mean ± SEM of gold particles per phagosome. The number above each bar denotes the number of phagosomes counted for each fungus.

**TABLE 5. Mφ and DC kill *C. albicans* under anaerobic conditions**

<table>
<thead>
<tr>
<th>Target</th>
<th>Time (h)</th>
<th>% <em>Candida</em> killed by:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mφ</td>
</tr>
<tr>
<td>Candida</td>
<td>1</td>
<td>64 ± 7</td>
</tr>
<tr>
<td>Candida</td>
<td>2</td>
<td>75 ± 8</td>
</tr>
<tr>
<td>Op Candida</td>
<td>1</td>
<td>59 ± 4</td>
</tr>
<tr>
<td>Op Candida</td>
<td>2</td>
<td>75 ± 4</td>
</tr>
</tbody>
</table>

<sup>a</sup> DC and Mφ were incubated with *Candida* in the absence or presence of 10% PHS (Op Candida) for 1 or 2 h at 37°C under anaerobic conditions. Fungicidal activity then was quantified as described in Materials and Methods. Data are the mean ± SEM of four individual experiments.

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**FIG. 5.** Phagocytosis of *Candida* was studied by incubating adherent human DC and murine-derived DC lines phagocytose bacteria such as *Bordetella bronchiseptica* (29, 31), *Listeria monocytogenes* (30), *Chlamydia trachomatis* (55, 75), *BCG mycobacterium* (35), *Mycobacterium tuberculosis* (76), and *Salmonella enterica* subsp. *enterica* (23), in addition to the protozoan *Leishmania major* (8, 39, 51, 80, 83). In vivo, murine DC containing internal
Candida as efficiently as human monocyte-derived Mφ. Fresh complement-preserved serum did not enhance the fungicidal activities of either DC or Mφ. Presumably, opsonization in serum would lead to recognition and ingestion of Candida via additional receptors including the Fc receptor for IgG and complement receptor type 1 (CR1) (40, 85, 86). Thus, engagement of a variety of cell surface receptors by both cell types leads to efficient activation of the cells’ microbicidal machinery.

The mechanism of DC and Mφ killing of C. albicans was mainly oxygen dependent, probably via lysosomal hydrolases. Thus, killing was almost as efficient under anaerobic conditions as under aerobic conditions. Furthermore, PL fusion did take place in Candida-infected DC, albeit only at one-third of that observed after infection with H. capsulatum. No evidence was obtained for an oxidative killing mechanism, even though phagocytosis of Candida by DC stimulated the production of superoxide anion. Furthermore, there was no evidence that NO was involved. In contrast, murine FSDC did produce NO upon phagocytosis of Candida and hyphae, but no definitive evidence was presented to demonstrate that NO actually was responsible for killing the Candida cells (19). As our immature DC were derived from peripheral blood monocytes, these data fit in with the general idea that the preponderance of human neutrophil candidacidal activity is mediated via the production of toxic oxygen metabolites (16, 77, 81), whereas killing of Candida by human monocytes and Mφ occurs predominantly though nonoxidative mechanisms (17, 44, 77).

Human DC also have been reported to kill and degrade L. monocytogenes (38). In contrast, T. cruzi survives and multiplies intracellularly within human DC (78), and L. monocytogenes (30), B. bronchiseptica (29), and S. enterica subsp. enterica (23) apparently survive within the murine DC line CB1. Electron microscopy reveals both viable and degraded Chlamydia in murine DC (55, 75) and both viable and degraded B. burgdorferi in human DC (21). Further, murine DC, collected after in vivo infection with L. major, contained viable parasites that caused the development of lesions upon reinjection into BALB/c mice (51).

Although the murine DC line tsDC kills M. tuberculosis (76), these bacteria grow more rapidly within human DC than in human Mφ (22). Most interesting is the fact that IL-10, but not IFN-γ, TNF-α, IFN-γ in combination with TNF-α, IL-4, or transforming growth factor β, significantly suppresses the growth of M. tuberculosis in DC but not in Mφ (22). Thus, it is clear that the interactions of DC with microorganisms vary both with the species of DC and with the particular microorganism under investigation.

Although killing of a microorganism would seem to be a necessary prerequisite to obtain efficient presentation of antigens, Moll and colleagues (51) found that murine DC contained viable, virulent L. major parasites, but infected DC still were capable of stimulating lymphocyte proliferation. Because the DC that stimulated proliferation contained viable organisms, possibly a small number of intracellular parasites were actually degraded and antigen was processed and deposited on the surface of the infected DC prior to presentation to lymphocytes. Alternatively, antigens could be processed and regurgitated by other infected phagocytes and then transferred to DC for presentation. However, attempts to demonstrate that mycobacterial antigens could be transferred from infected Mφ to DC were unsuccessful (58). Whether DC might acquire antigens from other microbial pathogens via this route remains to be determined.

Lastly, we demonstrated that phagocytosis of both viable and HK Candida cells by human DC leads to the stimulation of...
T-cell proliferation. As we found in our studies of human DC and *H. capsulatum* (24), DC that had phagocytosed viable *Candida* cells actually were more efficient at stimulating T-cell proliferation than DC that had ingested HK cells. Thus, ingestion of 1,000-fold fewer viable yeast cells than HK cells led to equivalent stimulation of lymphocyte proliferation. These results are in agreement with the fact that mice develop better immunity when given a sublethal inoculum of a viable organism than they do with HK organisms. Thus, these data again suggest that the heat-inactivation process may destroy important immunogenic antigens and have implications for the design and use of DC in vaccine strategies.

Although we did not skin test our donors for sensitivity to *Candida* antigens, we presume that the proliferation observed was mostly a secondary response, as *C. albicans* is a commensal organism in humans. Likewise, in our other studies with *H. capsulatum* and human DC (24) we presumed that the T-cell proliferation was a secondary response, as *Histoplasma* is indigenous in Cincinnati. In those studies, we did identify three *Histoplasma* and human DC (24) we presumed that the T-cell sign and use of DC in vaccine strategies.

**REFERENCES**

DENDRITIC CELL INTERACTION WITH CANDIDA ALBICANS


