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Topics: Candida albicans, Interleukin and Corpus albicans

Short paper

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Protection of neutropenic mice from lethal *Candida albicans* infection by recombinant interleukin 1*

Natural and synthetic immunomodulators that increase nonspecific resistance to infection are also known to induce interleukin 1 (IL 1) production. Previous studies have demonstrated a protective effect of recombinant human IL 1β against death from infection caused by *Pseudomonas aeruginosa*. In the present study we investigated the effect of IL 1β or IL 1α on the survival of neutropenic mice with a lethal *Candida albicans* infection. Mice with cyclophosphamide-induced neutropenia were injected with 3×10^5 *C. albicans* i.v. When 80 ng IL 1β was given as a single i.p. injection 24 h before the infection, survival compared to that in control animals was as follows: 100% *vs.* 97% at 24 h, 83% *vs.* 70% at 48 h and 70% *vs.* 23% at 72 h after the infection ($p < 0.01$). The effect of IL 1 was also apparent when it was given ½ h before or 6 h after the infection. The results obtained with 80 ng IL 1α given at 24 h before infection were similar to that obtained with IL 1β. The numbers of *Candida* cultured from the blood, liver, spleen, and kidney were not significantly different in IL 1β-treated and control animals. Passive transfer of serum obtained from mice pretreated with IL 1 to recipient mice did not provide protection against a subsequent lethal candidal infection. In conclusion, the present study demonstrates that IL 1β and IL 1α prolong survival in neutropenic mice with a lethal *C. albicans* infection.

1 Introduction

Disseminated infection caused by *Candida* species is a major infectious complication in the compromized host [1]. Treatment of these infections with antifungal drugs generally yields disappointing results, especially in neutropenic patients [2]. Thus, there is a clear need for other forms of therapy, such as those enhancing natural resistance to infection.

A variety of substances, such as bacterial lipopolysaccharide and its derivatives, BCG, and muramyl peptides have been shown to increase natural resistance mechanisms [3–5]. In systemic candidal infection in experimental animals, these substances were able to improve survival [6–8], but because of their toxicity they have not been appealing as a therapy in humans. These immunomodulatory drugs are able to induce synthesis and secretion of interleukin 1 (IL 1), a family of cytokines mediating the acute-phase response [9]. Previous investigations have shown that recombinant IL 1 is able to increase survival in mice with a lethal infection caused by *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* [10, 11]. The mechanism of this protection is unclear [11], and it is not known whether these findings are limited to an effect on infections due to Gram-negative bacteria. Therefore, we investigated the effect of IL 1 on an experimental candidal infection of mice. Efficacy of IL 1 in candidal infection could not only offer some insight into the mechanisms of protection against death due to infection, but might also create new modalities for treatment of candidal infections in humans.

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Abbreviations: **IL 1:** Interleukin 1 **cfu:** Colony-forming unit(s)

2 Materials and methods

2.1 Mice

Female 25 g Swiss Webster mice (Taconic Farms, Germantown, NY) were kept in cages (6 mice per cage) with filter lids, and were fed standard lab chow and water *ad libitum*.

2.2 IL 1

Recombinant human IL 1β (kindly supplied by Dr. Alan Shaw, Biogen, Geneva) and recombinant human IL 1α (kindly supplied by Dr. Peter Lomedico, Hoffmann-La Roche, Nutley, NJ), which contain less than 20 pg of endotoxin/mg of protein, were used. The IL 1 was given as a single i.p. injection in 2% (v/v) heat-inactivated normal mouse serum in 0.1 ml pyrogen-free saline. Control mice received heat-inactivated IL 1 (100 °C for 20 min) in 2% heat-inactivated normal mouse serum.

2.3 Candida albicans

A strain of *C. albicans*, termed UC 820, maintained on agar slants at 4 °C was inoculated into 100 ml of Yeast Mould agar (Difco Laboratories, Detroit, MI) and cultured for 24 h at 37 °C. After being washed twice by centrifugation at 1500 × g, the number of yeast cells in the suspension was counted in a

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hemocytometer, and the viability was confirmed by inoculating serial dilutions onto agar plates.

2.4 Infection model

Mice were rendered neutropenic ($< 0.2 \times 10^9$ granulocytes/liter) by means of two s.c. injections of cyclophosphamide (Bristol Myers, Syracuse, NY), 150 and 100 mg/kg of body weight, respectively, 4 days and 1 day before the i.v. injection of 3×10^5 *C. albicans* in 200 µl phosphate-buffered saline into the lateral tail vein. The mice in each cage were randomized to receive either IL 1 at different times and in different dosages, or heat-inactivated IL 1. Survival was scored at intervals of 6–8 h over a period of 72 h.

2.5 *In vitro* antifungal effects of IL 1

We examined the possibility that IL 1 might have a direct antifungal effect. Using an automated spiral plater (Spiral Systems Inc., Cincinnati, OH) we prepared a concentration gradient of IL 1β ranging from 0.15 to 42 ng/ml on the agar surface of Mueller-Hinton plates. Radial streaks of *C. albicans* from a suspension containing 1×10^5 colony forming units (cfu)/ml were made on the plates. After incubation overnight at 37 °C the distance from the center to the most central point of growth inhibition was measured.

2.6 Quantitation of *C. albicans* in blood and organs

Neutropenic mice, treated either with IL 1β or heat-inactivated IL 1β 24 h earlier, received an i.v. injection of 3×10^5 cfu of *C. albicans*. Blood for cultures was taken by cardiac puncture after CO_2 asphyxia 15, 30 and 90 s and 3 and 6 min after the i.v. injection. In another series of experiments, mice were killed by CO_2 asphyxia 24 h after the *C. albicans* injection. Immediately after death, blood cultures were taken by cardiac puncture, and the spleen, the left kidney and the liver were removed aseptically, weighed and homogenized in sterile saline in a tissue grinder. Blood and suspensions of tissue were plated on Sabouraud's dextrose agar. After overnight incubation at 37 °C, the number of cfu's was counted.

2.7 Serum for passive transfer

Twenty-four hours after an i.p. injection of either 80 ng IL 1β or pyrogen-free saline in 2% (v/v) normal mouse serum, mice were exsanguinated by cardiac puncture after CO_2 asphyxiation. The blood was pooled and allowed to clot, and the serum, separated by centrifugation, was either used immediately or stored at −70 °C.

2.8 Statistical analysis

Survival curves were analyzed using the Kaplan Meier/log rank test [12]. Student's t-test was used for the results of the microbiological studies.

3 Results

3.1 Survival of mice

Recombinant human IL 1β was given 24 h before infection as a single i.p. injection in dosages of 800 ng, 80 ng or 8 ng. IL 1 induced a dose-dependent enhancement of survival in infected mice (Fig. 1a). The differences in survival between mice treated with IL 1β and control animals receiving heat-inactivated IL 1β became most prominent between 36 and 72 h after the injection of *C. albicans*. If the survival curves are considered together, the survival differences compared to control animals were significant for the 800 ng dose ($\chi^2 = 15.6$,

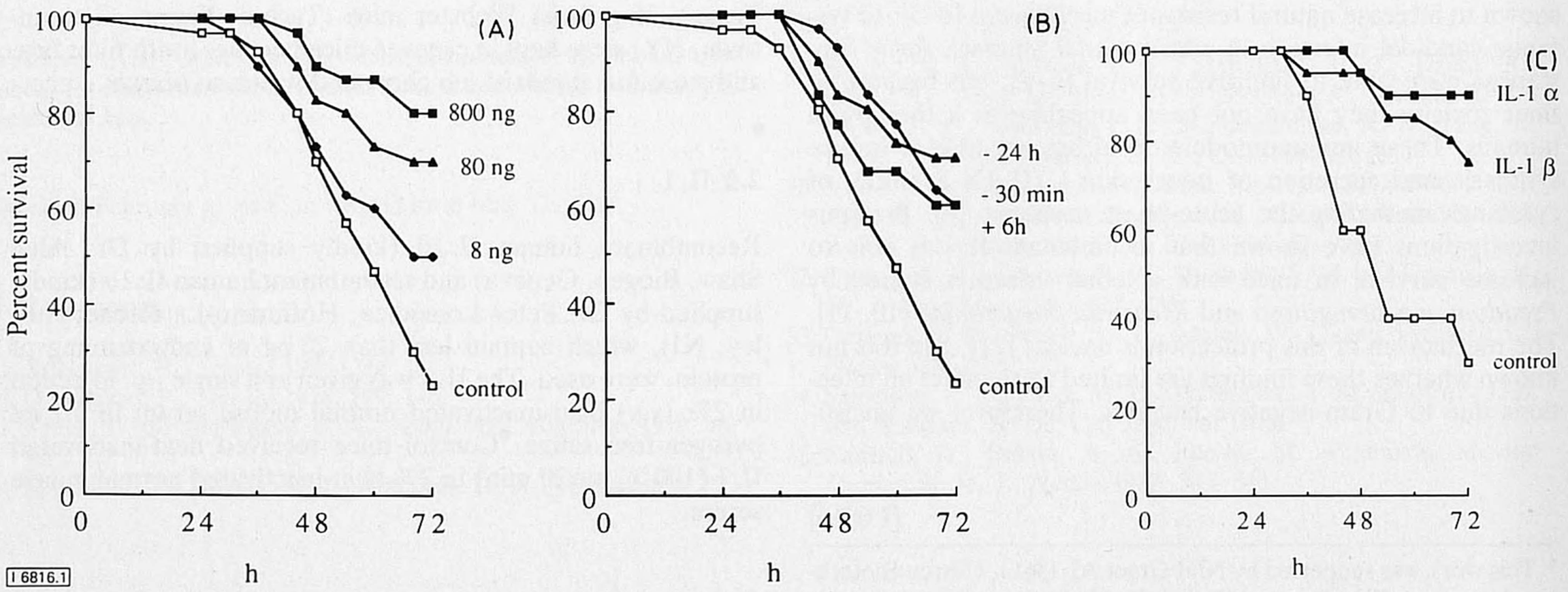

Figure 1. The effect of IL 1β treatment on the survival of granulocytopenic mice with a candidal infection. Recombinant human IL 1β was given as a single i.p. injection. Control mice received heat-inactivated IL 1 (100 °C for 20 min). (A) The effect of different dosages of IL 1β given 24 h before infection. Each group represents 30 mice. The differences in survival compared to control animals are significant for the 800 ng dose and for 80 ng, but not for the 8 ng dose. (B) Survival with a single injection of 80 ng IL 1β given at different times. There were 30 mice in each group. The controls and the group receiving 80 ng IL 1β 24 h before infection are the same as presented in (A). The protective effect of IL 1β was similar whether it was given 24 h before, shortly before or 6 h after infection. (C) 80 ng IL 1α given 24 h before infection had a similar protective effect as 80 ng IL 1β (20 mice per group).

$p < 0.001$) and for 80 ng ($\chi^2 = 9.2$, $p < 0.01$), but not for the 8 ng dose ($\chi^2 = 2.3$). To investigate the efficacy of IL 1 given at different time points in relation to the infection, the relatively low dose of 80 ng (3.0 μg/kg, which equals 1.2 mg/m^2) was used. The protective effect of this dose of IL 1 was similar whether it was given 24 h before, shortly before ($\chi^2 = 7.4$, $p < 0.01$) or 6 h after infection ($\chi^2 = 5.3$, $p < 0.02$ compared to controls) (Fig. 1b). IL 1α given at a dose of 80 ng/mouse 24 h before infection appeared to have the same protective effect as 80 ng IL 1β (Fig. 1c).

3.2 *In vitro* assessment of antimicrobial activity

No direct antifungal effect of IL 1 could be demonstrated when *C. albicans* were incubated *in vitro* with concentrations of IL 1β up to 42 ng/ml.

3.3 Effect of IL 1 on the number of *C. albicans in vivo*

The number of *C. albicans* in the bloodstream of neutropenic mice up to 6 min after i.v. injection of 3×10^5 cfu was similar in mice pretreated with 80 ng of IL 1β 24 h earlier and those that had received heat-inactivated IL 1 (data not shown). We infer from these data that the rates of clearance of organisms from the bloodstream were the same in the two treatment groups. Using the same experimental conditions, no differences were found in the number of *Candida* cultured from blood, liver, spleen, or kidney of IL 1-treated and control animals 24 h after infection (Fig. 2). When the data were expressed as number of microorganisms per organ rather than per gram of tissue, the data from two groups also did not differ.

3.4 Passive transfer of serum

To investigate whether a humoral factor was responsible for the protective effect, neutropenic mice were injected i.p. with 0.5 ml of serum obtained from mice pretreated 24 h earlier with either 800 ng IL 1β or saline containing 2% normal mouse serum. When the transfer was performed either at time of the

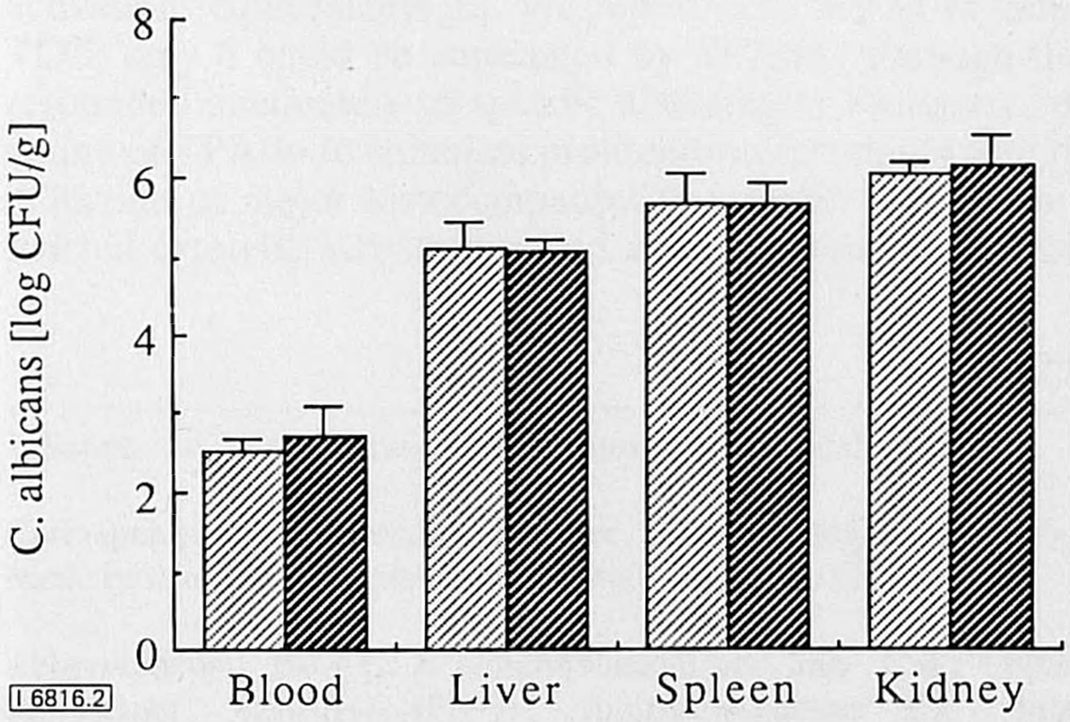

Figure 2. Counts of colonies of *C. albicans* in the blood and organs of granulocytopenic mice 24 h after an i.v. injection of 3×10^5 cfu of *C. albicans*. Mice received either 80 ng human recombinant IL 1β (light bars) or heat-inactivated IL 1β (dark bars) 24 h before injection. Each bar represents the mean ± SD of log cfu/g tissue in 6 mice.

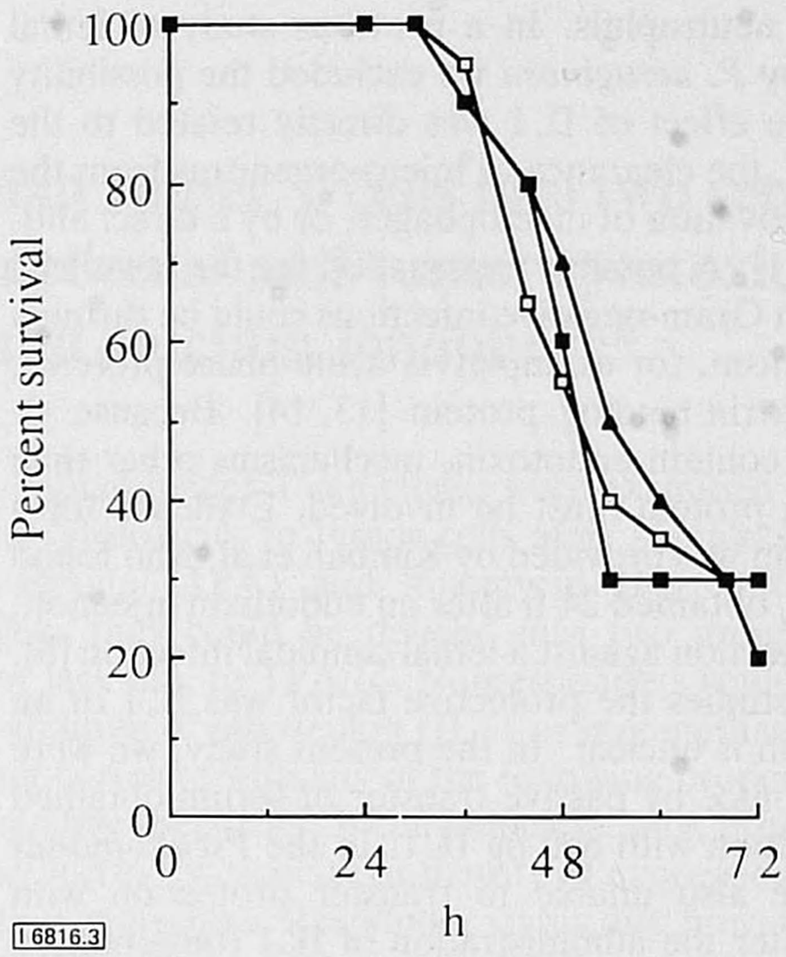

Figure 3. Passive transfer of serum. Serum from mice that received 800 ng IL 1β or saline 24 h before was injected i.p. into recipient mice that were infected with 3×10^5 cfu of *C. albicans*. The mice were injected with the serum either shortly before the infection (■; n = 10) or 24 h after infection (▲; n = 10). The survival of these mice does not differ from the controls (□; n = 20).

i.v. injection of *C. albicans* or 24 h later, no protection was obtained (Fig. 3).

4 Discussion

In these studies, we report the efficacy of a single injection of a low dosage of either IL 1β or IL 1α in prolonging survival in neutropenic mice with disseminated *C. albicans* infection. These results extend those we have obtained for *Pseudomonas aeruginosa* in a previous study [11]. In contrast with this Gram-negative infection, protection against a candidal infection was obtained not only when IL 1β was given 24 h before, but also when it was given simultaneously with or 6 h after the injection of *C. albicans*. This lack of a so-called "negative phase" (*i.e.* a period in which no protection can be induced [6]) may be due to the fact that the course of the candidal infection in these mice was somewhat more protracted than that of the Gram-negative bacterial infection, thus allowing more time for a possible beneficial effect of IL 1 to be established. Alternatively, there might be differences between the mechanisms of protection to death from bacterial and fungal infections. It is of interest that Kimball et al. [6] demonstrated a negative phase for protection by bacterial endotoxin in a *C. albicans* infection. The accelerated death rate observed in their studies might be due to the combined adverse effect of the infection and the endotoxin.

The protection against candidal infection obtained by prior administration of bacterial endotoxin has been attributed to enhancement of the clearance of *C. albicans* from the blood stream and inhibition of the outgrowth in the kidney [7]. We found no differences in the rate of clearance of fungi from the bloodstream (shortly after injection and 24 h later) and their outgrowth in the tissues between IL 1-treated and control animals. Since all our experiments were performed in severely neutropenic mice, it seems unlikely that the beneficial effect

was mediated by neutrophils. In a previous study of lethal infection caused by *P. aeruginosa* we excluded the possibility that the protective effect of IL 1 was directly related to the induction of fever, the clearance of micro-organisms from the bloodstream by activation of macrophages, or by a direct antimicrobial effect [11]. A possible explanation for the beneficial influence of IL 1 in Gram-negative infections could be through a humoral mechanism, for example via acute-phase proteins, such as an endotoxin-binding protein [13, 14]. Because *C. albicans* does not contain endotoxin, mechanisms other than endotoxin-binding protein must be involved. Evidence for a humoral mechanism was provided by Kimball et al. who found that mouse serum, obtained 24 h after an endotoxin injection, could transfer protection against a lethal candidal infection [6]. Whether in their studies the protective factor was IL 1 or an acute-phase protein is unclear. In the present study, we were unable to protect mice by passive transfer of serum obtained 24 h after an injection with 800 ng IL 1. In the *Pseudomonas* infection we were also unable to transfer protection with serum obtained after the administration of IL 1 (unpublished data).

Thus, the present study indicates that the beneficial effect of IL 1 is not mediated through a transferable factor and cannot be explained by an endotoxin-antagonizing effect. It has been suggested that cytokines like IL 1 and tumor necrosis factor (TNF) contribute to death from infection [15–17]. How early treatment with IL 1 could reduce the lethal effects of these cytokines is not yet known. One could speculate that this could be effected by occupation of cytokine receptors on cells or by an inhibition of endogenous cytokine production. Further studies are needed to elucidate these possibilities. Whichever the mechanism is, the protective effect could have important implications for the future treatment of serious candidal infections in neutropenic patients.

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