We investigated the effects of recombinant human granulocyte-macrophage colony-stimulating factor (rGM-CSF) administered by the subcutaneous route, first alone and then alternating with azidothymidine (AZT), in leukopenic patients with severe human immunodeficiency virus (HIV) infection. Ten patients with acquired immunodeficiency syndrome (AIDS) or related disorders, five of whom could not tolerate conventional doses of AZT, were administered rGM-CSF subcutaneously for 12 days. They then were administered an alternating regimen using AZT for 1 week, followed by 5 days of subcutaneous rGM-CSF and 2 days without any medication. During the initial 12 days of GM-CSF administration, there was an increase in the mean white blood cell (WBC) value. In addition, rGM-CSF stimulated circulating monocytes as evidenced by an increase in superoxide anion production and expression of surface HLA-DR antigen. However, at the same time rGM-CSF increased the serum HIV p24 antigen in each of the six evaluable patients from 189 X 10^3 to 2.02 pg/mL (geometric mean X 1 SEM) at entry to 375 X 10^3 to 2.11 pg/mL (P < .05). During the subsequent period of alternating AZT and rGM-CSF treatment, serum HIV p24 antigen fell below the day 14 value in most patients, particularly after the weeks of AZT administration. The mean T4 cell value increased in patients who had not previously received AZT, but generally did not change in those who had prior AZT exposure. Hematologic toxicity appeared to be somewhat reduced compared with continuous full-dose AZT therapy, and two patients with previous AZT hematologic toxicity tolerated this alternating regimen for 25 weeks. Additional regimens simultaneously combining these two agents are worth exploring.

This is a US government work. There are no restrictions on its use.

The thymidine analogue 3'-azido-2',3'-dideoxycytidine (also known as azidothymidine, AZT, or zidovudine) inhibits the replication of human immunodeficiency virus (HIV) in T cells and monocytes in vitro and in vivo. This drug has been shown to reduce the morbidity, improve survival, and reduce dementia in certain patients with severe HIV infection. More recently, AZT has been shown to confer clinical benefits even in early HIV infection (M. Fischl, personal communication, February 1990). However, the use of AZT is often associated with hematologic toxicity, particularly macrocytic anemia and leukopenia. This toxicity is most severe in patients who have had opportunistic infections, have low numbers of T4 cells, or have pre-existing anemia or neutropenia from their underlying disease. While the anemia in patients receiving AZT can often be managed by transfusions and sometimes the administration of erythropoietin, the leukopenia often necessitates a decrease in dosage or cessation of AZT therapy. Thus, leukopenia represents a major limitation of the use of AZT in some patients.

Granulocyte-macrophage colony-stimulating factor (GM-CSF), a physiologic cytokine produced by certain T cells, stimulates the formation and biologic function of myeloid cells in humans. Recombinant human GM-CSF (rGM-CSF) expressed in mammalian cells, bacteria, or yeast has been found to have biologic activity essentially identical to that of human GM-CSF (hGM-CSF). Initial clinical trials of rGM-CSF in patients with HIV infection or undergoing cytotoxic chemotherapy showed that it induced dose-dependent increases in the circulating neutrophil, eosinophil, and monocyte values, but had no significant effect on erythropoiesis. In this study we investigated the use of rGM-CSF to ameliorate the leukopenia induced by AZT in a group of patients with severe HIV infection. Specifically, we administered rGM-CSF for 2 weeks to leukopenic patients with acquired immunodeficiency syndrome (AIDS) or AIDS-related complex (ARC), followed by an alternating regimen of AZT and rGM-CSF. An alternating regimen was chosen because of evidence that rGM-CSF might have anti-HIV activity itself in a mononuclear cell line, and because of concerns that rGM-CSF might enhance the myelotoxicity of AZT if the two drugs were administered simultaneously. This concern was based on the line of thinking that bone marrow progenitor cells driven to divide by rGM-CSF might have increased levels of thymidine kinase (the cell cycle-dependent enzyme responsible for the initial phosphorylation of AZT) and therefore greater toxicity from AZT-triphosphate. Indeed, our group has recently shown that monocyte/macrophages (M/M) induced to divide by rGM-CSF have increased susceptibility to AZT toxicity. While there has since been some evidence that bone marrow toxicity from AZT might not be increased by rGM-CSF in vitro, much work still needs to be done regarding this issue. At the time we initiated the study, we felt that alternating AZT and rGM-CSF would be the most conservative approach to combination therapy with these agents.

We found that rGM-CSF administered for 2 weeks as a single agent increased the number of circulating granulocytes and monocytes, and increased monocyte function. At the same time, we found that rGM-CSF used alone also...
enhanced HIV replication. This latter observation is consistent with recent findings from our laboratory and others that rGM-CSF enhances HIV replication in peripheral blood M/M in vitro. Finally, the regimen of alternating AZT and rGM-CSF was tolerated for over 25 weeks, including certain patients who previously could not tolerate AZT, and appeared to induce some immunologic or virologic improvements in certain patients.

MATERIALS AND METHODS

Patient selection. Ten moderately leukopenic (total white blood cell [WBC] count less than 4,500 cells/mm³) patients, ages 25 to 56 with severe HIV infection, were entered into the study. All were homosexual men; one patient also was a hemophiliac who had previously received factor VIII replacement therapy. Two of the patients had had Pneumocystis carinii pneumonia (PCP); the other eight had ARC and oral candidiasis or constitutional symptoms. All patients had less than 200 T4 cells/mm³. The hematologic criteria for entry into the study included a platelet count ≥75,000 cells/μL, hematocrit ≥30% or hemoglobin ≥11.0 g/dL, and absolute neutrophil count of greater than 1,000 cells/mm³. Patients with an active opportunistic infection, malignancy (including Kaposi’s sarcoma), or threefold increases in transaminase levels were not eligible. Five of the patients (nos. 1, 2, 4, 5, 7) had never received AZT, whereas five (nos. 3, 6, 8, 9, 10) could not tolerate full-dose AZT by virtue of having developed either anemia (hemoglobin less than 8 g/dL) or leukopenia (absolute neutrophil count less than 750 cells/mm³) while receiving 200 mg of AZT every 4 hours. The patients who had previously received AZT did not take this medication for at least 4 weeks before entry. All patients were treated on an outpatient basis at the Warren G. Magnuson Clinical Center of the National Institutes of Health, Bethesda, MD, after giving written informed consent. The protocol for this study was approved by the Institutional Review Board of the National Cancer Institute.

Therapeutic agents. Lyophilized rGM-CSF (Sandoz/Schering-Plough Pharmaceuticals, East Hanover and Kenilworth, NJ) was supplied in vials containing 0.216 mg of glycoprotein with a specific activity of 5.6 x 10⁶ chronic myelogenous leukemia (CML) units per milligram of glycoprotein, as measured by minor modifications of a chronic myelogenous leukemia (CML) assay previously published by Griffin et al. The lyophilized rGM-CSF was reconstituted with 2 mL of preservative-free sterile water to a final concentration of 0.108 mg/mL, refrigerated at 4°C, and used within 24 hours. AZT was supplied by Burroughs-Wellcome Company (Research Triangle Park, NC) in 100 mg capsules.

Treatment schema. Patients were administered an initial course of rGM-CSF subcutaneously, 2 μg/kg/d, in three divided doses for 12 days. The drug was then stopped, and patients received no treatment for 2 days. This rest period allowed the GM-CSF–induced proliferation to diminish before AZT was administered; in this way, it was thought that levels of thymidine kinase might be reduced by the time AZT was given, and thus that AZT toxicity might be decreased. The patients then received AZT 200 mg orally every 4 hours for 7 days, followed by 5 days of rGM-CSF at the same dose, followed by 2 days off treatment. This 14-day cycle of 7 days of AZT, 5 days of rGM-CSF, and 2 days of no drug was repeated for up to 6 months. If at any point the absolute neutrophil count was less than 1,400 cells/mm³ after the week on AZT, the rGM-CSF was increased by 2 μg/kg/d to a maximum of 6 μg/kg/d. Then, on 6 μg/kg/d of rGM-CSF, the AZT dose was reduced during subsequent cycles if the absolute neutrophil count fell below 750 cells/mm³ after treatment with AZT. Patients were transfused as necessary for anemia.

Bone marrow evaluations were performed in seven patients at entry and at week 14. Lymphocyte subsets were measured every 1 to 2 weeks by fluorescence-activated cell sorting (FACS) on populations gated to exclude monocytes. Serum samples for HIV p24 antigen measurement using an antigen capture assay were also obtained every 1 to 2 weeks. These serum samples were stored at −20°C and then tested blindly in one large assay. Delayed cutaneous hypersensitivity reactions were assessed after subcutaneous injections of 0.1 mL of candida extract (Hollister-Stier, Elkhart, NY; 1/100 dilution), intermediate strength (STU) purified protein derivative (PPD) (Connaught Labs, Willowdale, Ontario, Canada), tetanus toxoid (Lederle Labs, Pearl River, NY; 10 LF/mL), and trichophyton extract (Hollister-Stier; 1/30 dilution) at entry and every 6 weeks while on study.

Superoxide anion assay. Peripheral blood mononuclear leukocytes obtained from patients at entry and after the 12-day course of rGM-CSF were separated by density sedimentation on Ficoll-Paque (Pharmacia Fine Chemicals, Piscataway, NJ). Contaminating granulocytes were further depleted by overnight incubation in suspension in Dulbecco’s modified Eagles’ medium (GIBCO, Quality Biologicals, Gaithersburg, MD) containing penicillin 100 U/mL, streptomycin 100 μg/mL, glutamine 2 mmol/L, and 10% fetal calf serum (FCS) (less than 0.06 ng/mL endotoxin, GIBCO). The monocyte functions assessed in these studies were not substantially altered after this overnight incubation. The percentage of monocytes was estimated by Coulter counter channelizer size profile analysis (Coulter Electronics, Hialeah, FL) normalized for that individual patient, and the cells suspended at a concentration of 1 x 10⁶ monocytes/mL. Previous studies have documented a correlation between cell size determination and phenotypic analysis, morphology, and esterase staining of monocyte populations. Monocytes, 1 x 10⁵, were then added to each of quadruplicate wells in microtiter plates (96 wells/plate) and incubated for 1 hour at 37°C, followed by washing to remove nonadherent cells. Earle’s balanced salt solution (GIBCO) containing ferriochrome c was then added to each well, and superoxide anion production by l-formylpeptide (FMLP 10⁻⁴ mol/L) was measured in quadruplicate wells by the reduction of ferricytochrome c as previously described.

Analysis of monocyte surface antigens. Monocyte surface antigens were analyzed at entry and at the end of 12 days of rGM-CSF. Single cell suspensions (50 mL) of freshly isolated mononuclear leukocytes diluted to 10 x 10⁶ cells/mL in phosphate-buffered saline with 0.1% sodium azide and 2% FCS were exposed to 10% human AB serum for 10 minutes to prevent nonspecific binding, and then incubated at 4°C for 30 minutes with fluorescein isothiocyanate-conjugated (FITC) anti-Leu M3 (CD14) and phycoerythrin-conjugated (PE) anti-HLA-DR (Becton-Dickinson Monoclonal Center, Inc, Mountain View, CA) monoclonal antibodies (MoAbs). After staining, the cells were washed twice with buffer without FCS, immediately resuspended in 0.25 mL of 2% paraformaldehyde, and stored at 4°C until they were analyzed by two-color immunofluorescence flow microfluorometry using a FACStar cell sorter (Becton-Dickinson). In some experiments, the monocytes were also identified based on their electronically gated forward and side scatter profiles. Parallel studies showed a more than 90% correlation between scatter profile and the expression of CD14 surface antigen. Statistical analysis. The clinical data were analyzed for statistical significance using the Wilcoxon rank sum and Wilcoxon signed rank tests. During the initial 2-week treatment with rGM-CSF, the hypothesis did not specify in advance whether the p24 antigen levels would increase or decrease; therefore, a two-tailed test was used. For the other measurements, we stated an expected change in a particular direction as the hypothesis in advance, and a one-tailed test was chosen. To evaluate geometric means of the changes in HIV p24 antigen, a value of 31 pg/mL (the lowest limit of detection of the
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assay) was assigned for samples in which antigen could not be detected.

RESULTS

Results of initial treatment with rGM-CSF. Nine patients (nos. 1 through 9) completed the 2-week initial treatment period with 2 μg/kg/d of rGM-CSF. One patient (no. 10) withdrew at day 3 because of fevers, myalgias, and arthralgias. The mean number of total WBCs, neutrophils, monocytes, and eosinophils all increased during rGM-CSF administration, with the greatest percentage increase occurring in the eosinophil lineage (Fig 1). Also there was a small rise in the mean number of absolute T4 cells (mean change in T4 value of 28 ± 19 cells/mm³ at week 2) that was attributable to a nonspecific increase in the absolute lymphocyte count. The mean WBC value then decreased rapidly during the 2 days after cessation of GM-CSF.

Four of the nine evaluable patients had detectable serum HIV p24 antigen at entry (week 0). In each of these four patients, the HIV serum p24 antigen increased during the 2-week initial treatment with rGM-CSF (Fig 2). In addition, HIV p24 antigen became detectable by day 14 in two other patients who had no detectable serum p24 antigen at entry (Fig 2). In total, six patients either had increased serum p24 antigen levels or had their p24 antigen become detectable during 2 weeks of continuous treatment with rGM-CSF (mean increase in the six from 189 × ± 2.02 pg/mL to 375 × ± 2.11 pg/mL [geometric mean × ± SEM] [P < .05, two-tailed]), the other three patients remained p24-negative throughout. Of these six patients who had increases in p24 antigen, four had prior treatment with AZT (nos. 3, 6, 8, 9) but had discontinued its use 6, 8, 12, and 12 weeks before entry. In three of these four patients for whom information is available, p24 antigen levels were either undetectable or slightly decreased while off AZT during the 4-week period before beginning the protocol (Fig 2). In view of the time that these four patients had been off AZT and the stability of their p24 antigen levels during the 4 weeks before exposure to rGM-CSF, it is doubtful that these changes were simply the result of a rebound after cessation of AZT.

In vitro M/M function. Previous studies have shown that the in vitro treatment of human monocytes with rGM-CSF enhances the secretion of both spontaneous and inducible hydrogen peroxide and superoxide anion, and also enhances the expression of class II histocompatibility antigen. In light of these in vitro observations, monocytes from four of the nine patients who completed the 2-week initial course of rGM-CSF were obtained before and after rGM-CSF therapy, and evaluated for superoxide anion production in response to FMLP stimulation and for HLA-DR expression. We found that FMLP-induced superoxide anion production increased after 12 days of in vivo therapy with rGM-CSF in each of the four patients tested (Fig 3). (Monocytes from other patients were not evaluated for superoxide anion production because of insufficient cell numbers.) This effect was most apparent in patients with low levels of inducible superoxide anion before receiving rGM-CSF. In addition, there was a trend upward in HLA-DR expression in circulating monocytes during the initial 2 weeks of rGM-CSF therapy (Fig 4). This effect was most noticeable in those patients who initially had low levels of surface HLA-DR before therapy. Thus, in addition to increasing the number of circulating monocytes, the studies suggest that rGM-CSF activated these cells.

![Fig 1. Mean changes in WBCs of the nine patients who completed the initial 12-day period of subcutaneous rGM-CSF administration. (a) Neutrophils; (b) monocytes; (c) eosinophils; (d) other cells.](image-url)
While those patients who had never received AZT before entry had a substantial increase in their mean T4 cells (maximum increase of 90 cells/mm³ at week 7, P < .05), those patients who had previously developed AZT myelotoxicity before entry did not overall have an increase.

In general, the patients had a decrease in their HIV p24 antigen after the weeks they received AZT and then had an increase after rGM-CSF administration (Fig 6). In the four patients who had detectable serum antigen at entry, there was an overall decline in detectable antigen after the weeks of AZT administration. In two patients (nos. 4 and 9), p24 antigen increased somewhat when AZT doses were reduced after the development of bone marrow suppression. One of the two patients who became p24-positive during the initial rGM-CSF therapy was intermittently positive on alternating treatment, while the other remained negative. Finally, the three patients who were p24-negative during the initial 2 weeks remained negative during alternating therapy.

Generally, the mean number of WBCs increased during the period of rGM-CSF administration, but then decreased during AZT administration (Fig 7). No patient was removed from the study during the first 25 weeks as a result of myelotoxicity. However, during this period two patients (nos. 1 and 2) had their rGM-CSF increased to 4 µg/kg/d, and four patients (nos. 3, 4, 6, 9) had it increased to 6 µg/kg/d due to neutropenia. Of the four patients whose doses were raised to 6 µg/kg/d, two (nos. 4 and 9) subsequently had their AZT dose reduced to 200 mg every 6 hours (beginning weeks 21 and 9, respectively), and one of these (no. 4) subsequently had the dose reduced to 100 mg every 6 hours. The other three evaluable patients who did not require increases in their dose of rGM-CSF tolerated this drug combination well.

Three patients (nos. 3, 4, 9) required red blood cell (RBC) transfusions while on study. Two of these patients also had developed neutropenia necessitating AZT dose reductions, as described above. One patient requiring transfusions had been transfusion-dependent on AZT before entry, while another patient had also developed anemia on AZT before entry. A third patient who had never taken AZT before the study developed symptomatic anemia at week 25 and received an RBC transfusion.

Seven patients had bone marrow evaluations at entry and after 14 weeks. Initial evaluations in most patients showed nonspecific changes with varying degrees of lymphocytosis and plasmacytosis. Biopsies performed after 14 weeks of therapy showed mild eosinophilia in all patients. Three of the seven patients had increases in granulocyte or myeloid precursors and also had increased cellularity.

Skin test reactivity for delayed-type hypersensitivity (DTH) was performed in nine patients, and no clear trends in this parameter were observed. Most patients reported an increase in energy and sense of well-being. However, there were no significant changes in patient weights while on study.

Other toxicity. All patients enrolled developed fevers of at least 37.5°C (maximum 39.4°C) while on rGM-CSF; these were often accompanied by myalgia and arthralgia. The fevers and associated symptoms were controlled with ibuprofen or acetaminophen, and usually could be prevented.
by taking ibuprofen orally before the injection of rGM-CSF. The fevers generally subsided at the end of the rGM-CSF injections. A mildly pruritic, macular, erythematous cutaneous eruption, which on biopsy showed a nonspecific, perivascular, lymphohistiocytic infiltrate, developed at the rGM-CSF injection site in 7 of the 9 patients. The rashes faded rapidly on discontinuation of the injections, but recurred when the injections were resumed. Mild elevations in hepatic transaminases occurred in three patients, all of whom had slight elevations at entry (mean entry aspartate aminotransferase for the three patients was $52 \pm 17$ IU/L, peak was $167 \pm 23$ IU/L, mean entry alanine aminotransferase for the three patients was $70 \pm 25$ IU/L, peak was $257 \pm 39$ IU/L). During the weeks on AZT, eight patients reported some malaise, headache, or nausea. In general, most patients reported resolution of these AZT-related symptoms during the weeks they received rGM-CSF.

**DISCUSSION**

While AZT has been shown to be beneficial in patients with severe HIV infection, the development of myelosuppression limits its use over a 6-month period of time in a substantial percentage of AIDS patients. In a recent open trial, only 21% of such patients could tolerate 6 months of AZT without dose interruption or cessation of therapy. Moreover, the incidence of myelosuppression increases beyond the initial 6 months. The marrow suppression associated with AZT therapy appears to be multifactorial, with the underlying HIV infection itself playing a contributing role. While myelosuppression is less problematic with reduced doses of AZT (e.g., 600 mg/d), many patients with underlying bone marrow suppression cannot tolerate even these reduced doses.

Several bone marrow stimulating cytokines, including GM-CSF, are presently being explored clinically for their potential to stimulate myelopoiesis in AIDS patients with AZT toxicity. One possible use of such substances is in patients who have low endogenous cytokine production (e.g., erythropoietin in patients with renal failure). Another possible use is in reducing the toxicity of bone marrow suppressive drugs (e.g., with intermittent cytotoxic cancer chemotherapy). However, AZT therapy for HIV infection differs from that of most cytotoxic chemotherapy in that it is usually administered continuously, and it is not obvious a priori how one should optimally use GM-CSF in this setting.

In this study we have explored the use of rGM-CSF to...
ameliorate the leukopenia associated with AZT therapy in HIV infection. rGM-CSF has been shown to affect certain monocyte functions in vitro, and this provided a second rationale for its use. We elected to alternate rGM-CSF with AZT (with a 2-day rest period after the rGM-CSF administration) because rGM-CSF had been reported to have anti-HIV activity in a monocytoid cell line and had not been reported to enhance HIV replication (as assessed by culture) when administered as a single agent to AIDS patients. Also, we were initially concerned that if rGM-CSF was administered at the same time as AZT, we might conceivably see enhanced bone marrow toxicity from AZT. As discussed above, this was based on the concern that the intracellular levels of thymidine kinase, the enzyme responsible for the initial phosphorylation of AZT to a 5'-monophosphate, might increase with rGM-CSF-induced cell proliferation; this subsequently has been shown to occur at least in monocytes exposed to AZT.

Fig 5. Mean change in T4 cells (ΔT4) of patients on the alternating regimen of rGM-CSF and AZT. (A) Mean change in T4 cells of all nine patients. Asterisks denote one-tailed statistical significance from baseline with \( P < .05 \). (B) Mean change in T4 cells of the five patients with no prior AZT and the four with prior AZT treatment. Asterisks denote one-tailed statistical significance between the two groups of patients with \( P < .05 \). G, rGM-CSF; A, AZT.

Fig 6. HIV p24 antigen in the six patients who demonstrated detectable levels on the alternating regimen of rGM-CSF and AZT. (A) HIV p24 antigen in patients 4, 5, and 8. In patient 4, the AZT dose was reduced to 200 mg orally every 6 hours beginning at week 21, 100 mg every 4 hours beginning week 23, and 100 mg every 6 hours beginning week 25. (B) HIV p24 antigen in patients 3, 6, and 9. In patient 9, the AZT dose was reduced to 200 mg orally every 6 hours beginning week 9. G, rGM-CSF; A, AZT.
During an initial 2-week period of subcutaneous administration of rGM-CSF, the patients exhibited a 4-fold increase in their total WBCs. The increases in leukocytes were comparable with those previously observed by Groopman et al., who administered rGM-CSF intravenously, and except for mild erythematous rashes around the injection sites in some patients, the toxicities were comparable. Thus, for outpatient therapy with rGM-CSF, subcutaneous self-administration appears feasible. However, during the initial period of rGM-CSF administration we also found that each of six evaluable patients had significant increases in their circulating serum HIV p24 antigen. This increase could not be explained by a rebound from prior AZT therapy; two of these patients had never been treated with AZT, while the other four had been off AZT for 6, 8, 12, and 12 weeks, and when evaluable had stable p24 antigen levels in the 4-week period before entry into the protocol. These findings are consistent with the in vitro enhancement of HIV replication in monocytes after their exposure to GM-CSF, as observed by Perno et al in our lab and others. It is possible that the increase in the numbers of monocytes may also have contributed to this effect. It should be noted that Groopman et al. did not observe an increase in HIV as detected by cell culture in patients receiving rGM-CSF. However, cell culture techniques often preferentially measure T cells infected by HIV, while p24 antigen would provide a measure of HIV replication in both T cells and monocytes. While the number of patients studied here is small and this phenomenon needs further study, the data suggest that the long-term use of rGM-CSF without antiretroviral therapy in patients with AIDS be undertaken with regard to the possibility that this agent might potentiate viral replication in vivo.

As is frequently observed in patients with AIDS and low initial T4 cells who are treated with AZT, the increases in T4 cells in patients receiving AZT were transient, and were not statistically significant beyond week 8. Interestingly, increases in T4 cells appeared to be less pronounced in patients who had previously developed bone marrow toxicity on AZT, despite their being off AZT for at least 4 weeks before entry into the trial. While this observation may not necessarily extend to other dideoxynucleoside or anti-HIV drugs, it suggests that immunologic endpoints might be difficult to evaluate in AZT-intolerant patients who are administered other anti-HIV agents.

The finding that rGM-CSF augmented monocyte superoxide anion production and HLA-DR expression in some patients parallels previous observations of changes in monocyte function after their exposure to rGM-CSF in vitro. This may be particularly relevant for patients with AIDS since their monocytes are functionally defective and express reduced levels of HLA-DR. Reactive oxygen intermediates play an important role in monocyte effector responses against certain opportunistic pathogens common in AIDS patients (eg, Mycobacterium avium-intracellulare). In addition, HLA-DR expression is critical for optimal monocyte accessory cell function, and recent evidence also suggests that HIV-1 can impair this function. Therefore, the ability of GM-CSF to augment monocyte superoxide anion production and the number of cells expressing HLA-DR might possibly have implications for the treatment of patients with AIDS.

In this pilot study, while hematologic toxicity was reduced compared with full-dose continuous AZT, we could not definitely conclude whether the alternating regimen of AZT
and rGM-CSF was superior to continuous low-dose AZT therapy or even intermittent AZT therapy in leukemic patients. Although 3 of the 9 patients did eventually develop toxicity on the regimen, two of these patients had been unable to tolerate full-dose AZT therapy; the other six patients (including two with prior AZT intolerance) tolerated the regimen without substantial hematologic toxicity. However, it should be noted that the reduced toxicity may simply have resulted from the intermittent AZT administration, and a delineation of the role of rGM-CSF in this regimen will require further study.

In our lab, Perno et al\textsuperscript{13} have recently found that while rGM-CSF by itself can increase HIV replication in vitro in monocyte/macrophages, it can at the same time enhance the net in vitro anti-HIV effect mediated by AZT and other thymidine dideoxynucleoside congeners. (Interestingly, this effect persisted for 7 days after the rGM-CSF was washed out; thus it is possible that it would apply in an alternating regimen as used here.) This enhancement of the anti-retroviral activity of AZT by rGM-CSF is in part related to increased cell-entry and phosphorylation. However, a similar enhancement of activity does not occur with 2',3'-dideoxycytidine or 2',3'-dideoxyadenosine.\textsuperscript{33} While there remains the theoretic concern that combined use of these agents may potentiate cytopenias from bone marrow toxicity, there is in vitro evidence that rGM-CSF may actually reduce the toxic effects of AZT on bone marrow progenitor cells.\textsuperscript{34} Whether this will occur in patients remains to be seen.

While rGM-CSF potentially holds promise for use in AIDS patients in combination with AZT, there are a number of complex interactions between these two agents in patients with HIV infection. In this study we have shown that an alternating weekly regimen of these two agents can be well-tolerated for 6 months in patients who previously could not tolerate AZT. However, it is unclear whether these findings may have simply resulted from intermittent AZT therapy, and some patients had persistently elevated p24 antigen on this regimen. Pilot studies are presently underway at the National Cancer Institute (Bethesda, MD) and elsewhere to evaluate the simultaneous use of these two drugs.\textsuperscript{35} At the time that such studies are completed, it will be possible to assess the cumulative experience with these agents and move toward larger controlled trials of these two agents.

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