Immunomodulatory Treatment of *Mycobacterium avium* Complex Bacteremia in Patients with AIDS by Use of Recombinant Granulocyte-Macrophage Colony-Stimulating Factor

Carol A. Kemper, Luiz E. Bermudez, and Stanley C. Deresinski

Eight AIDS patients with *Mycobacterium avium* complex (MAC) bacteremia were randomized to receive azithromycin with or without granulocyte-macrophage colony-stimulating factor (GM-CSF) for 6 weeks to examine the effect of GM-CSF administration on clearance of mycobacteremia and on monocyte function. Superoxide anion production was significantly increased ex vivo in monocytes from patients receiving GM-CSF but not in those from patients receiving azithromycin alone. Relative to monocytes obtained from untreated healthy controls, median differences in viable intracellular MAC at 2, 4, and 6 weeks were $-0.76, -0.94$, and $-0.47 \log_{10}$ cfu/mL of lyase for cells from patients receiving GM-CSF versus $-0.15, -0.11$, and $-0.19 \log_{10}$ cfu/mL for cells from patients receiving azithromycin alone. Although no effect on mycobacteremia was detected, the administration of GM-CSF to AIDS patients with MAC bacteremia resulted in activation of their blood monocytes, as evidenced by increased superoxide anion production and enhanced mycobactericidal activity. GM-CSF deserves further investigation in the treatment of mycobacterial infections.

Despite significant strides in our ability to prevent and treat *Mycobacterium avium* complex (MAC) infections, MAC remains a significant cause of morbidity and premature mortality in patients with advanced human immunodeficiency virus (HIV) disease. The annual incidence of MAC infection, at least before the availability of more effective antiretroviral therapy, was $\sim 24\%$ in patients with CD4 cell counts $<100/mm^3$ who did not receive antiretroviral prophylaxis [1, 2]. In those who do develop MAC bacteremia, optimal antituberculosis therapy results in suppression of mycobacteremia in only $\sim 70\%$, and the risk of relapse remains high ($\sim 5\%$–$60\%$) in patients who initially respond [3–5]. Unfortunately, $86\%$–$100\%$ of those who develop recrudescent infection do so with organisms that demonstrate high-level macrolide resistance [3]. Additional approaches, such as the use of immunomodulatory agents that may enhance the ability of infected monocytes and macrophages, the usual site of residence of MAC, to suppress intracellular growth of this organism are needed. Previous studies both in vitro and in an experimental mouse model of disseminated MAC infection have shown that the use of recombinant granulocyte-macrophage colony-stimulating factor (GM-CSF) resulted in a significant decrease in the number of viable bacteria in macrophages, blood, liver, and spleen [6–8]. We therefore evaluated the effect of GM-CSF administration to AIDS patients with MAC bacteremia in a small, randomized trial.

Materials and Methods

Patients with HIV infection and newly diagnosed, previously untreated MAC bacteremia were randomized to receive orally administered azithromycin, 600 mg daily, with or without subcutaneously administered GM-CSF for 6 weeks. Two patients received GM-CSF in a dose-escalating fashion as follows: They first received azithromycin alone for 2 weeks, then GM-CSF at 50 $\mu$g/m$^2$/day for 7 days, followed by GM-CSF at 125 $\mu$g/m$^2$/day for 7 days, and then GM-CSF at 250 $\mu$g/m$^2$/day for 14 days. The remaining 2 patients randomized to GM-CSF received 250 $\mu$g/m$^2$/day for the duration of the study. Clinical symptoms and quantitative mycobacterial colony counts in blood (limit of detection, $\leq 0.1$ cfu/mL of whole blood), obtained by the lysis centrifugation method [1, 9], were assessed at least every 2 weeks. Mean individual logarithmic changes in baseline colony counts were compared with the last culture obtained while receiving therapy at either week 4 or week 6.

Human monocyte function was assessed ex vivo at least every 2 weeks by one of the investigators who was blinded to the randomization. Heparinized human peripheral venous blood was processed.
with Histopaque (Sigma, St. Louis) as previously described [6, 10]. Briefly, the suspension was adjusted to 10^6 cells/mL in RPMI 1640 supplemented with 10% heat-inactivated fetal bovine serum (Sigma), incubated for 2 h at 37°C in 5% CO_2 in moist air to permit the adherence of monocytes, and washed with Hank’s balanced salt solution warmed to 37°C to remove unattached cells. Residual monolayers contained ~5 x 10^5 cells/well. More than 97% of the cells in the monolayers were monocytes as assessed by their ability to ingest neutral red. The number of monocytes in the monolayers was observed daily as previously described [6]. No preferential detachment was observed in comparisons of experimental and control groups.

Histopaque, horse heart type III ferricytochrome c, superoxide dismutase, and PMA were purchased from Sigma. Recombinant human GM-CSF (yeast-derived) was donated by Immunex (Seattle) and had specific activity of 2 x 10^7 U/mg of protein. Lipopolysaccharide from Escherichia coli O111:B6 was purchased from List Research Laboratories (Campbell, CA). The O_2^- release was assessed spectrophotometrically by measuring the superoxide dismutase–inhibitable reduction of ferricytochrome c as previously described [6]. Macrophage monolayers (5 x 10^5 cells/well) were infected with a dispersed suspension of M. avium strain 101 (serovar 1) (5 x 10^6 cells) for 4 h, the extracellular bacteria were removed by washing, and the intracellular growth was monitored for 4 days as previously described [10]. Monocytes from 2 healthy non–HIV-infected volunteers were used as controls for each assay. After 4 days, the monolayers were lysed and the lysate was plated onto Middlebrook media 7H10 as previously described [6, 11]. The number of viable intracellular bacteria are reported as mean colony-forming units per milliliter of macrophage lysate suspension. Duplicate plates were prepared for each well.

Each experiment was repeated twice. The values were obtained in duplicate wells, and mean ± SD were calculated. The significance of the comparisons was analyzed with Student’s t test. Significance was set at P < .05 for the two-tailed tests.

**Results**

Nine HIV-infected patients were enrolled in the study, including 8 who had MAC bacteremia and 1 who was enrolled on the basis of a report of a positive blood culture that subsequently proved erroneous. One patient was female. Five patients were white, 3 were Hispanic, and 1 was African American. The mean age was 35 years. The mean CD4 cell count obtained within 2 months before enrollment was 10 cells/mm^3.

Four patients were randomized to receive azithromycin alone and 5 were randomized to receive the combination (including the patient who was subsequently found not to have MAC bacteremia); 2 of the latter group who were diagnosed with MAC bacteremia received their GM-CSF in a dose-escalating fashion. The duration of GM-CSF therapy for the 4 patients randomized to the combination ranged from 15 to 42 days (median, 30). Modest increases in white blood cell counts, particularly monocyte cell numbers, were observed in patients receiving GM-CSF.

Mean pretreatment MAC colony counts were, respectively, 0.5 ± 0.6 and 1.7 ± 1.6 for patients who received azithromycin with GM-CSF or azithromycin alone. One patient in each arm had sterilization of blood cultures during the 6-week study. The mean individual changes in the baseline logarithmic colony counts compared with the last culture obtained while receiving therapy (at either week 4 or 6) were, respectively, −0.9 and −1.0 log_{10} cfu/mL for patients who received azithromycin with GM-CSF (n = 3) or without (n = 3) (table 1). The median changes, expressed logarithmically, were −0.7 and −1.0 log_{10} cfu/mL, respectively. No significant difference between treatment groups in the decrease in mycobacterial colony counts could be detected in this small number of patients.

The study drugs were well-tolerated, and no patient required permanent discontinuation of therapy. Two of 5 patients who received GM-CSF developed possible side effects attributable to this agent, including 1 patient who received full-dose GM-CSF (250 µg/m^2) and who developed severe shaking, chills, fever, and sweats after study drug administration, which required withholding drug for 1 week and eventual dose reduction. The other patient complained of myalgia but continued to receive full-dose GM-CSF. Two of 9 patients had possible reactions to azithromycin: 1 developed nausea, requiring the withholding of azithromycin for 1 week and then dose reduction to 300 mg/day, whereas the other developed anemia, possibly related to azithromycin but more likely as a result of his underlying disease.

Mean superoxide anion production significantly increased in monocytes from all 4 patients receiving GM-CSF (53% to 119% increase in response to PMA stimulation relative to control monocytes) compared with that in those from patients who received azithromycin alone (−13% to 35% increase in response to PMA) (figure 1).

The mycobactericidal capacity of monocytes obtained from patients with MAC bacteremia increased in 3 of the 4 subjects who received GM-CSF (figure 2), as well as in the non–MAC-infected subject who received this agent. No such finding was observed in monocytes obtained from the patients who received azithromycin alone. At baseline, before administration of GM-CSF, the ability of cells to suppress intracellular growth of MAC after 4 days of incubation was not significantly different from that of cells obtained from the 2 healthy non–HIV-infected control subjects (table 2). However, by days 14 and 21 of study (data combined, by which time 2 subjects had received 7 days of lower-dose GM-CSF (50 µg/m^2/day) and 2 subjects had received 14 days of full-dose GM-CSF (250 µg/m^2/day), the mean intracellular mycobacterial colony counts were 0.56 ± 0.43 log_{10} cfu/mL lower in cells from the 4 GM-CSF–treated subjects than in cells from control subjects (ranging from a reduction of 0.80 to an increase of 0.08 log_{10} cfu/mL) (P = .40). In contrast, the mean reduction in monocyte inhibition of intracellular MAC in patients who received 14 days of azithromycin alone was 0.17 ± 0.17 log_{10} cfu/mL relative to that of the control cells (ranging from a reduction of 0.10 to 0.39 log_{10} cfu/mL).

A statistically significant increase in mycobacterial activity in response to GM-CSF was found by the fourth and sixth weeks of study. By day 28, the mean reduction in intracellular
Table 1. Effect of azithromycin with or without GM-CSF on median log_{10} CFU/mL of blood. No apparent difference was seen in concentration of MAC cfu in bloodstream during treatment.

<table>
<thead>
<tr>
<th>Group</th>
<th>Day 0</th>
<th>Day 14</th>
<th>Day 28</th>
<th>Day 42</th>
<th>Change from baseline*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Azithromycin plus GM-CSF</td>
<td>(n = 4)</td>
<td>(n = 4)</td>
<td>(n = 2)</td>
<td>(n = 2)</td>
<td>(n = 3)</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>0.15</td>
<td>-0.5</td>
<td>-1.0</td>
<td>-0.7</td>
</tr>
<tr>
<td>Azithromycin alone</td>
<td>(n = 4)</td>
<td>(n = 1)</td>
<td>(n = 3)</td>
<td>(n = 1)</td>
<td>(n = 3)</td>
</tr>
<tr>
<td></td>
<td>1.6</td>
<td>3.3</td>
<td>2.3</td>
<td>-1.0</td>
<td>-1.0</td>
</tr>
</tbody>
</table>

NOTE. Data are median log_{10} CFU/mL.

* Calculated using week 4 or week 6 data (last culture obtained while patient was receiving therapy). 1 patient on each arm became culture-negative (negative cultures were designated as .099 log_{10} CFU/mL, just below ability to detect presence of bacteremia).

Discussion

MAC is a facultative intracellular parasite found predominantly within cells of the monocyte-macrophage series [12]. In

![Figure 1. Mean superoxide anion production (nM) in response to PMA stimulation relative to control monocytes for patients receiving azithromycin (AZM) alone or in combination with GM-CSF. Because of pattern of dose escalation, data at day 14 and 21 were combined; by this time 2 patients had received 7 days of lower-dose GM-CSF (50 μg/m²/day) and 2 had received 14 days of full-dose GM-CSF (250 μg/m²/day).](https://academic.oup.com/jid/article-abstract/177/4/914/929172)

![Figure 2. Ability of monocytes from patients with AIDS and MAC bacteremia receiving azithromycin (AZM) alone or in combination with GM-CSF to suppress intracellular growth of MAC strain 101 ex vivo relative to that of monocytes obtained from untreated normal controls. Statistically significant increase in mycobacterial activity in response to GM-CSF was found by 4th and 6th weeks of study.](https://academic.oup.com/jid/article-abstract/177/4/914/929172)
Table 2. Relative in vitro intracellular suppression of MAC strain 101 by ex vivo infected monocytes from patients with AIDS and MAC bacteremia who were receiving azithromycin alone or in combination with GM-CSF compared with that of monocytes obtained from untreated normal controls.

<table>
<thead>
<tr>
<th>Group</th>
<th>Day 0</th>
<th>Day 14/21*</th>
<th>Day 28</th>
<th>Day 42</th>
</tr>
</thead>
<tbody>
<tr>
<td>Azithromycin plus GM-CSF</td>
<td>(n = 4)</td>
<td>(n = 4)</td>
<td>(n = 2)</td>
<td>(n = 2)</td>
</tr>
<tr>
<td></td>
<td>-0.06 ± 0.20</td>
<td>-0.56 ± 0.43</td>
<td>-0.94 ± 0.09</td>
<td>-0.47 ± 0.34</td>
</tr>
<tr>
<td></td>
<td>(-0.32 to +0.16)</td>
<td>(-0.80 to +0.08)</td>
<td>(-1.0 to -0.88)</td>
<td>(-0.70 to -0.23)</td>
</tr>
<tr>
<td>Azithromycin alone</td>
<td>(n = 4)</td>
<td>(n = 4)</td>
<td>(n = 3)</td>
<td>(n = 2)</td>
</tr>
<tr>
<td></td>
<td>-0.04 ± 0.22</td>
<td>-0.17 ± 0.17</td>
<td>-0.15 ± 0.11</td>
<td>-0.19 ± 0.27</td>
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<tr>
<td></td>
<td>(-0.37 to +0.12)</td>
<td>(-0.39 to -0.01)</td>
<td>(-0.28 to -0.07)</td>
<td>(-0.38 to +0.01)</td>
</tr>
</tbody>
</table>

NOTE. Data are mean ± SD (range) intracellular log_{10} cfu/mL at each week of therapy (patient log_{10} cfu/mL – control log_{10} cfu/mL). Monolayers were incubated with MAC for 4 days.

* Because of pattern of dose escalation, data at day 14 and day 21 were combined; by this time 2 patients had received 7 days of lower-dose GM-CSF (50 μg/m²/day) and 2 patients had received 14 days of full-dose GM-CSF (250 μg/m²/day).

³ Difference in mean reduction in intracellular organisms between treatment arms.

at least one study, macrophages coinfected with HIV and MAC have been reported to exhibit both decreased viability and enhanced MAC replication [13]. The decreased number and function of T cells characteristic of advanced HIV disease, with resultant defects in the production of cytokines, may lead to impaired monocyte function. In addition, glycopeptidolipids derived from MAC inhibit lymphocyte proliferation, an effect that could secondarily lead to the inhibition of macrophage

![Figure 3](https://academic.oup.com/jid/article-abstract/177/4/989/929172)
activation [14]. It has also been reported that an inhibitor of intracellular MAC growth, present in the serum of HIV-infected persons without AIDS as well as non–HIV-infected persons, is absent in AIDS patients [15, 16].

The mechanism by which monocytes/macrophages inhibit the growth of intracellular MAC remains uncertain, since the production of neither reactive oxygen nor nitrogen intermediates is responsible [17]. Phagosome and lysosome fusion appears to be impaired, and the undamaged organism proliferates within the mycobacterial vacuole, which fails to acidify as a result of exclusion of the vesicular proton ATPase [18, 19]. It has been suggested that the bacteriostasis associated with exposure to cytokines, such as interferon (IFN)-\(\gamma\), tumor necrosis factor (TNF)-\(\alpha\), and GM-CSF, is the result of acidification of the phagosome [20].

In vitro studies indicate that the activity of cytokines, such as TNF-\(\alpha\) and GM-CSF, is, in fact, critical to the ability of monocytes to restrict MAC replication. GM-CSF activates human monocytes to inhibit intracellular MAC, whether it is added to the in vitro system before or after infection [6, 21]. The addition of TNF-\(\alpha\) enhances the anti-MAC effect of GM-CSF, while IFN-\(\gamma\) antagonizes it. GM-CSF and TNF-\(\alpha\) each cause inhibition of intracellular growth of MAC within human alveolar macrophages, while both interleukin-2 and IFN-\(\gamma\) are ineffective [22]. The inhibition of growth of MAC within macrophages mediated by vitamin D\(_3\) is dependent on the activity of either TNF or GM-CSF [23].

The administration of GM-CSF to patients with fungal infections and malignancies results not only in increased peripheral blood leukocyte concentration but also in functional alterations of those cells [24, 25, 25a]. Administration of GM-CSF at 250 \(\mu\)g/m\(^2\)/day by continuous infusion to patients with refractory testicular cancer receiving autologous bone marrow transplantation resulted in increased monocyte major histocompatibility complex class I and II expression, as well as increased ex vivo monocyte-mediated cytotoxicity against U937 tumor cells [26]. We recently reported that GM-CSF administration to a non–HIV-infected patient with disseminated *Mycobacterium kansasii* infection who had abnormal monocyte function resulted in activation and enhanced mycobactericidal activity of those cells studied ex vivo [27]. The administration of GM-CSF, 300 mg three times weekly for 4 weeks, appeared to result in the reduction of abscesses due to *Blastoschizomyces capitatus* infection in a patient with acute myeloid leukemia [28]. Its use, however, probably limited by the need for parenteral administration and the potential for dose-related toxicity, although the drug was tolerated in this study. Lower dosages of GM-CSF, which may be better tolerated, appeared to result in ex vivo activity comparable to that achieved by the later larger dosages in the 2 patients who underwent escalation of their dosages.

Therapy with other cytokines, alone or in combination, may also be worthy of exploration [29]. IFN-\(\gamma\) administration to members of a family with disseminated MAC infection in the absence of HIV infection was therapeutically successful [30]. On the other hand, administration of this cytokine had no effect on mycobacteremia in a small number of AIDS patients with disseminated MAC infection [31].

Azithromycin has been demonstrated to have efficacy in the treatment of disseminated MAC infection in AIDS patients [32, 33]. In one study, MAC colony counts fell 1.55–2.0 log\(_{10}\), and sterilization of blood cultures occurred in 42%–56% of patients who received up to 6 weeks of azithromycin as a single agent (600–1200 mg/day) [33]. Nonetheless, recrudescent mycobacteremia, associated with high-level macrolide resistance in vitro, may occur within 16 weeks in a majority of patients receiving macrolide monotherapy [34]. For this reason, macrolide monotherapy is not recommended. Although the optimal antimycobacterial regimen for the treatment of MAC bacteremia in AIDS has not been determined, the combination of clarithromycin and ethambutol has been central to the most effective (i.e., most durable) combination regimens studied to date [3–5]. Whether a third agent—and which of the remaining agents—can enhance the durability of the response has not been determined, although clofazimine no longer appears to be a viable third choice, and its use may be associated with increased mortality [5].

Azithromycin achieves high concentrations within phagocytic cells, especially within acidic vacuoles, as a result of its dibasic amphophilic structure [35]. The pH of the mycobacterial vacuole (pH 6.9–8.6) may therefore result in lack of azithromycin accumulation at this critical site, as is the case with *Toxoplasma gondii*–infected cells [36]. The proposed acidification of the mycobacterial vacuole by GM-CSF may therefore result in increased accumulation of the azalide at this site. Acidification, unfortunately, also reduces the antibacterial activity of azithromycin [37, 38]. Nonetheless, in vitro and in vivo studies using the beige mouse demonstrate that the combination of GM-CSF with either azithromycin or amikacin resulted in greater anti-MAC effect than seen with any of these compounds used alone [7, 39]. Furthermore, with the exception of roxithromycin, the macrolide antibiotics can function as immunomodulators by stimulating phagocytosis, macrophage chemotaxis, and cytoidal activity, at least against certain organisms, such as *Candida albicans* [40]. Liposome-associated GM-CSF is more potent than free GM-CSF against MAC residing within human monocyte-derived macrophages, and the addition of GM-CSF significantly augments the activity of azithromycin compared with the effects of the agents alone [41].

We report here that administration of GM-CSF to AIDS patients with disseminated MAC bacteremia also receiving azithromycin results in activation of their monocytes, as reflected ex vivo in increased oxidative burst, and more important, increased mycobactericidal activity. We were unable, however, to demonstrate a statistically significant mycobactericidal effect in vivo in this small short-term study. Such an effect may have been masked by the potent anti-MAC effect of azithromycin. It is possible that a larger, more prolonged study evaluating the effect of GM-CSF, particularly in a cir-
cumstance in which less effective antibiotic therapy is available, such as in patients infected with macrolide-resistant strains of MAC, might demonstrate in vivo mycobactericidal activity. In addition, inhibition of replication by GM-CSF could theoretically delay or prevent the emergence of MAC resistance to the macrolides.

Acknowledgments

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References


