Treatment of Intra-Abdominal Abscesses Caused by *Candida albicans* with Antifungal Agents and Recombinant Murine Granulocyte Colony-Stimulating Factor

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The aim of the present study was to assess the influence of immunomodulation of host defense with recombinant murine granulocyte colony-stimulating factor (rmG-CSF) on intra-abdominal abscesses caused by *Candida albicans*. Mice received prophylaxis or therapy with 1 μg of rmG-CSF/day in the presence or absence of antifungal treatment consisting of amphotericin B (0.75 mg/kg body weight/day) or fluconazole (50 mg/kg/day). The number of *Candida* CFU in abscesses was significantly reduced (P < 0.05) in mice receiving rmG-CSF prophylaxis (day −1 or day −1 through 2) compared with controls on day 8 of infection. Administration of rmG-CSF therapy alone (for 5 days starting on day 4 of infection) had no influence on the number of *Candida* CFU in abscesses. Amphotericin B treatment was significantly more effective than fluconazole treatment (3.41 log CFU/abscesses; 95% confidence interval [CI], 3.17 log CFU/abscesses; 3.65 versus 3.90 log CFU/abscesses; 95% CI, 3.66 log CFU/abscesses; 4.16 log CFU/abscesses; P < 0.05). Therapeutic administration of rmG-CSF in conjunction with an antifungal agent showed a tendency towards a further reduction of *Candida* CFU in abscesses than antifungal treatment only. In conclusion, in this experimental model of intra-abdominal *Candida* abscesses, rmG-CSF administration did not have a detrimental influence on the course of infection. Amphotericin B treatment was most effective, and additional rmG-CSF therapy did not antagonize the effect of antifungal treatment. In contrast, addition of rmG-CSF therapy to antifungal treatment might further enhance the beneficial effect of the antifungal agent.

During the past three decades, the incidence of *Candida* species as a cause of nosocomial infections has steadily increased. Colonization of the gastrointestinal tract with *Candida* is common, and gastrointestinal perforation or surgery may thus be complicated by abdominal candidiasis with abscess formation. The mortality rate among patients with abdominal candidiasis is high (4, 21, 22).

The polyene compound amphotericin B has been the mainstay of antifungal therapy for critically ill patients with invasive candidiasis. However, due to its severe and dose-limiting adverse effects, alternatives to amphotericin B are being used, either alone or as combination therapy. The triazole antifungal agent fluconazole has proven to be equally effective in treating candidiasis as amphotericin B (18) and has become the drug of choice for treatment of candidiasis and disseminated *Candida albicans* infection (1, 19). Furthermore, fluconazole is considered the drug of choice for patients with peritoneal candidiasis (5), and fluconazole prophylaxis is able to prevent abdominal candidiasis in high-risk surgical patients (6). However, despite antifungal treatment, mortality remains high and additional therapy with agents that augment host defense, such as growth factor granulocyte colony-stimulating factor (G-CSF) may be of potential therapeutic benefit.

Pretreatment with recombinant murine G-CSF (rmG-CSF) beneficially influences the course of acute disseminated candidiasis (11) or bacterial peritonitis (2, 14) in mice. Combined therapy with an antifungal agent and rG-CSF has an additive effect against disseminated candidiasis in non-neutropenic mouse models when compared with antifungal treatment alone (9, 12). However, recent findings from a randomized, double-blind study have suggested that patients with intra-abdominal candidiasis treated with recombinant human G-CSF may have had a less favorable outcome than patients treated with fluconazole alone (B. J. Kullberg, K. Vandewoude, R. Herbrecht, F. Jacobs, and P. Kujath, Abstr. 38th Intersci. Conf. Antimicrob. Agents Chemother., p. 479, 1998). One of the explanations for the observed trend towards worse outcome of G-CSF treatment in intra-abdominal candidiasis may be that G-CSF down-regulates tumor necrosis factor alpha (TNF-α) production (7, 11, 14). Previous research by our group has shown that the clearance of intra-abdominal abscesses caused by *C. albicans* is delayed in TNF-α- and lymphotoxin-α-deficient mice, primarily by inducing a T helper (Th) 2 response (24). To explore whether G-CSF application may indeed have detrimental consequences in intra-abdominal candidiasis, we assessed the effect of immunomodulation with rmG-CSF in the presence or absence of amphotericin B or fluconazole on intra-abdominal abscesses caused by *C. albicans* in mice.

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TREATMENT OF ABDOMINAL ABSCESS WITH rmG-CSF AND ANTIFUNGALS

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MATERIALS AND METHODS

Animals. Female, 6- to 8-week-old CBA mice were allowed to acclimatize to laboratory conditions for 1 week prior to infection. The animals were housed under specific-pathogen-free conditions and were fed standard laboratory chow (Hope Farms, Woerden, The Netherlands) and water ad libitum. The ethics committee on animal experiments of the University Medical Center Nijmegen had approved the experiments.

Compounds. Amphotericin B was obtained as Fungizone (Bristol-Myers Squibb, Woerden, The Netherlands), containing 50 mg of amphotericin B, 41 mg of sodium deoxycholate, and 20.2 mg of sodium phosphate per vial, and was reconstituted with 10 ml of distilled water to obtain a standard solution of 5 mg/ml. Further dilutions were prepared in pyrogen-free 5% dextrose.

Flucanazole was purchased from Pfizer Nederland, as a stock solution containing 2 mg of flucanazole/ml rmG-CSF, provided by Amgen (Thousand Oaks, Calif.) was diluted in pyrogen-free saline to obtain a final concentration of 10 µg/ml.

MICs. MICs were determined according to the M27-A broth microdilution method as described by the National Committee for Clinical Laboratory Standards (15). Briefly, C. albicans strain UC820 was cultured for 24 h at 35°C and resuspended in 0.9% NaCl. The suspension was measured (530 nm) and was set between 75 and 77% (1 x 10⁶ to 5 x 10⁶ CFU/ml). The suspension was further diluted with distilled water (1:10) and RPMI 1640 containing 0.165 M 3-N-morpholinepropanesulfonic acid (MOPS) (1:100, pH 7.0) to obtain a final suspension of 1 x 10⁶ to 5 x 10⁶ CFU/ml. One hundred microliters of the suspension was incubated for 18 h at 35°C in the presence or absence of the antifungal agent at concentrations. The MIC of amphotericin B was defined as the lowest concentration of amphotericin B which resulted in an opticaly clear tube, and the MIC of flucanazole was defined as the lowest concentration of flucanazole which resulted in a turbidity reduction of 50% compared with that of the growth control as determined spectrophotometrically.

Abdominal abscess induction. C. albicans strain UC820 was inoculated into 100 ml of Sableourad broth and cultured for 24 h at 37°C. After three washes with pyrogen-free saline by centrifugation at 1,500 x g, the number of yeast cells was counted in a hemacytometer; occasional strings of two or more yeast cells were counted as 1 CFU of C. albicans. Pyrogen-free saline was used to dilute the suspension to the requested concentration. The viability of the yeast was at least 98%, as determined by plating serial dilutions on Sabouraud dextrose agar plates. Mouse feces were ground in a tissue grinder, suspended in 0.9% pyrogen-free saline to produce a 5% (wt/vol) mixture, and sterilized in a steam autoclave (15 min, 2 bar, 120°C). The sterility of the preparation was confirmed by plating aliquots on blood agar plates. To induce abdominal abscess formation, mice received an intraperitoneal injection of 100 µl of sterile fecal suspension containing 5 x 10⁶ live C. albicans CFU.

Treatment regimens. Antifungal treatment started on day 3 of infection, with doses of flucanazole (50 mg/kg of body weight/day) or amphotericin B (0.75 mg/kg/day) that had proven to be equally effective in reducing the number of abscesses of C. albicans strain UC820. Flucanazole was administered orally every 12 h by gavage at a dosage of 25 mg/kg/dose. Control animals received 100 µl of sterile pyrogen-free saline by gavage 1 h after infection; regimen B, prolonged rmG-CSF prophylaxis was administered once daily for 4 days from day -1 to day 2 of infection; regimen C, rmG-CSF therapy was given once daily for 5 days commencing on day 4 of infection.

For assessment of antifungal treatment combined with rmG-CSF on the number of Candida CFU in abcesses, rmG-CSF was administered according to regimen A or C starting on day 3 of infection, either alone or in combination with amphotericin B or flucanazole, with the same doses and dosing schedules described above.

Outcome assessments. The number of circulating granulocytes was determined on blood obtained from the retro-orbital plexus from subgroups of mice prior to or at different points in time during infection. On different days of infection, subgroups of animals were anaesthetized with ether and bled from the retro-orbital plexus for measurement of circulating TNF-α, interleukin-1 alpha (IL-1α), IL-6, gamma interferon (IFN-γ), and IL-10 concentrations. Thereafter, these mice were sacrificed, and the abdominal cavity was explored for the presence and number of abscesses of ≥1 mm. All abscesses of ≥1 mm were removed, measured, rinsed with 70% ethanol, and subsequently rinsed with saline to remove external microorganisms. Since it was hypothesized that rmG-CSF might alter the containment of Candida within the abscesses and lead to spread of the infection throughout the peritoneum with subsequent hematogenous dissemination, we removed a 10- by 10-mm sample of the peritoneum for assessment of peritonitis and the left kidney for assessment of Candida dissemination.

The tissues were homogenized in sterile saline in a tissue grinder, and Candida blastoconidia were enumerated by plating serial dilutions (abscesses, kidneys) or the complete homogenate (peritonium) on Sabouraud dextrose agar plates, as described previously (13). The CFU were counted after 24 h of incubation at 37°C and expressed as log CFU/organ. Bacterial confluence of the abscesses was evaluated by plating aliquots on blood agar. Cultures yielded no aerobic or anaerobic bacteria. For histology, tissue samples were fixed in buffered formalin (4%) and embedded in paraffin. Sections were stained with periodic acid-Schiff and hematoxylin-eosin and examined microscopically.

Stimulation of splenic lymphocytes. To determine the effect of rmG-CSF on cell responses, splenes of groups of mice that received either rmG-CSF therapy (1,000 ng once daily for 5 days) or control vehicle were removed on day 8 of infection. Spleen cells were obtained by gently squeezing spleens in a sterile 200-µm-pore-size filter chamber. Microscopic examination of Giemsa-stained cytospin preparations showed that splenocytes consisted of 95% lymphocytes, 2% monocytes, and 3% granulocytes. Splenocytes were washed and resuspended in RPMI-dm and counted in a Burker counting chamber, and the number was adjusted to 5 x 10⁶/ml. One milliliter of the cell suspension was stimulated with 1 x 10⁷ heat-killed C. albicans UC820 blastoconidia (effector-to-target cell ratio, 2:1). The measurement of IFN-γ and IL-10 concentrations was performed in supernatants collected after 48 h of incubation at 37°C in 5% CO₂ in 24-well plates (Greiner, Alphen a/d Rijn, The Netherlands).

Cytokine assays. The concentrations of TNF-α and IL-10 were determined by specific radioimmunoassays, as described previously (16). The detection limit with a 100-µl sample was 40 pg/ml for TNF-α and 20 pg/ml for IL-10. IL-10, IFN-γ, and IL-6 concentrations were determined by a commercially available enzyme-linked immunosorbent assay kit (Biosource Europe), according to the guidelines of the manufacturer. The detection limits were 8, 1.5, or 150 pg/ml, respectively.

Statistical analysis. Values were expressed as means ± standard deviation or, in case of logarithmic data, as means and 95% confidence intervals (95% CIs). The differences between two groups were analyzed by the Mann-Whitney U test. For these comparisons, the level of significance was set at a P value of <0.05. For comparison of three or more groups, the data were analyzed by using the Kruskal-Wallis one-way analysis of variance. For posttest comparisons, the Bonferroni method was used. To ascertain reproducibility, most experiments were performed at least twice, and the data represent the average results of all experiments performed.

RESULTS

In vitro susceptibilities and pharmacokinetics. The MICs of both amphotericin B and flucanazole for C. albicans strain UC820 were 0.25 mg/liter. A dose of up to 1.5 mg of amphotericin B/kg/day was well tolerated. No abnormalities of renal and hepatic function were detected after 5 days of amphotericin B administration, as measured by serum urea, creatinine, aspartate aminotransferase, and alanine aminotransferase concentrations compared to those of mice receiving control vehicle (data not shown). Peak concentrations of flucanazole in plasma (25 mg/kg) given by gavage were reached 0.5 h after administration (25.57 ± 1.36 mg/liter), and trough concentrations measured before the next dose (12 h after administration) amounted to 6.82 ± 0.21 mg/liter. Thus, for flucanazole, the time above the MIC in the circulation was 100% of the dosing interval.

Intra-abdominal abscesses and antifungal therapy. All animals survived the acute phase of infection and consistently produced abscesses, which could be detected from day 3 of infection. No differences in the number of abscesses were observed between any groups on any day of infection (Table 1).
On day 8 of infection, quantification of yeast cells recovered from intra-abdominal abscesses from amphotericin B-treated mice showed a significantly reduced outgrowth of *C. albicans* CFU compared to controls or fluconazole-treated mice ($P < 0.05$) (Table 1). Fluconazole treatment did not reduce the number of CFU in the abscesses compared to the controls. Combination therapy of amphotericin B with fluconazole did not further reduce outgrowth from abscesses compared to amphotericin B treatment alone. Seven days after the termination of antifungal treatment, on day 14 of infection, no differences in the number of *Candida* CFU in the abscesses were observed (Table 1).

**Immunomodulation with rmG-CSF.** At the time of intraperitoneal infection, the mean number of granulocytes in mice that received rmG-CSF 24 h earlier was $1.6 \times 10^9 \pm 0.2 \times 10^9$/liter, whereas in untreated mice, the number amounted to $0.8 \times 10^9 \pm 0.1 \times 10^9$/liter ($P < 0.05$). On day 3 of infection, the circulating numbers of granulocytes were unchanged in control mice ($0.8 \times 10^9 \pm 0.1 \times 10^9$/liter), whereas the numbers of granulocytes in mice that had received rmG-CSF prophylaxis ($2.2 \times 10^9 \pm 0.6 \times 10^9$/liter; $P < 0.05$) or prolonged rmG-CSF prophylaxis ($2.4 \times 10^9 \pm 1.2 \times 10^9$/liter; $P < 0.05$) were higher than those in controls.

On the third day of infection, mice that had received rmG-CSF prophylaxis had significantly fewer *Candida* CFU in their abscesses than untreated mice did ($P < 0.05$) (Table 2). Both prophylaxis groups showed a reduced outgrowth of *Candida* CFU compared to controls on day 9 ($P < 0.05$) (Table 2). Therapy with rmG-CSF that started on day 4 of infection did not influence the numbers of CFU in the abscesses on either day of infection compared to controls. The number of abscesses did not differ between the groups on any day of infection (Table 2).

Since administration of rmG-CSF might alter the containment of *Candida* CFU in the abscesses, the amount of peritonitis was determined. Although the amount of *Candida* in the peritoneum was around the detection limit, i.e., was negligible, mice that received prolonged rmG-CSF prophylaxis had a significantly larger number of *Candida* CFU/square centimeter of peritoneum than did controls (day 3, $82 \pm 27$ versus $37 \pm 22$, $P < 0.01$; day 9, $9 \pm 6$ versus $2 \pm 2$ CFU/cm$^2$, $P < 0.05$) (data are means ± standard deviations of at least 10 mice per group, the experiment was performed thrice).

In addition, whether rmG-CSF increases hematogenous dissemination of *Candida* was assessed. Again, the number of *Candida* CFU was around the detection limit at any point in time, and no differences in the number of *C. albicans* CFU, i.e., dissemination of *Candida*, in the kidneys were observed between groups (data not shown).

**TABLE 1. Effect of antifungal treatment on number of abscesses and outgrowth of *C. albicans* in abscesses**

<table>
<thead>
<tr>
<th>Agent</th>
<th>Result on day:</th>
<th>3</th>
<th>8</th>
<th>14</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of abscesses/mouse</td>
<td>Log CFU in abscesses/mouse (95% CI)</td>
<td>No. of abscesses/mouse</td>
<td>Log CFU in abscesses/mouse (95% CI)</td>
</tr>
<tr>
<td>Control vehicle</td>
<td>5 ± 2</td>
<td>5.25 (4.67–5.82)</td>
<td>13 ± 5</td>
<td>3.96 (3.70–4.21)</td>
</tr>
<tr>
<td>Amphotericin B</td>
<td>12 ± 4</td>
<td>3.41 (3.17–3.65)</td>
<td>9 ± 1</td>
<td>2.75 (2.42–3.09)</td>
</tr>
<tr>
<td>Fluconazole</td>
<td>10 ± 4</td>
<td>3.90 (3.66–4.16)</td>
<td>7 ± 2</td>
<td>2.71 (2.52–2.89)</td>
</tr>
<tr>
<td>Amphotericin B + fluconazole</td>
<td>10 ± 4</td>
<td>3.60 (3.43–3.77)</td>
<td>6 ± 3</td>
<td>2.45 (2.12–2.78)</td>
</tr>
</tbody>
</table>

\(^a\) Effects were determined on different days after intraperitoneal injection of 100 μl of sterile fecal suspension containing $5 \times 10^5$ viable *C. albicans* blastoconidia. Treatment with amphotericin B (0.75 mg/kg/day) or fluconazole (50 mg/kg/day) commenced on day 3. Data are the cumulative results of experiments performed three times and are expressed as means and 95% CI for at least 8 mice per group. Treatment groups were compared to controls only.

\(^b\) $P < 0.05$.

\(^c\) $P < 0.01$.

**TABLE 2. Effect of immunomodulation with rmG-CSF on number of abscesses and outgrowth of *C. albicans* in abscesses**

<table>
<thead>
<tr>
<th>Agent</th>
<th>Result on day:</th>
<th>3</th>
<th>9</th>
<th>15</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of abscesses/mouse</td>
<td>Log CFU in abscesses/mouse (95% CI)</td>
<td>No. of abscesses/mouse</td>
<td>Log CFU in abscesses/mouse (95% CI)</td>
</tr>
<tr>
<td>Control vehicle</td>
<td>4 ± 1</td>
<td>5.01 (4.72–5.29)</td>
<td>8 ± 3</td>
<td>3.84 (3.64–4.04)</td>
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<tr>
<td>rmG-CSF prophylaxis (day -1)</td>
<td>6 ± 2</td>
<td>4.72 (4.51–4.93)</td>
<td>8 ± 5</td>
<td>3.46 (3.23–3.74)</td>
</tr>
<tr>
<td>Prolonged rmG-CSF prophylaxis (days -1 through +2)</td>
<td>5 ± 2</td>
<td>5.01 (4.83–5.18)</td>
<td>9 ± 6</td>
<td>3.36 (3.15–3.58)</td>
</tr>
<tr>
<td>rmG-CSF therapy (days +4 through +8)</td>
<td>10 ± 5</td>
<td>3.91 (3.71–4.12)</td>
<td>6 ± 3</td>
<td>3.14 (2.73–3.54)</td>
</tr>
</tbody>
</table>

\(^a\) Effects were determined on different days after intraperitoneal injection of 100 μl of sterile fecal suspension containing $5 \times 10^5$ viable *C. albicans* blastoconidia. rmG-CSF (1,000 ng/day) was given as indicated. Data are the cumulative results of experiments performed three times and are expressed as means and 95% CI for at least 7 mice per group. Treatment groups were compared to controls only.

\(^b\) $P < 0.05$.

\(^c\) $P < 0.01$. 
Stimulation of splenic lymphocytes. To assess whether rmG-CSF induces a Th1 or a Th2 type immune response, $5 \times 10^6$ splenic lymphocytes obtained on day 9 of infection from untreated mice and mice that received rmG-CSF therapy were stimulated with $10^7$ CFU of heat-killed C. albicans in vitro. Lymphocytes of mice that were treated with rmG-CSF produced significantly more IL-10 ($901 \pm 304$ pg/ml) than control lymphocytes ($532 \pm 263$ pg/ml; $P < 0.05$). No significant differences in IFN-γ production between rmG-CSF-treated mice ($560 \pm 390$ pg/ml) and controls ($710 \pm 475$ pg/ml) was observed. The IL-10/IFN-γ ratio was twofold higher for mice treated with rmG-CSF (1.9 ± 0.58) than for the control group (1.0 ± 0.54; $P < 0.05$).

Immunomodulation combined with antifungal agents. On day 8 of infection, amphotericin B treatment significantly reduced the number of CFU compared to controls (Fig. 1; $P < 0.05$). Combination treatment of rmG-CSF therapy with fluconazole or amphotericin B was slightly more effective in reducing the number of CFU in abscesses, although the differences between mice treated with an antifungal agent alone and mice treated with the agent in combination with rmG-CSF therapy were not significant (Fig. 1B; $P > 0.05$). Histopathology of the abscesses on day 8 of infection showed that treatment with rmG-CSF either alone or in conjunction with antifungal treatment increased the number of granulocytes present in the abscesses. This was observed for both prophylactic (data not shown) and to a larger extent for therapeutically administered rmG-CSF (Fig. 2). On day 15 of infection, no differences between groups were observed, neither by histopathological evaluation nor in outgrowth (data not shown), although the combination of amphotericin B with rmG-CSF therapy (3.02 log CFU/abscesses; 95% CI, 2.43 to 3.61 log CFU/abscesses) showed a tendency to reduce the outgrowth of Candida CFU from abscesses most compared to controls (3.39 log CFU/abscesses; 95% CI, 2.92 to 3.86 log CFU/abscesses; $P > 0.05$). No difference in the numbers of abscesses was observed between the groups at any point in time.

Mice that received rmG-CSF prophylaxis had significantly higher circulating IL-10 concentrations than controls ($45.8 \pm 33.6$ pg/ml for rmG-CSF prophylaxis versus $16.8 \pm 1.5$ for controls; $P < 0.05$) on day 3 of infection. No further differences in the concentrations of circulating cytokines on any day of infection were detected (data not shown).

DISCUSSION

The results of the present study indicate that modulation of host defense with rmG-CSF prophylaxis significantly reduces the number of Candida CFU in intra-abdominal abscesses, whereas rmG-CSF therapy (administered on days 4 through 8 of infection) had neither a beneficial nor an adverse effect. Antifungal treatment of intra-abdominal Candida abscesses with amphotericin B was significantly more effective than treatment with fluconazole. The addition of rmG-CSF therapy to conventional antifungal treatment did not antagonize the individual effect of the antifungal agents. In contrast, it showed a trend towards further reduction of Candida CFU in abscesses compared to antifungal treatment alone.

After injection of a sterile fecal suspension containing viable Candida blastocandida, abscesses developed following a period of peritonitis. Administration of rmG-CSF during either of these periods had different effects on outgrowth from abscesses; rmG-CSF prophylaxis significantly reduced the number of CFU in abscesses compared to that in abscesses of control mice, whereas administration rmG-CSF therapy induced a slight increase in the number of CFU in abscesses compared with that in abscesses of control mice. Mice that had received rmG-CSF prophylaxis had large numbers of rmG-CSF-primed polymorphonuclear leukocytes (PMN) present at the time of infection and thus an enhanced capacity to clear the injected Candida blastocandida from their abdominal cavities compared to that of controls, as was demonstrated by a reduced outgrowth from abscesses from these mice.

Although mice that had received prolonged rmG-CSF prophylaxis showed an increased severity of diffuse peritonitis, the data were around the detection limit and do not seem to be of biological relevance, since it was not accompanied with an increased hematogenous dissemination of Candida into the kidneys. At the time of rmG-CSF therapy, which commenced not earlier than on day 3 of infection, abscesses were already well established, and at this point in time, inflammatory cells other than granulocytes, such as macrophages and lymphocytes, gradually play a central role in host defense against Candida abscesses. G-CSF inhibits the TNF production by macrophages and monocytes (7, 23) and has been shown to induce a Th2-type cytokine pattern via attenuation of monokine release (3, 8). Furthermore, our laboratory has previously

FIG. 1. Effect of amphotericin B (AMB) or fluconazole (FCZ) combined with rmG-CSF on the outgrowth of C. albicans in intra-abdominal abscesses per mouse on day 8 of infection. (A) Mice received rmG-CSF on day −1 of infection. (B) Mice received daily rmG-CSF on days 3 through 7 of infection. Horizontal bars indicate the means. a, $P < 0.05$ compared with control mice; b, $P < 0.01$ compared with control mice. The data were obtained from one experiment with 4 animals per group.

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shown that TNF is important for the clearance of *Candida* from intra-abdominal abscesses, primarily through induction of Th1 responses and enhancement of the extracellular killing capacity of granulocytes (24). Hence, therapeutic administration of rmG-CSF was expected to have detrimental effects in this model of intra-abdominal *Candida* abscesses. However, rmG-CSF therapy had no adverse influence on the course of infection. Although the IL-10/IFN-γ ratios of mice treated with rmG-CSF were significantly increased compared with controls, this may not have been of biological relevance. G-CSF not only increases the number of PMN but it also augments the expression of adhesion molecules, recruitment, and capacity to kill *Candida* blastoconidia (25–27). These effects of G-CSF on anticandidal activity have probably compensated for the anti-inflammatory effect of G-CSF through its effect on macrophages and T cells.

Intra-abdominal abscesses are difficult to treat, and therapy consists primarily of drainage of the infected cavity with or without antimicrobial treatment. To date, no guidelines for the use of antifungal agents for treatment of intra-abdominal candidiasis exist. In an international conference of investigators with extensive experience in the treatment of candidal infections, 60% of the investigators would treat patients with peritoneal candidiasis with fluconazole alone and 5% would treat patients with amphotericin B lipid formulation (5). In our model, fluconazole had no beneficial influence on the course of experimental intra-abdominal *Candida* abscesses in mice, whereas amphotericin B improved the clearance of *Candida*.
blastococci from abscesses. This is contradicts the study by Sawyer et al., which showed that fluconazole was as effective as amphotericin B in reducing the number of Candida recovered from experimental intra-abdominal abscesses (20). In that study, however, fluconazole therapy was started at the time of infection and thus represents the effect of fluconazole on Candida peritonitis rather than on well-established Candida abscesses. Indeed, fluconazole prophylaxis has proven to prevent development of abdominal candidiasis in high-risk surgical patients (6).

Simultaneous administration of combination therapy of fluconazole with amphotericin B was not as effective as amphotericin B treatment alone in reducing the number of CFU in abscesses in our experiments.

Since combination therapy of an antifungal agent with G-CSF has proven to be more efficacious than antifungal treatment alone against disseminated candidiasis (9, 12), it was hypothesized that the combination of rmG-CSF therapy with antifungal agents may have a synergistic effect, despite the observation that therapy with rmG-CSF alone was not as effective in our model of intra-abdominal abscesses. The addition of rmG-CSF therapy to either amphotericin B or fluconazole did not significantly improve the outcome, although there was a slight tendency towards further reducing the number of Candida CFU per abscess. Both amphotericin B and fluconazole accumulate within PMN (17), and their concentrations might have been increased by G-CSF, which has been shown to increase the intracellular concentration of antibiotics (10). In addition, histopathological examination of abscesses obtained from mice treated with rmG-CSF showed a larger number of granulocytes than in control mice. Serving as secondary transcytosing elements, these cells might have contributed to achieving higher drug concentrations in the abscesses. In our experiments, however, these putative effects have not led to significant synergistic effects of rmG-CSF and antifungal agents. In conclusion, amphotericin B was effective in treating experimental intra-abdominal Candida abscesses, and concurrent administration of G-CSF was suggested to further improve the outcome.

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REFERENCES


