Endogenous Interleukin (IL–1α and IL–1β Are Crucial for Host Defense against Disseminated Candidiasis

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Background. Interleukin (IL–1α and IL–1β are protective proinflammatory cytokines involved in host defense against Candida albicans. It is, however, unknown whether they provide protection through similar mechanisms. We investigated the effect of endogenous IL–1α and IL–1β on disseminated C. albicans infection.

Methods. Mice deficient in the genes encoding IL–1α (IL–1α−/−), IL–1β (IL–1β−/−), or both molecules (IL–1α−/−β−/−) were used. Survival and C. albicans outgrowth in the kidneys was assessed after intravenous injection of C. albicans.

Results. Both mortality and C. albicans outgrowth in the kidneys were significantly increased in IL–1α−/− and IL–1β−/− mice, compared with those in control mice, with the IL–1α−/−β−/− mice being most susceptible to disseminated candidiasis. The host defense mechanisms triggered by IL–1α and IL–1β differed from one another. IL–1β−/− mice showed decreased recruitment of granulocytes in response to an intraperitoneal C. albicans challenge, and generation of superoxide production was diminished in IL–1β−/− granulocytes. IL–1α−/− mice had a reduced capacity to damage C. albicans pseudohyphae. Protective type 1 responses were deficient in both IL–1α−/− and IL–1β−/− mice, as assessed by production of interferon-γ by splenocytes in response to heat-killed C. albicans.

Conclusion. Although IL–1α and IL–1β have differential effects on the various arms of host defense, both cytokines are essential for mounting a protective host response against invasive C. albicans infection.

Despite the availability of potent antifungal agents, acute disseminated candidiasis remains a life-threatening disease that occurs mainly in immunocompromised patients [1]. Immunotherapies with cytokines have great potential to augment host resistance and as adjunctive treatment for invasive candidiasis. For further development of these strategies, a better understanding of the protective immune mechanisms against invasive candidiasis is needed.

Interleukin (IL–1α and IL–1β are proinflammatory cytokines that exert similar biological activities after interaction with the IL–1 type 1 receptor (IL–1RI) and the IL–1R accessory protein [2]. Exogenous recombinant human IL–1α or IL–1β has been administered in studies of disseminated murine candidiasis, and these studies have clearly indicated a protective role for IL–1 in this infection model [3, 4]. The mechanisms of this beneficial effect have been only partly elucidated: IL–1 has no direct antifungal effect, and the protective effect of IL–1 in host defense against Candida albicans does not depend on the presence of granulocytes or humoral factors, such as acute-phase proteins [3, 4]. To characterize the role of endogenous IL–1α and IL–1β in disseminated candidiasis and to gain further insight into the mechanisms through which both IL–1 molecules confer protection against disseminated candidiasis, mice in which the genes encoding IL–1α (IL–1α−/−), IL–1β (IL–1β−/−), or both (IL–1α−/−β−/−) had been disrupted were used in the present study. The IL–1−deficient mice and their immunocompetent littermates were subjected to experimental disseminated C. albicans infection.
MATERIALS AND METHODS

Mice. IL-1α−/−, IL-1β−/−, and IL-1α−/−β−/− mice were produced as described elsewhere [5]. As control mice for the IL-1–deficient mice, age-matched C57Bl/6 (IL-1α+/−β+/−) mice were used. The mice were allowed to become accustomed to laboratory conditions for 1 week before experimental use as described below.

Infection model. Mice were injected intravenously with 1 × 10⁶ C. albicans (ATCC MYA-3573; UC820) blastoconidia, and survival was assessed. For measurement of circulating granulocytes and cytokines, mice were bled from the retroorbital plexus on day 1, 3, or 7 of infection. To quantify fungal outgrowth, the kidneys were removed aseptically, weighed, and homogenized, and serial dilutions were plated on Sabouraud agar, as described elsewhere [3]. Colony-forming units were counted, and results were expressed as log colony-forming units per kidney. For histologic analysis, kidneys of subgroups of mice (5 mice/group) were fixed in buffered formaldehyde (4%). Paraffin-embedded sections were stained with periodic acid–Schiff or hematoxylin-eosin.

To investigate whether the role of endogenous IL-1 in candidiasis is mediated by polymorphonuclear neutrophils (PMNs), mice were rendered granulocytopenic by use of cyclophosphamide (Bristol-Myers Squibb), administered subcutaneously at a dose of 150 mg/kg on day 4 and at a dose of 100 mg/kg on days 1 and 2 of infection with 1 × 10⁶ C. albicans blastoconidia [3]. Daily differential counts in peripheral blood smears confirmed granulocytopenia (<100 × 10⁶ cells/L; data not shown). Hyperuricemia-induced tumor necrosis factor (TNF) production was prevented by gastric instillation of sodium bicarbonate at a dose of 100 mg/kg twice per day, starting on day −4 and continuing until the end of the experiment [6]. C. albicans outgrowth in the kidneys on day 1 or 3 of infection was determined as described elsewhere [3].

PMN recruitment. PMN recruitment to an infection site was determined after intraperitoneal injection of 1 × 10⁷ cfu of heat-killed C. albicans in uninfected mice. After 4 h, mice were killed by CO₂ asphyxiation, and peritoneal exudates were obtained by washing with ice-cold PBS. The numbers of PMNs were assessed in Giemsa-stained cytocentrifuge preparations.

Superoxide production. Superoxide production was studied in a luminol-enhanced peroxidase-catalyzed chemiluminescence assay [7]. Briefly, peritoneal exudate PMNs were suspended at a concentration of 2 × 10⁵ cells/mL of Hanks’ balanced salt solution without phenol red (Gibco), supplemented with 0.25% human serum albumin. Cells (2 × 10⁷/well) were incubated in 96-well microtiter plates (Costar Corning) with 50 μmol/L luminol and 4.5 U/mL horseradish peroxidase (Sigma) and were stimulated with either medium (as a negative control) or 2 × 10⁷ heat-killed blastoconidia and PMA (50 ng/mL). Chemiluminescence was measured on a Victor2 1420 counter and expressed as the total amount of superoxide produced in 35 min, by integrating the area under the curve per PMN.

Phagocytosis and intracellular killing of C. albicans. Exudate peritoneal phagocytes were collected 4 h (PMN) or 72 h (macrophages) after intraperitoneal injection of 10% protease peptone. Cells were centrifuged at 550 g, counted, and resuspended in RPMI 1640 Dutch modification (with 20 mmol/L HEPES, without glutamine; RPMI-dm; ICN Biomedicals) supplemented with 5% heat-inactivated fetal calf serum.

Phagocytosis and intracellular killing were studied in an adherent monolayer of phagocytes, as described elsewhere [8]. Briefly, monolayers were incubated with opsonized blastoconidia (ATCC 10261) in modified Eagle’s medium (Gibco) (effector-to-target cell [E:T] ratio, 40:1). After 15 min, supernatants containing noningested blastoconidia were plated on Sabouraud agar. The percentage of phagocyted blastoconidia was calculated as [1 – (number of uningested cfu/cfu at the start of incubation)] × 100. To assess the percentages of C. albicans blastoconidia internalized versus those only attached to the membrane, fluorescein isothiocyanate–labeled C. albicans was opsonized and incubated for 15 min on a macrophage monolayer (E:T ratio, 40:1, as described above). After washing with sterile medium to remove the extracellular nonadherent yeasts, the total fluorescence was measured in the monolayer to enumerate the total of internalized and adherent C. albicans cells. The fluorescence of the extracellular, membrane-adhered C. albicans was quenched by adding methylene blue, the cells were washed, and the number of fluorescent internalized blastoconidia was assessed. In 3 separate experiments, 90%–97% of the total number of internalized and adherent blastoconidia were shown to be intracellular, and no differences in the ratios that were adherent to phagocyted C. albicans cells between wild-type and IL-1–deficient mice were apparent.

After removal of the nonphagocyted blastoconidia, killing of blastoconidia by PMNs was assessed in the same monolayers in fresh medium. After 3 h of incubation at 37°C and 5% CO₂, the wells were gently scraped with a plastic paddle and washed with 200 μL of distilled H₂O to achieve lysis of macrophages. This procedure was repeated 3 times, after which the pooled washes were adjusted to a final volume of 1 mL with distilled water. Microscopic examination of the culture plates showed that there was a complete removal of phagocytes. Viable intracellular blastoconidia were quantified as described elsewhere [8]. The percentage of phagocyte-killed yeasts was determined as [1 – (cfu after incubation/number of phagocyted cfu)] × 100. Phagocyte-free incubations of blastoconidia were included as controls for yeast viability.

Assessment of PMN-mediated pseudohyphal damage. PMN-mediated pseudohyphal damage was determined by the XTT dye assay, as described elsewhere [9]. ATCC MYA-3573 blastoconidia were suspended at 1 × 10⁶ cfu/mL of RPMI-dm (pH
synergized with culture medium as a negative control, heat-
impaired survival, compared with that of IL-1
assay.

Production of cytokines and NO. Resident peritoneal macro-
phages were obtained aseptically with ice-cold PBS. Cells were
resuspended in RPMI 1640 without phenol red and t-glutamine (RPMI-wp; ICN Biomedicals). Pseudo hyphae
(1 × 10⁵) and PMNs in RPMI-wp were added to the wells in the presence of 10% fresh IL-1αβ serum (E:T ratio, 8:1). Control wells contained pseudohyphae or PMNs only. After incubation for 2 h, PMNs were lysed with sterile H₂O. After 15 min, sterile XTT (400 μg/mL; Sigma Chemical) and coen-
zyme Q₉ (50 μg/mL; Sigma) were added. After 1 h of incubation at 37°C, the plate was centrifuged (770g), the supernatants were
transferred to a microtiter plate, and the absorbance was mea-
sured in a spectrophotometer at 450 nm. The percentage of fungal
damage was calculated as 1−[(A₄₅₀ hyphae and PMNs−A₄₅₀ PMNs)/A₄₅₀ hyphae] × 100.

Cytokine assays. TNF-α, IL-1α, and IL-1β concentrations were
determined using specific radioimmunoassays, as described
elsewhere [11]. The detection limit was 40 pg/mL for TNF-α
and 20 pg/mL for IL-1α and IL-1β. IL-10, IFN-γ, and IL-6
concentrations were determined by ELISA (Biosource), and the
detection limits were 8, 15.6, and 150 pg/mL, respectively. Mu-
rine keratinocyte-derived chemokine (KC), macrophage inhibi-
tory protein (MIP)-2, and monocyte chemotactic protein–1
(MCP-1) were measured by ELISA (R&D Systems).

Statistical analysis. Parametric data are expressed as mean ± SD, and data that showed normal distribution after log trans-
formation are expressed as means and 95% confidence intervals
(CIs). Nonparametric values are expressed as medians. Since ≥3 groups were compared, parametric data were analyzed using 1-way analysis of variance (ANOVA), and nonparametric data were analyzed using the Kruskal-Wallis 1-way ANOVA. For post-
test comparisons of nonparametric data, the Mann-Whitney U
test was applied. The Kaplan-Meier log rank test was used to
analyze survival data. The data represent the pooled results of
all experiments performed.

RESULTS

Disseminated candidiasis in nonneutropenic mice. Whereas
72% of the IL-1α−/−β−/− mice survived infection with 1 × 10⁵
cfu of C. albicans, only 41% of IL-1α−/− and 38% of IL-1β−/−
mice survived (P < .05, control mice vs. IL-1α−/− or IL-1β−/−).
mice, Kaplan Meier log rank test) (figure 1). Moreover, survival was even further impaired (29%) in mice lacking both IL-1α and IL-1β; this difference was significant, in comparison with IL-1α−/−β−/− mice (P < .001) and with IL-1α−/− mice and IL-1β−/− mice (P < .05) (figure 1).

Since the kidneys are the main target organs in disseminated candidiasis [3, 12], C. albicans outgrowth in the kidneys was determined. On day 7 of infection, C. albicans outgrowth was increased 51-fold in IL-1α−/− mice (P < .01), 3-fold in IL-1β−/− mice (P > .05), and 125-fold in IL-1α−/−β−/− (P < .001) (figure 2), when means of outgrowth in knockout and IL-1α−/−β−/− mice were compared. At this point in time, the C. albicans burden in IL-1β−/− mouse kidneys was significantly higher than that in IL-1α−/− mouse kidneys (P < .05) (figure 2). Circulating concentrations of IL-1α, IL-1β, TNF-α, IFN-γ, IL-10, and IL-6 in blood obtained from mice on day 7 of infection were under the detection limit (data not shown).

To determine the difference in outgrowth between IL-1α−/− and IL-1β−/− mice at a later point in time during infection, subgroups of IL-1α−/−, IL-1β−/−, or IL-1α+/−β+/− mice received an intravenous injection of 5 × 10^4 cfu of C. albicans. On day 14 of infection, outgrowth in the kidneys of IL-1α−/− mice (mean, 6.03 log cfu [95% CI, 5.50–6.57 log cfu]) and IL-1β−/− mice (mean, 6.10 log cfu [95% CI, 5.44–6.77 log cfu]) was significantly increased, compared with that in IL-1α+/−β+/− mice (mean, 4.25 log cfu [95% CI, 2.54–5.96 log cfu]) (P < .05). However, no difference between the numbers of C. albicans colony-forming units recovered from the kidneys of IL-1α−/− mice and IL-1β−/− mice was observed at this time point.

**Histopathologic assessment.** Seven days after an intravenous injection of 1 × 10^4 C. albicans blastoconidia, the inflammatory lesions in the kidneys of IL-1α−/−β−/− mice were healing, showing few PMNs and fibroblasts (figure 3D). In the kidneys of IL-1β−/− and IL-1α+/−β+/− mice, a granulomatous response was observed without the presence of yeast cells (figure 3B and 3C). In IL-1α−/− mice and IL-1α+/−β−/− mice however, a large amount of C. albicans had accumulated in the collecting ducts, surrounded by PMNs and lymphocytes (figure 3A and 3C).

**Disseminated candidiasis in neutropenic mice.** To assess whether the protective effect of IL-1 is PMN mediated, mice were rendered granulocytopenic by use of cyclophosphamide. Three days after infection with 1 × 10^4 C. albicans blastoconid-
IL-1α and IL-1β are produced and released from infected cells and play a role in the pathogenesis of disseminated candidiasis. IL-1 influences both the fungicidal activity of PMNs and the recruitment of these cells to the site of infection.

**Table 1. No. of circulating polymorphonuclear neutrophils (PMNs) before or on day 3 of infection with Candida albicans blastoconidia (1 × 10^6 cfu administered intravenously), and recruitment of PMNs 4 h after intraperitoneal injection of heat-killed C. albicans blastoconidia (1 × 10^6 cfu).**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Circulating PMNs, no. 10^6/L</th>
<th>Intraperitoneal PMN recruitment</th>
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<tr>
<td></td>
<td>Before infection</td>
<td>On day 3 of infection</td>
</tr>
<tr>
<td>IL-1α+/β+/+</td>
<td>0.89 ± 0.31</td>
<td>0.84 ± 0.71</td>
</tr>
<tr>
<td>IL-1α−/−</td>
<td>0.42 ± 0.10</td>
<td>0.86 ± 0.30</td>
</tr>
<tr>
<td>IL-1β−/−</td>
<td>1.38 ± 0.47</td>
<td>3.20 ± 0.99</td>
</tr>
<tr>
<td>IL-1α−/β−/−</td>
<td>0.67 ± 0.35</td>
<td>2.95 ± 0.52</td>
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</table>

**NOTE.** Data are the mean ± SD of at least 3 mice/group, were obtained from 1 experiment, and were analyzed using 1-way analysis of variance and the Mann-Whitney U test for posttest comparisons. 

a Percentage of the total no. of peritoneal exudate cells

b P < 0.005, vs. control or IL-1α−/− mice.

c P < 0.01, vs. control or IL-1α−/− mice.

The largest quantity of IL-1 was recovered from the kidneys of IL-1α−/−β−/− mice (mean ± SD, 5.23 ± 0.37 log cfu), and the difference was significant in comparison with IL-1α+/β+/+ mice (mean ± SD, 5.64 ± 0.51 log cfu) (P < 0.01). Outgrowth in the kidneys of IL-1α−/− mice (mean ± SD, 4.27 ± 1.31 log cfu) was similar to that in the kidneys of IL-1β−/− mice (mean ± SD, 4.13 ± 0.67 log cfu). (Data are cumulative results of 2 experiments for at least 6 mice/group.)

**PMN circulating numbers and recruitment.** To study the role of endogenous IL-1α and IL-1β in the recruitment of PMNs to the site of a localized C. albicans infection, peritoneal PMNs were collected after intraperitoneal injection of heat-killed C. albicans (table 1). Whereas the recruitment of PMNs in IL-1α−/− mice did not differ from that in IL-1α+/β+/+ mice, significantly lower numbers of PMNs were recruited to the site of infection in IL-1β−/− mice, indicating that IL-1β is most important for PMN recruitment to the site of infection. In contrast, during the early days of infection, increased numbers of circulating PMNs were observed in IL-1β−/− mice and IL-1α−/β−/− mice, compared with those in IL-1α−/− and IL-1α+/β+/+ mice (table 1), underscoring the importance of IL-1β in the migration of PMNs from the bloodstream to the site of infection. The mean numbers of peripheral blood PMNs were similar for all groups on day 7 of infection (data not shown).

**Anticandidal response of PMNs against C. albicans blastoconidia and pseudohyphae.** To further characterize the influence of endogenous IL-1α and IL-1β on the activity of PMNs against C. albicans, the fungicidal capacity of PMNs was determined in vitro. Whereas IL-1α−/− PMNs showed a tendency only toward reduced superoxide production (mean ± SD, 58.3 ± 10^3 ± 15.4 ± 10^3 cps), IL-1β−/− PMNs produced significantly less superoxide than did control PMNs (mean ± SD, 30.6 ± 10^3 ± 15.9 ± 10^4 vs. 71.5 ± 10^3 ± 10.3 ± 10^3 cps) (P < 0.01). However, this observation was not accompanied by an altered capacity of IL-1−/− deficient PMNs to phagocytose or kill internalized C. albicans blastoconidia; all groups phagocytosed ∼25% of the blastoconidia and killed ∼75% of internalized blastoconidia.

In contrast, IL-1β modulated the capacity of PMNs to damage pseudohyphae (figure 4). Pseudohyphal damage caused by IL-1α−/− or IL-1α−/β−/− PMNs was significantly less efficient than that caused by IL-1α+/β+/+ or IL-1β−/− PMNs (P < 0.001) (figure 4). Deficiency of both IL-1 molecules showed a tendency to further impair PMN antipseudohyphal resistance, compared with IL-1α deficiency alone. Deficiency of IL-1β alone did not diminish the capacity of PMNs to damage C. albicans pseudohyphae.

**Macrophage function and stimulation.** Macrophage recruitment was determined by harvesting peritoneal exudate cells 72 h after an intraperitoneal injection of 1 × 10^6 cfu of heat-killed C. albicans. No differences in the mean ± SD numbers of macrophages that were recruited into the peritoneal cavity were observed, compared with those in control mice (IL-1α+/β+/+, 1.01 × 10^6 ± 0.12 × 10^6; IL-1α−/−, 1.10 × 10^6 ± 0.02 × 10^6; IL-1β−/−, 0.96 × 10^6 ± 0.19 × 10^6; IL-1α−/β−/−, 1.18 × 10^6 ± 0.15 × 10^6). IL-1 influenced neither the mean ± SD capacity of exudate peritoneal macrophages to phagocytose C. albicans blastoconidia (IL-1α+/β+/+, 41% ± 5%; IL-1α−/−, 50% ± 4%; IL-1β−/−, 56% ± 3%; IL-1α−/β−/−, 41% ± 16%) nor their capacity to damage C. albicans pseudohyphae (data not shown). No differences between NO production by macrophages from the mouse strains were observed in response to either heat-killed C. albicans (all around the detection limit) or heat-killed C. albicans blastoconidia.
LPS (control mice, 1.37 ± 0.54 ng/mL; IL-1β−/− mice, 0.78 ± 1.35 ng/mL; IL-α−/−β−/− mice, 5.28 ± 0.2 ng/mL; P> .05).

IL-1−/−deficient resident peritoneal macrophages showed impaired production of IL-6 and of the chemokine KC in response to heat-killed C. albicans blastoconidia or pseudohyphae. The difference in IL-6 production was significant for IL-1α−/− macrophages and IL-1α+/−/β−/− macrophages, in comparison with IL-1α+/+ β−/− macrophages, and the difference in KC production was significant for IL-1β−/− macrophages and IL-1α−/− β−/− macrophages, in comparison with IL-1α+/+ β−/− macrophages (P< .05) (table 2). No differences between groups were observed in the production of TNF-α, MCP-1, or MIP-2.

**Stimulation of splenic lymphocytes.** To assess whether IL-1 induces a type 1 or a type 2 immune response, splenocytes were stimulated with heat-killed C. albicans. Whereas IL-1α+/+ β−/− splenocytes produced IFN-γ, neither IL-1α−/− nor IL-1β−/− splenocytes did (P< .05) (table 3), indicating that both IL-1α and IL-1β are important for protective IFN-γ production. Whereas IL-1β−/− and IL-1α−/− β−/− splenocytes did not produce any IL-10, both IL-1α+/+ β−/− splenocytes and IL-1α−/− β−/− splenocytes did (P< .05) (table 3), indicating that both IL-1α and IL-1β are important for protective IFN-γ production, whereas the residual IL-10 production in IL-1α−/− mice further contributed to a type 2 response.

**DISCUSSION**

The results of the present study indicate that deficiency of endogenous IL-1α or IL-1β has deleterious effects on the outcome of disseminated candidiasis. Both IL-1α−/− mice and IL-1β−/− mice showed increased mortality associated with an increased outgrowth of C. albicans in the kidneys. IL-1β proved to be important for PMN recruitment and generation of superoxide production. IL-1α was essential for the capacity of PMNs to damage C. albicans pseudohyphae, and both IL-1α and IL-1β were required for the induction of protective Th1 responses.

One of the mechanisms through which IL-1 has been suggested to confer protection is the enhancement of granulopoiesis and influx of PMNs to the site of infection. Although IL-1α−/− mice had 50% fewer circulating granulocytes at the time of infection than control mice, their number in the circulation on day 3 of infection and PMN recruitment to the site of infection did not differ from that of control mice. IL-1β−/− mice, however, a trend toward an increased number of circulating granulocytes during infection was observed, and this coincided with significantly decreased PMN migration to the site of infection in a standardized model of intraperitoneal C. albicans challenge. This is in line with earlier evidence that exogenous administration of IL-1α or IL-1β induces peripheral blood granulocytosis [13, 14]. Our data on PMN recruitment in response to heat-killed C. albicans also suggest an important role for endogenous IL-1β in the early PMN influx, in line with studies showing that exogenous administration of IL-1 induces PMN accumulation [15, 16]. Moreover, PMN recruitment in IL-1−/−deficient mice may be further impaired as a result of decreased production of neutrophil chemoattractants. Whereas IL-1α−/− mice showed a tendency toward reduced production of the CXC chemokine KC, KC production by IL-1β−/− macrophages was significantly reduced, thus likely contributing to impaired granulocyte recruitment in IL-1β−/− mice. Whereas the differences in chemokine production between IL-1α−/− and IL-1β−/− mice may not be impressive, the major difference between the mouse strains was the complete absence of IL-1α−/− in the IL-1β−/− mice. Since IL-1β has been shown to be an important mediator of

<table>
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<tr>
<th>Stimulation, strain</th>
<th>TNF-α, ng/mL</th>
<th>IL-6, pg/mL</th>
<th>MCP-1, pg/mL</th>
<th>MIP-2, pg/mL</th>
<th>KC, pg/mL</th>
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<td>Blastocodia</td>
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<td>IL-1α+/+ β−/−</td>
<td>0.56 ± 0.14</td>
<td>926 ± 547</td>
<td>87 ± 45</td>
<td>2190 ± 1633</td>
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<td>IL-1α−/−</td>
<td>0.88 ± 0.41</td>
<td>154 ± 9</td>
<td>40 ± 1</td>
<td>2660 ± 1630</td>
<td>380 ± 197</td>
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<td>IL-1β−/−</td>
<td>0.46 ± 0.22</td>
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<td>IL-1α−/− /β−/−</td>
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<td>159 ± 21</td>
<td>1674 ± 753</td>
<td>265 ± 254</td>
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<td>IL-1α+/+ β−/−</td>
<td>1.32 ± 0.82</td>
<td>1378 ± 796</td>
<td>221 ± 135</td>
<td>3350 ± 2699</td>
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<td>IL-1α−/−</td>
<td>0.95 ± 0.42</td>
<td>366 ± 203</td>
<td>152 ± 154</td>
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<td>435 ± 196</td>
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<td>IL-1β−/−</td>
<td>0.74 ± 0.29</td>
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<td>98 ± 56</td>
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<td>99 ± 87</td>
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<tr>
<td>IL-1α−/− /β−/−</td>
<td>0.98 ± 0.39</td>
<td>233 ± 187</td>
<td>1295 ± 510</td>
<td>303 ± 326</td>
<td></td>
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</table>

**NOTE.** Data are the mean ± SD production in 5 mice/group, obtained from 1 experiment, and were analyzed using 1-way analysis of variance and the Mann-Whitney U test for posttest comparisons. IL, interleukin; KC, keratinocyte-derived chemokine; MCP, monocyte chemotactic protein; MIP, macrophage inhibitory protein; TNF, tumor necrosis factor.

* P< .05, vs. IL-1α+/+ β−/− mice.
Table 3. In vitro cytokine production by splenocytes (5 x 10^6 cells/mL) stimulated with heat-killed Candida albicans blastoconidia (10^6/mL).

<table>
<thead>
<tr>
<th>Strain</th>
<th>IFN-γ, pg/mL</th>
<th>IL-10, pg/mL</th>
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<tr>
<td>IL-1α+/β−/−</td>
<td>47.4 ± 30.7</td>
<td>32.3 ± 8.8</td>
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<tr>
<td>IL-1α−/−</td>
<td>&lt;15.6a</td>
<td>30.8 ± 12.2</td>
</tr>
<tr>
<td>IL-1β−/−</td>
<td>&lt;15.6a</td>
<td>&lt;8b</td>
</tr>
<tr>
<td>IL-1α+/β−/−</td>
<td>&lt;15.6a</td>
<td>&lt;8b</td>
</tr>
</tbody>
</table>

*NOTE.* Data were obtained from 1 experiment and are expressed as the mean ± SD production in 5 mice/group. IFN, interferon; IL, interleukin.

a P<.05, vs. IL-1α+/β−/− mice.
b P<.05, vs. IL-1α+/β−/− and IL-1α−/− mice.

granulocyte recruitment [17, 18], this effect most likely is instrumental in explaining the difference in recruitment between the IL-1α−/− and IL-1β−/− mice. However, it has to be taken into account that assessment of PMN recruitment to a peritoneal inflammatory stimulus is, at best, an approximation of early parenchymal PMN recruitment at the various sites of infection.

Transcription of the genes encoding MIP-2 and KC is induced through signals mediated by Toll-like receptor 4 [19], and IL-1 has been shown to selectively stabilize KC mRNA [20]. Hence, it is hypothesized that the normal production of IL-1 on KC mRNA.

The effect of endogenous IL-1 on PMN function has not been investigated previously. The results of the present study indicate that IL-1 affects PMN function and that the modes of action differ for IL-1α and IL-1β. Whereas IL-1β−/− PMNs showed impaired superoxide production, IL-1α−/− PMNs showed a decreased capacity to damage C. albicans pseudohyphae, indicating that IL-1α is important for PMN degranulation. In addition, production of IL-6, which is induced by IL-1 and known to stimulate PMN function [21–23], was reduced only in IL-1β−/− mice, and this may further contribute to impaired PMN function and reduced anticandidal defense. Since the C. albicans burden in IL-1α−/− kidneys was significantly higher than that in IL-1β−/− kidneys on day 7 of infection, it is suggested that the initially reduced PMN recruitment in IL-1β−/− mice is overridden by the effect of the reduced capacity to kill C. albicans pseudohyphae in IL-1α−/− mice. However, despite the difference in outgrowth between the 2 mouse strains on day 7, IL-1β−/− mice showed susceptibility to infection similar to that of IL-1α−/− mice. Most of the apparent discrepancy is explained by the fact that, on day 14 of infection, the fungal burden in both IL-1α−/− mice and IL-1β−/− mice was equally increased. Another possible explanation for this observation is that, in addition to fungal outgrowth, the additional inflammatory damage in the organs of IL-1β−/− mice may also have contributed to mortality.

If the beneficial effect of endogenous IL-1 is exerted only through PMNs, the differences in outgrowth between the groups, as observed in nongranulocytic peritoneal mice, should disappear in granulocytopenic mice. However, the observed differences persisted, indicating that the effect of endogenous IL-1 is at least partly mediated through cells or mechanisms other than modulation of PMN recruitment and function, which is in line with previous findings [3, 4]. Nevertheless, because cyclophosphamide also reduces lymphocyte numbers, it cannot be excluded that a minor beneficial effect of IL-1 on C. albicans outgrowth may be mediated through PMNs.

Macrophages and lymphocytes are other cells that are likely to mediate the protective effect of IL-1 [24, 25]. Most importantly, IFN-γ production was found to be absent in IL-1α−/− splenocytes. This is in agreement with previous data showing that endogenous IL-1 is important for the production IFN-γ during C. albicans stimulation of whole blood [26]. The crucial role of IFN-γ in host defense has become apparent from studies showing that IFN-γ-deficient mice are highly susceptible to disseminated candidiasis and that administration of recombinant IFN-γ reduces outgrowth [12, 27, 28]. Recently, we observed that delayed development of a type 1 response—that is, delayed IFN-γ production—contributed to increased outgrowth in localized C. albicans infection [9]. Furthermore, we observed foreign body giant cells (FBGCs) in the kidneys of IL-1−/− mice. The transition of macrophages to functionally inactive FBGCs is favored in the presence of type 2 cytokines [29]. Therefore, the presence of FBGCs points to an anti-inflammatory type 2 response, which has been shown to be detrimental to anticanidial host defense [30].

In conclusion, the present study has further clarified the mechanisms through which IL-1α and IL-1β enhance host resistance against candidiasis. Interestingly, the absence of either IL-1α or IL-1β had divergent consequences. Although they are products of different genes, IL-1α and IL-1β are highly homologous and have similar tridimensional structures and common receptors. It is unclear at this point what determines the functional differences in IL-1α−/− and IL-1β−/− mice—a different distribution of cytokines in the organs, the known dichotomy between cell-bound IL-1α and secreted IL-1β [31], or differential receptor affinity. Although a functional difference between IL-1α and IL-1β was observed in this study, both IL-1α and IL-1β were shown to be essential for anticanidial host defense. The complementary roles of IL-1α and IL-1β in host defense against C. albicans infection are further underscored by the even higher susceptibility to disseminated candidiasis of mice lacking both IL-1α and IL-1β. These data constitute an additional body of evidence that IL-1-dependent mechanisms play an important role in innate host defense against C. albicans.
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References


