Combined Therapy With Recombinant Granulocyte Colony-Stimulating Factor and Erythropoietin Decreases Hematologic Toxicity From Zidovudine

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Twenty-two patients with acquired immunodeficiency syndrome (AIDS) or severe AIDS-related complex and multileg hematopoietic defects were treated with recombinant granulocyte colony-stimulating factor (G-CSF) and erythropoietin (EPO) in a phase I/II trial. All patients were neutropenic and anemic after withdrawal of all bone marrow-suppressive drugs. Daily, G-CSF was subcutaneously self-administered therapy was continued with dose modification to maintain granulocyte colony-stimulating factor (G-CSF) and erythropoietin (EPO) was added to the regimen and the dose increased until an increase of 15 g/L of hemoglobin was observed. Groups of patients were administered increasing doses of zidovudine to determine their tolerance. G-CSF and EPO therapy was continued with dose modification to maintain an ANC greater than 1,500/µL and hemoglobin greater than 100 g/L. The dose of zidovudine was not altered. All 22 patients responded to G-CSF with a mean 10-fold increase in neutrophils occurring in less than 2 weeks. Significant increases in CD4 and CD8 cell number, lymphocyte proliferative response, and bone marrow cellularity were seen. EPO therapy increased hemoglobin in all 20 evaluable patients within 8 weeks. Sixteen patients received 1,000 mg and four patients received 1,500 mg of zidovudine per day. The reinstitution of zidovudine resulted in a decline in reticuloocytes and hemoglobin and the reappearance of transfusion requirements in eight of the 20 patients, six of whom had the study medications stopped. No patient had the study medications stopped because of neutropenia or thrombocytopenia. Toxicities were mild and did not require dose modifications. Limiting dilution plasma and lymphocyte co-cultures for HIV as well as serum p24 antigen levels did not change significantly during G-CSF or combined G-CSF and EPO therapy. HIV p24 antigen decreased significantly with zidovudine therapy. Opportunistic infections occurred in 14 patients but were successfully treated with myelosuppressive antimicrobial agents, including ganciclovir, without the development of neutropenia. These results suggest that combined therapy with G-CSF and EPO may improve the neutropenia and anemia of AIDS. Combined therapy may allow the resumption of full-dose zidovudine in most patients intolerant of the hematologic effects of zidovudine without apparent alteration of HIV expression or the efficacy of zidovudine.

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Hematopoietic failure and poor hematologic tolerance of therapies for acquired immunodeficiency syndrome (AIDS) and its complications are serious impediments to the successful treatment of patients with HIV infection. Decreased hematopoietic reserve may necessitate the use of less efficacious antiviral therapy or dose and schedule modifications of other myelosuppressive agents for HIV-related complications. For example, the anemia and neutropenia associated with zidovudine use requires reductions in the dose of zidovudine and may prevent or limit the simultaneous use of zidovudine with other effective agents such as ganciclovir, trimethoprim-sulfamethoxazole, α-interferon, or pentamidine. Because of the potential for hematopoietic suppression, alternative regimens for the treatment of Pneumocystis carinii pneumonia, such as aerosolized pentamidine, were developed and chemotherapy regimens for lymphomas were substantially altered. Even in patients who initially tolerate myelosuppressive therapy, long-term use of these agents in patients with AIDS is frequently associated with periods of dose reduction or discontinuation for hematologic toxicity. Unfortunately, dose reductions of effective medications or the use of alternative therapies may result in decreased clinical effectiveness, may result in an increase in the likelihood of early development of resistance and relapse, or allow premature death from the inability to treat adequately the complications of HIV infection.

The use of hematopoietic stimulants to overcome drug-related hematopoietic suppression is one of several approaches to treatment of patients with HIV infection and bone marrow failure. Granulocyte-macrophage colony-stimulating factor (GM-CSF) has been shown to restore normal neutrophil levels in patients with severe neutropenia and HIV infection. GM-CSF is well tolerated in long-term administration and can be safely administered by daily subcutaneous injection. GM-CSF has been shown to potentiate the anti-HIV activity of zidovudine in peripheral blood mononuclear cells as well as to stimulate monocyte function in vitro and in vivo in the setting of HIV infection. Studies of GM-CSF with zidovudine and ganciclovir suggest that GM-CSF may ameliorate the neutropenia associated with some myelosuppressive agents.

Similarly, erythropoietin (EPO) is effective in the treatment of anemia associated with AIDS. Placebo-controlled trials show that exogenously administered EPO significantly reduces transfusion requirements in patients with AIDS receiving zidovudine. Despite the reduction in transfusion requirements, the mean monthly dose of zidovudine taken by all patients decreased over time. Other toxicities including progressive leukopenia and thrombocytopenia may have required use of reduced doses of zidovudine.

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Clearly, the multiple hematopoietic defects in AIDS will require a multifactorial approach to treatment. Because each hematopoietin has unique, isolated biologic activities, finding the right combination of bone marrow stimulants that augment hematopoiesis without altering the course of HIV infection may be difficult. In this study, we examined whether two recombinant hematopoietins, granulocyte CSF (G-CSF) and EPO, could be safely administered together and whether combined therapy could decrease zidovudine hematologic toxicity.

MATERIALS AND METHODS

Study population. Twenty-two patients with AIDS or severe AIDS-related complex, leukopenia, and anemia were enrolled in the study. Two recombinant human hematopoietins, G-CSF (Amgen, Thousand Oaks, CA) and EPO (AmGen), and commercially available zidovudine were used. There were no competing trials and all eligible patients were enrolled. Approval was obtained from the UCLA Human Subjects’ Protection Committee and informed consent was obtained from each patient before initiating therapy.

Patients stopped all bone marrow suppressive drugs including zidovudine and cotrimoxazole for more than 4 weeks before study entry. Entry criteria required a neutrophil count of less than 2.5 X 10^9/L, hemoglobin of less than 120 g/L, and platelet count of greater than 0.5 X 10^9/L after this washout period. Patients did not have active opportunistic infections requiring therapy with myelosuppressive medications at the start of therapy. Extensive evaluations were performed in patients with fever to exclude those patients with unrecognized infections. Two patients with *mycobacterium avium intracellulare* infection not receiving active therapy for this infection were enrolled in the study.

Treatment regimen. In the first stage of treatment, patients self-administered 3.6 mg/kg G-CSF by a daily subcutaneous injection. The G-CSF dose was escalated in 1.2-mg/kg increments each week until an absolute neutrophil count of greater than 6 X 10^9/L was achieved and maintained for 2 weeks. The maximum dose of G-CSF allowed was 7.2 mg/kg per day. Because the starting dose of G-CSF resulted in very high neutrophil counts in some patients (>25 X 10^9/L), the dose of G-CSF was adjusted after the first 11 patients were entered to maintain a lower target neutrophil range (1.5 to 5.0 X 10^9/L).

After correction of their neutropenia, patients began self-administration of EPO at 150 IU/kg three times a week by subcutaneous injection while continuing G-CSF therapy. The dose of EPO was increased 50 to 100 IU/kg every other week until the patient's hemoglobin level increased by at least 15 g/L over the baseline value or a maximum dose of 500 IU/kg per day was attained.

After both the leukopenia and anemia had been corrected, successive groups of eight patients were given zidovudine at 1,000 or 1,500 mg per day in five divided doses. Two additional patients were enrolled for each patient experiencing a grade 3 or greater (Eastern Cooperative Oncology Group [ECOG]) toxicity. The dose of zidovudine was not altered during the initial 12 weeks of the trial, during which time the dose of G-CSF was increased or decreased by 1 mg/kg weekly to maintain a target absolute neutrophil count (ANC) of greater than 1.5 X 10^9/L. The dose of EPO was adjusted biweekly to maintain the hemoglobin level greater than 100 g/L. The maximum dose of EPO allowed was 500 IU/kg/d. The dose of zidovudine at which 50% of the patients experienced grade 3 or greater toxicity was defined as the maximum tolerated dose (MTD).

Duration of treatment and dosage modifications. Therapy was stopped because of hematologic intolerance of zidovudine (grade 4 ECOG hemoglobin toxicity or the persistence of grade 4 ECOG neutropenia without correction with EPO and G-CSF). Patients who required myelosuppressive, anti-infective therapy for opportunistic infections (primarily ganciclovir) and transiently required packed red blood cell support were allowed to continue. No other indication for discontinuation was encountered.

Concomitant medications. All patients received aerosolized pentamidine, 300 mg administered via Respigard II nebulizer (Marquest, Chicago, IL), once a month as prophylaxis for *Pneumocystis carinii* pneumonia. Patients who developed opportunistic infections during the trial were treated with the best therapy available even if this medication was myelosuppressive. Iron supplementation was administered to all patients. This administration consisted of 325 mg orally per day. In those patients who developed iron deficiency despite of this supplementation, intravenous dextran was administered in one dose to correct their iron deficit.

Study evaluations and follow-up. Hematologic and clinical monitoring was performed twice a week during the G-CSF dose finding period. Patients were seen weekly during the first 24 weeks of study and bimonthly after that. Complete blood counts were obtained with each visit and a complete chemistry panel was obtained each month. Leukocyte alkaline phosphatase (LAP) score, ferritin, iron, iron-binding capacity, and urinalysis were performed before and after each stage of treatment. The first eight patients underwent bone marrow examination at study entry. Bone marrow examinations were repeated after treatment with G-CSF and after combined therapy with EPO and G-CSF in these patients.

Peripheral blood burst forming unit erythroid (BFU-E) assays were performed on the first 13 patients. Eight of these patients had these assays performed before cytokine therapy. The standard BFU-E assay was modified to incorporate 0, 0.01 µmol/L, 0.1 µmol/L, 1.0 µmol/L, and 10 µmol/L zidovudine in the semi-solid agar. This assay allowed determination of the 50% inhibitory concentration (ID₅₀) of zidovudine for BFU-E.

Standard assays for neutrophil function using *Staphylococcus aureus* phagocytosis and intercellular killing were performed on the first eight consecutive patients before and during G-CSF therapy. Opportunistic infections were evaluated using recommended guidelines of the NIAID AIDS Clinical Trials Group (ACTG) for evaluations of patients with fever, night sweats, weight loss, persistent diarrhea, or ocular complaints. Standard tests were used to document infections.

All patients had limiting dilution HIV cultures of both plasma and peripheral blood mononuclear cells performed at baseline, after treatment with G-CSF, after treatment with G-CSF and EPO, and after 12 weeks of combined G-CSF, EPO, and zidovudine therapy or at the time of discontinuation from study. These cultures were done by a modification of the technique of Coombs et al. In brief, 1 x 10⁶ to 4 x 10⁷ peripheral blood lymphocytes were cultured with 1 x 10⁸ phytohemagglutinin (PHA)-stimulated normal lymphoblasts in 2 mL of Iscove’s modified Dulbecco’s media containing 10% fetal bovine serum and 10 U/mL of interleukin-2 (AmGen). For the plasma cultures, fresh plasma was diluted 1:2 with media and filtered through 0.45-µm filters. This diluted plasma was added (400 µL, 80 µL, 40 µL, and 4 µL) to 1 x 10⁶ of the PHA-stimulated normal lymphoblasts. These dilutions are equivalent to 5, 25, 50, and 500 tissue culture infective doses (TCID) per milliliter. Additional PHA lymphoblasts (1 x 10⁷) were added on day 7. The cell-free supernatant was assayed for HIV antigens by the Abbott HIV Antigen ELISA (Abbott Laboratories, Abbott Park, IL) on day 14. Samples with more than 300 pg of antigen were scored as positive. In addition, sera was obtained...
HIV antigen kit according to the manufacturer's instructions. Only weekly and frozen for later analysis of HIV antigens using the same batch-tested HIV antigen results are used for purposes of data analyses.

Complete T-lymphocyte subset analyses, PHA-proliferative responses, and delayed hypersensitivity testing were performed at baseline, after each stage of therapy, and at discontinuation of study medications.

Data management and statistical analyses. Patients who were hospitalized at outside institutions during the study had their complete hospital records obtained and analyzed for the purposes of this study. Follow-up is complete on all enrolled patients.

Data analysis was done using SAS software version 6.02 (SAS Institute, Inc, Cary, NC). Data sets were reviewed by three individuals for accuracy before analysis. For continuous variables, a two-sided sign rank test was performed. For discontinuous variables, either a two-sided Fisher exact test or $\chi^2$ test was used. For intervening pretreatment and posttreatment values, a mean weighted slope and variance analysis was performed and compared with a line with zero slope for statistical significance. All $P$ values cited in the text are two-sided sign rank values unless otherwise stated.

For analysis of the limiting dilution lymphocyte and plasma cultures, the lowest dilution titer (in TCID) that was positive was used. If the culture was negative at the lowest titer, the lowest TCID minus one was used. This dose underestimates the value of developing a negative culture but also may underestimate the peak of infectious virus. This is a conservative approach for analysis of left censored data. Given the wide range in measurable values, both the titer and the log of the titer were used in separate analyses.

**RESULTS**

Twenty-two patients with severe HIV infection and Center for Disease Control-defined AIDS were enrolled on this study over 9 months. Two patients were discontinued from study medications before the reinstitution of zidovudine (one death and one repeated suicide attempt) and are not evaluable. The patients uniformly had very advanced stages of HIV infection. All but three patients had a history of opportunistic infections and over half the patients had previously been on zidovudine for more than 18 months. Four of the patients had been unable to take zidovudine for more than 3 months before study entry because of severe bone marrow hypoplasia. The remainder of the patients had received doses of zidovudine between 100 and 400 mg/d before study entry. These medications were stopped 4 weeks before study entry. The median CD4 value for the group was only 16.5 cells/µL (range, 3 to 116). All patients were anergic by delayed hypersensitivity skin testing and 71% had detectable HIV serum antigens at entry.

**Hematologic effects of therapy.** Within 48 hours of initiation of G-CSF therapy, significant increases in leukocyte count with normalization of neutrophil number were seen in all patients (Fig 1). In the first 11 patients the G-CSF dose was held constant despite steadily increasing neutrophil counts. These patients were maintained at 3.6 µg/kg for up to 13 weeks without untoward side effects. The mean neutrophil count in this subpopulation was $19.7 \times 10^9$/L (range, $3.6$ to $37.4 \times 10^9$/L). Over 85% of these cells were segmented neutrophils and neutrophil precursors.

Reduced to $<5.5 \times 10^9$/L, these patients, decreases in the doses of G-CSF were required. For most patients, doses of 0.30 to 1.2 µg/kg/d were adequate to maintain the target neutrophil count while on zidovudine and, in some cases, ganciclovir.

Neutrophil function studies were performed on the first eight patients. One patient did not have enough circulating neutrophils before study for testing. Of the remaining seven patients, all had normal phagocytosis and six had normal intracellular killing of *S aureus* before G-CSF therapy. In the single patient with an intracellular killing defect before G-CSF therapy, this defect was corrected during administration of G-CSF.

All 20 evaluable patients who were given EPO increased their hemoglobin more than 15 g/L in the absence of zidovudine. The mean dose required to achieve the target increase in hemoglobin was $238 \pm 84.4$ IU/kg administered.
by a single subcutaneous injection three times a week. The mean time to observe the target increase was 5 ± 3.4 weeks. This increase in hemoglobin was associated with changes in other markers of iron use including significant decreases in serum iron, iron saturation, and ferritin levels (Table 1) despite oral iron supplementation. Two patients with adequate iron stores at entry became iron deficient with a decline in reticulocytosis while on oral iron and required parenteral supplementation.

Twenty patients were treated with both the recombinant hormones and zidovudine. Sixteen patients received 1,000 mg of zidovudine and four patients received 1,500 mg of zidovudine for greater than 12 weeks. Combined therapy with G-CSF and EPO was successful in preventing neutropenia but was only partially successful in ameliorating the anemia associated with the reinstitution of zidovudine therapy. Although neutrophil counts declined significantly with the addition of zidovudine, the mean nadir neutrophil count (5.4 ± 0.8 × 10^9/L; range, 1.5 to 15.0 × 10^9/L) was equal to the target value and was significantly greater than the mean prestudy neutrophil count in the absence of zidovudine. Thus, the decline in neutrophil count may be a result of a readjustment of the target neutrophil count. As shown, the dose of G-CSF after zidovudine was lower than after G-CSF alone, although this was higher than after G-CSF and EPO (Fig 2).

While no patient was removed from therapy because of neutropenia, six patients were taken off the study after the reinstitution of zidovudine therapy for anemia that required transfusions. No pretreatment factor, including serum EPO levels, accurately predicted the development of anemia while on EPO and zidovudine. This result is in disagreement with prior studies of EPO^15 and may reflect the fact that patients in this study were not receiving any myelosuppressive therapy at the time of study entry. However, we noted that all the patients who failed to maintain the EPO-induced reticulocytosis in the first week of zidovudine therapy became transfusion dependent.

To discover the mechanism of severe zidovudine intolerance, we measured the sensitivity of red cell precursors in the peripheral blood for inhibition by zidovudine at various stages of therapy. At baseline, the mean circulating peripheral blood BFU-E was significantly reduced to 50% ± 23% of normal controls (P < .0001, n = 8), although two patients had normal values. In addition, the mean 50% inhibitory concentration of BFU-E for zidovudine (ID50) at baseline was 0.43 ± 0.13 μmol/L. This value is well below the expected 1.0 μmol/L mean serum concentration ex-

| Table 1. Laboratory Values After Each Stage of Drug Treatment |
|---------------------------------|-----------------|-----------------|-----------------|
| | Baseline | After G-CSF | After G-CSF and EPO | After G-CSF, EPO, and AZT |
|---------------------------------|-----------------|-----------------|-----------------|
| Hematology Studies              |                 |                 |                 |
| Leukocyte count (× 10^9/L)      | 2.2 ± 0.1       | 14.6 ± 1.9*     | 12.6 ± 2.3*     | 6.3 ± 0.9**††     |
| Absolute neutrophils (× 10^9/L) | 1.4 ± 0.1       | 12.8 ± 1.9*     | 10.8 ± 2.1*     | 5.4 ± 0.9**††     |
| Hemoglobin (g/L)                | 105 ± 2         | 106 ± 3         | 125 ± 3*†       | 105 ± 6†         |
| Absolute reticulocytes (g/L)    | 0.65 ± 0.09     | 0.76 ± 0.07     | 1.96 ± 0.23††   | 0.81 ± 0.22†     |
| Lymphocyte count (× 10^9/L)     | 0.48 ± 0.06     | 0.86 ± 0.11*    | 0.86 ± 0.11*    | 0.70 ± 0.13      |
| Platelets (g/L)                 | 1.78 ± 1.2      | 1.61 ± 1.1      | 1.75 ± 1.7      | 1.87 ± 2.3       |
| Immune Studies                  |                 |                 |                 |
| CD4 count (cells/μL)            | 23 ± 6          | 41 ± 9*         | 33 ± 8          | 12 ± 7           |
| CD8 count (cells/μL)            | 287 ± 46        | 547 ± 81*       | 597 ± 96*       | 331 ± 174        |
| Viral Studies                   |                 |                 |                 |
| HIV antigens (serum) (pg/mL)    | 173 ± 58        | 192 ± 56        | 251 ± 115       | 69 ± 28**‡       |
| HIV lymphocyte co-cultures†††   | 7.5 ± 4.0       | 6.0 ± 2.6       | 3.4 ± 1.2       | 17.5 ± 4.6       |
| HIV plasma culture (TCID/mL)    | 174 ± 72        | 375 ± 105       | 223 ± 90        | 196 ± 136        |
| Miscellaneous Chemistry Studies |                 |                 |                 |
| Iron (μg/dL)                    | 72 ± 5          | 88 ± 7          | 66 ± 8*         | 107 ± 18†        |
| Iron binding (μg/dL)            | 2,621 ± 125     | 2,637 ± 130     | 2,570 ± 155††   | 2,501 ± 212‡     |
| Ferritin (ng/mL)                | 1,766 ± 419     | 2,545 ± 912     | 863 ± 240†      | 1,580 ± 626      |
| Cholesterol (mg/dL)             | 142 ± 8         | 115 ± 7*        | 120 ± 8*        | 120 ± 8*         |
| Triglycerides (mg/dL)           | 226 ± 31        | 223 ± 25        | 310 ± 37        | ND               |
| Albumin (g/dL)                  | 4.2 ± 0.1       | 4.2 ± 0.1       | 4.3 ± 0.1       | 4.1 ± 0.1        |
| Lactate dehydrogenase (U/L)     | 213 ± 14        | 287 ± 27*       | 231 ± 17†       | 338 ± 54**†      |
| Uric acid (mg/dL)               | 4.6 ± 0.2       | 6.6 ± 0.5*      | 6.5 ± 0.4*      | 6.4 ± 1.0*       |

All values are mean ± SE. Abbreviation: ND, not done.

*Significantly different from baseline value (P < .05, two-tailed, sign rank test).
†Significantly different from after G-CSF value (P < .05, two-tailed, sign rank test).
‡Significantly different from after G-CSF and EPO value (P < .05, two-tailed, sign rank test).
§The number of tissue culture infective cells per 10⁶ in the peripheral blood mononuclear cell fraction.
G-CSF AND EPO DECREASE TOXICITY FROM ZIDOVUDINE

Nonetheless, the maximum tolerated dose of zidovudine in patients in this study was 1,000 mg/d in five divided doses. 

Effect of therapy on HIV replication. In vitro studies have suggested that hematopoietins may alter HIV expression. We used three methods to evaluate the effects of treatment on the numbers of circulating infected cells and HIV expression. Peripheral blood mononuclear cell-limiting dilution co-cultures and plasma-limiting dilution cultures for HIV showed no significant difference after treatment with G-CSF alone or with combined G-CSF and EPO therapy (Table 1). Also, there was no significant difference in HIV antigens detected in the plasma or serum after G-CSF alone or combined G-CSF and EPO therapy (Table 1). As pretherapy and posttherapy values may obscure trends that occur during the interval period, we used a mean weighted slope and variance analyses for the entire study population for each interval. Using this analysis, there were no significant differences in the level of HIV antigens over any treatment interval.

When zidovudine therapy was restarted, significant reductions in the level of HIV antigens but not lymphocyte or plasma virus levels were seen (Table 1). Analyses of the viral culture and antigen data from the combined G-CSF and EPO and zidovudine treatment phase showed two patterns of response. For most patients, HIV antigens in the blood rapidly decreased with the use of zidovudine and remained suppressed throughout the 12-week treatment interval. A subset of patients lacked suppression of HIV antigens when they were restarted on zidovudine. In general, these later patients were previously on zidovudine for a longer period although this difference was not significant. We are currently measuring the zidovudine sensitivity of these isolates to see if these patients had virus isolates that were resistant to the effects of zidovudine.

Effects on immune system. Absolute CD4 cell number and CD8 cell number increased significantly during treatment with G-CSF (Table 1, Fig 2). Because the CD4:CD8 ratio declined over this interval, there appears to be a disproportionate increase in CD8 cells during therapy with G-CSF. While this increase in CD4 cells may in part be from monocytes, the percentage of monocytes declined with G-CSF therapy (mean decrease, 6.7% ± 1.5%; *P* = .0002) and the absolute number was unchanged (mean increase, 45 ± 50 cells/μL; ***P* = .3889) during treatment. Accompanying the increases in lymphocytes were significant increases in PHA proliferation (mean increase, 9.38 ± 6.03; **P** = 0.012). This increase persisted with continued drug treatment. No significant increases in subsets were observed during subsequent treatment with EPO nor zidovudine therapy. Despite these changes in lymphocyte subsets, skin testing showed persistent anergy in all patients throughout the trial.

Effects on other cell populations. Treatment with G-CSF did not reduce the production of other cell lineages as there was no significant change in platelet count or hemoglobin level during G-CSF therapy (Fig 1). Instead, we observed significant increases in bone marrow megakaryocytes and cellularity in all eight patients in whom bone marrow

Fig 2. Immunologic and virologic effects of G-CSF, EPO, and zidovudine. Each graph is a bar graph of observed values. The mean and 95% confidence intervals are presented. The bottom panels are the doses of study medications during the trial. The symbol (*) indicates that the mean value is significantly different (*P* < .05, two sided) from the baseline mean value. The symbol (**) indicates that the mean value is significantly different from the mean value after G-CSF therapy. The symbol (***) indicates that the mean value is significantly different from the mean value after G-CSF and EPO therapy.

Expected for the 1,000 mg/d dose of zidovudine.23 None of these eight patients had a BFU-E IDₙ₀ for zidovudine of greater than 1.0 μmol/L at baseline and all became transfusion dependent with the reintroduction of zidovudine therapy.

Twelve patients have died since the start of the trial and eight patients remain on therapy more than 1 year later. The doses of the recombinant hormones required in these later patients have tended to decrease over time despite continued daily dosing with 1,000 mg or more per day of zidovudine. Because we do not have a measure of drug dose compliance, it is not known whether the decreased need for hematopoietic stimulation is due to increases in hematopoietic reserve or noncompliance with the zidovudine dosage.
The authors observed in the first 13 patients during G-CSF therapy. These effects were seen to a lesser magnitude in the later patients in whom lower doses of G-CSF were used. Similarly, treatment with G-CSF did not alter the response to EPO nor did the combined therapy reduce platelet counts.

**Drug-related toxicity.** One patient diedunexpectedly during hospitalization for electrolyte disturbances while receiving G-CSF and EPO alone and no etiology for her death was found on autopsy. Whether treatment with recombinant hormones contributed to her death is unknown as significant electrolyte disturbances were not seen in other patients.

Mild bone pain was the most consistent complaint observed (14 of 22 patients) with G-CSF therapy. Significant increases in uric acid and decreases in cholesterol were seen but were not associated with clinical symptoms. These results may be secondary to the marked increase in cell proliferation (Table 1). Transient, mild local skin irritation at the site of injection was the only side effect observed during EPO administration. This result was seen in seven of 20 patients. Headache, nausea, and agitation were the most common side effects during zidovudine therapy. No patient had dose modifications of any of the study medications because of toxicity. Six patients were taken off the study because of the development of anemia.

**Opportunistic infections.** Opportunistic infections occurred in 21 of 22 patients during this study. The most common infection was oral candidiasis (20 of 22 patients) that responded to topical or systemic antifungal therapy. No patient developed a disseminated candida infection.

Cytomegalovirus (CMV) retinitis or gastritis was diagnosed premortem in seven patients and was a contributory factor in the death of five of these patients. One patient had CMV encephalitis diagnosed on autopsy. Five of these seven patients who were previously intolerant of zidovudine monotherapy tolerated full induction doses of ganciclovir (5 mg/kg twice a day) and at least 1,000 mg/d of zidovudine. These patients also received long-term maintenance therapy (5 mg/kg once a day) with continued resolution of their retinitis or gastritis without hematopoietic toxicity. Two patients had progressive anemia while on ganciclovir and one was removed from therapy because of transfusion requirements. No patient receiving ganciclovir required modifications of either ganciclovir or zidovudine because of leukopenia.

Five patients had mycobacterium avium intracellulare at entry or diagnosed during study. Three patients were unsuccessfully treated with five drug antituberculous regimens. Two patients developed chronic, disseminated varicella infection that was treated successfully with intermittent intravenous or oral acyclovir. No patient developed pneumocystis carinii pneumonia while on study medications.

**DISCUSSION**

Hematologic toxicity is the major dose-limiting toxicity of zidovudine therapy in patients with AIDS. Most patients will be unable to tolerate maximum doses of zidovudine for more than 1 year. Because zidovudine therapy is associated with an increase in survival, multiple strategies have been tried to reduce hematologic toxicity.

One such approach is the use of reduced doses of zidovudine. Recent studies demonstrated that reduced doses of zidovudine were at least as effective as the standard dose and were less toxic. Suppression of HIV antigens was demonstrated at the lowest dose used in one study, suggesting that the lowest effective dose of zidovudine may not have been found. Despite the reduction in toxicity, anemia remained the major toxicity for patients with AIDS. In one of the studies, the hemoglobin level declined to less than 80 g/L in 39% of the standard-treatment group and 29% of the low-dose group. Twenty-six percent of patients (24.8% in standard-dose and 26.3% of low-dose, P = .69) received transfusions despite dose reduction and the mean amount of blood transfused was substantial (5.1 ± 4.5 units in the standard-dose and 6.1 ± 7.5 units in the low-dose group, P = .37). In the other study, significant reductions in hemoglobin levels were seen within 12 weeks at all dose levels studied including the 300 mg/d dose. Thus, dose reduction is not an effective strategy for the prevention of zidovudine-related anemia, and therapies that reduce hematologic toxicity would be a significant advance in the treatment of patients with HIV infection.

In this study we show that the co-administration of two recombinant human hematopoietic hormones, G-CSF and EPO, is well tolerated and partially corrects the neutropenia and anemia seen in patients with AIDS. Therapy with G-CSF was associated with a prompt increase in circulating neutrophils in all 22 patients and the increase in neutrophils was not associated with any measurable stimulation of HIV viral expression. In the patients who received the highest doses of recombinant G-CSF, statistically significant increases were seen in hemoglobin levels, CD4 cell number, lymphocyte proliferative responses, and bone marrow cellularity.

The addition of recombinant EPO to the regimen resulted in the correction of anemia in all 20 evaluable patients. With the reinstitution of zidovudine, two distinct populations of patients were identified. Despite high doses of EPO, one group of patients immediately had suppression of reticulocytosis and rapidly developed transfusion-dependent anemia. In vitro assays of zidovudine inhibition of red cell progenitors suggests that this group of patients may have an abnormal sensitivity of erythropoietic precursors to zidovudine. While G-CSF therapy markedly increased the number of erythroid progenitors, at the doses of EPO used in this trial, we were unable to overcome this inhibition of erythropoiesis. A larger group of patients appeared to derive benefit from combined G-CSF and EPO therapy. More than half of these previously intolerant patients resumed daily doses of 1,000 mg or higher of zidovudine. The significant increases in CD4 cell number and proliferative responses suggest that some immunologic improvement may occur with future combinations of cytokines.
Fig 3. Sequential bone marrow examinations from one patient on study. (A) Baseline examination of the first patient enrolled (ID No. 001). (B) Performed at the conclusion of G-CSF alone therapy. Please note the marked increase in cellularity of all cell lineages. This increase in cellularity is maintained with the addition of EPO (C) and after 12 weeks of combined therapy with zidovudine (D).
Despite these improvements, patients continued to have complications of HIV infection and immune suppression that resulted in the deaths of 12 of the 22 patients. All but one death was the result of opportunistic infections. We believe that this continued high mortality is the result of two processes. First, these patients had advanced-stage HIV infection. Recurrent opportunistic infections in this population are expected and survival of these patients was anticipated to be short. Second, many of these patients had previously received zidovudine for periods of up to 2 years before study entry. When we examined the effect of the reinstitution of zidovudine in patients with detectable HIV antigens, several patients did not have the expected suppression of HIV antigens despite the use of high doses of zidovudine. It is possible that these patients harbor HIV isolates that are resistant to the antiviral effect of zidovudine. As such, hematologic reconstitution and reinstitution of zidovudine would not likely be of benefit to these patients as the major problem is ineffective HIV suppression.

Nonetheless, clinical benefit of these hematopoietic growth factors for the majority of the patients studied was shown. The ability to overcome neutropenia and, in some patients, anemia from multiple agents including tri-methoprim-sulfamethoxazole, ganciclovir, pentamidine, and zidovudine has relevance to the treatment of HIV-infected patients in the community setting. Although the doses of zidovudine used in this trial are high by current standards, there is mounting evidence that doses of zidovudine used in this trial may be more effective than lower doses in the treatment of patients with neurologic disease and advanced AIDS.$^{28,30}$ The use of lower doses of zidovudine with G-CSF and EPO would only magnify the effectiveness of this form of treatment.

The impact of this therapy on the survival of patients is difficult to quantitate from this study. Moreover, this approach to the treatment of hematopoietic failure is only one of several therapeutic regimens under study. Alternative therapies including reduction in the dose of zidovudine, combination therapy with agents such as the interferons, and the use of the newer, less myelosuppressive nucleoside analogues such as dideoxyinosine (ddI) and azidodideoxynucleoside (AZdU) are being evaluated. The unique feature of G-CSF and EPO therapy is that it may allow the use of multiple, myelosuppressive agents simultaneously. Future trials should compare this approach with other treatments for patients who have hematologic failure. These trials will correctly define the place of G-CSF and EPO in the treatment of patients with AIDS.

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Combined therapy with recombinant granulocyte colony-stimulating factor and erythropoietin decreases hematologic toxicity from zidovudine

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