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

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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-$\gamma^{-/-}$ 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; 10^5 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 antiserum 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 ruthenilated 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-$\gamma^{+/+}$ 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

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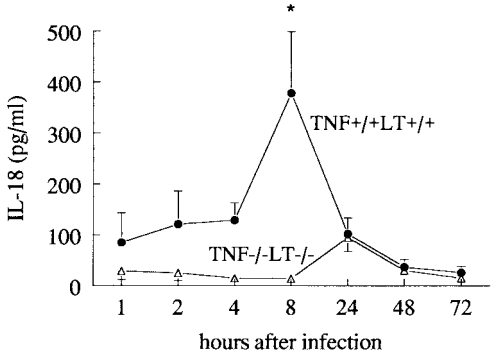

FIG. 1. Circulating IL-18 during disseminated candidiasis. Groups of 10 $TNF^{+/+}$ $LT^{+/+}$ and $TNF^{-/-}$ $LT^{-/-}$ mice were injected i.v. with 10^5 *C. albicans* CFU. IL-18 concentrations in the serum of $TNF^{+/+}$ $LT^{+/+}$ (closed circles) and $TNF^{-/-}$ $LT^{-/-}$ (open triangles) mice 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, $P < 0.05$

neutralization of endogenous TNF and LT using soluble TNF p55 receptors reduced circulating IL-18 following i.v. treatment with concanavalin A (2).

The role of endogenous IL-18 for the defense against *C. albicans* infection was investigated by administering neutralizing 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 increased 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

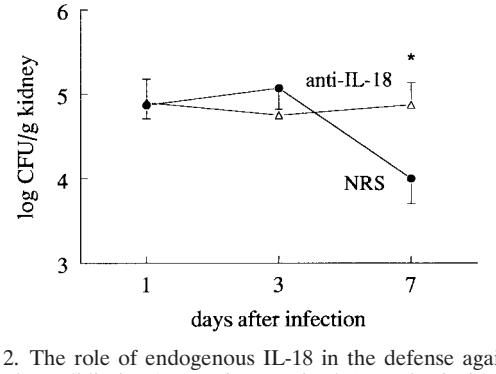

FIG. 2. The role of endogenous IL-18 in the defense against disseminated candidiasis. CBA mice received 200 μl of either NRS (closed circles) or anti-IL-18 antiserum (open triangles) and were thereafter injected i.v. with 10^5 *C. albicans* CFU. Outgrowth of the microorganism in the kidneys was assessed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

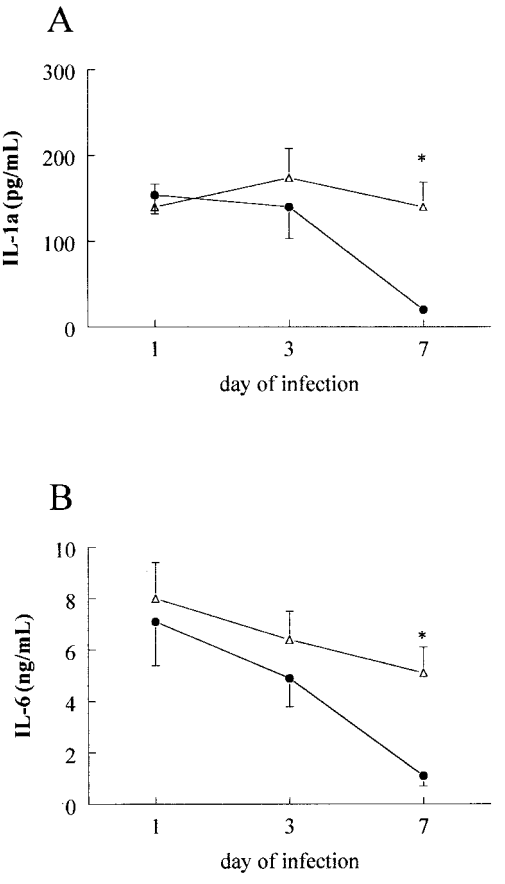

FIG. 3. Cytokine concentrations during disseminated candidiasis. CBA mice received 200 μl of either NRS (closed circles) or anti-IL-18 antiserum (open triangles) and were thereafter injected i.v. with 10^5 *C. albicans* CFU. Circulating concentrations of IL-1α (A) and IL-6 (B) were measured on days 1,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

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 inactive 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* outgrowth in the kidneys of $IFN\text{-}\gamma^{-/-}$ mice (94% compared to the outgrowth in IFN-γ-deficient 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

stimulation of macrophages, which is known to occur at least 7 days after infection, as has been shown previously with IFN-$\gamma^{-/-}$ 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-$\gamma^{-/-}$ 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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