Cytokines Enhance Neutrophils from Human Immunodeficiency Virus–Negative Donors and AIDS Patients to Inhibit the Growth of *Mycobacterium avium* In Vitro


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*Mycobacterium avium* is one of the most prevalent opportunistic infections in AIDS patients, and neither prophylaxis nor treatment against *M. avium* is effective. To evaluate host defense mechanisms against mycobacterial infections, studies investigated whether neutrophils from AIDS patients could inhibit the growth of *M. avium* in vitro and what cytokines enhance neutrophil function against *M. avium*. Peripheral blood neutrophils from human immunodeficiency virus–negative and AIDS patients were incubated with media, granulocyte colony-stimulating factor (G-CSF), granulocyte-macrophage colony-stimulating factor (GM-CSF), interleukin-8, or macrophage-inhibitory proteins and infected with *M. avium*, and the inhibition of bacterial growth was determined. G-CSF (1000 U/mL) and GM-CSF (2000 U/mL) stimulated neutrophils from AIDS patients to significantly inhibit *M. avium* growth. These results demonstrate that neutrophils from AIDS patients can respond to exogenously supplied G-CSF or GM-CSF by inhibiting the growth of *M. avium*.

The neutrophil is one of the first cells involved in mediating innate host resistance to both bacterial and fungal infections, and the interactions with neutrophils and mycobacteria are thought to be important in initiating general host resistance against mycobacterial infection. In mouse strains susceptible to *Mycobacterium avium* infections, neutrophil infiltrates are reduced compared with infiltrates in resistant mice following intraperitoneal inoculation of *M. avium* [1]. Furthermore, macrophages within the regional lymph nodes and lungs in *M. avium*–infected mice contain large numbers of intracellular acid-fast bacteria and neutrophil-derived lactoferrin [2, 3]. Neutrophils may, thus, facilitate macrophage-mediated killing of mycobacteria by initially phagocytizing *M. avium*, causing partial degradation of the bacteria and releasing enzymatic granules that macrophages then phagocytize, thereby increasing the macrophage’s effectiveness in eliminating the bacteria [2]. In vitro systems using human neutrophils have shown that isolated neutrophil molecules, defensins, kill *M. avium* [4], and activation of neutrophils enhances killing of *Mycobacterium tuberculosis* [5], indicating that human neutrophils appear to have antimycobacterial capabilities. Neutrophils are thought to contribute to the initial host inflammatory responses to *M. avium* infection and, thus, may be a primary factor in resistance to infection by *M. avium* in immunocompetent persons.

AIDS-associated neutrophil disorders may place these patients at risk for developing mycobacterial infections, which occur in >60% of AIDS patients, leading to morbidity and mortality [6, 7]. Many AIDS patients are neutropenic, resulting from human immunodeficiency virus (HIV) infection itself [8] or secondarily to the side effects of zidovudine or ganciclovir treatment or both [9, 10], and this neutropenia may render these patients more susceptible to *M. avium* and other opportunistic infections.

In addition to neutropenia, neutrophil function may also be diminished by HIV infection, although it remains controversial as to whether their function in AIDS patients is defective [8]. Alternatively, insufficient production of cytokines modulating neutrophil function may be the underlying cause of decreased neutrophil activity. The cytokines granulocyte colony-stimulating factor (G-CSF), granulocyte-macrophage colony-stimulating factor (GM-CSF), interleukin (IL)-8, and macrophage-inhibitory proteins (MIPs) have been shown to mediate effective antimycobacterial activity by recruiting neutrophils and increasing their bactericidal activity against mycobacteria [11–13]. HIV infection is known to either reduce or increase the amounts of cytokines produced by macrophages [14], and we have demonstrated in previous studies that HIV infection dramatically increases cytokine production induced by *M. avium* and lipopolysaccharide (LPS) in macrophages [15].

These studies were designed to gain a better understanding of the contribution of neutrophils to host defense against *M. avium* infections in HIV-negative persons and in AIDS patients. We also wanted to identify factors that enhance neutrophil-mediated inhibition of *M. avium* growth and to determine whether HIV infection of macrophages could alter production of neutrophil-modulating factors.
Methods

Donor and patient characteristics. Seven healthy HIV-negative donors used for this study were skin-tested and were tuberculosis-negative. This group consisted of 5 men and 2 women with a mean (±SD) age of 37 ± 10 years.

The HIV-positive patient population was composed of 31 men and 4 women with a mean (±SD) age of 34.5 ± 11 years. Each person had AIDS; The mean (±SD) CD4 T cell count was 79.2 ± 98 cells/mm³ (range, 0–280). Thirty-two persons (92%) had an AIDS-defining illness that was first diagnosed a mean of 9 ± 14 months before the study. These illnesses included: Pneumocystis carinii pneumonia (11 patients), candidal esophagitis (9 patients), cerebral toxoplasmosis (3 patients), cytomegalovirus retinitis (3 patients), Kaposi’s sarcoma (2 patients), cryptococcal meningitis (2 patients), recurrent bacterial infections (1 patient), and cryptosporidiosis (1 patient). No patient had documented infection due to *M. avium*, and all patients had negative cultures for this organism 6 months before the study or immediately after it. Twenty-nine patients (80%) were receiving antiretroviral therapy at the time of the study; this included zidovudine monotherapy (12 patients), didanosine monotherapy (9 patients), and combination therapy (80%) were receiving antiretroviral therapy at the time of the study; this included zidovudine monotherapy (12 patients), didanosine monotherapy (9 patients), and combination therapy (5 patients) or zidovudine plus didanosine (5 patients). Thirty-three persons (94%) were receiving prophylaxis against *P. carinii* pneumonia, and the regimens included trimethoprim-sulfamethoxazole (16 patients), dapsone (14 patients), trimethoprim-sulfamethoxazole (5 patients), aerosolized pentamidine (4 patients), or aerosolized pentamidine (4 patients). Five patients were receiving prophylaxis against *M. avium* infection.

Cytokines and cytokine ELISAs. Recombinant (r) G-CSF (activity: 5000 pg/mL = 150 U/mL) and a truncated placebo form of G-CSF were provided by B. Henderon (Amen, Thousand Oaks, CA, and Genetics Institute, Cambridge, MA). MIP-1α, MIP-1β, MIP-2α, and MIP-2β were provided by B. Sherry (Picower Institute of Medical Research, Manahasset, NY), rIL-8, both monocyte- and endothelial cell–derived, were purchased from Collaborative Biomedical Products (Bedford, MA). G-CSF, GM-CSF, and IL-8 ELISAs were purchased from R&D Research (Minneapolis).

Isolation of peripheral blood mononuclear cells (PBMC), neutrophils, and macrophages. Peripheral blood from consenting donors was collected using acid citrate dextrose as the anticoagulant; 1 mL of 6% Dextran 70 (Baxter Healthcare, Deerfield, IL) was added to 8 mL of blood to sediment the red cells. The buffy coat was removed, washed twice in Ca⁺⁺- and Mg⁺⁺-free Hank’s balanced salt solution (HBSS; BioWhittaker, Walkersville, MD), and layered onto Ficoll-Paque (Pharmacia, Piscataway, NJ). The mononuclear cell layer was removed from the interface, washed twice, and resuspended in Iscove’s modified Dulbecco’s medium (IMDM) (Hyclone, Logan, UT) containing 10% pooled human male serum (Lampire Biologicals, Pipersville, PA) and L-glutamine (Life Technologies Gibco BRL, Gaithersburg, MD).

Neutrophils were isolated from the red blood cell pellet by washing the pellet twice in HBSS and then lysing the red blood cells by adding 0.2% NaCl. The cells were washed three times with HBSS to remove the red blood cell debris and restore isotonicity. The neutrophils were resuspended in complete IMDM and counted. The preparations contained between 95% and 98% neutrophils identified by Wright’s stain.

Macrophages were isolated by adherence to culture plates. PBMC, 10⁶/well in a 48-well plate (Costar, Cambridge, MA), were cultured for 5–7 days in complete IMDM. The nonadherent cells were removed by two vigorous washings with HBSS, and then fresh medium was added to the cultures. By 7 days, a complete monolayer was present, which contained ~10⁵ adherent cells, of which 98%–100% were macrophages, as demonstrated by nonspecific esterase staining.

Growth of *M. avium*. Virulent stocks of *M. avium* were maintained by passaging *M. avium*, serovar 4, in cultures of human macrophages. *M. avium* was plated from macrophage cultures, and smooth transparent colony types were selected for growth in broth. The organisms were cultured for 10–14 days in Middlebrook 7H9 broth (Difco, Detroit) made with endotoxin-free water (Baxter Healthcare) containing albumin, dextrose, and catalase (Difco). The organisms were harvested, centrifuged, and washed twice in HBSS. The cells were resuspended in fresh 7H9 broth, aliquoted, and frozen at ~70°C. An aliquot was thawed and plated in Middlebrook 7H11 agar with oleic acid, albumin, dextrose, and catalase (Difco) to enumerate organisms per milliliter of medium.

Infection of neutrophils, PBMC, and macrophages with *M. avium* and monitoring of bacterial growth. Neutrophils and PBMC were inoculated with *M. avium* immediately after isolation from peripheral blood, while macrophages were infected after 7 days of in vitro culture. Isolated neutrophils or PBMC were plated at a concentration of 10⁶ cells/well in a 48-well plate. All cells were inoculated with 10⁵ *M. avium* well. Individual cytokines were then immediately added to the *M. avium*–infected neutrophils, and the cells were incubated for various time periods. The growth of *M. avium* in tissue culture medium without cells was also determined by adding 10⁵ organisms to 0.5 mL of complete medium in a 48-well plate. The cells were harvested and lysed by the addition of 0.2% SDS to release *M. avium* from the cells. Lysate (100 µL) was inoculated into Bactec 12B vials (Becton Dickinson Diagnostic Systems, Sparks, MD) and incubated for 24 h. The samples were then monitored by the Bactec 460TB system for growth of the organisms. This system determines the amount of [14C]palmitic acid that has been converted to 14CO₂ by metabolism of the bacteria and reports this conversion as the growth index (GI) on a scale of 0–999. The vials were read 24 h after inoculation of the cell lysate and the GI at this time point was recorded. The percentage of reduction in *M. avium* growth was determined by the formula [1 – (GI of treated neutrophils / GI of untreated neutrophils)] × 100.

Determination of neutrophil viability. Neutrophil viability was determined by trypan blue dye exclusion on days 0, 1, and 2. Cytospin cell preparations were also made and stained with Wright’s stain and an acid-fast stain to determine the number of intact neutrophils and the percentage of neutrophils containing intracellular bacteria.

Isolation, culture, and HIV infection of macrophages. Mononuclear cells from the Ficoll-Paque gradients were washed twice with HBSS, resuspended in complete IMDM, and plated in 25-cm² tissue culture flasks (Costar) with vented caps at a concentration of 10⁴ cells/flask. The macrophages were isolated by adherence to plastic for 4–5 days, and the nonadherent mononuclear cells were removed by two vigorous washings with HBSS. Fresh IMDM was added, and the cells were incubated for a total of 7 days before infection with HIV-1.

HIV stocks were maintained by harvesting supernatants from human macrophages infected with HIV-1 in vitro (NIH AIDS Research
and Reference Repository), a monocytotropic strain of HIV-1. Supernatants from infected macrophages were pooled, and the amount of HIV-1 was titrated by diluting the stocks and reinfecting human macrophages with the different dilutions. The amount of virus was determined by the amount of p24 present in the supernatants of infected macrophages using an ELISA (NEN/DuPont Research, Cambridge, MA). Stock HIV-1 for these experiments was titrated at a TCID50 of 5.25, and macrophages were infected at an MOI of 0.1–0.01.

Macrophages were infected with stock HIV-1 for 4 h at 37°C. The monolayers were then washed three times with HBSS, and fresh IMDM was added to the cells. The macrophage cultures were incubated for a total of 14 days before stimulation with M. avium or LPS, which allows chronic HIV-1 infection to occur. After the 14 days, the macrophages were stimulated with M. avium at a ratio of 10 organisms/cell or LPS (Escherichia coli O55:85) (Sigma, St. Louis) at a concentration of 100 ng/mL. The supernatants were harvested at various times after stimulation, filtered through a 0.22-μm filter, and stored at −70°C until assayed for the presence of G-CSF or IL-8 by ELISA.

Effects of macrophage supernatants on the ability of neutrophils to inhibit growth of M. avium. Supernatants from HIV-infected and uninfected treated macrophages, as described above, were added to freshly isolated neutrophils from HIV-negative donors at dilutions of 1:2 and 1:4. The neutrophils were then infected with M. avium at a ratio of 1:1; cells were incubated for 4 days before harvesting to determine the amount of bacteria present.

Statistical analysis. Significant differences in responses between HIV-positive and -negative donors were determined by nonparametric Wilcoxon rank sum analysis. Differences between treatment groups were determined by nonparametric Wilcoxon signed rank analysis.

Results

M. avium growth is inhibited when cultured in the presence of neutrophils. To compare the differences in growth inhibition of M. avium by untreated neutrophils, PBMC, and macrophages, the different peripheral blood cell populations were infected with M. avium. Growth of M. avium was monitored to determine which cell population would best inhibit its growth. The neutrophils significantly inhibited the growth of M. avium at 7 days after infection compared with both PBMC (P = .000) and macrophages (P = .043) (figure 1) when the cells were inoculated with the same amount of M. avium.

rG-CSF stimulates neutrophils to inhibit M. avium growth. To determine whether activation of the cells by various neutrophil-modulating agents could increase the M. avium growth-inhibiting response, neutrophils were incubated with various concentrations of the following factors: rG-CSF, GM-CSF, IL-8 (either monocyte- or endothelial cell–derived), or MIP-1α, -1β, -2α, or -2β. Cells were incubated in the presence of the cytokines and M. avium for 3 days before being harvested. Of the factors, only G-CSF significantly (P = .018) augmented the ability of neutrophils to inhibit the growth of M. avium (figures 2, 3). Optimal stimulating concentrations of G-CSF were 2000 and 1000 U/mL. Comparisons between G-CSF preparations were also made and showed that both rG-CSF for research use and the pharmaceutical grade (Neupogen; Amgen) had similar effects on reduced growth of M. avium, while the truncated form of G-CSF, pG-CSF, did not affect neutrophil growth inhibition of M. avium growth (figure 2). In contrast to the effect of G-CSF on neutrophil function against M. avium, MIP-2β, at concentrations of 200, 100, and 50 U/mL, significantly enhanced growth of M. avium (P = .0001, .0001, and .001, respectively) (figure 3). No significant differences in growth reduction of M. avium were seen between monocyte- or endothelial cell–derived IL-8. Of the 7 HIV-negative donors tested, 6 responded to G-CSF treatment.

In kinetic experiments, we also investigated G-CSF–mediated inhibition of M. avium growth by neutrophils from HIV-negative donors. Neutrophils were incubated with rG-CSF or rGM-CSF at concentrations of 2000 U/mL or medium alone and with M. avium. Maximal differences in the growth of M. avium occurred at 3 days after infection for all treatments (figure 4); therefore, in all other experiments, the neutrophils were incubated with M. avium for 3 days before the cells and bacteria were harvested.

G-CSF enhances neutrophil viability. Viability of G-CSF–treated neutrophils was examined as a mechanism of growth inhibition of M. avium. G-CSF significantly enhanced neutrophil viability in both M. avium–infected and noninfected cells at 24 h after infection (table 1). G-CSF at 1000, 2000, and 4000 U/mL significantly enhanced viability of M. avium–infected neutrophils (P = .039, .044, and .001, respectively) compared with M. avium–infected, non–G-CSF–treated cells. G-CSF
Figure 2. Effect of treatment with recombinant granulocyte colony-stimulating factor (G-CSF), granulocyte-macrophage colony-stimulating factor (GM-CSF), or interleukin (IL)-8 on capacity of neutrophils to limit growth of M. avium. Freshly isolated neutrophils from HIV-negative donors were treated with various concentrations of cytokine and immediately infected with M. avium. Cells were incubated for 3 days and harvested; amount of M. avium was determined by Bactec 460TB system 24 h after inoculation of vials. Endo, endothelial cell–derived; mono, monocyte-derived; pG-CSF, truncated form of G-CSF. Data represent 4 different experiments.

also significantly enhanced neutrophil viability in cells not infected with M. avium at 1000, 2000, and 4000 U/mL ($P = .001, .001, .0001$, respectively) compared with non–G-CSF–treated cells (table 1). By 48 h, viability of both treated and untreated neutrophils was <10%. No significant difference in viability occurred between control noninfected and M. avium–infected neutrophils at any time point.

Light microscopic examination of neutrophils treated with G-CSF demonstrated that many more cells had intact cell membranes and the nuclei were not pyknotic compared with cells not treated with G-CSF. M. avium was associated with 95%–98% of the neutrophils, and no differences in the percentage of infected neutrophils were observed between any of the treatment groups.

G-CSF enhances neutrophils from AIDS patients to inhibit the growth of M. avium. We then investigated whether G-CSF also stimulated neutrophils from AIDS patients to inhibit the growth of M. avium. Neutrophils were isolated and treated with various concentrations of G-CSF, GM-CSF, or monocyte-derived IL-8. At a concentration of 1000 U/mL, G-CSF significantly reduced the growth of M. avium in neutrophil cultures ($P = .008$) (figure 5); at 2000 U/mL, GM-CSF significantly ($P = .019$) enhanced growth inhibition (figure 6). IL-8, however, did not significantly activate neutrophils to reduce the growth of M. avium (figure 6).
Figure 3. Effect of macrophage-inhibitory proteins (MIPs) on \textit{M. avium} growth in neutrophils. Freshly isolated neutrophils from HIV-negative donors were treated with various concentrations of cytokine and immediately infected with \textit{M. avium}. Cells were incubated for 3 days and then harvested; amount of \textit{M. avium} was determined by Bactec 460TB system 24 h after inoculation of vials. % growth represents amount of growth of \textit{M. avium} in treated neutrophils versus growth in non-treated neutrophils and was calculated by formula: (growth index of treated neutrophils ÷ growth index of untreated neutrophils) × 100. Data represent 4 different experiments.

No significant differences were observed between cells from HIV-negative and positive donors in the ability to kill \textit{M. avium} with or without G-CSF treatment.

Neutrophils from 30 (86%) of 35 patients responded to G-CSF. It was of interest to determine whether different clinical parameters would correlate with either responsiveness or nonresponsiveness to G-CSF. We examined the clinical data from the two extreme patterns of responsiveness: patients whose neutrophils responded to all concentrations of G-CSF and patients whose neutrophils did not respond to any concentration of cytokine. Clinical data from a total of 10 patients were examined, 4 responders and 6 nonresponders. Responsiveness to G-CSF was not dependent on CD4 T cells, percentage of neutrophils, total number of neutrophils, or neutrophils per milliliter of peripheral blood (table 2). No statistical correlations were observed between responsiveness to G-CSF and age, ethnic background, risk factor for HIV infection, drug regimens, or existence of other opportunistic infections (table 3). Of note, however, 4 of the 6 nonresponders but none of the G-CSF responders had clinical diagnoses of \textit{P. carinii} infection.

\textit{M. avium} and LPS induce increased production of G-CSF and IL-8 by HIV-infected macrophages. Since G-CSF stimulated neutrophils from both HIV-negative and positive donors to inhibit \textit{M. avium} growth, it appeared that neutrophils from AIDS patients could respond to G-CSF and GM-CSF. We then investigated if in vivo susceptibility to \textit{M. avium} infection was associated with a failure of cells to produce G-CSF or GM-CSF or both.

![Figure 4](https://academic.oup.com/jid/article-abstract/175/4/891/943910)

**Table 1.** % viability of neutrophils treated with different concentrations of granulocyte colony-stimulating factor (G-CSF) and infected with \textit{M. avium}.

<table>
<thead>
<tr>
<th>G-CSF concentration (U/mL)</th>
<th>Uninfected</th>
<th>\textit{M. avium}–infected</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>67.8 ± 4.6</td>
<td>60.1 ± 1.0</td>
</tr>
<tr>
<td>500</td>
<td>72.5 ± 5.8</td>
<td>67.8 ± 3.8</td>
</tr>
<tr>
<td>1000</td>
<td>84.4 ± 6.3</td>
<td>77.0 ± 1.4</td>
</tr>
<tr>
<td>2000</td>
<td>81.2 ± 1.1</td>
<td>76.2 ± 1.0</td>
</tr>
<tr>
<td>4000</td>
<td>91.5 ± 1.6</td>
<td>85.8 ± 7.1</td>
</tr>
</tbody>
</table>

**NOTE.** Data are % viability based on trypan blue uptake. Data represent mean ± SE of 3 different experiments using 3 different sets of donor cells.
HIV infection induced the macrophages to constitutively express both G-CSF and IL-8, although a concurrent HIV and M. avium infection and HIV and LPS stimulation induced significantly more of both cytokines than HIV infection alone. Supernatants from HIV- and M. avium–infected and HIV- and LPS-treated macrophages do not augment neutrophil inhibition of M. avium growth. Because both G-CSF and IL-8 were present in the supernatants of HIV-infected macrophages, we then determined if these supernatants could augment the reduction in M. avium growth by neutrophils isolated from HIV-negative donors. When these neutrophils were incubated with macrophage supernatants containing G-CSF, no enhancement or decrease in the growth of M. avium was seen. Although HIV-1–infected macrophages produced significantly more G-CSF than did non–HIV-infected cells, the amount of G-CSF was considerably less, 88–100 U/mL, than that required to activate neutrophils in vitro, 1000–2000 U/mL.

**Discussion**

Disseminated M. avium infection is one of the most common opportunistic infections associated with AIDS [16]. M. avium

**Figure 5.** Comparison of M. avium growth in neutrophils from HIV-negative and -positive donors treated with and without granulocyte colony-stimulating factor (G-CSF). Freshly isolated neutrophils were incubated with and without 2000 U/mL G-CSF and immediately infected with M. avium for 3 days. After incubation, cells were lysed, and amount of M. avium was determined by radiometric Bactec 460TB system 24 h after inoculation of vials. Neutrophils from HIV-positive donors responding (○) and not responding (●) to G-CSF are shown.

Human macrophages were infected in vitro with HIV-1, then stimulated with either live M. avium, 1 organism per cell, or LPS, 100 ng/mL. Surprisingly, HIV infection increased the quantity and kinetics of production of both G-CSF and IL-8 induced by either M. avium or LPS over that of non–HIV-infected macrophages. HIV-infected cells stimulated with M. avium produced significantly more G-CSF, 2963 ± 124 pg/mL (mean ± SD) (P = .0001), for the longest period of time, 5 days, compared with LPS, 1265 ± 125 pg/mL (figure 7). G-CSF was detectable in non–HIV-infected macrophage supernatants stimulated with M. avium at 5 days, but in low quantities, 339 ± 20 pg/mL.

IL-8 was induced by M. avium and LPS in both HIV-infected and noninfected macrophages. The quantity and kinetics of IL-8 induction, however, were altered by HIV infection (figure 8). HIV infection significantly increased the amount of IL-8 induced by M. avium at 4 (P = .0001), 24 (P = .0001), and 48 h (P = .0001) after infection and also significantly enhanced the quantity of LPS-induced IL-8 at 4 (P = .004), 24 (P = .004), and 48 h (P = .045) and 5 days (P = .0001) after treatment compared with LPS-stimulated, HIV-negative macrophage cultures. In addition, HIV infection induced the macrophages to constitutively express both G-CSF and IL-8

**Figure 6.** Growth of M. avium in neutrophils from HIV-positive donors treated with granulocyte-macrophage colony-stimulating factor (GM-CSF) or interleukin (IL)-8. Freshly isolated neutrophils were treated with 2000 U/mL GM-CSF or 1000 U/mL monocyte-derived recombinant IL-8 and immediately infected with M. avium. Cells were incubated for 3 days and then lysed; amount of M. avium was determined by radiometric Bactec 460TB system 24 h after inoculation of vials. Neutrophils responding (○) and not responding (●) to GM-CSF or IL-8 are shown.
Inhibition of *M. avium* Growth by Neutrophils

Table 2. Comparison of patient data of donors responding and not responding to granulocyte colony-stimulating factor (G-CSF) treatment—reduction in *M. avium* growth, CD4 cell numbers, and neutrophil numbers.

<table>
<thead>
<tr>
<th>Patient group, patient no.</th>
<th>% reduction in <em>M. avium</em> growth* after G-CSF treatment</th>
<th>CD4 cells/mm³</th>
<th>Neutrophils/mL³</th>
</tr>
</thead>
<tbody>
<tr>
<td>Responders</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>−24.5</td>
<td>−32.4</td>
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</tr>
<tr>
<td>3</td>
<td>−23.5</td>
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<td>16</td>
</tr>
<tr>
<td>4</td>
<td>−39.3</td>
<td>−28.2</td>
<td>7</td>
</tr>
<tr>
<td>Nonresponders</td>
<td></td>
<td></td>
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<tr>
<td>5</td>
<td>11.6</td>
<td>60.2</td>
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<td>6</td>
<td>58.4</td>
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<td>80</td>
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<td>7</td>
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<tr>
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<td>14.8</td>
<td>11</td>
</tr>
<tr>
<td>9</td>
<td>66.0</td>
<td>22.6</td>
<td>280</td>
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<tr>
<td>10</td>
<td>40.4</td>
<td>2.1</td>
<td>29</td>
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<tr>
<td>Normal³</td>
<td>−21.1 ± 11.9</td>
<td>−15.2 ± 12.0</td>
<td>&gt;500</td>
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</table>

* % reduction in *M. avium* growth derived by formula: \[ \left( \frac{\text{growth index of treated}}{\text{growth index of control}} \right) \times 100 \]. Negative values, reduction in growth; positive values, increased growth; ND, not done due to insufficient numbers of cells.

³ Isolated neutrophils/mL of blood used for G-CSF studies.

³ Mean ± SE (n = 7).

Some infections occur during late-stage HIV infection (with CD4 T cell numbers usually <100/mm³), whereas *M. avium* is usually nonpathogenic in immunocompetent persons [16]. This organism is ubiquitous, being found in air, soil, and local water supplies [17–19]. The exact mechanisms involved in the breakdown of host resistance to *M. avium* infection in AIDS patients are unclear but may involve innate host resistance mediated by neutrophil function. The results of our study indicate that neutrophils are capable of reducing the growth of *M. avium*. Treatment of neutrophils with rG-CSF enhances the neutrophil function.

Table 3. Comparison of patient data of donors responding and not responding to granulocyte colony-stimulating factor (G-CSF) treatment—clinical data.

<table>
<thead>
<tr>
<th>Patient group, patient no.</th>
<th>Age</th>
<th>EB*</th>
<th>Risk¹</th>
<th>AIDS¹</th>
<th>Other§</th>
<th>HIV infection¹</th>
<th>Other¹</th>
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<td>Responders</td>
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</tr>
<tr>
<td>1</td>
<td>32</td>
<td>W</td>
<td>ST</td>
<td>iCD4</td>
<td>OC</td>
<td>AZT, ddC</td>
<td>TMS, ACY</td>
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<tr>
<td>2</td>
<td>34</td>
<td>W</td>
<td>ST</td>
<td>CMV (6/93)</td>
<td>Diarrhea</td>
<td>AZT, ddC</td>
<td>TMS</td>
</tr>
<tr>
<td>3</td>
<td>52</td>
<td>W</td>
<td>ST</td>
<td>CMV (1/93)</td>
<td>Diarrhea</td>
<td>TMS, G-CSF, RIF, FLU</td>
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</tr>
<tr>
<td>4</td>
<td>36</td>
<td>W</td>
<td>ST</td>
<td>iCD4</td>
<td>OC</td>
<td>AZT, ddI</td>
<td>TMS, CLA</td>
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<td>Nonresponders</td>
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<td>PCP (2/93)</td>
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<td>FLU</td>
</tr>
<tr>
<td>8</td>
<td>36</td>
<td>B</td>
<td>IVDU</td>
<td>PCP (11/88)</td>
<td>CMV</td>
<td>CIP, FLU, AP</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>38</td>
<td>B</td>
<td>IVDU</td>
<td>RBI</td>
<td>Sal</td>
<td>AZT</td>
<td>DAP, FLU, RIF, PRED</td>
</tr>
<tr>
<td>10</td>
<td>38</td>
<td>W</td>
<td>ST</td>
<td>KS (6/92)</td>
<td>M. a sputum</td>
<td>AZT</td>
<td>DAP, FLU, RIF, PRED</td>
</tr>
</tbody>
</table>

* Ethnic background: W = white, B = black, H = Hispanic.

¹ Risk factor for HIV infection: ST = sexually transmitted, IVDU = intravenous drug use.

First clinical diagnosis of AIDS-related disease: CMV = cytomegalovirus, PCP = *P. carinii* pneumonia, RBI = recurrent bacterial infection, KS = Kaposi’s sarcoma, iCD4, decline in CD4 cells. Parentheses indicate month/year diagnosed.

§ Other AIDS-related diseases: OC = oral candidiasis, CMV = cytomegalovirus infection, fungal = fungal sepsis, Sal = salmonella infection, *M. a sputum* = *M. avium* isolated from sputum.

¹ Other concurrent medication: TMS = trimethoprim-sulfamethoxazole, ACY = acyclovir, RIF = rifabutin, FLU = fluconazole, CLA = clarithromycin, DAP = dapsone, CIP = ciprofloxacin, AP = aerosolized pentamidine, PRED = prednisone.
may be related to the relative differences in virulence between *M. avium*, serovar 4, isolated from an AIDS patient and *M. fortuitum*. Mobilization of neutrophils to a specific site by IL-8 and MIPs in mycobacterial infections, however, may play a major role in resistance to infection. Resistance to *M. avium* infection in mice requires T cell–derived MIP-1 and MIP-2, which recruit neutrophils to the affected area [20, 21], and purified protein derivative from *M. tuberculosis* induces IL-8 [22], a potent chemotactic factor for neutrophils; thus, in AIDS patients, MIPs, IL-8, and neutrophils may be deficient.

*M. avium* infection in AIDS patients is a major cause of morbidity, causing persistent fevers, night sweats, weight loss, and diarrhea [23]. Survival of AIDS patients with *M. avium* infection is significantly shorter than for those without *M. avium* infection [6]. There are limitations in the current therapeutic and prophylactic regimens for *M. avium* infections in AIDS patients. Multidrug treatment regimens are only partially effective in eradicating mycobacteremia and improving symptoms but do not extend the shortened life span associated with *M. avium* infection [24, 25]. Furthermore, agents used as prophylaxis are only partially protective in preventing mycobacteremia and may induce development of resistant organisms [26]. Rifabutin, a prophylactic agent, in some cases induces neutropenia and may interact with other drugs that are simultaneously administered, thereby abrogating their therapeutic effects [27].

**Figure 7.** Production of granulocyte colony-stimulating factor (G-CSF) by HIV-infected and noninfected macrophages. Cells were infected with HIV-1_Ba-L_, then incubated with media (○), infected with *M. avium*(1:1)(▲), or stimulated with lipopolysaccharide (100 ng/mL) (■); supernatants were harvested at 4 h or 1, 2, or 5 days after treatment. Amount of cytokine was determined by ELISA. Data are mean ± SD of triplicate samples and are representative of experiments using macrophages from 5 different donors.

**Figure 8.** Production of interleukin (IL)-8 by HIV-infected and noninfected macrophages. Macrophages were infected with HIV-1_Ba-L_, incubated with media (○), and infected with *M. avium*(1:1)(▲), or stimulated with lipopolysaccharide (100 ng/mL) (■). Supernatants were harvested at 4 h or 1, 2, or 5 days after treatment. Amount of cytokine was determined by ELISA. Data are mean ± SD of triplicate samples. Supernatants were collected from same 5 macrophage donors used to generate granulocyte colony-stimulating factor protein data in figure 7.
In addition, the high concentrations of drugs required for antimycobacterial activity are often toxic to the patient and result in discomfort [27]. Combined cytokine plus antibiotic therapy in the treatment of *M. avium* infections in AIDS patients may be of considerable benefit to the patient. Use of G-CSF with clarithromycin [28] and GM-CSF with azithromycin or amikacin [29] in mice has been effective in decreasing *M. avium* bacteremia. G-CSF in combination with quinolone has also been shown to enhance resistance to infection in cancer patients [30]. Cytokine therapy combined with traditional antibiotics may increase the numbers of cells required to elicit a protective host response, thereby decreasing the amount of antibiotics required for effective clearance of the bacteria. Specifically, the use of G-CSF in the treatment of *M. avium* infections could increase both the numbers and the half-life of circulating neutrophils [31]. Many of the antibiotics used to treat mycobacterial infections are taken up by phagocytes and exert their antimycobacterial activity intracellularly [32]. If the viability of the neutrophil in circulation was prolonged, the quantity of antibiotic administered could be decreased because its effect within the neutrophil would be prolonged. In addition, the underlying cause of increased *M. avium* infections in AIDS patients may be the limited numbers of neutrophils able to effectively respond to *M. avium* infection rather than a functional defect of the neutrophil. Use of G-CSF in AIDS patients at risk for *M. avium* infection may also be beneficial by increasing the numbers of circulating neutrophils.

Other studies have shown both normal and dysfunctional activity of neutrophils from AIDS patients in their chemotactic, phagocytic, and bactericidal activity and oxidative metabolism processes [33–41]. G-CSF and GM-CSF have been shown in vitro to enhance neutrophils isolated from AIDS patients to both phagocytize and kill *Staphylococcus aureus* by oxidative metabolism [42, 43]. Mycobacteria may or may not be killed by an oxygen-dependent pathway [44, 45] and, thus, G-CSF may stimulate other metabolites, such as nitric oxide, that may mediate effective mycobacterial killing. In our studies, addition of both G-CSF and GM-CSF to neutrophils from HIV-positive patients inhibited the growth of *M. avium*, although GM-CSF had no effect on neutrophils from HIV-negative donors. These results suggest that neutrophils from AIDS patients may be functionally different from those from HIV-negative donors. In addition, neutrophils from 22% of our AIDS patients did not respond to G-CSF treatment by inhibiting the growth of *M. avium*, whereas neutrophils from 14% of the HIV-negative population did not respond. The increased number of patients not responding to G-CSF compared with healthy donors may also reflect the effects of HIV infection.

The macrophage can be infected with HIV, and although no cytopathic effect is visible, macrophage function is dramatically altered by HIV infection. We have previously shown that a concurrent *M. avium* and HIV infection dramatically increases the production of tumor necrosis factor-α, IL-6, and IL-1β [15]. Results of these studies now also demonstrate that induction of G-CSF and IL-8 by HIV-1–infected macrophages stimulated with either *M. avium* or LPS is also significantly increased and therefore, HIV infection does not inhibit the production of neutrophil-stimulating factors by macrophages. These supernatants did not increase neutrophil antimycobacterial activity, possibly because of the low concentration of G-CSF present. ~100 U/mL. In vivo, however, concentrations of cytokine produced locally and affecting neutrophils are probably much higher.

G-CSF is used clinically to both increase neutrophil numbers and increase their viability while in circulation in both neutropenic cancer and AIDS patients. However, it has not been used specifically to improve host defense against microbial infections, although G-CSF has been shown to decrease the incidence of infections in neutropenic patients [10, 30, 46, 47]. G-CSF is well tolerated by AIDS patients with few side effects [31], allows continuation of myelotoxic drugs [46, 47], and does not increase HIV replication [46, 47]. Use of G-CSF, therefore, in nonneutropenic AIDS patients at risk for *M. avium* infection warrants further consideration as a possible therapeutic agent.

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References

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