Recombinant Human Granulocyte-Macrophage Colony-Stimulating Factor Ameliorates Zidovudine-Induced Neutropenia in Patients With Acquired Immunodeficiency Syndrome (AIDS)/AIDS-Related Complex

By James D. Levine, J. Davis Allan, Judy H. Tessitore, Nathalie Falcone, Frank Galasso, Robert J. Israel, and Jerome E. Groopman

To evaluate the effect of recombinant granulocyte-macrophage colony-stimulating factor (GM-CSF) on patients with acquired immunodeficiency syndrome (AIDS) or AIDS-related complex (ARC) who were intolerant to zidovudine because of neutropenia, we performed a randomized, open-label study in which patients were assigned to one of two groups. Zidovudine was discontinued in group A patients before instituting GM-CSF treatment and was restarted in a graduated fashion over 4 weeks. Group B patients continued on full-dose (1,200 mg/d) zidovudine therapy while beginning GM-CSF therapy. A total of 17 patients were entered, eight in group A and nine in group B. Five of eight patients in group A and seven of nine in group B had a history of Pneumocystis carinii pneumonia (PCP). All were homosexual males, except one female in group A who was the sex partner of a bisexual male with AIDS. All patients had neutropenia (absolute neutrophil count [ANC] < 1,000/µL) while taking full-dose zidovudine. The mean CD4 (± SD) lymphocyte level was 37 (± 29)/µL and 39 (± 44)/µL in groups A and B, respectively. After randomization, patients were begun on subcutaneous GM-CSF at a dose of 1.0 µg/kg/d. Patients in group A received 2 weeks of daily GM-CSF, at which time zidovudine was restarted if the ANC was greater than 1,000/µL; if the ANC was less than 1,000/µL, the dose of GM-CSF was increased to 3.0 µg/kg, and at 2-week intervals either zidovudine was restarted or the dose of GM-CSF was increased to 5 µg/kg and then 10 µg/kg, to maintain the ANC greater than 1,000/µL. Group B patients received full-dose zidovudine concurrently with GM-CSF administration. The dose of GM-CSF was increased every 2 weeks if necessary to keep the ANC greater than 1,000/µL while maintaining full-dose zidovudine therapy. Patients in each group showed an increase in total white blood cell (WBC) count. Neutrophils and eosinophils were responsible for the majority of this increase. Patients in group A had a more rapid increase in WBC than those in group B; however, by week 8, the WBC in each group was essentially equal. Viral replication as measured by human immunodeficiency virus (HIV) p24 antigen (Ag) was decreased in four patients in each group, increased in one patient in each group, and remained unchanged in the remainder. The ability to culture virus from peripheral blood mononuclear cells was not changed by the regimen. The major toxicities of the regimen were fever and malaise. We conclude that daily subcutaneous GM-CSF at relatively low dose is capable of ameliorating neutropenia in patients with AIDS or ARC and can sustain leukocyte counts during concomitant zidovudine therapy.

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ZIDOVUDINE is currently the primary antiretroviral agent that has demonstrated effectiveness in patients with acquired immunodeficiency syndrome (AIDS) and AIDS-related complex (ARC). More than half of the patients who take zidovudine eventually discontinue the drug because of toxicity. Bone marrow suppression resulting in neutropenia and anemia is often the dose-limiting toxicity of zidovudine treatment. Severe anemia can be managed with red blood cell (RBC) transfusion, but the neutropenia cannot be managed in a similar fashion. In addition to the quantitative decreases in leukocytes due to

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Submitted August 27, 1990; accepted August 27, 1991.

Supported in part by Grants No. HL37774-04, HL41374-01, AI24747-02, HL42112-02, and HL43510-01 from the National Institutes of Health, Bethesda, MD; and by DAMD 17-87-C-7017, US Department of Defense.


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0006-4971/91/7812-0010$3.00/0

GM-CSF AND ZIDOVUDINE-INDUCED NEUTROPENIA

was made at the end of every 2-week period (eg, week 2, 4, 6, etc). was supplied by Schering, Kenilworth, NJ, as a lyophilized powder,

week's duration; platelet count greater than 75,000/µL; hemoglobi-

duration of the protocol was 12 weeks. At the end of 12 weeks, the goal was to keep the ANC greater than 1,000/µL and maintain the patient on a zidovudine dose of 1,200 mg/d.

In group A, zidovudine was discontinued and patients were then begun on subcutaneous GM-CSF at a dose of 1 µg/kg/d. The dose of GM-CSF was increased only at the end of weeks 2, 4, 6, 8, 10, and 12 and then only if the ANC was less than 1,000/µL. In group A patients, a decision was made every other week (weeks 2, 4, 6, etc) to either increase the GM-CSF dose or increase the zidovudine, depending on the ANC. For example, if at the end of week 2 the ANC was greater than 1,000/µL, zidovudine was restarted—first at 600/ mg/d and then 2 weeks later, if the ANC was still greater than 1,000/µL, full-dose zidovudine (1,200 mg/d) was begun. If the ANC was less than 1,000/µL at these times, the dose of GM-CSF was increased from 1 µg/kg according to the following dose escalation scheme: 3 µg/kg/d, 5 µg/kg/d, and 10 µg/kg/d. The goal was to keep the ANC greater than 1,000/µL and maintain the patient on a zidovudine dose of 1,200 mg/d.

In group B, patients were continued on full-dose zidovudine (1,200 mg/d) and were begun on GM-CSF at 1 µg/kg/d with the goal of continuing full-dose zidovudine while maintaining an ANC of greater than 1,000/µL. A decision to increase the GM-CSF dose was made at the end of every 2-week period (eg, week 2, 4, 6, etc). For example, if the ANC at week 2 was less than 1,000/µL, the dose of GM-CSF was increased to 3 µg/kg/d. If at week 4 it was still less than 1,000/µL, the GM-CSF dose was increased to 5 µg/kg/d; the next, and last, dose escalation was 10 µg/kg/d. The duration of the protocol was 12 weeks. At the end of 12 weeks, patients who tolerated the zidovudine/GM-CSF combination, and elected to, were continued on their regimen.

The CBC was monitored weekly for the first 4 weeks and then at intervals of every other week. Bone marrow aspirates and biopsies were obtained at baseline and at weeks 4 and 12. HIV p24 antigen, CD4 and CD8 lymphocyte quantitation, and HIV viral cultures were obtained at regular intervals during the course of the study. Prophylaxis for Pneumocystis carinii pneumonia (PCP) was allowed, including aerosolized pentamidine.

RESULTS

Patient characteristics for both groups are shown in Table 1. There were 17 patients enrolled, 16 men and one woman. All but five patients had a history of at least one episode of PCP. The mean (±SD) CD4 helper lymphocyte level was 37 (±29)/µL in group A and 39 (±44/µL) in group B. Ten patients had HIV p24 antigen levels greater than the limit of detection (50 pg/mL) (five in group A, five in group B) at enrollment in the study.

Hematologic and immunologic effect. The hematologic response to GM-CSF is shown in Fig 1. Within 1 week of commencing once daily subcutaneous GM-CSF, the leukocyte count increased significantly in both groups. Increases in neutrophilic and eosinophilic granulocytes were responsible for the majority of the increase in the white blood cell (WBC) count (Figs 2 and 3). There was no consistent or sustained change in either lymphocyte (including CD4 and CD8 cells) or monocyte cell counts over the course of the study. The increase in neutrophil count was chronologically earlier and of greater magnitude in the patients who discontinued zidovudine on institution of GM-CSF therapy (group A). However, the difference was only significant at week 2 (P = .028) and thereafter there were no significant differences in ANC between the groups.

The mean platelet counts (Fig 4) in patients remaining on the study did not change in either group. However, this may be misleading, as a total of five patients were withdrawn from the study because of significant thrombocytopenia (platelet count <50,000/µL), which was a protocol criteria for removal from study and discontinuation of both GM-CSF and zidovudine. Most of these patients had resolution of their thrombocytopenia after discontinuing

<table>
<thead>
<tr>
<th>Table 1. Patient Characteristics on Entry to Study</th>
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<tr>
<td>Male</td>
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<tr>
<td>Female</td>
</tr>
<tr>
<td>Total</td>
</tr>
<tr>
<td>Age (yr)</td>
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<tr>
<td>History of previous OI (PCP)</td>
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<tr>
<td>Kaposi sarcoma</td>
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<td>CD4 cells (per µL)</td>
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<td>Performance status</td>
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<td>ECOG 1</td>
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<td>ECOG 2</td>
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Abbreviations: OI, opportunistic infection; ECOG, Eastern Cooperative Oncology Group.
Fig 1. Change in total WBC count during the course of subcutaneous GM-CSF administration. The results shown are the means (±SD) of the patients in each group. (●) Group A; (○) group B. Differences between groups are not significant.

Fig 2. Change in the ANC during the course of subcutaneous GM-CSF administration. The results shown are the means (±SD) of the patients in each group. Differences between the groups are not significant, except at week 2: $P = .028$.

Fig 3. Change in the eosinophil count during the course of subcutaneous GM-CSF administration. The results shown are the means (±SD) of the patients in each group. Differences between groups are not significant.

Fig 4. Platelet counts during course of subcutaneous GM-CSF. Differences between groups are not significant.

Both zidovudine and GM-CSF. The baseline platelet count did not predict the later development of significant thrombocytopenia. Significant thrombocytopenia occurred at weeks 5 and 10 in group A patients and at weeks 4, 7, and 12 in group B patients. Hemorrhagic manifestations did not occur in any of the significantly thrombocytopenic patients. There were adequate numbers of megakaryocytes seen in the bone marrow in the only patient who had a bone marrow examination at the time of development of thrombocytopenia.

Neither the reticulocyte count nor the hematocrit was affected by GM-CSF treatment. A total of 15 patients (seven of eight in group A and eight of nine in group B) required packed RBC transfusion for symptomatic anemia.

Bone marrow aspirates and biopsies did not show any significant changes by light microscopy other than for previously described increases in overall cellularity on GM-CSF. There were adequate numbers of megakaryocytes present in the one patient who had a bone marrow examination performed within 7 days of developing thrombocytopenia. No patient that demonstrated anergy during baseline evaluation regained the ability to respond to skin test antigens when retested.

Subjects in group B were more likely to require an increase in GM-CSF dose to maintain the ANC greater than 1,000/µL (Table 2), although in the majority of patients in both groups a GM-CSF dose of 1 µg/kg was adequate. At week 4, three subjects required dose escalation to 3 µg/kg. Only one patient required dose escalation to 5 µg/kg, and this occurred at week 12 after an initial satisfactory response that lasted 8 weeks. At the same time, this individual developed fever and significant malaise and subsequently discontinued both drugs with gradual resolution of symptoms. However, he was diagnosed with his first episode of PCP a few weeks later.

GM-CSF effects on HIV. All patients had serum HIV p24 Ag levels measured at baseline. The serum HIV p24 Ag levels in group A measured at end of study or at discontinuation from study remained essentially unchanged when
compared with baseline levels in four of eight patients (Fig 5). Levels were increased in two of eight patients and decreased in two of eight patients. In group B patients, HIV p24 Ag levels remained below the detectable limit of 50 pg/mL in four of nine patients, decreased in four of nine patients, and increased in one patient (Fig 5).

During the first 4 weeks of the study, at the time interval when group A patients were not taking zidovudine, one patient in group A demonstrated an increase in HIV P24 Ag after discontinuing zidovudine. The HIV p24 Ag was 295 pg/mL at baseline (on zidovudine alone). Then, after GM-CSF was started, it decreased to 51 pg/mL at week 1, 88 pg/mL at week 2, and 96 pg/mL at week 4.

The frequency of recovery of HIV from cultures of patients' peripheral blood monocytes did not change over the course of the study. Thus, the combination of GM-CSF and zidovudine did not appear to consistently increase HIV P24 Ag or other measures of HIV activity in these study patients and may have resulted in a decrease in viral activity.

**Infections on-study.** Patients in both groups either had recurrent or persistent viral and/or fungal infections. Documented bacterial infections occurred in four patients, two in each group (Table 3). The ANC in the 2 group A patients was greater than 1,500/µL in each at the time of diagnosis of infection. The ANC in the group B patient with the rectal abscess was 4,480/µL when he went to surgery for drainage. The patient who developed *Pseudomonas* pneumonia and bacteremia had an ANC of 2,937/µL the week before his diagnosis, which decreased to 220/µL at diagnosis, resulting in a dose increase of GM-CSF to 3 µg/kg. In addition to these culture-documented bacterial infections, three infections clinically diagnosed as bacterial in origin occurred.

**Table 3. Infections on-Study**

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Diagnosis</th>
<th>Treatment</th>
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<tbody>
<tr>
<td>Group A</td>
<td></td>
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</tr>
<tr>
<td>02</td>
<td>Oral thrush</td>
<td>Clotrimazole troche</td>
</tr>
<tr>
<td>04</td>
<td>CMV retinitis</td>
<td>Gancyclovir</td>
</tr>
<tr>
<td>06</td>
<td>Herpes simplex virus</td>
<td>Ayclovir</td>
</tr>
<tr>
<td>07</td>
<td>Herpes zoster</td>
<td>Ayclovir</td>
</tr>
<tr>
<td>05</td>
<td>Herpes simplex virus</td>
<td>Clotrimazole troche</td>
</tr>
<tr>
<td>09</td>
<td>Sinusitis</td>
<td>Cefalosporin, clindamycin</td>
</tr>
<tr>
<td>13</td>
<td>Herpes simplex virus</td>
<td>Ayclovir</td>
</tr>
<tr>
<td>15</td>
<td>Pneumonia/bacteremia</td>
<td>Ayclovir, cefazolin, tobramycin</td>
</tr>
<tr>
<td>17</td>
<td>Perirectal abscess</td>
<td>Surgery, penicillin, flagyl,</td>
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<td>ciprofloxacin</td>
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Additionally, one patient in group B demonstrated a decrease in HIV P24 Ag after starting GM-CSF. The HIV p24 Ag was 295 pg/mL at baseline (on zidovudine alone). Then, after GM-CSF was started, it decreased to 51 pg/mL at week 1, 88 pg/mL at week 2, and 96 pg/mL at week 4.
These infections included two episodes of cellulitis and one episode of sinusitis. All of the bacterial infections responded to antibiotic therapy.

Side effects. The majority of patients tolerated treatment with GM-CSF and zidovudine well. Side effects, such as fever and malaise, appeared to increase with increasing GM-CSF dose. Table 4 illustrates the major side effects we observed. Most patients experienced mild to moderate malaise. This appeared to resolve as the study progressed and patients' complaints decreased over time unless progression of disease became an issue. Bone pain occurred in five patients, but was not sufficiently severe to warrant withdrawal from study. Fever, malaise, and bone pain usually were alleviated with ibuprofen. Local inflammation at the injection site was common in all patients at initiation of GM-CSF therapy, but rapidly decreased in severity within a few weeks. No episodes of phlebitis were observed.

Table 2 lists the reasons patients were discontinued from the study. After thrombocytopenia, the major reason was either progression of their underlying HIV-related disease or the need for additional drugs that could be marrow suppressive (such as gancyclovir for CMV infections). Four patients, two in each group, remained on study after the initial 12 weeks. Three of these four were on a GM-CSF dose of 1.0 μg/kg/d. The fourth patient had his dose escalated to 5 μg/kg/d at week 12 and was discontinued at week 15 because of fever and fatigue. He was diagnosed as having his first episode of PCP pneumonia shortly after dropping the protocol.

DISCUSSION

We have studied the safety and efficacy of self-administered subcutaneous recombinant human GM-CSF in AIDS and ARC patients who were neutropenic because of their disease and/or zidovudine therapy. As opposed to patients with neoplasia receiving GM-CSF to alleviate chemotherapy-induced marrow suppression, our patients required relatively low doses of GM-CSF to maintain a neutrophil count greater than 1,000/μL. Patients receiving chemotherapy often require five to 10 times the 1 μg/kg dose necessary in the majority of our HIV-infected patients to overcome myelosuppressive therapy. The explanation for this observation may simply be that zidovudine and HIV are not as marrow toxic as the chemotherapy regimens given to patients with cancer.

Discontinuation of zidovudine before the administration of GM-CSF (group A) resulted in a more rapid increase in WBC count than in patients who continued zidovudine (group B). However, by 6 weeks (2 weeks after group A patients were on full-dose zidovudine [1,200 mg/d]), there was no difference in either the total WBC count or the ANC in either group. This demonstrates both the significant myelotoxicity of zidovudine and the ability of relatively low doses of GM-CSF to overcome this toxicity without first discontinuing zidovudine.

The subcutaneous route for administration of GM-CSF allows for self-administration and was tolerated well in our study. Almost all of our subjects developed local erythema at the injection site, but this was not a significant problem at the doses given and, in most patients, erythema resolved as the subjects became more facile with self-injection. The constitutional symptoms that led to discontinuation of GM-CSF may result from several factors. Our cohort of patients was in a poor prognostic group with a high incidence of opportunistic infection. They had advanced HIV infection and significant immune suppression as indicated by past medical history, and low numbers of CD4 lymphocytes and neutrophils. Many of these patients had persistent low-grade fever and impaired performance status before entry into the study, and it was often difficult to differentiate progression of AIDS from drug toxicity. A larger group of patients will be required to determine this.

One possible explanation for fever, bone pain, and malaise may be GM-CSF's stimulation of monocytes to release cytokines such as tumor necrosis factor (TNF) and interleukin-1, which are capable of inducing these symptoms. Increases in serum TNF have been demonstrated in cancer patients receiving continuous infusion GM-CSF, although it remains to be demonstrated that the low doses of GM-CSF given in this study are also capable of increasing the secretory activity of monocytes in AIDS patients.

In the absence of concomitant zidovudine, thrombocytopenia has not been observed in our previous intravenous and subcutaneous studies of GM-CSF treatment of AIDS and ARC patients. In light of the observed improvement in thrombocytopenia in many HIV-infected patients with zidovudine therapy, the development of significant thrombocytopenia (<50,000/μL) in our patients was an unexpected occurrence. There are several possible explanations for this observation. One is simply that platelet counts in HIV-infected patients may wax and wane due to idiopathic thrombocytopenic purpura (ITP). Because of intense hematologic scrutiny of patients in this study, declining platelet counts may have been more frequently detected and resulted in withdrawal from the study as per protocol requirement. Another possible explanation is that thrombocytopenia resulted from zidovudine-induced marrow toxicity that was not observed in previous zidovudine studies because neutropenia would precede thrombocytopenia in patients not receiving GM-CSF, and zidovudine treatment would be discontinued before significant thrombocytopenia could develop. A third and more intriguing hypothesis is that the administration of GM-CSF stimulated macrophage phagocytosis so that clinically significant ITP was either induced or accelerated and zidovudine-induced marrow suppres-
GM-CSF and Zidovudine-Induced Neutropenia

GM-CSF and zidovudine-induced neutropenia

sion prevented increased platelet production. Platelet-bound immune complexes and antiplatelet antibody have both been implicated in the pathogenesis of thrombocytopenia in patients with HIV infection.\(^{13,14}\) Stimulation of tissue macrophages by GM-CSF could result in accelerated clearance of immunoglobulin coated platelets. In support of this possibility is the report of relapse of ITP in a patient receiving GM-CSF for advanced malignancy.\(^{15}\)

As has been observed in other reports on the clinical use of GM-CSF, eosinophils make up a significant part of the WBC response. We could not discern any toxicity resulting from this eosinophilia, although the duration of the study may not have been sufficient to result in clinically evident side effects.

Bacterial infections occurred in these patients despite maintaining the ANC greater than 1,000/µL. The study was not designed to demonstrate efficacy in preventing infections, as we did not compare the rate of infection to a control group that was neutropenic and not given GM-CSF. The use of GM-CSF did not appear to prevent migration of neutrophils, as demonstrated by abscess and cellulitis formation.

The observation that infections, particularly opportunistic bacterial infections, occurred in these patients despite normal neutrophil counts is not surprising and is further evidence that the deficit in the ability to defend against microbial infection in HIV-infected patients involves other factors such as specific defects in antibody production or qualitative neutrophil abnormalities and not simply neutrophil numbers.

Recombinant GM-CSF elevated the ANC in neutropenic patients and allowed continued use of full-dose zidovudine in patients who otherwise would have possibly had to discontinue or alter their antiretroviral therapy. Our study was not designed to demonstrate efficacy of the combination of zidovudine plus GM-CSF in changing the natural history of AIDS or ARC. We did not observe a consistent change in serum HIV p24 Ag levels or increased recovery of HIV from monocytes cultured in vitro during the study. In vitro data suggest that GM-CSF may increase replication within macrophages of certain isolates of HIV particularly tropic for these cells, making the addition of GM-CSF to the regimen of patients with HIV infection somewhat problematic and controversial.\(^{16,17}\) However, both this study and our previous studies of GM-CSF in AIDS patients\(^{1,11}\) have failed to demonstrate consistent stimulation of viral production that could be attributed to GM-CSF alone and not to the discontinuation of an antiviral agent. In addition, Pluda et al\(^{18}\) have shown increases in HIV P24 Ag levels in patients on GM-CSF, but only when zidovudine had been discontinued. We observed a similar effect in one patient in whom an increase in serum HIV p24 Ag was noted when zidovudine was discontinued. This was followed by a decrease to pre-GM-CSF levels within 2 weeks of restarting zidovudine. There is also in vitro evidence that GM-CSF enhances the antiviral effect of zidovudine, probably by increasing drug entry into the monocyte and thereby increasing intracytoplasmic levels of zidovudine.\(^{19}\) We did not measure intracellular zidovudine levels; this phenomenon could possibly explain the decrease in HIV p24 Ag levels in patients on the combination. Studies should be performed to test whether this in vitro phenomenon is important clinically and whether increasing the ANC to normal in neutropenic AIDS/ARC patients changes infectious events, especially bacterial infections. In addition, the future use of the combination will also be determined by the efficacy of other less myelosuppressive antiretroviral agents now being studied.

We have demonstrated overall tolerance of the combination, as well as certain potentially limiting toxicities, particularly thrombocytopenia. The optimal use of GM-CSF in HIV-infected patients receiving zidovudine will require further clinical trials, especially now that the recommended dose of zidovudine has been decreased, apparently without loss of efficacy.

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