Interleukin-4 suppresses antifungal activity of human mononuclear phagocytes against *Candida albicans* in association with decreased uptake of blastoconidia

Emmanuel Roilides a, Isaac Kadiltsoglou a, Anastasia Dimitriadou a, Maria Hatzistilianou a, Anna Manitsa b, John Karpouzas a, Philip A. Pizzo c, Thomas J. Walsh c,*

a Department of Pediatrics, University of Thessaloniki, GR-54642 Thessaloniki, Greece  
b Transfusion Medicine Department, Hippokration Hospital, GR-54642 Thessaloniki, Greece  
c Pediatric Branch, National Cancer Institute, Bethesda, MD 20892, USA

Received 11 August 1997; accepted 28 August 1997

Abstract

Pathogenesis of invasive candidiasis may involve regulatory activities of Th2 immunity on phagocytic host defenses. The effects of interleukin (IL)-4 on antifungal capacity of human mononuclear phagocytes against *Candida albicans* were studied. Incubation of adherent mononuclear leukocytes from healthy donors with IL-4 (1–5 ng ml⁻¹) at 37°C for 2–4 days suppressed uptake of *C. albicans* blastoconidia in the presence of human serum (*P* ≤ 0.01), and anti-IL-4 inhibited its suppressive effect. The effect of IL-4 was protein synthesis-dependent. Interferon-γ (0.25–25 ng ml⁻¹), granulocyte-macrophage colony-stimulating factor (CSF, 20 ng ml⁻¹), macrophage-CSF (15 ng ml⁻¹) but not IL-10 (100 ng ml⁻¹) somewhat counteracted the suppressive effect of IL-4. In contrast, mannose receptor-mediated uptake of blastoconidia in the absence of serum was increased by IL-4. Killing of conidia was decreased after incubation of morphonuclear leukocytes with IL-4 for 2 days (*P < 0.05*). While superoxide anion production in response to phorbol myristate acetate was decreased by IL-4 (*P < 0.05*), it was not altered in response to blastoconidia and pseudohyphae. Morphonuclear leukocyte-induced pseudohyphal damage also remained unaltered. These findings suggest that IL-4 plays its detrimental role in invasive candidiasis by predominantly suppressing uptake and killing of blastoconidia by morphonuclear leukocytes. Anti-IL-4, IFN-γ, GM-CSF and M-CSF appear to counteract suppression of morphonuclear leukocyte phagocytic activity suggesting new approaches to the management of disseminated candidiasis.

**Keywords**: Interleukin-4; Monocyte/macrophage; Uptake; Cytokine; *Candida*

* Corresponding author. Tel: +1 (301) 402-0023; Fax: +1 (301) 402-0575; E-mail: walsh@pbmac.nci.nih.gov

Preliminary results of this study have been presented in part at the 8th Immunocompromised Host Society Meeting at Davos, Switzerland, June 1994.

1. Introduction

Interleukin (IL)-4 is a glycoprotein of MW 20 kDa that was originally described as a B cell growth and differentiation factor [1]. Initial studies demonstrated
that this cytokine has stimulatory properties in different compartments of the immune system such as macrophage cytotoxicity and expression of class II major histocompatibility antigen as well as adherence antigens [2–5]. More recent studies, however, have revealed that IL-4 is a multifunctional Th2 lymphocyte-derived cytokine that has predominantly immunosuppressive properties (reviewed in [6]). It has been found to inhibit production of IL-1 [7,8], tumor necrosis factor-α (TNFα) [9,10], granulocyte colony-stimulating factor (G-CSF), granulocyte-macrophage colony-stimulating factor (GM-CSF) [11], IL-6 [12–15] and IL-8 [16], as well as activation of macrophages by interferon-γ (IFN-γ), IL-1, TNFα and GM-CSF, resulting in a suppression of oxidative burst and intracellular killing [17,18]. IL-4 also has been shown to suppress adhesion [19], expression of CD14 [20] and monocyte-mediated cytotoxicity [21] as well as nitric oxide production and antifungal activity of murine macrophages [22].

Disseminated candidiasis is the most frequent fungal infection that occurs in immunocompromised patients. In particular, patients with defects in phagocytic host defenses, such as very low birth weight infants, surgical, debilitated, as well as neutropenic patients, are susceptible to invasive candidiasis [23]. In addition to polymorphonuclear and mononuclear phagocytes [24], the pathogenesis of candidiasis involves an interaction between Th1 and Th2 cellular immunities. For example, Romani et al. observed an association of Th1 responses with acquired resistance to Candida [25] and that neutralization of IL-4 induces systemic protection from Candida [26]. Little is known, however, about the direct role of IL-4 on the antifungal activities of mononuclear phagocytes specifically against Candida blastoconidia and pseudohyphae that underlie these in vivo phenomena. Moreover, the interaction between IL-4 and other Th1- and Th2-type cytokines, such as IFN-γ, IL-10, GM-CSF and macrophage colony-stimulating factor (M-CSF) on these antifungal activities of monocytes have not been comprehensively studied.

The purpose of this study was to evaluate the effects of IL-4 on antifungal activities of human monocytic phagocytes against blastoconidia and pseudohyphae of Candida albicans and on secretion of oxidative burst metabolites produced by MNCs. In addition, the impacts of neutralization of IL-4 with anti-human IL-4 antibody (ab) and of co-treatment of MNCs with other cytokines were also evaluated.

2. Materials and methods

2.1. Blood donors and effector cells

Buffy coats were prepared from anticoagulated venous blood of healthy adult volunteers regardless of sex at the Transfusion Medicine Department, Hippokration Hospital, Thessaloniki, Greece. Each experiment was performed with cells from a different blood donor. The donors had IgG, IgA, IgM, IgE serum levels and CD4/CD8 ratios within or slightly higher than normal limits.

Mononuclear cells were separated from buffy coats by centrifugation over Ficoll (Lymphocyte Separation Medium, 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) consisting of RPMI-1640, 25% pooled human serum (collected from healthy adult donors at Transfusion Medicine Department, Hippokration Hospital), 100 units ml⁻¹ penicillin, and 100 μg ml⁻¹ streptomycin. They were counted by trypan blue staining on hemocytometer and the percentage of monocytes was estimated by May–Grünwald–Giemsa staining. The viability of cells was greater than 95% and approximately 30–40% of them were monocytes (MNCs). Concentration of MNCs was adjusted at 5×10⁶ per ml. They were enriched in the cell preparations by adherence on plastic or glass as described below. More than 85% of adherent mononuclear leukocytes were monocytes as determined by staining with May–Grünwald–Giemsa and nonspecific esterase.

2.2. Reagents and pretreatment of MNCs

Recombinant human IL-4 with specific activity 5×10⁷ units mg⁻¹ and goat anti-human IL-4 polyclonal antibody were obtained from R and D Systems, Minneapolis, MN, USA. Human IFN-γ was kindly provided by Genentech Inc., South San Francisco, CA, at specific activity 4×10⁷ units mg⁻¹. Human M-CSF with specific activity 7×10⁷ units mg⁻¹ was a generous gift from Chiron Corp.,
Emeryville, CA, USA. Human GM-CSF was kindly provided by Immunex, Seattle, WA, at specific activity $>5 \times 10^7$ units mg$^{-1}$ of protein. Interleukin-10 was purchased from Pepro Tech Inc., Rocky Hill, NJ, USA. The cytokines used contained no detectable levels of endotoxin (manufacturer’s assay). Cycloheximide (CHX) was purchased from Sigma Chemical Co., St. Louis, MO, USA. All agents were further diluted in HBSS without Ca$^{2+}$ and Mg$^{2+}$ or in RPMI-1640 to appropriate stock concentrations.

One million MNCs in CM were placed on 18-mm sterile round glass coverslips in 12-well plates (Costar, Cambridge, MA, USA) for uptake assay or without coverslips in each well of 12-well plates for superoxide anion ($O_2^-$) assay. Both plates were incubated at 37°C with 5% CO$_2$ for 45 min. The wells were washed twice with warm HBSS not containing Ca$^{2+}$ and Mg$^{2+}$. Fresh CM was added into the wells and the glass- or plastic-adherent MNCs were further incubated in 1 ml CM at 37°C with 5% CO$_2$ with or without cytokines for 1–4 days. No significant changes in the number of MNCs adhering on glass due to IL-4 (up to 10 ng ml$^{-1}$) were observed as has been reported for adherence on plastic surfaces [19].

In addition, $25 \times 10^6$ MNCs were cultured in 25-cm$^2$ tissue culture flasks (Costar) with 10 ml CM containing 10% fetal calf serum (Gibco; CM-FCS) instead of 25% human serum for assays assessing killing of blastoconidia (hereafter referred to as conidioidal assays) and pseudohyphal damage assays. Flasks were placed at 37°C with 5% CO$_2$ for 2 h. They were then washed twice with warm HBSS not containing Ca$^{2+}$ and Mg$^{2+}$. Fresh CM-FCS was added into the flasks and the plastic-adherent MNCs were further incubated in 10 ml CM-FCS at 37°C with 5% CO$_2$ with or without cytokines for 2 days. IL-4 at its maximal concentration used in these studies had no effect on the viability of MNCs as assessed by trypan blue staining or on their size as evaluated microscopically.

### 2.3. Organism

Strain #86-21 of *C. albicans* isolated from a patient with disseminated candidiasis was used in these studies. It was preserved in skim milk aliquots at $-35^\circ$C, and was grown on Sabouraud dextrose agar (SDA) plates for 18–24 h at 37°C before each experiment. Under these conditions, *C. albicans* grows exclusively as blastoconidia. Blastoconidia were obtained directly from SDA plates and suspended in HBSS at a final concentration of $10^7$ cfu ml$^{-1}$. Pseudohyphae were obtained from blastoconidia as described below (Section 2.7).

### 2.4. Assay of uptake of blastoconidia

After treatment of MNCs with IL-4, supernatants were removed and 1 ml of CM containing $10^8$ *C. albicans* blastoconidia per ml was added to each well. Following 15 min incubation at 37°C with 5% CO$_2$ for uptake, coverslips were washed 3 times with warm HBSS without Ca$^{2+}$ and Mg$^{2+}$ to remove the extracellular and loosely bound blastoconidia, and the cells were fixed and stained with May-Grünwald-Giemsa. In the experiments where macrophage mannose receptor (MMR)-mediated uptake was studied, plain RPMI-1640 instead of CM was used. Uptake of blastoconidia by cells was assessed by light microscopy.

Both percent uptake and uptake index were calculated by counting 100 MNCs per coverslip for duplicate coverslips. Percent uptake was determined as the percentage of MNCs that had one or more blastoconidia ingested or attached after counting 100 of them. Uptake index was the average number of blas-
Table 1
Uptake of *C. albicans* blastoconidia by monocytes (MNCs) pre-treated with IL-4 for 1, 2 or 4 days*

<table>
<thead>
<tr>
<th>IL-4 (ng/ml)</th>
<th>% Uptakeb</th>
<th>Uptake indexc</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>65.3 ± 7.2</td>
<td>1.48 ± 0.16</td>
</tr>
<tr>
<td>5</td>
<td>47.0 ± 12.4</td>
<td>1.45 ± 0.17</td>
</tr>
<tr>
<td>Day 2</td>
<td>63.2 ± 2.8</td>
<td>1.63 ± 0.11</td>
</tr>
<tr>
<td>5</td>
<td>45.5 ± 5.5</td>
<td>1.59 ± 0.11</td>
</tr>
<tr>
<td>Day 4</td>
<td>66.0 ± 12.2</td>
<td>2.11 ± 0.29</td>
</tr>
<tr>
<td>5</td>
<td>46.2 ± 9.2</td>
<td>1.58 ± 0.14</td>
</tr>
</tbody>
</table>

*Mean ± standard error of mean of 3-10 donors tested in equal number of experiments.

bPercent uptake: number of yeast-uptaking MNCs divided by the total number of MNCs counted.

cUptake index: total number of MNC-associated *C. albicans* blastoconidia divided by the number of MNCs having blastoconidia ingested or attached on them.

dDifference from control, not significant.

eDifference from control, P = 0.007.

2.6. Conidiodal assay

MNCs that had been incubated with CM-FCS only or with IL-4-containing CM-FCS were mixed with 10⁶ blastoconidia in 1/1 effector-to-target cell (E/T) ratio and 10% pooled human serum in HBSS (final volume 1 ml in polypropylene tubes) [27]. From previous studies of conidiodal activity performed in our laboratory the MNC/yeast ratio 1/1 was considered to be the most appropriate to study the effects of IL-4. The mixture was incubated on a shaker at 37°C for 120 min. After complete lysis of monocytes with sterile H₂O at the end of incubation, dilutions were made, plated in duplicate on SDA plates, and incubated at 37°C for 18 h. Colonies were counted and conidiodal activity was calculated using the formula: % killing = ((C−X)/C) x 100, where X is the number of cfu with MNCs at 120 min, and C is the number of cfu without MNCs at 120 min.

2.7. Pseudohyphal damage assay

The colorimetric MTT assay [28] was adapted for *C. albicans* pseudohyphae [29]. Briefly, a suspension of 2.5 x 10⁶ blastoconidia per ml in RPMI-1640 was made, and 1-ml aliquots were plated in the wells of 24-well plates (Costar). The plates were

Table 2
Uptake of *C. albicans* blastoconidia by IL-4-pretreated monocytes (MNCs) and inhibition of IL-4 suppression by anti-IL-4 antibody (ab)*

<table>
<thead>
<tr>
<th>Pretreatment</th>
<th>% Uptakeb</th>
<th>0 h</th>
<th>6 h</th>
<th>24 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-4 (ng/ml)</td>
<td>ab</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>ND</td>
<td>76.0</td>
<td>60.0</td>
<td>86.5</td>
</tr>
<tr>
<td>1</td>
<td>ND</td>
<td>61.5</td>
<td>43.0</td>
<td>ND</td>
</tr>
<tr>
<td>5</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>60.0</td>
</tr>
<tr>
<td>0</td>
<td>ND</td>
<td>74.0</td>
<td>64.5</td>
<td>82.0</td>
</tr>
<tr>
<td>1</td>
<td>ND</td>
<td>77.0</td>
<td>56.0</td>
<td>ND</td>
</tr>
<tr>
<td>5</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>78.0</td>
</tr>
</tbody>
</table>

*Results are means of duplicate conditions from representative experiments among 2-5 similar experiments with different donors. Interleukin-4 was added to the MNCs 2 days (42 h) before the assay and 2 µg goat anti-IL-4 polyclonal antibody was added at the indicated times (0, 6, and 24 h) after addition of IL-4.

bPercent uptake: number of uptaking MNCs divided by the total number of MNCs counted.

ND: not determined.
incubated at 37°C for 4 h, by which time more than 95% of blastoconidia had germinated to pseudohyphae.

The supernatants were aspirated and MNCs that had been incubated with CM-FCS only or with IL-4-containing CM-FCS in flasks were added to the wells in E/T ratios 1/1, 5/1 and 10/1. After 2 h at 37°C and 5% CO₂, supernatants were aspirated, MNCs were lysed by adding 300 μl of 0.5% sodium deoxycholate and pseudohyphae were washed three times with sterile water. Subsequently, 1 ml of RPMI-1640 containing 0.5 mg ml⁻¹ 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 the wells of a 96-well plate (Costar), and the color was measured on a Perkin-Elmer microplate reader (Perkin-Elmer, Wifon, CT, USA) at 540 nm. A well containing only isopropanol was used as a blank. Control wells containing pseudohyphae and buffer only but not MNCs were included in each experiment. Antifungal activity (pseudohyphal damage) was calculated using the formula:

\[
\text{% pseudohyphal damage} = \frac{\text{OD of control wells} - \text{OD of test wells}}{\text{OD of control wells}} \times 100
\]

where control wells are those containing pseudohyphae only. Each condition was tested in duplicate or quadruplicate and the results were averaged.

2.8. Statistics

Differences between baseline levels and levels at individual concentrations were assessed with repeated measures analysis and paired Student’s t-test. All P values reported are two-sided. Bonferroni correction test was used in instances of multiple comparisons where P values were close to 0.05.

3. Results

3.1. Effect of IL-4 on MNC uptake activity

The percent uptake of blastoconidia by MNCs in the presence of human serum was significantly suppressed after incubation of MNCs with either 1 or 5 ng ml⁻¹ IL-4 at 37°C. This suppression was consistent in almost all donors and the differences from controls were significant at both 1 and 5 ng ml⁻¹ IL-4 (P=0.002 and 0.007, respectively; Fig. 1, left panel). Using 10 ng ml⁻¹ in 4 experiments, no further significant suppression of percent uptake was found (data not shown). The uptake index was not significantly altered by treatment with any concentration of IL-4 used (Fig. 1, right panel).

To define the time that is necessary for IL-4 to
Table 3
Uptake of *C. albicans* blastoconidia by monocytes (MNCs) pretreated with IL-4 in combination with other cytokines for 2 days

<table>
<thead>
<tr>
<th>MNC treatment</th>
<th>% Uptake $^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. IL-4 (ng/ml)</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>IFN-γ (ng/ml)</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>0.25</td>
</tr>
<tr>
<td>5</td>
<td>0.25</td>
</tr>
<tr>
<td>0</td>
<td>2.5</td>
</tr>
<tr>
<td>5</td>
<td>2.5</td>
</tr>
<tr>
<td>0</td>
<td>25</td>
</tr>
<tr>
<td>5</td>
<td>25</td>
</tr>
<tr>
<td>B. IL-10 (ng/ml)</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>5</td>
<td>100</td>
</tr>
<tr>
<td>GM-CSF (ng/ml)</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>M-CSF (ng/ml)</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>20</td>
</tr>
<tr>
<td>5</td>
<td>20</td>
</tr>
<tr>
<td>0</td>
<td>15</td>
</tr>
<tr>
<td>5</td>
<td>15</td>
</tr>
</tbody>
</table>

$^a$Mean±standard error of mean of 3–6 (A), 7 (B) or 3–7 (C) different donors tested in equal number of experiments performed in duplicate. The experiments of uptake of blastoconidia were performed in the presence of human serum.

$^b$Percent uptake: number of uptaking MNCs divided by the total number of MNCs counted.

$^c$The difference from control (0/0) is statistically significant with $P=0.025$. In A, the difference between 0/0.25 and 5/0.25 as well as that between 0/25 and 5/25 tend to be smaller than the difference between 0/0 and 5/0 with $P$ values of 0.08 each.

$^d$The difference from control (0/0) is statistically significant with $P=0.035$. None of the other differences in B is significant.

$^e$The difference from control (0/0) is slightly significant with $P=0.06$. In C, the difference between 0/20 and 5/20 as well as that between 0/15 and 5/15 tend to be smaller than the difference between 0/0 and 5/0 with $P$ values of 0.1 and 0.067 each.

Suppress uptake, MNCs were incubated with IL-4 for 1, 2 or 4 days and then uptake of *C. albicans* was evaluated in the presence of serum. IL-4 (5 ng ml$^{-1}$) significantly suppressed percent uptake after 2 days (Fig. 1 and Table 1) and 4 days ($P=0.01$; Table 1). The trend of suppression of uptake index after incubation for 4 days was only slightly significant ($P=0.08$). After 1 day incubation, the mean percent uptake was also decreased but the difference from control was not significant (Table 1). At days 1 and 2, the maximal degree of suppression of percent uptake was 28% and at day 4 it was 30%. Thus, incubation periods longer than 1 day and including 2 and 4 days were required by IL-4 to consistently exert its suppressive effect on uptake activity of MNCs.

3.2. Inhibition of IL-4 suppression of MNC uptake activity by anti-IL-4 antibody

To investigate whether the suppressive effect of IL-4 can be abrogated by IL-4 neutralization, MNCs were treated with IL-4 (1 or 5 ng ml$^{-1}$) for 42 h and 2 μg goat anti-IL-4 polyclonal antibody was added at the initiation of culture or 6 or 24 h later. At the end of 42 h incubation, uptake of *C. albicans* blastoconidia was assessed in the presence of human serum. As shown in Table 2, anti-IL-4 ab added simultaneously with IL-4 to MNCs inhibited the suppressive effect of IL-4 suggesting that the suppression of uptake is IL-4 specific. Similarly, anti-IL-4 ab added 6 or 24 h after addition of IL-4 inhibited IL-4-induced suppression.

3.3. Effect of protein synthesis inhibition on IL-4 activity

To test whether the suppressive effect of IL-4 on uptake requires new protein synthesis, the protein synthesis inhibitor CHX was used. Fig. 2 shows that treatment of MNCs with 10 ng ml$^{-1}$ CHX in-
hibited the IL-4-induced suppression of uptake activity against *Candida* blastoconidia by 58%. Similar results were obtained when concentration 100 ng ml⁻¹ of CHX was used (data not shown).

3.4. Effects of combinations of IL-4 and other cytokines on MNC uptake activity

As IFN-γ has been found to have immunoenhancing properties on MNC antifungal activities [29–31], the effect of 2 days incubation of MNCs with the combination of IL-4 and IFN-γ was evaluated. IL-4 had a moderately suppressive effect on percent uptake (*P* = 0.025; Table 3A), a finding which is consistent with the results of Fig. 1. IFN-γ alone at 0.25, 2.5 and 25 ng ml⁻¹ did not show any trend in percent uptake by MNCs. However, when combined with IL-4, IFN-γ tended to counteract the suppressive effect of IL-4 on uptake (Table 3A).

Incubation of MNC for 2 days with IL-10, alone at 100 ng ml⁻¹, did not significantly alter uptake of *Candida* blastoconidia. Moreover, while IL-4 (5 ng ml⁻¹) suppressed *Candida* uptake (*P* = 0.035), IL-10 did not significantly alter the suppressive effect of IL-4 when combined with it (Table 3B).

In these experiments, neither GM-CSF alone (20 ng ml⁻¹) nor M-CSF alone (15 ng ml⁻¹ equivalent to approximately 1000 units ml⁻¹) appeared to exert any effect on MNC percent uptake of *Candida* blastoconidia after incubation for 2 days (Table 3C). However, in combination with IL-4 (5 ng ml⁻¹), GM-CSF and M-CSF each counteracted the suppressive effect of IL-4 on MNC uptake.

3.5. Effect of IL-4 on MMR-mediated uptake

Because IL-4 has been found to upregulate the
expression of MMR [32,33] whereas it downregulates the expression of Fcγ receptors on the surface of macrophages [34], experiments in the absence of serum (opsonins) were performed to delineate the effect of IL-4 on MMR-mediated uptake of Candida blastoconidia. In contrast to the effect of IL-4 on uptake in the presence of serum, IL-4, at a concentration of 5 ng ml\(^{-1}\), increased both percent uptake and uptake index of unopsonized blastoconidia by MNCs significantly (Table 4).

3.6. Effect of IL-4 on superoxide production by MNCs

Because production and secretion of oxidative burst metabolites such as \(O_2^-\) are associated with antifungal activity of MNCs, the effect of IL-4 on \(O_2^-\) production was also investigated. IL-4-pretreated MNCs exhibited suppressed \(O_2^-\) production in response to PMA. Production of \(O_2^-\) was reduced after incubation of MNCs with IL-4 from 2.30 ± 0.34 nmol per 10\(^6\) MNCs per 1 h of controls to 1.42 ± 0.47 at 1 ng ml\(^{-1}\) (\(P = 0.026\)) and to 1.46 ± 0.27 at 10 ng ml\(^{-1}\) (\(P = 0.011\)) (Fig. 3, left panel).

In contrast, IL-4 did not appear to induce a significant or consistent alteration of \(O_2^-\) production in response to particulate fungus-specific stimuli, such as blastoconidia and pseudohyphae of C. albicans. For example, \(O_2^-\) production in response to Candida blastoconidia was 0.63 ± 0.35 nmol per 10\(^6\) MNCs per 1 h after treatment with 10 ng ml\(^{-1}\) IL-4 as compared to 0.88 ± 0.15 for controls. Similarly, \(O_2^-\) production in response to Candida pseudohyphae was 2.23 ± 0.53 nmol per 10\(^6\) MNCs per 1 h after treatment with 10 ng ml\(^{-1}\) IL-4 as compared to 1.54 ± 0.12 for controls (Fig. 3, right panel). Neither of these trends was significant.

3.7. Effect of IL-4 on MNC conidiodical activity

The conidiodical activity of MNCs was significantly decreased by IL-4 in a concentration-dependent manner (Fig. 4) from 61.4 ± 4.9% (controls) to 51.9 ± 6.4% (1 ng ml\(^{-1}\); \(P = 0.04\)) and to 47.5 ± 6.7% (10 ng ml\(^{-1}\); \(P = 0.019\)). In two of the five experiments, in which anti-IL-4 antibody was used in combination with 10 ng ml\(^{-1}\) IL-4, the antibody partially inhibited IL-4-induced suppression (data not shown).

3.8. Effect of IL-4 on MNC-induced pseudohyphal damage

The effect of IL-4 on antifungal activity of MNCs against Candida pseudohyphae (pseudohyphal damage) was also evaluated. Using the MTT assay and E/T ratios 5/1 and 10/1, no change in pseudohyphal damage induced by IL-4-treated MNCs was found as compared to controls (Table 5). Similar results were obtained by using E/T ratio 1/1 (data not shown).

4. Discussion

In this study, IL-4 has been shown to decrease the overall activity of human MNCs to uptake blastoconidia of C. albicans despite increasing the MMR-mediated uptake of the same target. This effect can be counteracted by anti-IL-4 antibody and by the cytokines IFN-\(\gamma\), GM-CSF and M-CSF. Additionally, IL-4 suppresses MNC \(O_2^-\) production in response to PMA but not to particulate fungus-specific stim-
uli. Moreover, IL-4 suppresses MNC conidiodical activity, but not pseudohyphal damage. The suppressive effects were achieved by concentrations of IL-4 in the range of 1–10 ng ml\(^{-1}\), concentrations which have been shown to induce modulatory effects on other immune functions, such as secretion of cytokines and antiparasitic activity [12,17,18,35–37].

The suppression of percent uptake of *C. albicans* in our study differs from the lack of suppression of uptake of *Saccharomyces cerevisiae* particles reported by Abramson and Gallin [35]. Differences in methods and in fungus-specific receptor factors may account for this discordance. That percent uptake but not uptake index was suppressed by IL-4 in our study suggests a heterogeneous population of MNCs differing in sensitivity to IL-4.

As killing of blastoconidia of *C. albicans* is predominantly an intracellular event, the IL-4-induced suppression of uptake of *Candida* appears to be related to the decreased killing of *Candida* blastoconidia in the presence of serum. Although O\(_2^\cdot\) production in response to blastoconidia was not affected by IL-4, the fact that uptake was decreased might have led to the suppressed intracellular killing of blastoconidia. The role of IL-4-induced suppression of uptake and impaired intracellular killing of blastoconidia is underscored by the absence of IL-4’s effect on MNC O\(_2^\cdot\) production in response to and damage of pseudohyphae, which are not ingestible.

The mechanism that underlies functional downregulation of uptake activity of MNCs is presently unclear. It is notable that IL-4 downregulates the expression of Fcy receptors on the surface of MNCs [34], although it is not clear how this relates to the effect of IL-4 on the uptake of *C. albicans* blastoconidia. Since human serum was present during uptake in our study, FcyR-mediated uptake was presumably operative. However, it is known that MMR also mediates uptake and intracellular killing of *Candida* blastoconidia [38], and thus, a combination of FcyR- and MMR-mediated phenomena may have been seen in our experiments with human serum. Since IL-4 downregulates FcyR [34] and upregulates MMR [32] and we have shown that IL-4 increases MMR-mediated uptake, the only moderate suppressive effect of IL-4 on overall uptake that has been found in this study may have been the sum of two opposite activities of IL-4 on the two kinds of receptors.

The effect of IL-4 on uptake activity of MNCs was inhibited by simultaneous treatment of the cells with CHX, a protein synthesis inhibitor, suggesting that new protein synthesis is required for the effect of IL-4 to occur. Similar protein synthesis-dependent inhibition of the IL-4 effect on the expression of IL-1, IL-8 and TNF\(\alpha\) has been found [7,9,16]. In this context, IL-4 induced expression of IL-1 receptor antagonist [39] as well as it inhibited mRNA and protein synthesis of IL-1 and TNF\(\alpha\) by MNCs in response to lipopolysaccharide [12,36,40]. Perhaps reduction of the autocrine function of these cytokines induced by IL-4 also results in reduced uptake of *C. albicans*.

As MNC-induced antimicrobial and tissue-destructive functions depend on secretion of oxidative burst metabolites, IL-4 may also suppress these activities via downregulation of NADPH oxidase or signal transduction pathway to oxidative burst. Studies of RNA transcription of critical host defense genes, such as NADPH oxidase, might shed more light on the IL-4/host defense interaction. Of note, only O\(_2^\cdot\) production in response to PMA but not to particulate stimuli was suppressed. The excitatory pathways leading to NADPH are complex and many different actions may have a regulatory impact on their various steps. IL-4 is known to cause a translocation of protein kinase C to a nuclear fraction [41] and inhibit the expression of two immediate early genes c-fos and c-jun [42]. Nevertheless, the lack of effect of IL-4 on O\(_2^\cdot\) production in response to *Candida* pseudohyphae may be related to the absence of suppression of IL-4 on MNC-induced pseudohyphal damage.

Nitric oxide production by macrophages has been found to contribute to antifungal activity in rodents [22] and IL-4 inhibits nitric oxide-dependent killing of *Candida* [22]. In contrast, however, the role of nitric oxide in macrophage killing of *Candida* has been debatable in humans [43,44].

The combinations of IL-4 with IFN-\(\gamma\), GM-CSF, M-CSF and IL-10 were also studied. Of note, at the conditions used in this study none of these cytokines alone significantly affected uptake of *C. albicans* blastoconidia by MNCs either positively or negatively. However, IFN-\(\gamma\), GM-CSF and M-CSF each
counteracted IL-4-induced modest suppression at some degree, a finding that is consistent with the fine balance of Th1 and Th2 cytokine networks [6,25]. In this regard, IL-4 has been found to antagonize the enhancing effect of IFN-γ on O₂⁻ and neutrophin generation [45–47], expression of Fc receptors [46], uptake of immune complexes [46], IL-1 and IL-6 production [13,48] as well as apoptosis [49]. Although IL-10 has suppressive effects on other antifungal functions of phagocytes [22,50,51], it was not found to suppress uptake of *C. albicans* blastoconidia significantly and was not interactive with IL-4, affirming the diversity of immunoregulatory properties of Th2 cytokines on uptake-related receptors on the surface of MNCs.

Hepatosplenic candidiasis or chronic disseminated candidiasis [52] is an infection that may involve IL-4 or other Th2-type cytokines. Becoming clinically overt upon recovery from neutropenia, hepatosplenic candidiasis has long been an immunologic enigma. Some patients with hepatosplenic candidiasis will continue to demonstrate progressive infection refractory to antifungal therapy despite having recovered from neutropenia. Perhaps IL-4-mediated suppression of uptake and conidiodal activity may be contributing to this infection. As demonstrated by Romani et al. [26], administration of anti-IL-4 antibody augments host response, increases anti-*Candida* activity in vitro and upregulates Th1 activity in murine candidiasis. Therefore, use of anti-IL-4 antibody may be beneficial in the management of hepatosplenic candidiasis. Our findings indicating that IFN-γ, GM-CSF and M-CSF also reverse the suppressive effects of IL-4 on MNCs against *C. albicans*, suggest that these cytokines may have beneficial effects in hepatosplenic candidiasis. Indeed, two open-label studies of M-CSF [53] and GM-CSF [54] found activity of these cytokines in the management of hepatosplenic candidiasis.

In conclusion, IL-4 has been shown to have moderate suppressive effects on overall uptake and fungicidal activities of human mononuclear phagocytes against *Candida* blastoconidia but not on O₂⁻ production in response to *Candida* particles or on MNC-induced damage of pseudohyphae. Anti-IL-4 antibody and the cytokines IFN-γ, GM-CSF and M-CSF appear to counteract this suppression of uptake.

Acknowledgments

We are grateful to Genentech Inc. for providing IFN-γ, Immunex for providing GM-CSF and Chiron Corp. for providing M-CSF. We are also indebted to Mrs. Niki Mega and Mrs. Voula Vasiadou of the Transfusion Medicine Department, Hippokration Hospital, for preparing buffy coats from blood of healthy volunteers.

References

ony-stimulating factor levels in stimulated human monocytes. Immunology 76, 566–571.


