Recombinant Murine Granulocyte Colony-Stimulating Factor Protects against Acute Disseminated Candida albicans Infection in Nonneutropenic Mice

Bart Jan Kullberg, Mihai G. Netea, Jo H. A. J. Curfs, Monique Keuter, Jacques F. G. M. Meis, and Jos W. M. van der Meer

Departments of Medicine and Medical Microbiology, Catholic University Nijmegen and University Hospital, Nijmegen, The Netherlands

The effect of recombinant granulocyte colony-stimulating factor (rG-CSF) on acute disseminated Candida albicans infection in nonneutropenic mice was investigated. Mice treated with a single dose of rG-CSF showed a significantly reduced mortality (28% vs. 90%; P < .001). The outgrowth of C. albicans from the kidneys, spleens, and livers of rG-CSF–treated mice was significantly reduced (log cfu/g of kidney, 5.54 vs. 7.13; P < .001), as were circulating tumor necrosis factor-α and interleukin-1β. After rG-CSF, the kidneys showed fewer infectious infiltrates, enhanced granulocyte influx, and almost complete absence of hyphal outgrowth. During peritoneal C. albicans infection, rG-CSF enhanced influx of granulocytes to the site of infection, and exudate granulocytes showed increased oxygen radical production. These results indicate that rG-CSF enhances host resistance to disseminated candidiasis in nonneutropenic mice through activation of granulocytes and their recruitment to the site of infection.

Disseminated candidiasis is being recognized with increasing frequency in patients with a variety of underlying diseases [1]. Although various new antifungal drugs have been developed recently, mortality due to disseminated candidiasis remains high, even in nonneutropenic hosts [2]. Therefore, it would be rational to focus on augmentation of host defense mechanisms in addition to conventional antifungal therapy [3]. There is vast evidence that neutrophils (PMNL) constitute the main mechanism of host defense against invasive and disseminated candidiasis [4]. Candida blastospores can be phagocytized and killed by phagocytic cells, and PMNL are able to kill Candida pseudohyphae and hyphae through both oxidative and nonoxidative mechanisms, even without ingesting the latter forms [3]. It is conceivable that augmenting either the number of PMNL or their microbicidal capacity will enhance the resistance to disseminated candidiasis in nonneutropenic hosts.

Granulocyte colony-stimulating factor (G-CSF) is a hematopoietic growth factor that selectively stimulates the proliferation and maturation of myeloid progenitor cells [5, 6]. In addition to its effect on PMNL numbers, G-CSF has been shown to enhance a variety of functional properties of PMNL, such as chemotaxis, expression of cellular adhesion molecules, and superoxide production [7, 8]. Previous studies have demonstrated a beneficial effect on the course of experimental infection with Candida albicans when recombinant (r) G-CSF was administered prophylactically to prevent neutropenia. Repeated administration of rG-CSF effectively precludes the development of severe cyclophosphamide-induced neutropenia and protects animals against a subsequent lethal challenge with C. albicans [9, 10]. However, very few data exist on the potential role of rG-CSF in disseminated candidiasis or other infections in nonneutropenic animals. In the present study, we have investigated the effect of prophylactic and therapeutic administration of recombinant murine G-CSF on experimental disseminated C. albicans infection in nonneutropenic mice. Thus far, studies have used human recombinant G-CSF in mice. Since the activity of the human molecule is considered suboptimal in mice, we performed our studies with recombinant murine G-CSF, which has recently become available. Furthermore, these studies address the effects of rG-CSF on circulating cytokines, the recruitment of PMNL to the site of infection, and on the induction of reactive oxygen intermediates during C. albicans infection.

Materials and Methods

Mice. Specific pathogen–free female CBA/J mice, weighing 20–25 g, were used. Animals were housed under standard laboratory conditions and fed sterilized laboratory chow and water ad libitum.

Materials. Recombinant murine G-CSF, provided by Amgen (Thousand Oaks, CA), was diluted in pyrogen-free saline to a final concentration of 5 μg/mL. C. albicans UC820, a clinical isolate well-described earlier [11], was maintained on agar slants at 4°C. Preliminary experiments had shown that strain UC820 is able to develop hyphae and pseudohyphae in vitro as well as in vivo to the same extent as a panel of virulent control C. albicans strains.
did. *C. albicans* UC820 was inoculated into 100 mL of Sabouraud broth and cultured for 24 h at 37°C. After three washes with pyrogen-free saline by centrifugation at 1500 g, the number of yeast cells was counted in a hemacytometer; occasional strings of ≥2 yeasts were counted as 1 cfu of *C. albicans*. The suspension was diluted to the appropriate concentration with pyrogen-free saline. The viability of the yeasts was at least 98%, as determined by methylene blue exclusion and confirmed by plating serial dilutions onto Sabouraud dextrose agar plates.

**Infection model.** Mice were injected intravenously (iv) with 5 × 10⁶ cfu of *C. albicans* in 100 μL of pyrogen-free saline. rG-CSF was given as a subcutaneous (sc) injection of 500 ng (25 μg/kg) in 100 μL of pyrogen-free saline at various time points before or during infection. Control mice received 100 μL of pyrogen-free saline sc. Survival was assessed twice daily. Subgroups of animals were anesthetized with ether and killed by cervical dislocation on days 1 or 3 of infection, and blood was collected for the measurement of plasma cytokine concentrations. To assess the outgrowth of the microorganisms in the liver, spleen, and kidneys, the organs were removed aseptically, weighed, and homogenized in sterile saline in a tissue grinder. The number of viable *C. albicans* cells in the tissues was determined by plating serial dilutions on Sabouraud dextrose agar plates as described previously [12], and colony-forming units (cfu) were counted after overnight incubation at 37°C. The results were expressed as the log cfu per gram of tissue. From other groups of animals, the kidneys and the liver were removed and fixed in buffered formalin (4%), and serial sections were examined microscopically after staining with periodic acid–Schiff or with hematoxylin-eosin. To assess the role of PMNL in the rG-CSF–mediated protection against disseminated candidiasis, a subgroup of animals was rendered granulocytopenic by pretreatment with cyclophosphamide (Bristol-Myers Squibb, Weesp, Netherlands): 150 mg/kg of body weight in 100 μL of PBS sc 4 days before injection of *C. albicans* and 100 mg/kg sc 1 day before as well as on days 1, 3, 5, 7, and 9 after injection of *C. albicans* [13]. To investigate the role of PMNL respiratory burst, groups of nonneutropenic mice were treated with catalase (Sigma, St. Louis), an inhibitor of generation of reactive oxygen intermediates by PMNL, 32,000 U in 100 μL of pyrogen-free saline intraperitoneally (ip), rG-CSF (500 ng in 100 μL of pyrogen-free saline sc), or both, 30 min after the injection of *C. albicans*. The recruitment of neutrophils to the site of infection and their activation was investigated after injection of 10⁶ cfu of *C. albicans* into the peritoneal cavity of nonneutropenic mice. rG-CSF was given as a single sc injection of 500 ng either 24 h before or at the time of infection. Four hours after the injection of the microorganisms, the mice were killed, and peritoneal cells were harvested by rinsing the peritoneal cavity with cold PBS containing 0.38% (wt/vol) sodium citrate. After centrifugation, cells were counted in a hemacytometer, and the percentage of PMNL was determined in Giemsa-stained cytospin preparations. Peritoneal cells were resuspended in PBS for chemiluminescence assay.

**Blood samples.** Blood samples (20 μL) taken from the retroorbital plexus were collected, and the leukocytes were counted in a Coulter Counter (Coulter Electronics, Mijdrecht, Netherlands). The total numbers of granulocytes, lymphocytes, and monocytes per cubic millimeter were calculated from the total number of leukocytes per cubic millimeter and the differential counts of 200 leukocytes in two Giemsa-stained blood smears.

**Cytokine assays.** Blood (100 μL) was obtained by orbital bleeding into Eppendorf tubes containing 2 μg of EDTA. The blood was centrifuged (5 min, 7500 g), and the plasma was pooled and stored at −20°C until assayed for cytokines. Interleukin (IL)-1α, IL-1β, and tumor necrosis factor (TNF)-α were determined by an RIA as described earlier [14].

**Chemiluminescence assay.** Luminol-enhanced chemiluminescence was used as a measure of the respiratory burst as described previously [15]. In brief, peritoneal exudate cells were obtained from mice that had received an ip injection of *C. albicans* 4 h earlier, and 1 × 10⁶ cells were resuspended in 100 μL of PBS in polypropylene chemiluminescence cuvettes (LKB; Bio-Orbit, Turku, Finland) with 4 mg of opsonized zymosan (Sigma) in 100 μL of PBS and 700 μL of luminol, 10⁻⁷ M (Sigma). Chemiluminescence was read at 37°C during 2 h with an LKB-Wallac type 1251 chemiluminometer (Bio-Orbit) and expressed as the total amount of superoxide produced during the assay period by integrating the area under the curve (in millivolts × second) per PMNL.

**Statistical analysis.** The results obtained for the various treatment groups were analyzed by the Mann-Whitney U test and, when appropriate, by analysis of variance. Survival curves were analyzed by the Kaplan-Meier log-rank test. For all comparisons, the level of significance between groups was set at *P* < .05. All experiments were done at least twice.

**Results**

**Effect of rG-CSF treatment on acute disseminated candidiasis in nonneutropenic mice.** After iv injection of 5 × 10⁶ *C. albicans*, 90% of the untreated control animals died between days 6 and 8 of infection (figure 1). A single iv injection of 500 ng of rg-CSF 1 day before infection resulted in a significantly increased survival. Only 28% of the G-CSF–treated animals died (*P < .001*), without further mortality during the next weeks of follow-up. Prolonged treatment of mice with rG-CSF, 500 ng daily, from 1 day before infection through day 3 of infection, yielded similar results (figure 1). As described previously [12, 16], the main target organ of *Candida* infection was the kidney, from which increasing numbers of *C. albicans* cfu were cultured during the course of the infection, whereas in the spleen and the liver the number of *C. albicans* cfu decreased from day 1 of infection (figure 2). A single iv injection of 500 ng of rg-CSF significantly reduced the numbers of *C. albicans* cfu in the kidneys (*P < .001*) and the spleen (*P < .01*) on day 1 of infection (figure 2). On day 3, the numbers of cfu in the kidneys (*P < .001*), spleen (*P < .05*), and liver (*P < .01*) of G-CSF–treated animals were significantly lower than in controls. Repeated administration of rG-CSF yielded similar results (figure 2). Administration of a lower dose of rG-CSF (200 ng/mouse; 10 μg/kg) had an intermediate effect; mortality after 21 days amounted to 40% of the mice that received either a single dose of rG-CSF 1
Figure 1. Effect of rG-CSF on survival of mice after intravenous infection with $5 \times 10^5$ cfu of C. albicans. Mice (10 animals/group) were injected with single dose of 500 ng of rG-CSF (○) or saline (■) 1 day before infection or with daily injections of 500 ng of rG-CSF from 1 day before infection through day 3 of infection (△). Significant difference between controls and treatment groups: $P < .001$ (Kaplan-Meier log-rank test).

Figure 2. Outgrowth of C. albicans in kidneys, spleen, and liver of mice after intravenous injection of $5 \times 10^5$ cfu of C. albicans. Mice were injected subcutaneously with 500 ng of rG-CSF (solid bars) or with saline (open bars) on day −1 or with daily dose of 500 ng of rG-CSF on day −1 through day 3 of infection (shaded bars). Each column represents mean ± SE for 9 animals. Significant differences between rG-CSF−treated mice and control mice: * $P < .05$; ** $P < .001$; Mann-Whitney $U$ test.

day before infection or repeated doses of 200 ng of rG-CSF, compared with 90% mortality in untreated animals ($P < .01$).

Time of administration of rG-CSF. In the model of rapidly lethal infection, the effect of a single dose of rG-CSF was greatest when given 24 h before injection of C. albicans (table 1). Mice treated with a single dose of 500 ng of rG-CSF 30 min after injection of $5 \times 10^5$ cfu of C. albicans showed a reduced 21-day mortality (56%) compared with control mice ($P < .05$). The outgrowth of C. albicans in the kidneys and spleen of mice treated with rG-CSF 30 min after infection was significantly lower than in controls on day 1 and 3 of infection, although the differences were quantitatively rather small (table 1). When administration of rG-CSF was delayed until 24 h after injection of C. albicans, neither a significant effect on mortality (data not shown) nor an effect on the outgrowth of microorganisms was found (table 1).

Effect of rG-CSF on the histopathology of the kidneys during acute disseminated candidiasis. On gross examination, the kidneys of untreated control animals showed multiple abscesses at day 3 of infection. Microscopically, numerous infectious foci were found in periodic acid–Schiff–stained sections (figure 3A), with extensive hyphal outgrowth of C. albicans as well as yeast forms (figure 3B). In hematoxylin-eosin–stained sections, relatively few PMNL could be found at these sites (figure 3C). In animals that had received a single dose of rG-CSF 30 min after infection, the infectious foci were less numerous than in controls (figure 3D). In rG-CSF−treated animals, the inflammatory infiltrates contained larger amounts of PMNL (figure 3F), with fewer yeasts present. In contrast to untreated controls, candidae were almost completely present in the yeast form, and in most infiltrates, hyphae were absent (figure 3E). Although administration of a single dose of rG-CSF to mice as late as 24 h after infection neither enhanced survival of mice nor significantly reduced the number of cfu in their organs, a reduced outgrowth of pseudohyphae, as well as an enhanced influx of PMNL at the sites of infection, was seen on microscopic examination (figure 3G, H).

Circulating cytokines during acute disseminated candidiasis in nonneutropenic mice. The concentrations of circulating TNF-α and IL-1α are shown in figure 4. On day 1 of infection, TNF-α plasma concentrations were significantly lower in animals that had received a single dose of rG-CSF 24 h before infection compared with levels in control mice (72 ± 36 pg/mL vs. 252 ± 95 pg/mL, $P < .01$). On day 3 of infection, circulating TNF-α in rG-CSF−treated animals was also lower than in control animals (figure 4; $P = .06$). In animals that received repeated injections of rG-CSF on days −1 through +3 of infection, circulating TNF-α was also reduced on day
1 (P < .01) and day 3 (P = .06) of infection. Circulating IL-1α concentrations were lower in mice treated with rG-CSF than in controls on day 1 of infection only, both in animals treated with a single dose of rG-CSF (P = .07) and in those having received repeated doses (P < .05; figure 4). No differences in IL-1α plasma concentrations were observed on day 3 of infection. Circulating concentrations of IL-1β were below the detection limit in all treatment groups (data not shown).

Effect of rG-CSF on the numbers and function of granulocytes during infection. In untreated mice, the mean number of peripheral blood PMNL before infection was 2.1 ± 0.7 
\times 10^9/L. At the time of injection of C. albicans, the number of PMNL was 2.6 ± 1.3 \times 10^9/L in mice given rG-CSF 1 day earlier (P > .05). Twenty-four hours after infection of C. albicans, no significant changes had occurred in the numbers of PMNL in control mice (2.5 ± 1.9 \times 10^9/L), mice pretreated with a single dose of rG-CSF (2.6 ± 2.4 \times 10^9/L), or mice that had received daily injections of rG-CSF (3.2 ± 2.6 \times 10^9/L; P > .05). On day 3 of infection, the numbers of PMNL were unchanged in control mice (2.5 ± 0.7 \times 10^9/L) and mice that had received a single dose of rG-CSF (2.6 ± 0.6 \times 10^9/L), whereas the numbers of PMNL in mice treated with repeated daily doses of 500 ng of rG-CSF were significantly increased (5.5 ± 2.4 \times 10^9/L; P < .005). The mean numbers of blood lymphocytes and monocytes did not change significantly during the course of the infection in either rG-CSF–treated mice or controls (data not shown).

Peritoneal cell exudates harvested from uninfected control mice consisted of >98% macrophages and elicit no detectable chemiluminescence on stimulation with zymosan (data not shown). After intraperitoneal injection of 10^7 cfu of C. albicans, the number of peritoneal PMNL showed a sharp rise, to 1.5 ± 0.3 \times 10^9/L at 4 h after injection. The number of exudate PMNL in mice that had received 500 ng of rG-CSF 1 day before the infection was significantly higher than that in control mice or in mice that had received rG-CSF 4 h earlier (2.6 ± 0.3 \times 10^9/L vs. 1.1 ± 0.6 \times 10^9/L; P < .001). The total chemiluminescence as well as the chemiluminescence expressed per exudate PMNL were significantly increased in the animals pretreated with rG-CSF 1 day before peritoneal infection (0.27 vs. 0.15 mV·s/PMNL; P < .05).

Table 1. Effect of time of administration of single subcutaneous injection of 500 ng of rG-CSF on acute disseminated candidiasis.

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Kidney</th>
<th>Liver</th>
<th>Spleen</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 1</td>
<td>Day 3</td>
<td>Day 1</td>
</tr>
<tr>
<td>Untreated controls</td>
<td>6.32 ± 0.19</td>
<td>6.76 ± 0.50</td>
<td>4.82 ± 0.23</td>
</tr>
<tr>
<td>rG-CSF 24 h before infection</td>
<td>5.11 ± 0.29*</td>
<td>5.54 ± 0.34*</td>
<td>4.66 ± 0.09</td>
</tr>
<tr>
<td>rG-CSF 30 min after infection</td>
<td>5.56 ± 0.32*</td>
<td>6.00 ± 0.68*</td>
<td>4.70 ± 0.14</td>
</tr>
<tr>
<td>rG-CSF 24 h after infection</td>
<td>5.96 ± 0.39</td>
<td>7.16 ± 0.25</td>
<td>4.92 ± 0.22</td>
</tr>
</tbody>
</table>

NOTE. Data are log cfu of C. albicans/g of tissue (mean ± SD for 10 animals) assessed on day 1 and day 3 of infection. Mice were injected intravenously with 5 \times 10^7 cfu of C. albicans.

* Significantly lower than in control mice (P < .01).

† Significantly lower than in control mice (P < .05).

NOTE: Data are log cfu of C. albicans/g of tissue (mean ± SD for 10 animals) assessed on day 1 and day 3 of infection. Mice were injected intravenously with 5 \times 10^7 cfu of C. albicans.

* Significantly lower than in control mice (P < .01).

† Significantly lower than in control mice (P < .05).
Figure 3. Histopathology of kidneys of mice 3 days after intravenous injection of $5 \times 10^7$ cfu of *C. albicans*. In control animals, infiltrates are numerous (A) and consist of large amounts of *C. albicans* both in yeast form and as pseudohyphae (B). Only moderate influx of neutrophils (PMNL) is found (C). In mice treated with rG-CSF 30 min after infection, fewer infiltrates are present (D), with *Candida* blastospores but few or no pseudohyphae (E) and large amounts of PMNL (F). Administration of rG-CSF as late as 24 h after infection resulted in reduced outgrowth of pseudohyphae as well as enhanced influx of PMNL at sites of infection compared with control animals (G–I). Periodic acid–Schiff staining (top and middle rows); hematoxylin-eosin staining (bottom row). Original magnification, $\times 25$ (top row); $\times 1000$ (middle and bottom rows).

Discussion

The results of the present study show that a single injection of rG-CSF beneficially influences the course of potentially lethal acute disseminated candidiasis in nonneutropenic mice. Treatment with rG-CSF reduced the mortality and significantly decreased the outgrowth of *C. albicans* in the organs of the animals. Although the low dose of rG-CSF that was administered in our study had no effect on the numbers of PMNL in the circulation during acute disseminated candidiasis, the histopathology of the kidneys showed a noticeable increase in numbers of PMNL at the actual sites of infection, indicating that PMNL that were mobilized by rG-CSF were rapidly directed to the infected organs. The difference in numbers of PMNL at these sites between control mice and rG-CSF–treated animals suggests that the inability to attain a sufficient mobilization of PMNL at the site of infection may be an important determinant of the course of invasive candidiasis and that rG-CSF may be able to improve the outcome by enhancing PMNL recruitment. G-CSF not only increases the numbers of PMNL, their chemotaxis, and expression of cellular adhesion molecules but also has been shown to enhance their microbicidal function in vitro [8, 17]. When mice were infected ip with *C. albicans*, treatment with rG-CSF not only increased the influx of PMNL to the site of infection but also augmented the production of reactive oxygen intermediates by these cells, as was demonstrated by the increased chemiluminescence expressed per cell. Moreover, catalase, which is an inhibitor of the respiratory burst [18], completely abrogated the protective effect of rG-CSF in our model. It has been demonstrated earlier that stimulation of PMNL with rG-CSF in vitro is able to augment the intracellular killing of *C. albicans* [19] and to increase the release of reactive oxygen intermediates on stimulation with *Candida* blastospores or pseudohyphae in vitro [20].

Although the effect of rG-CSF in this model of acute overwhelming candidiasis was maximal when given 24 h before the infection, a significant beneficial effect was also found when given 30 min after the injection of the microorganisms.
When treatment with rG-CSF was begun as late as 24 h after infection, no significant improvement in survival was found. Although the actual experiment shown in table 1 suggests that there was a tendency toward increased outgrowth of C. albicans at day 3 after infection when rG-CSF was given 24 h after infection, this trend has not been observed in repeated experiments or at other time points. Moreover, the histopathology of the infected kidneys suggested a beneficial effect of this treatment schedule on the course of infection. It is conceivable that rG-CSF may be effective when given at later time points in models of subacute and less overwhelming disseminated candidiasis or when combined with antifungal drugs, which is currently under study in our laboratory.

Although very few data have been published on the application of rG-CSF in nonneutropenic animals with infection, several authors have studied the effect of rG-CSF on disseminated candidiasis in neutropenic animals. In these studies, animals have been rendered neutropenic by relatively low doses of cyclophosphamide and were subsequently treated with rG-CSF, leading to at least partial recovery of the numbers of PMNL before the microorganisms were injected [10, 21–24]. Therefore, in those studies, the susceptibility of rG-CSF–treated nonneutropenic mice was compared with that of control animals that were still neutropenic at the moment of infection. The results of such studies clearly indicate the importance of early PMNL recovery to prevent the development of fungal infections. From our experiments, it is clear that rG-CSF does not improve survival in the sustained absence of PMNL. These findings are in accordance with studies on chronic disseminated candidiasis in rabbits with a persisting and profound neutropenia, in which no effect of the administration of rG-CSF was found [25].

Few studies have been published on the role of rG-CSF in acute or chronic disseminated candidiasis in nonneutropenic animals. In one study, rG-CSF had a beneficial effect on mortality due to acute candidiasis only when given prophylactically as early as 3 days before infection but not when given later or in multiple doses [10]. Others recently have reported a beneficial effect on the mean survival time of mice when repeated administration of a high dose (50 µg/kg/day) of rG-CSF was begun before infection [26]. In similar experimental models, rG-CSF alone was not effective, whereas combination therapy with fluconazole or itraconazole, but not amphotericin B or flucytosine, appeared to be synergistic, but only when begun before or immediately after infection and only when small amounts of C. albicans were injected [23, 27].

In the present study, we have demonstrated a beneficial effect of rG-CSF on survival of mice with acute disseminated candidiasis and also in reducing the outgrowth of candidae from the target organs and the histopathologic damage to the kidneys, as well as a beneficial effect on recruitment of PMNL to the site of infection. Remarkably, treatment with rG-CSF led to a reduction in the concentrations of circulating TNF-α and IL-1α during the infection, in spite of the increased influx of inflammatory cells at the sites of infection. Although G-CSF is capable of directly inhibiting the production of TNF-α [28], we have shown earlier that mortality due to acute disseminated candidiasis is mediated through outgrowth of the microorganisms, leading to organ destruction, rather than through lethal circulating concentrations of proinflammatory cytokines [29]. It may be hypothesized that the reduced cytokine concentrations are at least in part a result of the reduced outgrowth of C. albicans in the tissues.

Other hematopoietic growth factors may as well have a beneficial effect on disseminated candidiasis. Granulocyte-macrophage colony-stimulating factor has been shown to augment the intracellular killing of C. albicans and to enhance superoxide production by PMNL in vitro [19]. Incubation with purified murine macrophage colony-stimulating factor (M-CSF) inhibits the outgrowth of C. albicans in murine peritoneal exudate macrophages [30]. Treatment of chronic disseminated candidiasis in rats with rM-CSF has been shown to reduce the outgrowth of C. albicans [31], but others found that administration of rM-CSF either before infection or commencing at the moment of infection aggravated the course of acute overwhelming candidiasis in mice [32].

In conclusion, we have demonstrated a beneficial effect of rG-CSF on acute disseminated candidiasis in mice, significantly
improving survival and reducing the outgrowth of *Candida* organisms in the tissues as well as the induction of proinflammatory cytokines. It is suggested that both activation of candidacidal functions of PMNL and their recruitment to the site of infection are the mechanisms through which rG-CSF yields its beneficial effect. Further studies should address issues such as late onset of treatment after the infection has been well established or effects in subacute or chronic rather than acute overwhelming infection. These results provide a strong support for double-blind, randomized, phase II trials that are currently being conducted to assess the safety and preliminary efficacy of G-CSF in combination with fluconazole in treating candidemia in nonneutropenic patients.

References