Monocyte-mediated Serum-independent Damage to Hyphal and Pseudohyphal Forms of Candida albicans In Vitro

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ABSTRACT Human peripheral blood monocytes attached to Candida albicans hyphae in the absence of serum and damaged the hyphae without completely ingesting them. Attachment and damage was not augmented by the addition of serum. Damage to hyphae was quantitated by a previously developed metabolic assay that measured leukocyte-induced reduction in uptake of [14C]cytosine by the hyphae. Use of cells from patients with hereditary disorders of leukocyte function, chronic granulomatous disease, and myeloperoxidase deficiency indicated that myeloperoxidase-independent and nonoxidative mechanisms could sometimes damage hyphae where oxidative mechanisms were impaired. Damage to hyphae by normal monocytes was inhibited by concentrations of sodium azide and sodium cyanide that primarily affect myeloperoxidase activity, as well as by halide-free conditions, catalase, and putative antagonists of hypochlorous acid or singlet oxygen. Iodination of hyphae, a myeloperoxidase and hydrogen peroxide-dependent process of monocytes, was similarly inhibited by sodium azide, sodium cyanide, and catalase. Under anaerobic conditions, damage to hyphae was reduced by 64.0–68.4%. In contrast, inhibitors of potential nonoxidative antifungal mechanisms, iron salts to saturate iron chelators, and polyanionic amino acid polymers to neutralize cationic proteins did not block damage to hyphae by monocytes. Preparations rich in lysosomal granules from fractionated normal monocytes also did not damage hyphae. Overall, it appeared that oxidative mechanisms were most important for damage to hyphae by normal monocytes.

Electron microscopy confirmed that Candida hyphae were damaged and probably killed by monocytes, but monocytes appeared to sustain significant damage in the process. In the absence of serum, monocyte cell membranes became closely approximated to Candida cell walls. It appeared that some Candida could escape this partial engulfment, as they were seen floating free with vesicular trilaminar membrane remnants covering hyphal surfaces. In general, monocytes appeared to be damaged by interactions with Candida hyphae more than neutrophils had been in previous studies.

INTRODUCTION Invasive lesions caused by Candida albicans characteristically contain hyphal and pseudohyphal forms that are too large to be ingested by phagocytic cells. However, our previous studies established that these large forms of Candida could be damaged and probably killed by human neutrophils in the absence of complete ingestion (1, 2). Damage to hyphae was largely attributable to products of oxidative metabolism of neutrophils (2). Like neutrophils, monocytes and macrophages derived from them have been demonstrated to ingest and kill yeastlike forms of Candida (3–7). Mononuclear phagocytes may be important components of host defense mechanisms against invasive candidiasis by contributing to prevention of dissemination or by limiting the extent of mucocutaneous lesions. Defective function of mononuclear phagocytes may be involved in patients with the chronic mucocutaneous form of candidiasis (8). Several important host defense functions have been attributed to mononuclear phagocytes, including direct phagocytic microbialic activity, antigen processing in the afferent limb of the immune response, interactions with lymphoid cells in cellular immune responses, and lysis of tumor cells.
by nonphagocytic killing mechanisms active at the cell surface (5). Though monocytes share many features with neutrophils, many differences between these cell types have become apparent. Unstimulated monocytes appear to have efficient intracellular microbicidal activity but have been found to be less actively phagocytic than neutrophils (9, 10). Though oxidative metabolism is largely responsible for microbicidal activity in both neutrophils and monocytes, production of oxidative products is not necessarily identical in both cell types (9, 11, 12), and nonoxidative bactericidal activity of monocytes may be important in some situations (3, 13). From this, it might be inferred that monocytes could play an important role distinct from neutrophils in host defenses against candidiasis. Furthermore, the capacity for intracellular killing of ingested yeasts may not reflect the ability to damage hyphae at the monocyte surface by nonphagocytic mechanisms.

Therefore, in these studies, we determined that monocytes could damage and probably kill hyphae and pseudohyphae of C. albicans. Hyphal damage by normal monocytes appeared to be primarily due to products of oxidative metabolism, especially the myeloperoxidase-H₂O₂-halide system, but myeloperoxidase-independent mechanisms were active as well when myeloperoxidase-deficient monocytes were tested.

METHODS

Hyphae and pseudohyphae. An isolate of C. albicans originally obtained from a patient with systemic candidiasis was germinated in Eagle’s minimal essential medium supplemented with nonessential amino acids (Gibco Laboratories, Grand Island Biological Co., Grand Island, N. Y.). Incubation at 37°C was continued until ≥95% of cells had formed germ tubes ≥30 μM in length (1, 2).

Monocytes. Mixed mononuclear cells were obtained from human peripheral blood by sedimentation on a Ficoll-(Pharmacia Fine Chemicals, Inc., Piscataway, N. J.) Hypaque (sodium and megam Lucin diatizatoes, Winthrop Laboratories, N. Y.) gradient, followed by dextran sedimentation to obtain neutrophils for some studies (14). Preparations of mononuclear cells were adjusted according to the relative percentages of monocytes, which varied from 18–42% as judged by neutral red ingestion and fluorescent staining with acridine orange. Mononuclear cell preparations used in these studies contained 1.0% neutrophils. For selected experiments, more purified preparations of monocytes were obtained by counterflow centrifugation (Beckman J21B with elutriator rotor, Beckman Instruments, Inc., Fullerton, Calif.) (15). These preparations contained 91–94% monocytes by nonspecific esterase staining (16). Monocytes were 98.5–100% viable as judged by trypsin blue exclusion. Monocytes were depleted from preparations by passage through nylon fiber columns (17) or by depletion of adherent cells by passage through three sets of plastic tissue culture dishes. Children with typical chronic granulomatous disease (three sex-linked, one autosomal recessive) and adults with hereditary myeloperoxidase deficiency were identified and blood was kindly supplied by Dr. Robert A. Clark (University Hospital, Boston, Mass.), Dr. Harvey Cohen (Children's Hospital, Boston, Mass.), and Dr. Richard K. Root (Yale-New Haven Hospital, New Haven, Conn.).

Sera. For selected studies, autologous sera were obtained from normal volunteers subjects at the same time as monocytes. Sera obtained from a patient with systemic candidiasis had anti-Candida antibodies detectable by immunodiffusion and a Candida whole cell agglutination titer of 1:128. Rabbit antisera to Candida had a 1:512 agglutination titer.

Separation of subcellular fractions of monocytes. Mononuclear cells were lyzed and subcellular fractions were isolated according to the method of Kimball et al. (18). Pelleted Hypaque-Ficoll separated mononuclear cells were washed in 5 ml 0.34 M cold sucrose, then centrifuged at 4°C for 5 min at 1,000 g. Cells were resuspended in 2 ml of 0.34 M cold sucrose containing 5,000 U heparin. Suspensions were repeatedly suctioned through Pasteur pipettes until ≥95% of cells were broken, as judged by phase contrast microscopy. An additional 10 ml of cold 0.34 M sucrose were added to lysates and gently but evenly mixed. 2 ml were removed and mixed with 4 ml of 0.34 M sucrose (designated as the lysate fraction). The remaining lysate was centrifuged at 800 g for 10 min., the supernate (S₁) saved, and the pellet resuspended in 5 ml 0.34 M sucrose and centrifuged at 400 g for 5 min. This supernate (S₂) was saved, and the pellet mixed with 6 ml 0.34 M sucrose (designated as the nuclear fraction). Supernates (S₁ and S₂) were combined and centrifuged at 27,000 g for 20 min, pellets were resuspended in 6 ml of 0.34 M sucrose (designated as the granular fraction), and remaining supernates that contained no free granules or other debris were designated as the post-granular supernate. For some experiments, heparin in fractions was neutralized by addition of protamine sulfate (Sigma Chemical Co., St. Louis, Mo.). Fractions were used fresh or stored at −20°C until assayed for their ability to damage Candida hyphae. For assays of damage to hyphae, some batches of fractions were sonicated or subjected to repeated freezng and thawing in dry ice and methanol in order to ensure lysis of granules and release of granule contents before use.

Assessment of hyphal damage by uptake of [³¹⁴C]leucine. Damage to hyphae was measured as in previous studies (1). Briefly, suspensions containing 5 × 10⁵ Candida albicans hyphae and 5 × 10⁵ monocytes in 0.5 ml Hanks’ balanced salt solution were placed in 15-ml plastic centrifuge tubes (Corning Glass Works, Science Products Div., Corning, N. Y.). In some studies, monocytes were replaced by separated subcellular fractions of monocytes. Control tubes contained 5 × 10⁵ hyphae without leukocytes or subcellular fractions. Triplicate tubes were rotated for 1 h at 37°C. Control tubes then received 5 × 10⁵ leukocytes or equivalent amounts of subcellular fractions present in experimental tubes. Selected assays were performed anaerobically in triplicate 15-ml polycarbonate plastic tubes with tightly screw cap stoppers and in triplicate anaerobic specimen collectors (Becton, Dickinson & Co., Rutherford, N. J.). Tubes were flushed with highly purified nitrogen that was passed first through an alkaline pyrogallol, then distilled water. Hanks’ balanced salt solution was kept under vacuum overnight, followed by bubbling through with purified nitrogen for 4 h before use. Sets of control tubes were included to monitor pH, oxygen content and oxygen consumption in response to ingestion of opsonized zymosan (YSI model 53 oxygen monitor, Yellow Springs Instrument Co., Yellow Springs, Ohio). Compared with air-saturated media, these conditions reduced oxygen content to <1%, and there was no stimulation in oxygen consumption with ingestion of opsonized zymosan by leukocytes. Leukocytes in all tubes were then lyzed with 2.5% sodium deoxycholate. Remaining hyphae were washed twice in distilled water and once in yeast nitrogen base broth that had been

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supplemented to include 1% dextrose and 0.15% asparagine. Each tube then received 0.25 µCi of [14C]cytosine (Research Products International Corp., Elk Grove Village, Ill.) dissolved in supplemental yeast nitrogen base broth. After incubating at 30°C for 1 h, hyphae were washed free of unbound [14C]cytosine using an automated multiple sample harvester (Otto Hillar Co., Madison, Wis.). Filters were dried and counted in a liquid scintillation counter. Percentage of reduced uptake of [14C]cytosine by hyphae induced by monocytes or subcellular fractions was calculated from (mean counts per minute in control tubes) – (mean counts per minute in experimental tubes)/(mean counts per minute in control tubes) × 100.

Iodination. Fixation of 125I to Candida by monocytes was measured according to the methods of Klebanoff and Clark (19) with the minor modifications outlined in previous studies (1). Triplicate incubation tubes each contained 1 × 10^6 monocytes and 5 × 10^6 Candida hyphae and 0.5 µM (0.2 µCi) Na^125I (New England Nuclear, Boston, Mass.) and were tumbled at 37°C for 1 h.

Inhibitors. Catalase (bovine liver, 6.1 mg/ml, 50,000 U/mg) was obtained from Worthington Biochemical Corp. (Freehold, N. J.) and dialyzed against water before use. Superoxide dismutase (bovine erythrocyte, lyophilized powder, 12,300 U/mg) obtained from Miles Laboratories Inc., Research Products (Elkhart, Ind.) was dissolved in water at a concentration of 5 mg/ml and stored at −20°C until used. For use in incubations, catalase and superoxide dismutase were first incubated with hyphae at 37°C for 1 h before addition of leukocytes, as these substances were noninhibitory without this preincubation. For some experiments, catalase was heat treated at 100°C for 15 min., and superoxide dismutase was autoclaved at 121°C for 30 min before use. Sodium azide, sodium cyanide, histidine, tryptophan, dimethyl sulfoxide (DMSO), mannitol, sodium benzoate, ferric chloride, ferric and ferrous ammonium sulfate, and polymerized amino acids (polyaspartic acid, polyglutamic acid, polyarginine, and polylysine) were obtained from Sigma Chemical Co. Triethylendenediamine, also known as 1,4-diazobicyclo[2,2,2]octane (DABCO) was supplied by Eastman Organic Chemicals Division (Eastman Kodak Co., Rochester, N. Y.).

Electron microscopy. After 60 min of incubation with monocytes, hyphae and monocytes were fixed in suspension by addition of phosphate-buffered glutaraldehyde/formaldehyde (final concentrations 1 and 4%, respectively). For preparation for transmission microscopy, the cells were spun at 450 g for 10 min and resuspended in the next solution for each of the following steps: 1% aqueous osmium tetroxide for 60 min, 0.1% aqueous uranyl acetate for 30 min, graded alcohols, propylene oxide and Epon 812 (Shell Chemical Co., Houston, Tex.). Toluidine blue-stained sections, 1-µm thick, were cut in both a plane perpendicular and a plane parallel to the axis of g-forces of centrifugation to examine the distribution of cells within the pellet. Ultrathin sections through the center of the pellet (including all cells from top to bottom) were cut with diamond knives, stained with lead citrate and uranyl acetate, and examined with a Philips EM300 at 80 kV (Philips Electronic Instruments Inc., Mahwah, N. J.).

Statistical methods. Mean±SEM were compared using the two-tailed two sample t test (30).

**RESULTS**

**Metabolic assays for damage to hyphae by monocytes.** C. albicans hyphae were damaged by human peripheral blood monocytes, as judged by monocye-induced reduction in uptake of [14C]cytosine by the hyphae (Fig. 1). As was the case with neutrophils (1), monocyte-mediated reduction in [14C]cytosine uptake correlated with changes in uptake of [PH]glucose and mannose, as well as electron-microscopic evidence to damage to the fungi. Monocytes rather than lymphocytes mediated the damage to hyphae, as lymphocytes depleted of monocytes by passage through nylon wool columns or by adherence to plastic dishes did not damage Candida at all. Furthermore, hyphal damage by preparations depleted of more than 90% of lymphocytes by elutriator (15) was no greater than by equal numbers of Hypaque-Ficoll separated monocytes consisting of 60–80% lymphocytes (45.0±5.9% damage by elutriator monocytes vs. 42.9±6.3% by Hypaque-Ficoll monocytes in three experiments). As in previous studies with neutrophils (1), damage to hyphae by monocytes was

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**Abbreviations used in this paper:** DABCO, 1,4-diazabicyclo[2,2,2]octane: DMSO, dimethyl sulfoxide.
irreversible and was complete within 30–60 min. Unlike previous observations using neutrophils (1), attachment to and damage of hyphae by monocytes was not augmented by the addition of autologous or homologous human sera (Fig. 1). Similarly, human and rabbit sera containing anti-Candida IgG antibodies affected neither attachment to hyphae as judged by phase contrast microscopy, nor damage to hyphae determined by cytosine uptake. Accordingly, all subsequent experiments were performed in the absence of serum.

Mechanisms of damage to hyphae by monocytes. Previous studies had shown that oxidative metabolism by neutrophils was critical in affecting damage to C. albicans hyphae (1). To study the importance of these processes in monocyte-mediated damage, cells were first used from patients with two well-characterized dysfunctions of phagocytic cells. In hereditary, chronic granulomatous disease, particles are ingested normally by neutrophils and monocytes. However, the normal postphagocytic burst in oxygen consumption does not occur and potentially microbicidal products are not produced in normal amounts, including hydrogen peroxide, superoxide anion, and perhaps other oxidative intermediates as well (12). In hereditary myeloperoxidase deficiency, particle ingestion is also normal and hydrogen peroxide is produced, but myeloperoxidase is not present to react with peroxide and a halide to produce potent microbicidal substances (12). Previous studies (21) showed that neutrophils from two patients with myeloperoxidase deficiency and three (two sex-linked, one autosomal) of four patients with chronic granulomatous disease failed to damage Candida hyphae at all. Neutrophils from the fourth patient with chronic granulomatous disease (typical, sex-linked) damaged hyphae minimally, below values obtained with leukocytes from normal control subjects (Fig. 2). Damage to hyphae by monocytes from this same patient was in the upper part of the range for normal subjects. Shortly after testing, this subject received a bone marrow transplant, preventing performance of repeat studies. Monocytes from the other three subjects with chronic granulomatous disease did not cause detectable damage to hyphae. In contrast, monocytes from the two patients with myeloperoxidase deficiency caused significant damage to Candida hyphae, below mean values for normal subjects, but within the normal range (Fig. 2). These results are consistent with a central importance for oxidative mechanisms in damage to Candida by monocytes. However, nonmyeloperoxidase-dependent mechanisms appeared to have been active in myeloperoxidase-deficient monocytes, and nonoxidative mechanisms may have played a role in damage to hyphae by monocytes from one of the four patients with chronic granulomatous disease.

To assess the relative roles of these mechanisms in damage to hyphae by normal monocytes, inhibitors of monocyte function were tested for their ability to block the damage to hyphae which occurred in the absence of inhibitors. In the concentrations used in these studies, the inhibitors impaired neither viability (as judged by trypan blue exclusion) nor oxygen consumption by monocytes. Sodium azide and sodium cyanide inhibited damage to hyphae by monocytes (Table 1). As shown by Klebanoff (22), the concentrations used primarily affect myeloperoxidase activity. Specificity of inhibition was confirmed using monocytes from one patient with myeloperoxidase deficiency, which damaged hyphae (≥36.2%) equally well in the presence or absence of 0.1 mM sodium azide. Omission of halide or addition of catalase inhibited monocyte-mediated
damage to hyphae but superoxide dismutase did not inhibit damage to hyphae at all. Other products may also be derived from oxidative metabolism of neutrophils and monocytes. Three putative quenchers of singlet oxygen (also antagonists of hypochlorous acid), DABCO (23), histidine (24), and tryptophan (25), all inhibited damage to hyphae. DABCO was least effective but could not be used in higher concentrations, where it caused damage to hyphae by itself, in the absence of monocytes. DMSO (26), a hydroxyl radical scavenger and antioxidant, had only a minimal, insignificant effect on damage to hyphae by monocytes. Other less potent putative hydroxyl radical scavengers (sodium benzoate, mannitol) were also noninhibitory. Prolongation of incubations beyond one hour did not change sensitivity of the inhibitors tested.

Further support for the importance of myeloperoxidase-mediated damage to hyphae by monocytes was provided by quantitation of iodination (Table II). On contact with hyphae, myeloperoxidase is released from monocyte granules and is free to react with hydrogen peroxide produced by the monocytes. This results in the fixation of added iodide to fungal proteins, measurable as trichloroacetic acid precipitable 125I. Hyphae were iodinated by monocytes and this process was inhibited by azide, cyanide and catalase. Anaerobic experiments provided additional evidence for the importance of oxidative mechanisms in damage to hyphae by monocytes. In three experiments, each performed in two different types of anaerobic environments, damage to Candida hyphae was significantly reduced compared with simultaneous aerobic control incubations (24.9±3.6% damage vs. 69.1±0.9% aerobically, and 10.6±7.8% vs. 33.4±5.4% aerobically, reductions in damage of 64.0 and 68.4%, respectively).

These results do not exclude the possibility that non-oxidative mechanisms may also be active in monocyte-mediated damage to fungi. Two potential antifungal mechanisms were studied using inhibitors: iron chelation and cationic proteins. Neither addition of excess iron salts (10 mM ferric chloride, ferric ammonium sulfate, and ferrous ammonium sulfate) nor of poly-anionic polymerized amino acids (10 μM polyaspartic and polyglutamic acids) significantly inhibited damage to hyphae by monocytes. Other polyanions, such as heparin and α1-antitrypsin, were nonspecific inhibitors of oxidative metabolism and could not be used (2). However, it remained possible that monocyte granule constituents had antifungal effects by other mechanisms or that inhibitors could not reach sites of action due to the close proximity of monocyte and hyphal surfaces. Therefore, monocytes were fractionated and granule-rich preparations were obtained. Neither whole cell lysates nor granule-rich fractions damaged hyphae. Nonspecific inhibition of cationic proteins was not apparent, as there was 88.2±3.3% damage to hyphae by the nuclear pellet, rich in cationic histones which have antifungal activity (27).

Ultrastructural observations of monocyte-hypha interactions. Electron microscopy was used to examine the effects of contact between monocytes and Candida hyphae. Contamination of specimens with neutrophils was 1.0% or less. When samples were taken 1 min after adding hyphae to the monocytes, no contact

### Table I: Effects of Potential Inhibitors of Oxidative Microbicidal Mechanisms of Leukocytes on Damage to Candida albicans Hyphae by Monocytes

<table>
<thead>
<tr>
<th>Inhibitor (concentration added)</th>
<th>Inhibition* (%)</th>
<th>Number of experiments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium azide (0.1 mM)</td>
<td>47.11</td>
<td>6</td>
</tr>
<tr>
<td>Sodium cyanide (1.0 mM)</td>
<td>49.81</td>
<td>4</td>
</tr>
<tr>
<td>Halide-free system (phosphate-bicarbonate buffer)</td>
<td>100.0</td>
<td>2</td>
</tr>
<tr>
<td>Catalase (2,100 U)</td>
<td>50.1±6.3</td>
<td>6</td>
</tr>
<tr>
<td>Superoxide dismutase (25 µg)</td>
<td>0.0</td>
<td>3</td>
</tr>
<tr>
<td>DABCO (0.1 mM)</td>
<td>19.01</td>
<td>2</td>
</tr>
<tr>
<td>Histidine (0.1 mM)</td>
<td>20.51</td>
<td>2</td>
</tr>
<tr>
<td>Histidine (1.0 mM)</td>
<td>39.81</td>
<td>2</td>
</tr>
<tr>
<td>Histidine (10.0 mM)</td>
<td>91.61</td>
<td>2</td>
</tr>
<tr>
<td>Tryptophan (1 mM)</td>
<td>2.4</td>
<td>2</td>
</tr>
<tr>
<td>Tryptophan (10 mM)</td>
<td>89.41</td>
<td>2</td>
</tr>
<tr>
<td>DMSO (14 mM)</td>
<td>12.7</td>
<td>2</td>
</tr>
<tr>
<td>DMSO (1.4 mM)</td>
<td>10.0</td>
<td>2</td>
</tr>
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</table>

* Calculated from results of [14C]cytosine uptake in the presence and absence of inhibitors. Values represent means of at least two separate experiments, each performed in triplicate.

† P < 0.05 by two sample t tests.

### Table II: Effects of Inhibitors of Myeloperoxidase-dependent Microbicidal Mechanisms of Leukocytes on Iodination of Candida Hyphae by Monocytes

<table>
<thead>
<tr>
<th>Inhibitor (concentration added)</th>
<th>Inhibition* (%)</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium azide (0.1 mM)</td>
<td>96.0±2.8†</td>
<td></td>
</tr>
<tr>
<td>Sodium cyanide (1.0 mM)</td>
<td>94.8±1.7†</td>
<td></td>
</tr>
<tr>
<td>Catalase (2,100 U)</td>
<td>61.6±8.9†</td>
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</tbody>
</table>

* Calculated from results using monocytes from four different subjects, where mean (±SEM) fixation of 125I to hyphae was 21,769 (±2,624) cpm above values for control tubes which contained hyphae but no monocytes. 
† P < 0.05 by two sample t test.
between hyphae and monocytes was seen and both monocytes and hyphal morphology appeared normal. After 1 h of incubation of monocytes with hyphae, the fungi were seen in various stages of partial engulfment by monocytes. Some of these partially engulfed fungi still appeared normal, with relatively intact internal structures (Fig. 3A). Other fungi had definite disruption of internal structures. At least 70% of monocytes that surrounded these damaged hyphae and some monocytes that surrounded intact hyphae were swollen and showed membrane disruption (Fig. 3B). Where no Candida were in contact, virtually none of the monocytes appeared swollen or damaged. The serum-independent contact between monocytes and hyphae involved close approximations between electron-dense portions of Candida cell walls with plasma membranes of monocytes (Fig. 3C). Some free-floating as well as engulfed Candida hyphae were covered with apparently surface-adherent vesicles and remnants (Fig. 3D) that had a trilaminar unit membrane structure (Fig. 3E). No such structures were observed on the surface of hyphae that had been incubated for only 1 min with monocytes. In contrast to monocytes, no damage to hyphae was attributable to contact with lymphocytes.

**DISCUSSION**

These studies have established that human peripheral blood monocytes could damage and probably kill the large tissue-invasive forms of *C. albicans* in the presence of complete ingestion. The mean amount and range of damage to hyphae by monocytes from normal subjects was almost identical to that observed in prior studies where normal neutrophils were used (1). With both cell types, attachment to hyphae occurred in the absence of serum. However, while IgG in serum aug-
mented attachment of hyphae to neutrophils (1), no con-
sistent serum effects on monocyte function were ob-
erved.

Experiments were performed using cells from pa-
tients with leukocyte dysfunction and leukocytes from
normal subjects incubated with and without various in-
hibitors. In many respects, mechanisms of damage to
hyphae by monocytes appeared to be similar to those
observed in our previous experiments using neutro-
phils (2, 21), but significant differences were observed
as well. Neutrophils and monocytes from patients with
chronic granulomatous disease are unable to produce
adequate amounts of hydrogen peroxide and other pro-
ducts of oxidative metabolism (12). Neutrophils and
monocytes from three of four patients with chronic
granulomatous disease did not damage Candida
hyphae at all. The fourth patient’s neutrophils caused
minimal damage to hyphae, but his monocytes dam-
aged hyphae in the high portion of the range for normal
leukocytes. This suggests that alternative nonoxidative
mechanisms for damage to hyphae by monocytes may
become operative in some circumstances. Com-
parable nonoxidative bactericidal activity by chronic
granulomatous disease monocytes has been observed
by others (13, 28). When neutrophils from patients with
myeloperoxidase deficiency were used, no damage to
hyphae was detected. However, monocytes from
these patients caused significant hyphal damage,
though below the mean level for normal monocytes.
Myeloperoxidase-mediated microbicidal activity oc-
curs through interactions with hydrogen peroxide
and a halide (22) and is of central importance in intra-
cellular killing of C. albicans yeasts (3, 29). In our pre-
vious studies using cell-free systems, the myeloperoxi-
dase-peroxide-halide system damaged Candida
hyphae efficiently (21). However, under some circum-
stances, such as use of other species of Candida yeasts,
myeloperoxidase-independent fungicidal mechanisms
may be operative (3, 30). Such mechanisms appeared to
be operative in our studies of damage to C. albicans
hyphae by myeloperoxidase-deficient monocytes.

Use of inhibitors of leukocyte function provided
additional insights into the mechanisms of hyphal
damage by normal monocytes. Azide and cyanide (used
in concentrations that primarily affected myeloperoxi-
dase [22]), as well as catalase and halide-free condi-
tions, significantly inhibited damage to hyphae and io-
dination of hyphae by monocytes. Superoxide dis-
mutase failed to inhibit hyphal damage, so superoxide
anion may lead more to production of hydrogen
peroxide rather than a direct antifungal effect. Both
superoxide anion and hydrogen peroxide are known to
be produced by monocytes and macrophages (12,
31–33). Other potential antimicrobial substances also
may be produced by oxidative metabolism of stimu-
lated phagocytic leukocytes, including hypochlorous
acid, singlet oxygen and hydroxyl-free radical (11, 12,
31, 34–36). Putative quenchers of singlet oxygen (also
antagonists of hypochlorous acid), DABCO (23), histi-
dine (24), and tryptophan (25), all caused significant in-
hibition of damage to Candida hyphae by monocytes.
While singlet oxygen produced by monocytes may
then contribute to damage of hyphae, the nonspecificity
of these quenching agents (34, 35) may likely reflect
damage by hypochlorous acid rather than by singlet
oxygen (34–36). DMSO, a potent hydroxyl radical
scavenger (26) and an antioxidant as well, did not sig-
nificantly inhibit damage to hyphae by monocytes.
In general, these results are similar to those previously
obtained in our laboratory when comparable inhibitors
were used to block hyphal damage by neutrophils (2)
and by cell-free systems consisting of myeloperox-
dase, halide, and hydrogen peroxide (or a peroxide-gener-
ating system) or photoactivated rose bengal (21).
Similarly, as was the case with neutrophils, two poten-
tial nonoxidative fungicidal mechanisms were not ap-
parent in normal monocytes; saturation of iron chelators
and addition of polyanions to neutralize cationic pro-
teins did not inhibit damage to hyphae by monocytes.
Leukocyte cationic proteins and other granule-assoc-
iated substances used by other investigators appeared
to be responsible for damage to Candida yeasts (3, 30,
37). In our own studies, partially purified fractions of
monocytes rich in lysosomal granules did not damage
Candida hyphae. Miller noted that comparable cell
homogenates from murine macrophages had no bac-
tericidal activity (38). However, cationic proteins and
perhaps other granule constituents as well might act as
ancillary antifungal mechanisms, enhancing effects of
oxidative mechanisms (39). While these studies have
not yet identified all mechanisms active in killing of
hyphae by normal monocytes, the data using monocytes
from both myeloperoxidase-deficient and one of the
four chronic granulomatous disease patients suggest
the existence of potent myeloperoxidase-independent
or nonoxidative antihyphal systems. Our results indi-
cated that anaerobiosis significantly reduced but
did not eliminate damage to hyphae by normal mono-
cytes. This is consistent either with the activity of non-
oxidative microbicidal mechanisms (40) or with a par-
tially anaerobic system, a possibility that cannot be
eliminated completely. In any case, whether or not
nonoxidative antifungal mechanisms are active in nor-
mal monocytes, results with myeloperoxidase-defi-
cient cells suggest that myeloperoxidase-independent
antifungal mechanisms may be more active in mono-
cytes than in neutrophils. Such mechanisms may be
more evident due to compensation in myeloperoxidase-
deficient cells compared with normal cells (3). When
additional patients with myeloperoxidase deficiency

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become available for study, further insights into alternative oxidative or nonoxidative antihyphal mechanisms should be obtainable using inhibition of myeloperoxidase-deficient monocytes.

Though results of damage to Candida hyphae by neutrophils in prior studies (1, 2) and by monocytes in current studies are similar in most respects, there are important distinctions which have been noted above. Defects in leukocyte function have been associated with candidiasis, but monocyte function and neutrophil function do not necessarily correlate (41). In addition, monocytes and monocyte-derived macrophages have important roles in the afferent limb of the immune response, in antigen processing, in secretory functions, and in modulating the immune response (5). Therefore, besides damaging hyphae, the interactions of monocytes with hyphae may have effects on the immune response to Candida that neutrophils do not share.

Electron microscopy also revealed similarities and important differences in interactions of hyphae with monocytes as compared with neutrophils. Both types of phagocytic cell attached to hyphae in the absence of serum. It appeared that monocyte plasma membranes adhered directly to structural portions of hyphal cell walls. Some partially engulfed hyphae were clearly damaged and appeared to be so disrupted that a fungicidal effect must have occurred. However, in contrast to neutrophils, partial engulfment, and damage to hyphae by monocytes appeared to be accompanied by damage to the monocytes themselves. These monocytes appeared swollen and many had disrupted membranes. In addition, many partially ingested and free-floating hyphae had surface-adherent vesicles that were bounded by three-layered unit membranes. Since these vesicular membrane fragments are located outside the hyphal cell wall, they derive most likely from destroyed monocytes, which must have been in contact with this fungal cell previously. These results are consistent with the observations of Cline (42), who noted the lability of human monocytes compared with mature macrophages.

The role of monocyte-mediated damage to Candida hyphae in the intact host remains to be established. However, the ability of leukocytes to damage and probably kill the tissue-invasive forms of this fungus is likely to be of major importance in defense mechanisms against candidiasis in vivo.

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