Role of Interleukin-18 in Host Defense against Disseminated Candida albicans Infection

Rogier J. L. Stuyt, Mihai G. Netea, Ineke Verschuuren, Giamila Fantuzzi, Charles A. Dinarello, Jos W. M. Van der Meer, and Bart Jan Kullberg

Department of Medicine, University Medical Center St. Radboud, Nijmegen, The Netherlands, and Division of Infectious Diseases, University of Colorado Health Sciences Center, Denver, Colorado

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In mice injected intravenously with Candida albicans, administration of anti-interleukin-18 (IL-18) antibodies increased the yeast load in the kidneys. There was no effect on the organ load with Candida when gamma interferon (IFN-γ)-deficient mice were treated with anti-IL-18 antibodies, suggesting that the protective effect of IL-18 is mediated through endogenous IFN-γ.

Candida albicans or mannoproteins derived from the yeast cell wall induce gamma interferon (IFN-γ) production by human mononuclear cells, and IFN-γ is a key cytokine for defense against candidiasis (8). The important role of endogenous IFN-γ in resistance to systemic candidiasis has been demonstrated in knockout mice deficient in IFN-γ, which are highly susceptible to C. albicans infection (1). Moreover, administration of recombinant IFN-γ to wild-type mice infected with C. albicans improves the outcome of the infection (8). Interleukin-18 (IL-18) serves as a costimulus for IFN-γ production in the context of costimulation with microbial products (14). When endogenous IL-18 is neutralized by administration of antibodies to IL-18 (3), there is little, if any, IFN-γ production after challenge with endotoxin. These data have led to the hypothesis that IL-18 is important for the host defense against disseminated candidiasis.

The aim of the present study was to investigate whether endogenous IL-18 is involved in host defense against Candida infection. IL-18 bioactivity was blocked by neutralizing anti-mouse IL-18 antibodies. In addition, we assessed whether the effects of IL-18 during disseminated candidiasis are mediated through production of IFN-γ by studying IFN-γ-deficient mice with disseminated candidiasis treated with anti-IL-18 antibodies. The role of tumor necrosis factor (TNF) in the subsequent IL-18 synthesis was investigated with mice deficient in TNF and lymphotoxin (LT).

CBA mice (females, 20 to 25 g, 6 to 8 weeks old) were purchased from Jackson Laboratories (Bar Harbor, Maine). IFN-γ−/− mice and their wild-type littermates (BALB/c genetic background) were generously provided by Organon (Oss, The Netherlands). Homozygous TNF−/− LT−/− and wild-type TNF+/− LT+/− mice (genetic background, C57BL/6J × 129sv) were obtained as mating pairs (kindly provided by F. Amiot, CEA, Fontenay-aux-Roses). Anti-mouse IL-18 polyclonal antibodies were produced in rabbits using recombinant mature murine IL-18 (Peprotech, Rocky Hill, N.J.) (3). Normal rabbit serum (NRS) was used in the control groups.

The mice were injected intravenously (i.v.) with C. albicans (strain UC 820; 106 CFU/mouse). EDTA-blood was collected from the retroorbital plexus for plasma IL-18 concentration measurements at various time points: 1, 2, 4, 8, 24, 48, and 72 h after infection. Animals received either 200 μl of anti-IL-18 antisera intravenously 10 min before infection and on days 2 and 4 after infection or a similar volume of NRS. Subgroups of 10 animals were killed on day 1, 3, or 7 of infection. The number of viable Candida cells in the kidneys was determined as previously described (7) and expressed as log CFU per gram of tissue.

IL-18 concentrations were determined by electrochemiluminescence, using a biotinylated rat anti-mouse IL-18 antibody (Igen, Gaithersburg, Md.) and a ruthenium goat antimouse antibody (Peprotech, Princeton, N.J.). The reaction was quantitated using the Origen 1.5 Analyzer (Igen) (15). IL-1β and TNF-α levels were determined by specific radioimmunoassays (11). Murine IL-6 and IFN-γ concentrations were measured using commercial enzyme-linked immunosorbent assay kits (Pelikine; CLB, Amsterdam, The Netherlands). Detection limits were 20 pg/ml (TNF, IL-1α, IL-1β, and IL-6) and 40 pg/ml (IL-18 and IFN-γ). The differences between groups were analyzed by the Mann-Whitney U test.

IL-18 concentrations were below the detection limit (40 pg/ml) in both uninfected TNF+/− LT+/− and TNF−/− LT−/− mice. Intravenous administration of C. albicans to TNF+/− LT+/− mice induced circulating IL-18 concentrations, which reached peak elevations at 8 h postinfection (279 ± 144 pg/ml) (Fig. 1). In Candida-infected TNF+/− LT+/− mice, the circulating IL-18 concentrations were significantly higher at 2, 4, 8, and 24 h postinfection than those in uninfected mice. Similar IL-18 concentrations were measured in CBA mice and IFN-γ+/− BALB/c mice (not shown). TNF and/or LT was required for the induction of IL-18, since the peak of circulating IL-18 levels was absent in TNF−/− LT−/− mice (Fig. 1). For TNF−/− LT−/− mice, the IL-18 concentrations were slightly increased above background levels for uninfected mice at 24 h after infection (98 ± 11 pg/ml), similar to the increase in TNF+/− LT+/− mice. These findings are in line with data showing that...
neutralization of endogenous TNF and LT using soluble TNF
p55 receptors reduced circulating IL-18 following i.v. treat-
ment with concanavalin A (2).

The role of endogenous IL-18 for the defense against
C. albicans infection was investigated by administering neutraliz-
ing anti-IL-18 antibodies to the mice prior to infection. C. albicans CFU in the kidneys decreased 10-fold within 7 days of
infection in mice injected with NRS, whereas neutralization of
IL-18 by anti-IL-18 antibodies prevented the elimination of the
microorganisms (Fig. 2). Circulating concentrations of IL-1α
and IL-6 on days 1 and 3 of infection were not influenced by
administration of anti-IL-18 antibodies. However, the in-
creased outgrowth of Candida in the organs of the anti-IL-18-
treated mice on day 7 was accompanied by higher circulating
concentrations of IL-1α and IL-6 than for NRS-treated mice
(Fig. 3). TNF, IL-1β, and IFN-γ concentrations were below the
detection limit in all samples on days 1, 3, and 7 after infection.

These data imply an important role of endogenous IL-18 in
the defense against disseminated candidiasis, and such findings
are supported by other studies showing that IL-18 is essential
for host defense against mycobacterial infections (16) and
Cryptococcus neoformans (5), Leishmania major (13), and Salmonella infections (9). In a recent study, it has been shown that
recombinant IL-18 restores the Th1 response to C. albicans in
caspase-1-deficient mice, which are unable to process the in-
active precursors in bioactive IL-18 and IL-1β (10). Promising
therapeutic properties of IL-18 in experimental infections with
C. neoformans (6) or Leishmania spp. (13) have also been
suggested.

To investigate whether the effect of IL-18 is mediated
through endogenous IFN-γ, neutralizing anti-IL-18 antibodies
were given to mice deficient in IFN-γ before infection with C.
 albicans. In contrast to the effects in wild-type mice (Fig. 2),
there was no effect of anti-IL-18 antibodies on Candida out-
growth in the kidneys of IFN-γ−/− mice (94% compared to the
outgrowth in IFN-γ−/− mice treated with NRS; $P > 0.05$), demonstrating that the effects of endogenous IL-18 during
disseminated candidiasis are mediated by IFN-γ. These
findings are consistent with previous data demonstrating the
importance of IFN-γ for the protective effects of IL-18 during infection with C. neoformans (6) or Salmonella enterica serovar
Typhimurium (9). The finding that IL-18 has a relatively late
effect, on day 7 of infection, is consistent with IFN-γ-mediated

**FIG. 1.** Circulating IL-18 during disseminated candidiasis. Groups of 10 TNF+/− LT+/− and TNF−/− LT−/− mice were injected i.v. with $10^5$ C. albicans CFU. IL-18 concentrations in the serum of TNF+/−
LT+/− (closed circles) and TNF−/− LT−/− (open triangles) mice were measured by enhanced chemiluminescence at various time points after
infection (the experiment was performed twice, with a total of 10 animals per time point). Asterisk, $P < 0.05$

**FIG. 2.** The role of endogenous IL-18 in the defense against dis-
seminated candidiasis. CBA mice received 200 μl of either NRS (closed circles) or anti-IL-18 antiserum (open triangles) and were thereafter injected i.v. with $10^5$ C. albicans CFU. Outgrowth of the
microorganism in the kidneys was assessed on days 1, 3, and 7 after
infection in groups of 10 animals. Asterisk, $P < 0.05$

**FIG. 3.** Cytokine concentrations during disseminated candidiasis. CBA mice received 200 μl of either NRS (closed circles) or anti-IL-18 antiserum (open triangles) and were thereafter injected i.v. with $10^5$ C. albicans CFU. Circulating concentrations of IL-1α (A) and IL-6
(B) were measured on days 1, 3, and 7 after infection in subgroups of
10 animals. Asterisk, $P < 0.05$. 

![Graph of IL-18 concentrations](image1)

![Graph of IL-1α and IL-6 concentrations](image2)

![Graph of cytokine concentrations](image3)
stimulation of macrophages, which is known to occur at least 7 days after infection, as has been shown previously with IFN-γ mice (1), whereas no effect of the anti-IL-18 antibodies was found during the first phase of infection, when neutrophil-mediated mechanisms are more important (1). However, since IFN-γ−/− mice are highly susceptible to disseminated candidiasis, it is possible that an additive effect of IL-18 on the anti-
Candida defense through IFN-γ-independent mechanisms is difficult to substantiate with these mice. IFN-γ-independent effects of IL-18 have been found in lethal endotoxemia and experimental models of streptococcal cell wall arthritis (4, 12).

In conclusion, endogenous IL-18 plays a protective role in the defense against disseminated infection with C. albicans. The production of IL-18 during disseminated candidiasis requires endogenous TNF and/or LT, and its protective effects are likely mediated through intermediary stimulation of endogenous IFN-γ synthesis.

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