Suppressive Effects of Interleukin-10 on Human Mononuclear Phagocyte Function against *Candida albicans* and *Staphylococcus aureus*

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The effects of interleukin (IL)-10, a potent antiinflammatory cytokine, on human monocyte functions against two medically important pathogens, *Candida albicans* and *Staphylococcus aureus*, were studied. Incubation with 20–100 ng/mL IL-10 for 2–3 days decreased the fungicidal activity of monocytes against serum-opsonized *C. albicans* blastoconidia (*P* ≤ .04), reduced their capacity to damage unopsonized hyphae (*P* ≤ .006), and suppressed superoxide anion production in response to phorbol myristate acetate (*P* = .019) and N-FMLP (*P* = .04) but not to serum-opsonized blastoconidia. Paradoxically, IL-10 enhanced phagocytic activity of monocytes against serum-opsonized blastoconidia (*P* < .01). In addition, IL-10–treated monocytes demonstrated decreased bactericidal activity (*P* = .046) but no change in bacterial phagocytosis. These findings demonstrate an overall suppressive role of IL-10 on human monocyte function against *C. albicans* and *S. aureus* and may have important implications in the use of this cytokine.

Invasive fungal infections, especially candidiasis, have been reported with increasing frequency during the past decade [1–3]. Moreover, *Staphylococcus aureus* and *Candida albicans* are now among the predominant causes of nosocomial infections [4]. Understanding regulation of the host immune response as an adjunct to antimicrobial therapy may offer new strategies for prevention and treatment of these infections [5–7].

Interleukin (IL)-10 may play an important role in response to bacterial and fungal infections by modulating excessive inflammation and antimicrobial defenses [8]. This cytokine is produced by CD4 and CD8 T lymphocytes, monocytes, macrophages, and B lymphocytes [9]. IL-10 plays a major role in the regulation of immunologic and inflammatory responses, including inhibition of T cell and macrophage functions. For example, it has been found to inhibit macrophage cytotoxicity [10, 11] as well as secretion of interferon (IFN)-γ and IL-2 by activated T helper (Th) 1 cells [12, 13], production of IL-1α and -β, tumor necrosis factor (TNF)-α, IL-6, and granulocyte-macrophage colony-stimulating factor (GM-CSF) by macrophages and other antigen-presenting cells [12, 14–22]. IL-10 has been also shown to suppress nitric oxide production and candidacidal activity of murine macrophages [23] and generally is considered a macrophage deactivator [14].

Because of its multiple suppressive activities on immune cells, IL-10 has the potential to be used as an antiinflammatory agent in septic shock [24] and in chronic inflammatory diseases [24] and as a specific immunosuppressive agent against rejection of transplanted organs [25]. As an application of these properties, clinical trials are currently being conducted for investigation of IL-10 in a number of diseases, including psoriatic arthritis, human immunodeficiency virus infection, and inflammatory bowel disease. However, administration of IL-10 to mice adversely affected host defenses during pneumonia due to *Klebsiella pneumoniae* [26] or *Streptococcus pneumoniae* [27], disseminated infection by *C. albicans* [28], and mycobacterial infections [29, 30]. To our knowledge, no study has examined the effects of IL-10 on human phagocyte effector function against *S. aureus* or *C. albicans*. We therefore investigated the effects of human IL-10 on selected antimicrobial activities of human monocytes (MNC) against these two important pathogens.

**Materials and Methods**

**Preparation of MNC.** Buffy coats were prepared from anticoagulated venous blood of healthy adult volunteers at the Transfusion Medicine Department of Hippokration Hospital, Thessaloniki, Greece. Mononuclear leukocytes were separated by centrifugation of buffy coats over ficoll (Lymphocyte Separation Medium; Life Technologies Gibco BRL, Paisley, UK). They were washed twice with Hanks’ buffered salt solution (HBSS) free of Ca²⁺ and Mg²⁺ and resuspended in complete medium (CM) con-
sisting of RPMI 1640, 25% pooled normal human serum (Trans-
fusion Medicine Department, Hippokration Hospital), 100 units/
ml penicillin, and 100 μg/mL streptomycin. They were counted by
trypsin blue staining on a hemocytometer, and the percentage of
MNC was estimated by May-Grunwald-Giemsa staining. Mono-
nuclear leukocytes were >95%, and 25%–45% of them were MNC.

Organisms. The isolates of S. aureus and C. albicans 86-21
used in this study have been well characterized and extensively
used in previous studies [31, 32]. They were preserved in skim milk
aliquots at −35°C. Preparative cultures of C. albicans were grown
on Sabouraud dextrose agar (SDA) plates, and of S. aureus
were grown on Mueller-Hinton agar plates at 37°C for 18-24 h. Blas-
toconidia of C. albicans were harvested from the SDA plate, in-
oculated into Sabouraud dextrose broth, and incubated at 37°C
for 18 h in a shaking water bath. Blastococidnia were washed with
HBSS and resuspended in HBSS at a final concentration of 107
colony-forming units (cfu) per milliliter. Similarly, individual S.
aureus colonies were inoculated and grown in trypticase-soy broth
for 2.5 h on a shaker at 37°C to obtain log-phase growth, washed
with HBSS, and adjusted to a final concentration of 2 × 107 cfu/
ml (bactericidal assay) or 2.5 × 109 cfu/ml (bacterial phagocy-
tosis) by optical density and cfu counts. For brevity, the term
hyphae is used to define pseudohyphae of C. albicans.

Reagents and pretreatment of effector cells. Human IL-10 with
a specific activity of 1–2 × 107 units/mg was a gift of DNAX (Palo
Alto, CA). IL-10 was diluted in HBSS without Ca2+ and Mg2+
and was stored frozen at −35°C. Rat-mouse hybridoma cell line JES3-
19F1.1.1 (producing antibody reactive with human IL-10) was ob-
tained from American Type Culture Collection (Rockville, MD).
The concentrations of IL-10 used to treat MNC were in the range
that can be achieved during certain infectious processes or phar-
macologically [24, 33, 34]. Anti-IL-10–containing supernatant of
1-week cultured hybridoma cell line in CM containing 10% fetal
calf serum (Life Technologies; CM-FCS) instead of 25% human
serum was centrifuged, filtered, and frozen at −35°C. In prelimi-
nary studies, IL-10 did not exert any direct effect on growth of C.
albicans blastococidnia, germination, or growth of hyphae nor was
there any effect on growth of S. aureus.

Briefly, we detached MNC from flasks in which they had been
incubated with IL-10; cells were washed, resuspended in HBSS
without Ca2+ and Mg2+, and transferred to tubes in which the
assays were performed. Two hundred microliters of a suspension
of MNC (5 × 107/mL) in CM were placed in 12-well plates con-
taining 18-mm sterile round glass coverslips (Costar, Cambridge,
MA) for the fungal phagocytosis assay or in 12-well plates without
coverslips for the superoxide anion (O2−) assay. Both plates were
incubated at 37°C with 5% CO2 for 45 min. The wells were then
washed twice with warm HBSS not containing Ca2+ and Mg2+.

Fresh CM was added to the wells, and the glass- or plastic-adherent
MNC were further incubated in 1 mL of CM at 37°C with 5% CO2
with or without IL-10 for 2–3 days.

In addition, 1–2 × 107 MNC were cultured in each of 25-cm2
primary culture flasks (Costar) with 10 mL of CM-FCS to assess
bacterial phagocytosis, bactericidal activity, killing of blastococidnia
(hereafter called conidioicidal activity), and hyphal damage. Flasks
were placed at 37°C with 5% CO2 for 2 h. They were then washed
twice with warm HBSS not containing Ca2+ and Mg2+. Fresh CM-
FCS was added to the flasks, and the plastic-adherent MNC were
further incubated in 10 mL of CM-FCS at 37°C with 5% CO2, with
or without IL-10 for 2–3 days. Adherent MNC were then detached
from the bottom by scraping, washed twice, and resuspended in
HBSS without Ca2+ and Mg2+ in order to be used in the assays.

Fungicidal assays. For the conidioicidal assay, a standard cfu
assay was used. MNC that had been incubated with CM-FCS only
or with IL-10–containing CM-FCS in flasks were transferred to
tubes and mixed with 105 blastococidnia at an effector-to-target cell
(E:T) ratio of 1:1 and 10% pooled human serum in 1 mL of HBSS
[31]. Control tubes containing blastococidnia, human serum, and
HBSS but not MNC were included in each experiment. The mixture
was rotated at 37°C for 2 h. After complete lysis of MNC with
sterile H2O at the end of incubation, dilutions were made, and
samples were plated in duplicate on SDA plates and incubated at
37°C for 18 h. Colonies were counted, and conidioicidal activity
was calculated using the formula: % killing = (1 − X/C) × 100,
where X is the number of cfu with MNC at 2 h and C is the number
of cfu without MNC at 2 h.

To assess hyphal damage, the colorimetric MTT assay [35] was
adapted for C. albicans hyphae [32]. Briefly, a suspension of
2.5 × 107 blastococidnia/mL in RPMI 1640 was prepared, and 1-mL
aliquots were plated in 24-well plates (Costar). The plates were
incubated at 37°C for 4 h, by which time >95% of blastococidnia
had germinated to hyphae.

The supernatants were aspirated, and MNC that had been incub-
ated with CM-FCS only or with IL-10–containing CM-FCS in
flasks for 2–3 days were added to the wells at E:T ratios of 2:1
and 5:1. After 2 h at 37°C and 5% CO2, supernatants were aspirated,
MNC were lysed by adding 300 μL of 0.5% sodium deoxycholate,
and hyphae were washed three times with sterile water. Subse-
quently, 1 mL of RPMI 1640 containing 0.5 mg/mL MTT was
added to each well, and the plates were further incubated at 37°C
and 5% CO2 for 3 h. The wells were then aspirated dry, 200 μL of
isopropanol were used to extract the dye in each well, volumes of
150 μL were transferred into a 96-well plate (Costar), and the color
(optical density, OD) was measured on a microplate reader (Perkin-
Elmer, Wilton, CT) at 540 nm. A well containing only isopropanol
was used as a blank. Control wells containing hyphae and buffer
only were included. Antifungal activity (hyphal damage) was cal-
culated using the formula: % hyphal damage = (1 − X/C) × 100,
where X is the OD of test wells at 2 h and C is the OD of control
wells containing hyphae only. Each condition was tested in dupli-
cate or quadruplicate, and the results were averaged.

Bactericidal assay. A standard cfu assay was used for assessing
bactericidal activity [31]. Monocytes were coincubated with
2.5 × 103 cfu of S. aureus at an E:T ratio of 1:1 in 1 mL of HBSS
containing 10% normal pooled human serum. Control tubes con-
taining bacteria, human serum, and HBSS but not MNC were
included in each experiment. Preparations were rotated at 37°C
for 30 min. Monocytes were lysed with sterile H2O, and serial dilutions
were plated on Mueller-Hinton medium and incubated at 37°C for
18 h. Colonies were counted, and bactericidal activity was assessed
by calculating the percent killing of bacteria compared with the
control. The following formula was used: % killing = (1 − X/C) × 100,
where X is the number of cfu with monocytes at 30
min and C is the number of cfu in the control at 30 min.

Phagocytosis of blastococidnia. After incubation of MNC with
IL-10 in wells, supernatants were removed and 1 mL of CM con-

Phagocytosis of bacteria. Phagocytosis of S. aureus by MNC was assessed by direct microscopy as previously described [31]. Briefly, 10⁶ serum-preopsonized S. aureus bacteria were mixed with 10⁶ MNC that had been previously incubated with CM-FCS alone or with CM-FCS containing various concentrations of IL-10 in 1 mL of HBSS, at a final E:T ratio of 1:100. Preparations were rotated at 37°C. Samples were obtained after 10 min and immediately mixed with cold N-ethylmaleimide (0.1 mM; Sigma, St. Louis) to inhibit additional phagocytosis. They were then incubated with lysozyme (20 U/mL; Sigma) at 37°C for an additional 10 min to lyse the nonphagocytosed, extracellular bacteria. Preparations were cytocentrifuged and stained with May-Grünwald-Giemsa. The percent phagocytosis was calculated as the proportion of MNC containing one or more bacteria among 100 MNC. A weighted phagocytic index was calculated by multiplying the number of MNC having either 1–10, 11–20, 21–30, 31–40 or >40 ingested organisms by either 1, 2, 3, 4, or 5, respectively, and dividing the total score by the number of MNC examined (usually 100).

Superoxide anion production assay. Superoxide anion (O₂⁻) production in response to soluble and particulate stimuli was assessed spectrophotometrically in a cytochrome c reduction assay [36]. One million MNC that had been incubated with CM only or with IL-10-containing CM in wells were mixed with 50 μM cytochrome c (Sigma) in 1 mL of HBSS. The mixture was incubated at 37°C for 15 min with one of the following stimuli: 100 ng/mL phorbol myristate acetate (PMA), 500 nM N-FMLP (both from Sigma), or serum-opsonized blastoconidia of C. albicans at an E:T ratio of 1:2. Control tubes not containing MNC but having the remaining constituents were included. After this incubation, O₂⁻ production was assessed as the difference in absorption from the control at 550 nm that was measured on a photometer (Micro Elisa Strip Reader 301; Bio-Tek, Winooski VT). Superoxide anion produced by 10⁶ MNC was then calculated using the millimolar extinction coefficient for reduced cytochrome c.

Statistics. Data were expressed as mean ± SE. Differences between values at individual IL-10 concentrations and baseline values were assessed by use of the paired Student’s t test or analysis of variance with Dunnett’s correction for multiple comparisons. The difference in bactericidal activity between IL-10 treatment and baseline value was evaluated by the Wilcoxon signed rank test with the Bonferroni correction. All P values were two-sided, and P<.05 was considered to be significant.

Results

Effect of IL-10 on fungicidal activity of MNC against blastoconidia of C. albicans. After incubation of MNC and blastoconidia at an E:T ratio of 1:1 in the presence of 10% pooled human serum for 2 h, concentrations of 20–100 ng/mL IL-10 were found to significantly decrease the percent killing of blastoconidia by MNC. For example, at 100 ng/mL IL-10, the percent killing was 27.6% ± 11.1% compared with 53.6% ± 4.3% for controls, P=.04; figure 1). At 20 ng/mL, IL-10 also suppressed conidiofacial activity from baseline to 42.5% ± 4.8% (P=.02; figure 1). In two duplicate experiments in which anti-IL-10-containing supernatant was used in combination with 20 ng/mL IL-10, the suppressive activity of IL-10 was partially inhibited (table 1).

Effect of IL-10 on MNC-induced damage of C. albicans hy-
The effect of IL-10 on antifungal activity of MNC against Candida hyphae (hyphal damage) was then evaluated by use of a 2-h MTT assay and E:T ratios of 2:1 and 5:1 (figure 2). After incubation of MNC with 20 or 100 ng/mL IL-10 at 37°C for 2–3 days, the capacity of MNC to damage unopsonized hyphae was significantly decreased. For example, at an E:T ratio of 5:1 (figure 2, hatched columns) and 100 ng/mL IL-10, hyphal damage was 10.8% ± 6.2% compared with 37.9% ± 6.6% for controls (P = .006). At the same concentration and at an E:T ratio of 2:1 (open columns), hyphal damage was 5.8% ± 4.2% compared with 26.8% ± 6.4% for controls (P = .002).

Effect of IL-10 on bactericidal activity of MNC against S. aureus. The effect of IL-10 on bactericidal activity of MNC against S. aureus (percent killing) was evaluated by use of a cfu assay at an E:T ratio of 1:1. After incubation of MNC with 20 ng/mL IL-10 at 37°C for 2–3 days, the capacity of IL-10–pretreated MNC to kill bacteria in the presence of serum was significantly decreased compared with control MNC. Percent killing was 6.5% ± 10.0% compared with 32.7% ± 9.4% for controls, P = .046 (figure 3). In two experiments using 2 ng/mL, no difference from untreated controls was found, and no further decrease at 100 ng/mL was observed (data not shown).

Effect of IL-10 on phagocytosis of opsonized and unopsonized blastoconidia of C. albicans. To evaluate the phagocytic activity of IL-10–treated MNC against serum-opsonized blastoconidia of C. albicans, glass coverslip–adherent MNC were incubated with 0, 2, 20, and 100 ng/mL IL-10 for 2–3 days. In these experiments, the MNC-induced percent phagocytosis of blastoconidia was not altered by 2 or 20 ng/mL IL-10. However, there was a small but significant increase after incubation of MNC with 100 ng/mL IL-10 at 37°C to 68.5% ± 3.4% compared with 60.4% ± 3.1% of controls (figure 4, P < .01). The increase was modest but consistent in all donors. The phagocytic index was not significantly altered by IL-10 treatment at any concentration used. For example, at 100 ng/mL of IL-10, the phagocytic index was 1.82 ± 0.16 versus 1.73 ± 0.11 for controls (P > .1).

Since C. albicans blastoconidia, unlike bacteria, may also be phagocytosed by monocytes and macrophages through serum-independent mechanisms utilizing mannose ligands [37], the effect of IL-10 on phagocytosis of unopsonized C. albicans blastoconidia was evaluated. Again, glass coverslip–adherent MNC were incubated with 0, 2, 20, and 100 ng/mL IL-10 at 37°C for 2–3 days. In these experiments, MNC-induced baseline (0 ng/mL) percent phagocytosis of blastoconidia was 38.0% ± 5.8%, significantly lower than that of serum-opsonized blastoconidia (P = .007). This percent phagocytosis was not altered after incubation of MNC with 2, 20, or even 100 ng/mL IL-10. Similarly, the phagocytic index was 1.46 ± 0.1, which was not quite significantly lower than that of serum-opsonized blastoconidia (P = .088). Again, this was not altered by IL-10 treatment at any concentration used.

Effect of IL-10 on phagocytosis of opsonized S. aureus. The phagocytic activity of IL-10–treated MNC against opsonized S. aureus also was evaluated. To this end, MNC were incubated with 0, 2, 20, and 100 ng/mL IL-10 for 2–3 days. In these experiments, the MNC-induced percent phagocytosis of bacteria was 83.0% ± 5.1% and was not altered significantly by incubation of MNC with 2, 20, or 100 ng/mL (at 100 ng/mL, the percent phagocytosis was 79.4% ± 4.7%; data from 5–8 experiments). Similarly, the weighted phagocytic index was 2.9 ± 0.1 and was not significantly altered by IL-10 treatment.

Effect of IL-10 on superoxide production by MNC. IL-10–pretreated MNC exhibit suppressed O$_2^-$ production in response to PMA and FMLP [36]. In the present study, we confirmed these findings with both stimuli used as controls. Concentrations of IL-10 within the range of 2–20 ng/mL were found to be the most significantly effective in suppressing O$_2^-$ production in response to these two stimuli. For example, with 20 ng/mL IL-10, O$_2^-$ production in response to PMA was 3.3 ± 0.2 nmol O$_2^-$ per 10$^6$ MNC per 15 min versus 5.0 ± 0.5 for control (34% decrease, P = .019) and in response to FMLP was 1.9 ± 0.3 versus 2.7 ± 0.2 nmol for control (30% decrease, P = .04). However, pretreatment of MNC with IL-10 was not shown to significantly alter O$_2^-$ production in response to opsonized C. albicans blastoconidia. Thus, baseline O$_2^-$ production was 1.2 ± 0.2 nmol versus 1.5 ± 0.3 nmol produced after treatment with 20 ng/mL IL-10 (results from 10 or 11 experiments).

Discussion

In this study, IL-10 was found to suppress the antifungal activity of MNC against blastoconidia and hyphae of C. albicans and antibacterial activity against S. aureus followed by a similar decrease of oxidative burst in response to soluble stimuli. In contrast, a high concentration of IL-10 exhibited a small albeit consistent increase of MNC phagocytic activity against serum-opsonized C. albicans but did not alter phagocytosis of S. aureus and O$_2^-$ production in response to blastoconidia. These effects were achieved by IL-10 concentrations within 2 and 100 ng/mL that can be easily achieved or even exceeded by exogenous administration of 10–25 µg/kg [24] or

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<th>IL-10 (ng/mL)</th>
<th>Anti-IL-10</th>
<th>% killing of blastoconidia</th>
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<tr>
<td>0</td>
<td>–</td>
<td>50.4 ± 1.8</td>
</tr>
<tr>
<td>20</td>
<td>–</td>
<td>34.6 ± 0.9</td>
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<td>0</td>
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<td>46.3 ± 0.7</td>
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<td>20</td>
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NOTE. Anti-IL-10 was added to MNC cultures with anti-IL-10-containing supernatant of rat-mouse hybridoma cell line diluted 1/2 in complete medium (see Materials and Methods). Fetal calf serum (carrier of anti-IL-10 antibody) had no effect on MNC-induced % killing of blastoconidia. IL-10 (20 ng/mL) was added at same time as anti-IL-10 to appropriate cultures, and incubation lasted for 2 days. Results are mean ± SE for 2 duplicate experiments.
Figure 2. Effect of interleukin (IL)-10 on % damage of unopsonized hyphae of C. albicans caused by human monocytes (MNC). MNC were pretreated with indicated concentrations of IL-10, added into wells containing 2.5 × 10³ hyphae/well, and incubated at 37°C for 2 h. Effector-to-target cell ratios were 2:1 (open bars) and 5:1 (hatched bars). Data are from 4–7 (0, 20, and 100 ng/mL) and 3 (2 ng/mL) experiments. Vertical bars indicate SE. Differences of IL-10–treated MNC from medium-pretreated MNC are significant (*, †). P < .05, † P ≤ .006.

by endogenous secretion of IL-10 in response to endotoxin during septic shock [33, 34].

Our study constitutes the first comprehensive report on the role of IL-10 on phagocytic host defenses against common bacterial and fungal pathogens of humans in vitro. That IL-10 mediates suppression of human monocyte effector functions such as conidiocidal activity and hyphal damage, as well as bactericidal activity, is consistent with previous findings of IL-10 effects on different immune functions [10–16, 18, 20, 21, 23, 38–41]. Recently, IL-10 has been reported to exert a deleterious effect on the outcome of established murine candidiasis, and this effect correlated with inhibition of nitric oxide–mediated candidacidal activity of macrophages [23]. As human mononuclear phagocytes do not generate sufficient nitric oxide for antimicrobial activity [42, 43], there was a critical need to investigate the effect of IL-10 on human MNC against C. albicans. Our findings correlate with the above effect [23].

These results are also consistent with and may serve as an explanatory basis for recent in vivo findings in pneumonia due to K. pneumoniae [26] and S. pneumoniae [27] as well as disseminated C. albicans infection [28]. Whereas IL-10 has an attenuating effect on septic shock [44, 45], its neutralization appears to decrease bacterial cfu counts and improve outcome of infected animals [26–28]. It appears that IL-10, like many other naturally occurring biologicals, is a two-sided sword and that, although IL-10 is very useful by attenuating excessive inflammatory reaction [44–47], it has also adverse inhibitory effects on antimicrobial functions of mononuclear phagocytes. Whether IL-10 has similar inhibitory effects on neutrophil effector functions is less well established [48].

The increased phagocytic activity of MNC against serum-opsonized blastoconidia of C. albicans found in this study is similar to the increased phagocytic activity of MNC against conidia of Aspergillus fumigatus found previously [36]. These increases are expected in the light of recent findings that IL-10 stimulates MNC FcγR surface expression and cytotoxic activity, which may imply a distinct regulation of phagocytosis and antibody-dependent cellular cytotoxicity [49]. Moreover, while our studies were in progress, Capsoni et al. [50] reported that the IL-10–induced receptor up-regulation, restricted to FcγRI expression, is correlated with augmentation of phagocytosis of erythrocytes and Saccharomyces cerevisiae. Taken together, these observations suggest that IL-10 is not only a macrophage “deactivating” cytokine [14] but that it has more diverse effects
Figure 3. Effect of interleukin (IL)-10 on bactericidal activity of human monocytes (MNC) against S. aureus in presence of human serum. MNC were pretreated with either buffer or with 20 ng/mL of IL-10 for 2–3 days, added into tubes containing 2.5 × 10⁶ bacteria at effector-to-target cell ratio of 1:1 and 10% pooled human serum, and incubated at 37°C for 30 min. Data are from 8 experiments. Vertical bars indicate SE. Difference of IL-10 (20 ng/mL)-pretreated MNC from control (0 ng/mL) MNC was significant (*P = .046).

Figure 4. Effect of interleukin (IL)-10 on phagocytic activity of human monocytes (MNC) against serum-opsonized blastoconidia of C. albicans. MNC were incubated with indicated concentrations of IL-10 on glass coverslips in wells in presence of 25% pooled human serum; 10⁶ blastoconidia were then added and incubated at 37°C for 15 min. Data were from 8 experiments. Vertical bars indicate SE. Difference of IL-10–pretreated MNC at 100 ng/mL from control (0 ng/mL) MNC was significant (*P < .01).

IL-10 was found to significantly decrease the percent killing of blastoconidia by MNC. This effect was partially neutralized by anti–IL-10 antibodies produced in the supernatant of a hybridoma cell line culture. The same supernatant containing the anti–IL-10 antibodies was utilized in a previous study, in which we demonstrated that anti–IL-10 inhibited superoxide anion production by MNC in response to FMLP [36]. The partial effect of neutralization of the IL-10 effect may have been due to low concentration or activity of antibody in the supernatant or non–IL-10 suppressive elements in the supernatant of the hybridoma cell line culture. The cell culture was endotoxin-free, and there was no contamination with endotoxin during preparation of supernatant. However, these contaminating materials are usually stimulatory for MNC function and not suppressive.

The immunomodulatory effects of IL-10 on phagocytic cells may vary with function and target organism. In this study, while not affecting phagocytosis of S. aureus, IL-10 inhibited bactericidal activity against this organism. Similarly, IL-10 did not affect phagocytosis of Legionella pneumophila, but it suppressed its intracellular killing by human monocytes and alveolar macrophages [53]. By comparison, IL-10 has been recently reported not to affect intracellular growth of Mycobacterium avium in human monocytes [54].

The IL-10–mediated increase of phagocytic activity of MNC against serum-opsonized blastoconidia at a high concentration is associated with suppressed fungicidal activity and may be
consistent with the Th2-type suppressive properties of IL-10. Since killing of blastoconidia predominantly takes place intracellularly, the enhancing effect of IL-10 on phagocytosis appears to be responsible for the only modest suppression (~50%) of conidiocidal activity. Intracellular killing, however, is probably overwhelmingly more important in the microbial clearance than phagocytosis and, thus, the overall in vivo effect of IL-10 is one of suppression. Along a similar line of investigation, IFN-γ, despite being one of the most potent activators of monocyte antimicrobial activity, reduced ingestion of erythrocytes and S. cerevisiae [50].

If phagocytosis of C. albicans blastoconidia is augmented but followed by suppressed conidialidal activity, as observed in the present study, then the intracellular fungal burden is increased, providing a sanctuary for phagocytosed intracellular C. albicans blastoconidia, and thus perpetuation of chronic infection. As the excessive viable intracellular blastoconidia overwhelm the suppressed capacity of MNC to kill them and grow, MNC are unable to stop the infection. Further, the IL-10–mediated suppression of extracellular hyphal damage permits more growth of hyphae and a net increase of invasive fungal elements.

Chronic disseminated candidiasis [55] is a refractory fungal infection in patients with cancer; the pathogenesis of this mycosis may involve IL-10 or other Th2-type cytokines. Becoming clinically overt upon recovery from neutropenia, this mycosis has long been an immunologic enigma, with some patients continuing to demonstrate progressive infection despite having recovered from neutropenia. That IL-10 mediates increased phagocytosis and suppressed conidialidal activity followed by increased susceptibility to disseminated candidiasis [23, 28] suggests that it may also contribute to the pathogenesis of this infection.

The mechanism of down-regulation of MNC antifungal and antibacterial activities is unclear. IL-10 has been previously found to have opposing effects to IFN-γ [22, 23]. In addition, IL-10 dramatically decreases production of TNF-α [20], a potent up-regulator of antimicrobial function of phagocytes [56]. The suppression of antifungal activity against blastoconidia, hyphae, and bacteria may be associated with down-regulation of such “immunoenhancing” cytokines at transcriptional and posttranscriptional levels. Similar effects of IL-10 on secretion of proinflammatory monokines TNF-α and IL-1β in response to C. albicans have been recently published [9]. Whereas our study addresses the effects of IL-10 on innate phagocytic response, IL-10 also modulates antibody responses, which occupy key roles in host defense against fungi and bacteria [57].

IL-10 may be produced at increased levels during the course of human immunodeficiency virus infection and correlate with Th cell dysfunction in these patients [58]. In addition, it has been shown that alterations in Th functional phenotype with decreased Th1-type cytokine levels are associated with macrophage dysfunction [59]. Of note, these antifungal defects were reversible by IFN-γ. Thus, the Th2 milieu may predispose human immunodeficiency virus–infected patients to develop chronic bacterial or fungal infections.

These effects of IL-10 on phagocytic host defenses may be particularly important in immunopathogenesis and management of respective infections (i.e., by blocking suppressive effects of endogenous IL-10) and in patients receiving IL-10 as an antiinflammatory cytokine. As IL-10 is being developed as a novel antiinflammatory cytokine [24, 25], our findings and those of others suggest that there may also be an increased risk for opportunistic bacterial and fungal infections. IL-10 may impair clearance of organisms during IL-10 antiinflammatory therapy. One potential approach that has been proposed is to simultaneously administer antibacterial and/or antifungal agents to control the infectious organism while IL-10 attenuates the detrimental inflammatory process [9, 46].

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