Effects of Cytokines and Fluconazole on the Activity of Human Monocytes against *Candida albicans*


Stratton Veterans Affairs Medical Center and Albany Medical College, Albany, New York

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This study evaluates the effects of cytokines, used singly and in combination, on the microbicidal activity of human monocyte-derived macrophages (MDM) against intracellular *Candida albicans* in the presence and absence of fluconazole. In the absence of fluconazole, the addition of tumor necrosis factor alpha (TNF-α), interleukin-1β (IL-1β), gamma interferon (IFN-γ), or IL-4 had no effect on the growth of *C. albicans*. In contrast, the addition of granulocyte-macrophage colony-stimulating factor (GM-CSF) resulted in decreased growth (*P* < 0.05), while the addition of IL-10 resulted in increased growth (*P* < 0.01). In the presence of fluconazole, only the addition of IFN-γ resulted in an increase in the growth of *C. albicans*. In the presence or absence of fluconazole, all cytokine combinations except IFN-γ plus GM-CSF caused significant decreases in growth (*P* < 0.01). IL-10 and IL-4 did not influence the activity of TNF-α or IL-1β. In the absence or presence of *C. albicans* the addition of fluconazole, all of the cytokines studied, and combinations of fluconazole and selected cytokines caused increases in nitric oxide (NO) production (*P* < 0.01). Similar observations were made for superoxide (O2•−) only in the presence of *C. albicans*. The greatest concentrations of NO and O2•− were produced when *C. albicans* alone was present in the assays. Our results demonstrate that in the presence of low concentrations of fluconazole (0.1 times the MIC), selected cytokines and their combinations significantly increase the microbicidal activity of MDM against intracellular *C. albicans*.

Invasive fungal infections, including disseminated candidiasis, are usually associated with high morbidity and mortality in debilitated and immunocompromised hosts, even when they are receiving appropriate antifungal agents (2, 5, 15, 22, 41). Phagocytic cells, using both oxidative and nonoxidative mechanisms, provide the primary host defense against microbial pathogens, including fungi (2, 8, 12, 13, 30, 32, 59). In addition, cell-mediated immunity and mechanical barriers protect the host. However, chemotherapeutic agents, corticosteroids, and radiation, which are often used in the treatment of malignant disease and AIDS, and for transplantation patients, disrupt these defense mechanisms. If patients are to survive infections associated with neutropenia and other compromises in host defense due to the use of these therapies, it is important to reverse or at least lessen immunosuppression in these patients. By doing so, serious infections such as disseminated candidiasis, which recent reports indicate has a mortality rate of 30 to 95%, may be avoided (5, 7, 22, 61).

Monocytes are an important component of cellular defense mechanisms in humans (8). When stimulated by microbes, monocyte eicosanoid metabolism is altered, arachidonic acid is produced and released, and there is an increase in the production of cytokines, including tumor necrosis factor alpha (TNF-α), interleukin-1β (IL-1β), IL-6, IL-8, granulocyte-macrophage colony-stimulating factor (GM-CSF), and interferons (8, 9, 11, 27). As a result, chemotaxis and phagocytosis are enhanced (8). The upregulated influx of activated inflammatory cells is an important mechanism by which the host can eliminate invading organisms. During the past decade there has been general agreement that GM-CSF and gamma interferon (IFN-γ) enhance the phagocytic function and increase the killing of *Candida albicans* (43, 48, 49, 52, 63). In addition, GM-CSF increases intracellular oxidative metabolism, as demonstrated by an increase in superoxide (O2•−) production (8). A monoclonal antibody against GM-CSF abolishes this effect. Thus, unless monocytes are activated by cytokines such as GM-CSF, their effectiveness in host defense is limited (8, 9). Because GM-CSF and IFN-γ have been shown to enhance phagocytic function, they are frequently considered for use in patients who are undergoing treatment for malignant disease, who are receiving bone marrow transplants, or who have AIDS (3, 11, 24, 31, 34–37, 40, 58). In addition, cytokines such as IL-1β, IFN-γ, and TNF-α can stimulate the production of GM-CSF (34). Finally, administration of white blood cells from donors given GM-CSF has been associated with increased survival of neutropenic patients undergoing cancer chemotherapy (1, 14, 36, 37). Although no clearly established recommendations have been published, the use of cytokines as therapeutic adjuvants in the prevention and/or treatment of invasive fungal infections has been advocated (1, 7, 14, 35–37).

The purpose of this study was to define the intracellular interaction between cytokines and subinhibitory concentrations of an antifungal agent, fluconazole, against *C. albicans*, and to determine which mechanisms are likely to be responsible for the observed candidacidal activity of human monocytes. We studied the antifungal effects of adding the cytokines TNF-α, IL-1β, IL-4, IL-10, IFN-γ, and GM-CSF, singly and in combination, to assays with human monocyte-derived macrophages (MDM) in the presence and absence of subinhibitory concentrations of fluconazole. In addition, nitric oxide (NO) and O2•− determinations were performed in order to define the oxidative capacity of the activated monocytes.

* Corresponding author. Mailing address: Infectious Disease Research, Stratton VA Medical Center, Albany, NY 12208. Phone: (518) 462-3311, ext. 3080. Fax: (518) 462-3350. E-mail: BALTCALDONA _@ALBANY.VA.GOV.
Cytokines, Fluconazole, and MDM Against C. albicans

MATERIALS AND METHODS

Microorganism. C. albicans strain T=6, referred to below simply as C. albicans, was originally isolated from the blood of a candidemic patient and was provided by the Wadsworth Laboratories, New York State Department of Health, Albany, N.Y. C. albicans was grown on Sabouraud dextrose agar for 48 h. For osmopsonized, several colonies were suspended in RPMI 1640 medium (Sigma Chemical Co., St. Louis, Mo.) containing 10% fresh pooled normal human serum and were incubated for 30 min at 37°C. The osmopsonized cells were then centrifuged and resuspended at a concentration of \(2 \times 10^5\) CFU/ml in RPMI 1640 medium containing 10% fetal calf serum (FCS).

Antimicrobial agent. Fluconazole was provided by Pfizer Laboratories, Groton, Conn. Antibiotic solutions were made fresh for each experiment in accordance with the supplier’s instructions, filter sterilized, and used immediately. The fluconazole MIC for C. albicans, determined according to NCCLS method M27-T, was 1 \(\mu\)g/ml (33).

Preparation of monocytes. MDM were prepared from the heparinized blood of healthy human donors who had signed the informed-consent form approved by the Institutional Review Board of the Albany Medical College and Stratton Veterans Affairs (VA) Medical Center, Albany, N.Y. Mononuclear cells were separated from whole blood by using Histopaque 1077 (Sigma). The resulting mononuclear cell preparation was \(\approx 98\%\) pure. The separated cells were resuspended at a concentration of \(2 \times 10^6\) cells/ml in RPMI 1640 medium containing 10% FCS, 100 U of penicillin G/ml, and 100 \(\mu\)g of streptomycin/ml. Cell viability, determined by using the trypan blue exclusion test, was \(\approx 98\%\).

Cytokines. All cytokines were obtained from R & D Systems, Inc., Minneapolis, Minn. TNF-\(\alpha\), IFN-\(\gamma\), GM-CSF, and IL-10 were used at a concentration of 100 \(\mu\)g/ml. IL-1\(\beta\), IL-4, and IL-10 were used at a concentration of 1,000 U/ml. IL-1\(\beta\), IFN-\(\gamma\), and IL-4 were used at a concentration of 2 \(\mu\)g/ml (33).

Study design. Human mononuclear cells (2 \(\times 10^6\)) were delivered to the wells of 24-well plates (Corning/Costar Corp., Cambridge, Mass.) in a 1-ml volume and allowed to adhere for 72 h. Monocytes adhered to the wells in a contiguous layer. Medium and nonadherent cells, including lymphocytes, were aspirated from the wells. The adherent monocyte layer was gently washed once with RPMI 1640. TNF-\(\alpha\), IL-1\(\beta\), IFN-\(\gamma\), and GM-CSF were added to duplicate wells singly or in combination, and the monolayers were incubated for 24 h in 5% CO2 at 37°C. In experiments where IL-4 and IL-10 were used, IL-4 and IL-10 were added to cells that had been pretreated with TNF-\(\alpha\). These cells were then incubated for an additional 24 h. Osmopsonized C. albicans (2 \(\times 10^6\) cells in a volume of 1 ml) suspended in RPMI 1640 plus 10% FCS was then added to the wells. One hour was allotted to allow phagocytosis to occur. Nonphagocytosed blastoconidia were removed by aspiration, and the cell layer was washed once with RPMI 1640. Cytokines were readministered to the monolayer, and 0.1 \(\mu\)g of fluconazole (0.1 times the MIC) was then added in a 1-ml volume to each well. Following incubation of the plates at 37°C in an atmosphere containing 5% CO2 for 0, 24, and 48 h, the supernatants were removed, the monocytes were lysed with distilled water, and the lysates were quantitatively plated in duplicate on Sabouraud dextrose agar. The plates were incubated for 24 h at 37°C, and the numbers of surviving organisms were determined. Control wells contained monolayers of monocytes and either C. albicans, fluconazole, C. albicans plus fluconazole, or appropriate cytokines singly or in combination. Each assay was repeated three to six times.

NO and \(O_2^-\) assays. NO concentrations were determined using a nitrate/nitrite colorimetric assay kit (Cayman Chemical, Ann Arbor, Mich.). This assay is based on the enzymatic conversion of nitrate to nitrite by nitrate reductase. Nitrite is detected by the production of a colored product using the Griess reaction. The concentration is then determined spectrophotometrically. \(O_2^-\) concentrations were determined using the method of Pick and Miezel (42). In this procedure the detection of \(O_2^-\) is based on the reduction of ferricytrome heme and measurement of the increase in absorbance at 550 nm. Each assay was repeated two to eight times.

Statistical methods. For time-kill curves (see Fig. 1 and 2), changes in the numbers of CFU per milliliter from 0 to 24 h, as well as from 0 to 48 h and from 24 to 48 h, were compared among experimental variables. The analysis of variance methodology (53) was applied to the yeast counts following conversion to log10 units. For NO and \(O_2^-\) concentrations (see Table 1 and Fig. 3 to 5), analyses were done at 24 and 48 h using the analysis of variance (53). Contrasts were tested intra-assay. In addition, in Table 1, interassay comparisons were made between live or heat-killed C. albicans and controls. Null hypotheses were specified a priori. The level of significance was 0.05.

RESULTS

Figure 1 shows the effects of TNF-\(\alpha\), IL-1\(\beta\), IFN-\(\gamma\), GM-CSF, IL-4, and IL-10, used singly, in the presence or in the absence of fluconazole, in MDM assays. The numbers of surviving yeasts in MDM treated with TNF-\(\alpha\), IL-1\(\beta\), IFN-\(\gamma\), or IL-4 alone were not significantly different from those in controls at either 24 or 48 h (Fig. 1A, B, C, and E). In contrast, addition of GM-CSF alone (Fig. 1D) resulted in a significant decrease in yeast survival at 24 h (\(P < 0.05\)), while addition of IL-10 resulted in an increase in yeast survival (Fig. 1F) (\(P < 0.01\)). In the presence of fluconazole, addition of IFN-\(\gamma\) resulted in an increase in the number of surviving yeasts at 24 h (Fig. 1C) (\(P < 0.01\)), and addition of GM-CSF, IL-4, or IL-10 (Fig. 1D through F) caused a decrease in the number of surviving yeasts at 48 h (\(P < 0.01\)). Fluconazole had no effect in assays containing TNF-\(\alpha\) or IL-1 (Fig. 1A and B). In the presence of fluconazole, addition of each of the six cytokines caused a reduction in the growth of the yeast from 24 to 48 h (\(P < 0.05\)).

The results in Fig. 2 demonstrate the effects of combinations of IFN-\(\gamma\) and GM-CSF, TNF-\(\alpha\) and IL-10, IL-1\(\beta\) and IL-10, IL-4 and IL-10, and TNF-\(\alpha\), IL-4, and IL-10, in the presence and absence of fluconazole, after 24 and 48 h of incubation. With or without fluconazole, all combinations of cytokines except IFN-\(\gamma\) plus GM-CSF caused a significant decrease in the growth of C. albicans from 0 to 48 h and from 24 to 48 h. On average, there was significantly less growth than in the controls (\(P < 0.01\)). In contrast, no significant differences were observed between the growth of C. albicans in control monolayers and that in monolayers treated with IFN-\(\gamma\) plus GM-CSF, with or without fluconazole (Fig. 2A). The anti-inflammatory cytokine IL-10 did not negate the activity of proinflammatory cytokines in our assay system, and there was no potentiation of anti-inflammatory cytokine activity when IL-4 and IL-10 were used together (Fig. 2D).

Table 1 shows the NO and \(O_2^-\) concentrations at 0, 24, and 48 h in wells containing MDM in the absence and presence of live or heat-killed C. albicans, with and without fluconazole. At 0 h the concentrations of NO and \(O_2^-\) in wells containing MDM but not C. albicans or fluconazole were very low. Under these conditions, the highest concentrations were reached at 24 h. Treatment of MDM with fluconazole caused an increase in the NO concentration but did not affect the concentration of \(O_2^-\). In the presence of either live or heat-killed C. albicans and with no fluconazole, NO and \(O_2^-\) concentrations increased dramatically at 24 h (\(P < 0.01\)). The greatest increases occurred in the presence of heat-killed C. albicans. In assays containing MDM and either live or heat-killed C. albicans, the NO concentrations at 24 h were 50% lower in the presence of fluconazole than in its absence (\(P < 0.01\)). However, the \(O_2^-\) concentration was lower (\(P < 0.01\)) in the presence of fluconazole only in the assay containing live C. albicans. At 48 h contrasts remained essentially the same, although in the presence of fluconazole, NO concentrations did not increase as markedly with the addition of C. albicans. \(O_2^-\) concentrations at 48 h were lower (\(P < 0.01\)) when fluconazole and heat-killed C. albicans were added to MDM.

Figures 3 to 5 show the concentrations of NO and \(O_2^-\) in MDM assays at 24 and 48 h in the presence of cytokines (singly), fluconazole, or combinations of cytokines and flucon-
FIG. 1. Time-kill curves demonstrating in vitro activity of fluconazole (at 0.1 times the MIC) against intracellular C. albicans, with and without cytokines. (A) TNF-α (100 U/ml); (B) IL-1β (1,000 U/ml); (C) IFN-γ (1,000 U/ml); (D) GM-CSF (100 U/ml); (E) IL-4 (1,000 U/ml); (F) IL-10 (100 U/ml). Solid circles, control; open circles, fluconazole alone; solid inverted triangles, cytokine alone; open inverted triangles, fluconazole plus cytokine.
azole, with and without *C. albicans*. The average concentrations for controls are also given. In the absence of *C. albicans*, TNF-α, IFN-γ, GM-CSF, and IL-10 each caused a significant increase in the concentration of NO at both 24 and 48 h (Fig. 3A) (*P* < 0.01). Fluconazole alone also caused a significant increase at both 24 and 48 h (Fig. 4A). Significant increases in the NO concentration also occurred at 24 and 48 h when fluconazole was combined with either TNF-α or IL-10 (Fig. 5A).

**TABLE 1.** Effects of live and heat-killed *C. albicans* on the production of nitric oxide and superoxide by MDM

<table>
<thead>
<tr>
<th>Assay component(s)</th>
<th>Conc of nitric oxide or superoxide (nM)* at the indicated time</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Without fluconazole</td>
</tr>
<tr>
<td></td>
<td>0 h</td>
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<tr>
<td><strong>Nitric oxide</strong></td>
<td></td>
</tr>
<tr>
<td>MDM</td>
<td>3.6 (1.9)</td>
</tr>
<tr>
<td>MDM + live <em>C. albicans</em></td>
<td>21.1 (3.9)</td>
</tr>
<tr>
<td>MDM + heat-killed *C.</td>
<td>193 (18)</td>
</tr>
<tr>
<td><em>albicans</em></td>
<td></td>
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<tr>
<td><strong>Superoxide</strong></td>
<td></td>
</tr>
<tr>
<td>MDM</td>
<td>0.4 (0.3)</td>
</tr>
<tr>
<td>MDM + live <em>C. albicans</em></td>
<td>0.3 (0.2)</td>
</tr>
<tr>
<td>MDM + heat-killed *C.</td>
<td>27.5 (4.4)</td>
</tr>
<tr>
<td><em>albicans</em></td>
<td></td>
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</tbody>
</table>

* Values in parentheses are standard errors of the means.
* a Significantly different from MDM alone (*P* < 0.01).
* b Significantly different from MDM plus fluconazole (*P* < 0.05).
* c Significantly different from MDM plus fluconazole (*P* < 0.01).
* d Significantly different from MDM plus *C. albicans* (*P* < 0.01).
* e Significantly different from MDM and no fluconazole (*P* < 0.01).
5A) \( P < 0.01 \). Addition of fluconazole and GM-CSF resulted in an increase in the NO concentration only at 48 h (Fig. 5A) \( P < 0.01 \). With the exception of fluconazole plus IL-10 at 48 h, the concentrations of NO were lower \( P < 0.01 \) in the presence of fluconazole and individual cytokines (Fig. 5A) than they were in the presence of the cytokines alone (Fig. 3A). In contrast to the changes in NO concentrations, no significant changes were observed in \( O_2^- \) concentrations in the absence of \( C. albicans \) (Fig. 3B, 4B, and 5B).

In MDM assays containing TNF-\( \alpha \), IFN-\( \gamma \), GM-CSF, IL-10, fluconazole alone, or combinations of the cytokines with fluconazole in the presence of \( C. albicans \), NO concentrations at 24 h were significantly lower \( P < 0.01 \) than those in controls containing only MDM and \( C. albicans \) (Fig. 3C, 4C, and 5C). However, except when MDM were treated with GM-CSF plus fluconazole, these same NO concentrations were higher \( P < 0.01 \) than those in control assays containing MDM but not \( C. albicans \) (Fig. 3A, 4A, and 5A). At 48 h, fluconazole alone (Fig. 4C) \( P < 0.01 \) and fluconazole plus IL-10 (Fig. 5C) \( P < 0.05 \) caused the NO concentration to decrease, while increases in NO concentrations were observed with the addition of IL-10 alone (Fig. 3C) \( P < 0.01 \) and fluconazole plus GM-CSF (Fig. 5C) \( P < 0.05 \). In contrast to the changes in NO concentrations, \( O_2^- \) concentrations did not change significantly in the presence of cytokines and \( C. albicans \). However, significant decreases in \( O_2^- \) concentrations were observed at both 24 and 48 h in assays containing fluconazole and \( C. albicans \) (Fig. 4D) \( P < 0.01 \) and in assays containing IFN-\( \gamma \) or IL-10, fluconazole, and \( C. albicans \) (Fig. 5D) \( P < 0.01 \). In assays containing TNF-\( \alpha \) or GM-CSF plus fluconazole and \( C. albicans \), the \( O_2^- \) concentrations were lower than those of the controls, although only the differences at 48 h were significant (Fig. 5D) \( P < 0.01 \). In the presence of \( C. albicans \), the addition of individual cytokines or fluconazole combined with either TNF-\( \alpha \) or GM-CSF caused the \( O_2^- \) concentration to increase at 24 h compared to that in controls containing MDM but not \( C. albicans \) \( P < 0.01 \).

Thus, in MDM assays lacking \( C. albicans \) but containing cytokines (Fig. 3A), fluconazole (Fig. 4A), or cytokines plus fluconazole (Fig. 5A), NO concentrations were higher than those in controls containing MDM alone. However, in assays containing \( C. albicans \) and cytokines, fluconazole, or cytokines plus fluconazole, NO concentrations were significantly lower than those in controls containing only MDM and \( C. albicans \). \( O_2^- \) concentrations were unaffected by cytokines or fluconazole in the absence of \( C. albicans \) (Fig. 3B, 4B, and 5B). However, when fluconazole alone (Fig. 4D) or fluconazole and cytokines (Fig. 5D) were present along with \( C. albicans \) in MDM assays, \( O_2^- \) concentrations were significantly lower than those in controls containing only MDM and \( C. albicans \).

**DISCUSSION**

Human monocytes and macrophages are important components of host defense against pathogens (7, 8, 18, 61). How-
ever, they require activation in order to achieve full antimi-
crobial activity (8). This activation occurs as a result of exposure
to mannan, an important component of the \textit{C. albicans} cell
wall, as well as following exposure to some cytokines (8, 9, 26,
55, 57, 62, 63). The microbicidal activity of monocytes is re-
lated to the oxygen burst mechanisms in the cell and may be
strain and species dependent (8, 10, 12, 17, 19, 23, 29, 30, 59,
61). Fluconazole is a fungistatic triazole drug that inhibits the
formation of \textit{C. albicans} hyphae and is concentrated in human
phagocytes (1, 2, 51, 56). Fluconazole interferes with 14-
\textit{a}-demethylation of sterols, resulting in the accumulation of 14-
\textit{a}-methylated sterols in the cell membrane. \textit{C. albicans} cells
with 14-\textit{a}-demethylated sterols are especially vulnerable to the
oxygen-dependent microbicidal activity of phagocytes in which
the initial oxygen product is $O_2^-$ (51). Furthermore, the in-
ability of the 14-\textit{a}-demethylation-deficient cells to form hy-
phae may allow the fungus to be more easily ingested by
phagocytes (51).

It has been clearly demonstrated in a murine model of dis-
seminated candidiasis that certain cytokines produced by
monocytes, for example TNF-\textit{\alpha}, increase survival (4, 6, 27, 28).
Furthermore, the use of GM-CSF in the treatment of neutro-
penic cancer patients with systemic, invasive fungal infections
has been shown to improve patient survival (14, 16, 18, 21,
34–37, 40, 43, 44, 48, 49, 61). Our study was undertaken in
order to define the activities of six cytokines used singly and in
combination, in the presence and absence of fluconazole, in a
human MDM assay against a fluconazole-sensitive \textit{C. albicans}

\begin{figure}
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\includegraphics[width=\linewidth]{figure4.png}
\caption{Concentrations of nitric oxide (A and C) and superoxide (B and D) produced by MDM pretreated with fluconazole (Flu) and either
not exposed (A and B) or exposed (C and D) to \textit{C. albicans}. *, $P < 0.05$; **, $P < 0.01$.}
\end{figure}
strain. In addition to evaluation of the importance of cytokines in candidacidal activity, the mechanism of this activity was evaluated by determining the O$_2^-$ and NO concentrations in cells following exposure to cytokines and fluconazole in the presence and absence of *C. albicans*.

Our results demonstrate that in the presence or absence of fluconazole, TNF-α and IL-1β had no effect on the microbicidal activity of the monocytes. In contrast, while IFN-γ had no effect on the intracellular killing of the yeast in the absence of fluconazole, in the presence of fluconazole IFN-γ stimulated intracellular killing at 24 h (*P* < 0.01). GM-CSF increased microbicidal activity at 24 h in the absence of fluconazole (*P* < 0.05). In addition, GM-CSF increased the candidacidal activity of MDM in the presence of fluconazole at 48 h (*P* < 0.01). Similar observations with GM-CSF at 24 h have been described previously, although the assay procedures described differed from ours (20, 31). Of interest was our observation that without fluconazole, microbicidal activity was lower at 24 h in the presence of IL-10 than in the controls (*P* < 0.01) but that at 48 h the presence of IL-10 was associated with increased microbicidal action (*P* < 0.01) in both the presence and the absence of fluconazole. Suppression of the microbicidal effect of macrophages by IL-4 and IL-10 in the absence of fluconazole has been described previously (25, 38, 46, 47, 54, 60, 61). We observed a 13-fold increase in NO concentration in the presence of MDM exposed to live *C. albicans*. In addition, we demonstrated that the NO concentrations in assays that included cytokines and/or fluconazole along with *C. albicans* were low compared to the NO concentrations in assays containing only MDM and *C. albicans*. Combinations of GM-CSF plus IFN-γ had no effect on microbicidal activity, regardless of the presence or absence of fluconazole. In contrast, combinations of TNF-α plus IL-10, IL-1β plus IL-10, IL-4 plus IL-10, and TNF-α plus IL-4 and IL-10 were associated with significantly increased microbicidal activity at 48 h and from 24 to 48 h (*P* < 0.01) in the absence or presence of fluconazole. In contrast to TNF-α, IL-1β, and IFN-γ, GM-CSF, IL-4, and IL-10 increased the candidacidal activity of MDM in the presence of fluconazole. Since this effect was significantly greater when fluconazole was present in the assay, our data support the use of selected cytokines when fluconazole is used for the therapy of disseminated candidiasis.

In this study we assessed the production of NO and O$_2^-$ by MDM activated by cytokines alone, fluconazole alone, *C. albicans* alone, and combinations of these. The roles of NO and O$_2^-$ during infection are complex. In contrast to the observations of Schneeman et al. (50), we detected NO production by MDM that had been stimulated by cytokines, fluconazole, and combinations of cytokines and fluconazole (Table 1; Fig. 3 to 5). The regulation of NO production by cytokines and the effect it has on the microbicidal activity of monocytes remain incompletely understood (12, 30). It is known, however, that NO production is enhanced in macrophages stimulated with TNF-α, IFN-γ, IL-1, and IL-2 (10, 23, 29, 32). We demonstrated increased NO production by MDM stimulated with TNF-α and IFN-γ, as well as by MDM stimulated with GM-
CSF. Although previous observations indicate that IL-4 and IL-10 do not induce NO synthetase production (29, 30), IL-10 stimulated NO production by MDM in our assays. Macrophages exposed to pathogens causing mycobacterial infections, malaria, viral hepatitis, and AIDS showed enhanced NO production (12). NO and its derivatives are important as antimicrobial effectors, with activity against parasitic, fungal, bacterial, and viral infection (12, 30). Evidence for the elaboration by C. albicans of a soluble factor inhibiting NO production has recently been described (10). Similarly to NO, O$_2^-$ production by MDM was greatly enhanced by exposure to live C. albicans. Although cytokines alone did not affect O$_2^-$ production by MDM exposed to live C. albicans (Fig. 3D), the presence of fluconazole or flucytosine plus cytokines resulted in a reduction in O$_2^-$ concentrations in MDM assays containing C. albicans (Fig. 4D and 5D).

In our assays there was no clear correlation between NO and O$_2^-$ production and intracellular killing of C. albicans by human monocytes. The fact that maximum production of NO and O$_2^-$ occurred when monocytes were exposed to C. albicans alone and that NO and O$_2^-$ production were suppressed in monocytes exposed to C. albicans in the presence of cytokines and/or flucytosine emphasizes the complexity of the interaction of C. albicans with host macrophages.

The results described in our study clearly indicate that the microbicidal activity of human monocytes is closely associated with the activities of selected cytokines. Furthermore, subinhibitory concentrations of fluconazole increase these cytokine-specific effects against fluconazole-sensitive intracellular C. albicans. These results support the use of selected cytokines as further investigation.

**ACKNOWLEDGMENTS**

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**REFERENCES**


