Stimulation of Hematopoiesis in Patients With Bone Marrow Failure and in Patients With Malignancy by Recombinant Human Granulocyte-Macrophage Colony-Stimulating Factor

By Saroj Vadhan-Raj, Stephen Buescher, Anne LeMaistre, Michael Keating, Ronald Walters, Carrie Ventura, Walter Hittelman, Hal E. Broxmeyer, and Jordan U. Gutterman

Granulocyte-macrophage colony-stimulating factor (GM-CSF) is a multipotential hematopoietin. To assess the toxicity and biological activity of recombinant human GM-CSF (rhGM-CSF) in vivo, 25 patients with malignancy or bone marrow failure were treated with rhGM-CSF (specific activity $-5 \times 10^7 \text{ U/mg}$) as part of a phase I trial. The treatment was administered by continuous intravenous (IV) infusion daily for 2 weeks at fixed dose levels and repeated after a 2-week rest period. Over the entire dose range tested (15 to 500 $\mu\text{g/m}^2/\text{d}$), rhGM-CSF treatment was associated with dramatic increases (two- to 70-fold) in total leukocyte counts, which consisted predominantly of neutrophils, bands, eosinophils, and monocytes. Furthermore, six of the 14 patients with one or more cytopenias that received at least two cycles of treatment had multi-lineage responses characterized by twofold or greater increases in platelet count to a level above 100,000, twofold or greater increases in corrected reticulocyte count, and a reduced requirement for red cell transfusions. Three of these patients became independent of both red cell and platelet transfusions for 17 to 37 weeks of follow-up. Treatment was associated also with an increase in bone marrow cellularity and frequency of cycling progenitor cells. The treatment was well tolerated; side effects included constitutional symptoms and bone pain. These results demonstrated that rhGM-CSF has a significant impact on hematopoiesis in patients with advanced malignancy and also in patients with bone marrow failure.

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COLONY-STIMULATING FACTORS (CSF) are a family of hematopoietic regulatory glycoproteins essential for survival, proliferation, and differentiation of hematopoietic progenitor cells in vitro.1,4 Classification of these growth factors is based on the types of mature cells seen in the colonies produced in response to these hormones. Thus, multi-CSF (also called interleukin-3) stimulates the production of mature cells of most of the hematopoietic lineages including granulocytes, macrophages, eosinophils, megakaryocytes, erythroid cells, and mast cells, and it stimulates self-renewal of multipotential stem cells. Granulocyte-CSF (G-CSF) and macrophage-CSF (M-CSF, also known as CSF-1) exhibit relative lineage-restricted specificity and stimulate the production of granulocytes and macrophages, respectively, while granulocyte-macrophage CSF (GM-CSF) stimulates the production of both granulocytes and macrophages.

GM-CSF is a T-cell-derived lymphokine with a broad range of biological activities. In addition to stimulating the proliferation of myeloid progenitor cells, it stimulates selected functions of mature cells. GM-CSF enhances superoxide production and lysozyme secretion in response to the bacterial chemo-attractant N-formyl-methionyl-leucyl-phenylalanine (FMLP), and phagocytosis of yeast by neutrophils.1,4 Furthermore, GM-CSF stimulates antibody-dependent killing of tumor target cells by neutrophils and eosinophils2 and tumoricidal activity of monocytes.10 The recent cloning of the gene encoding human GM-CSF11-14 and the expression of this recombinant protein in Escherichia coli and yeast made it possible to study the biological activities of this hormone in vitro and in vivo.15-17 Recombinant human GM-CSF (rhGM-CSF) not only stimulates granulocytes and macrophage formation but also formation of multipotent colonies of granulocytes, monocytes, erythroid cells, and megakaryocytes.14,15 Studies in primates demonstrated that rhGM-CSF is rapidly cleared from the circulation with a half-life of about seven minutes,16 induces leukocytosis,14,17 and activates neutrophils.17

On the basis of these observations, we conducted a phase I study of rhGM-CSF administered by continuous intravenous (IV) infusion to patients with malignancy or bone marrow failure with the objectives of examining its biological activities including its effects on hematopoiesis and its antitumor effects. In addition, we assessed the drug tolerance and established biologically active doses for humans.

PATIENTS AND METHODS

Eligibility. Twenty-five patients with cancer or bone marrow failure, or both, were treated in this study. All patients had histologically confirmed advanced malignancy and/or bone marrow failure. Eligibility criteria included a Karnofsky performance status of ≥50%, life expectancy of at least 12 weeks, preserved renal function (serum creatinine ≤2 mg/100 mL, proteinuria ≤2+), and hepatic function (bilirubin ≤1.5 mg/100 mL, prothrombin time ≤1.3 times control). Written informed consent was obtained from all patients before their participation in the study. Except for one patient, none

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had received radiation therapy, chemotherapy, or biologic therapy for at least 4 weeks before beginning this study; one patient, who had myelodysplastic syndrome and breast cancer, had been treated with tamoxifen for 2 months before rhGM-CSF treatment and was continued on the drug while on this study to avoid hormonal manipulation.

**Patient characteristics.** Of the 25 patients entered on this study, 14 were men and 11 were women. The median age was 58 years (range, 20 to 79 years) and median performance status was 70% (range, 50% to 90%). Seventy-five percent of the patients had undergone chemotherapy, and 25% had received biologic or hormonal therapy.

Six patients had diagnoses of solid tumors, and these included one case each of liposarcoma, breast cancer, colon carcinoma, squamous cell carcinoma of the lung, melanoma, and small cell carcinoma of the lung. The patient with small cell carcinoma of the lung had tumor involvement of bone marrow. In all six of these patients the malignancy was advanced and refractory to conventional therapy. Five of the six patients had normal blood counts, but the patient with liposarcoma had thrombocytopenia and required red cell transfusions before rhGM-CSF treatment.

The other 19 patients had either primary bone marrow failure from disease in the bone marrow or secondary bone marrow failure from chemotherapy or radiotherapy for malignancy, characterized by one or more cytopenias. These patients' diagnoses included myelodysplastic syndrome (MDS) in eight, smouldering leukemia in seven, and myelofibrosis, aplastic anemia, chronic myelogenous leukemia (CML), and multiple myeloma in one patient each. The two patients with CML and myeloma had received autologous bone marrow transplantation after intensive chemotherapy and total body irradiation more than 2 months before rhGM-CSF treatment and had shown no recovery of the bone marrow. Four of the eight patients with MDS had histories of other malignancies including multiple primary cancers (breast and ovarian cancer, and melanoma, one patient), liposarcoma (one), acute myelogenous leukemia (AML; one), and squamous cell carcinoma of skin (one). Before rhGM-CSF treatment, all of these 19 patients required red cell transfusions at doses of 10 to 3 weeks.

**rGM-CSF.** The rGM-CSF method used in this study was prepared and provided by Immunex Corporation (Seattle). Human GM-CSF cDNA was isolated from a library constructed by using mRNA from the human T cell line HUT-102, inserted into and expressed in yeast, and purified to homogeneity by reversed-phase high-performance liquid chromatography. The purified rhGM-CSF has a specific activity of \(-5 \times 10^7\) colony-forming units per milligram of protein. When analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, the purified rhGM-CSF protein reveals two bands (21.5 and 16 kd) that represent glycosylated and nonglycosylated forms. Endotoxin contamination was \(<1\) ng/mg of protein as measured by the Limulus amebocyte lysate assay.

**Study design.** Patients were treated with rhGM-CSF at fixed dose levels of 15, 30, 60, 120, 250, or 500 \(\mu\)g (corresponding to 0.75, 1.5, 3.0, 6.0, 12.5, and 25 \(\times 10^6\) units) per square meter of body surface per day. At least two patients participated at each of the three lower dose levels, and six patients were entered at each of the three higher levels. The drug was administered by continuous IV infusion daily for 14 consecutive days and was delivered at a constant rate by autosyringe pump through a central venous catheter. The treatment period was followed by a 2-week rest period, and then a second 2-week treatment cycle. For the study of pharmacokinetics, an IV bolus of rhGM-CSF was administered to the first 12 patients on the first day of cycle one, 24 hours before continuous infusion began.

Following the first two cycles of rhGM-CSF treatment, patients with solid tumors who showed an antitumor response and patients with hematologic diseases who showed a favorable hematologic response (increase in granulocyte count, platelet count, or corrected reticulocyte count twofold or more) without a significant increase (twofold or more) in marrow blast count, were eligible for maintenance therapy using the same schedule. This consisted of repeated cycles of IV infusion for 2 weeks at 2-week intervals. In patients eligible for maintenance therapy, the dose of rhGM-CSF was decreased appropriately (generally to 30 to 120 \(\mu\)g/m\(^2\)/d) to maintain the patient's WBC count in the normal range. Treatment was withheld from any patient who had a severe toxicity and was reinstituted at lower dose levels on full recovery. Appropriate dose adjustments were made when a patient's WBC count rose rapidly above 50,000/\(\mu\)L. Treatment was discontinued whenever disease progression was documented.

**Clinical and laboratory monitoring.** Before treatment, patients were evaluated with history and physical examination, complete blood cell count, WBC differential count, reticulocyte count, prothrombin time, serum biochemical profile including serum electrolytes and blood glucose, and urinalysis. A complete blood count was performed daily from days 1 to 8 and three times a week thereafter. All other tests were repeated at least once a week during each treatment cycle. All patients underwent chest x-ray and electrocardiographic examinations before and after the completion of the study. In addition, peripheral blood and bone marrow aspirate and biopsy specimens were obtained immediately before and after each treatment cycle and processed by conventional methods. The differential count and myeloid to erythroid cell ratio in the bone marrow of patients was determined with 500-cell differential counts performed on Wright-Giemsa stained smears of aspirated marrow. The cellularity was assessed on the biopsy specimen.

Subjective side effects were classified as mild, moderate, or severe. Mild referred to symptoms that did not require medication for relief. Moderate referred to symptoms that required medication for relief. Severe symptoms were those that were inadequately controlled with medications and caused a 25% drop in performance status. Tumor response in patients with solid tumor was evaluated by physical examination and appropriate radiologic and laboratory studies. Responses were graded according to standard criteria as was described earlier.

**RESULTS**

**Effects of rhGM-CSF treatment on hematological parameters in six patients with solid tumors.** Five of the six patients with solid tumors received two cycles of treatment, while in the sixth patient, treatment was discontinued on day 10 due to progression of disease. Treatment was associated with a marked increase in total leukocyte and absolute granulocyte counts (neutrophils and bands) in all six patients. The effects of increasing doses of rhGM-CSF on peripheral WBC and absolute granulocyte counts in the five patients who received two cycles of treatment are shown in Fig 1. The rise in WBC count was seen within 48 hours after the infusion was started. The elevation in WBC count was dose dependent, with up to threefold increase at a dose level as low as 30 \(\mu\)g/m\(^2\)/d, and increases up to sevenfold at a dose of 250 \(\mu\)g/m\(^2\). At doses of 60 \(\mu\)g/m\(^2\) or lower, the WBC count reached a plateau after the first week, whereas at doses of 120 \(\mu\)g/m\(^2\) or higher the WBC count continued to increase.
until the infusion was discontinued on day 14. The counts returned to the baseline levels on discontinuation of therapy. When the second cycle of rhGM-CSF was administered after a 2-week rest period, the patient's WBC count rose rapidly and often reached a level as high as the maximal level attained during the first cycle, and it continued to increase until the infusion was discontinued. The elevation in WBC count resulted predominantly from increases in neutrophilic granulocytes (2.8- to 5.3-fold), eosinophils (2.4- to >60-fold), and monocytes (1.6- to 7.5-fold). Substantial increases were observed also in absolute numbers of lymphocytes (1.9- to 6.6-fold).

Four of five patients with solid tumors who received two treatment cycles showed an increase in platelet count, and one patient experienced a transient decrease during the second cycle (from 282,000/μL to 89,000/μL). However, overall mean values of platelet counts were not significantly different before and after treatment.

Treatment was associated also with a more than twofold increase in corrected reticulocyte count above baseline to a level >1.5% in all patients. No significant changes were observed, however, in hemoglobin values and hematocrit levels at the end of treatment.

Effects of rhGM-CSF treatment on WBCs in 19 patients with hematologic disease. Responses in WBC and differential counts of 19 patients with hematologic diseases are shown in Table 1. A dose-response effect in the WBC count was observed, even in these patients with cytopenias. A wide range of responses was observed in patients treated at the same dose level: at the lowest dose level (15 μg/m²), a twofold increase in WBC cell count was seen, whereas a six- to 71-fold increase was seen at the highest level (500 μg/m²). Although the WBC count elevations were caused predominantly by increases in neutrophils, several patients also had marked increases in eosinophils and monocytes (Table 1).

Hematologic responses in patients with cytopenias. Among 20 patients who had one or more cytopenias, 19 patients had hematologic diseases and one patient had a solid tumor. Fourteen of these patients underwent at least two cycles of treatment and were considered evaluable for multilineage responses (Table 2).

Ten of the 14 patients had had granulocytopenia (granulocyte count of <1,000/μL) before rhGM-CSF treatment. All of them had twofold or greater increase in granulocyte count (to a level higher than 1,000/μL) in response to rhGM-CSF treatment. All 14 patients were anemic and had required RBC transfusions before starting rhGM-CSF treatment. Treatment was associated with significant reticulocytosis (two- to 20-fold increase) and in ten of these patients the corrected reticulocyte count rose twofold or more to a level >1.5% (normal range, 0.5% to 1.5%). This resulted in improvement in erythropoiesis and a decreased requirement for RBC transfusions in six patients. Three of these six patients (two

Table 1. Effects of rhGM-CSF on WBC Counts in Patients With Hematological Diseases (Peripheral Blood Counts* × 10⁹/μL)

<table>
<thead>
<tr>
<th>Dose (μg/m²)</th>
<th>WBCs</th>
<th>Neutrophils &amp; Bands</th>
<th>Monocytes</th>
<th>Eosinophils</th>
<th>Lymphocytes</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of Patients</td>
<td>Pre</td>
<td>Post</td>
<td>Pre</td>
<td>Post</td>
<td>Pre</td>
</tr>
<tr>
<td>15-60/n = 5</td>
<td>1.7</td>
<td>5.3</td>
<td>0.27</td>
<td>2.73</td>
<td>0.11</td>
</tr>
<tr>
<td>(1.2-3.6)</td>
<td>(2.2-9.5)</td>
<td>(0.26-2.7)</td>
<td>(0.92-8.17)</td>
<td>(0.27-2)</td>
<td>(0.09-1.768)</td>
</tr>
<tr>
<td>120/n = 4</td>
<td>1.9</td>
<td>10.0</td>
<td>0.67</td>
<td>7.17</td>
<td>0</td>
</tr>
<tr>
<td>(1.7-2.4)</td>
<td>(9.3-26.1)</td>
<td>(0-1.63)</td>
<td>(0.28-13.86)</td>
<td>(0-0.10)</td>
<td>(0-1.0)</td>
</tr>
<tr>
<td>250/n = 5</td>
<td>1.9</td>
<td>13.0</td>
<td>0.89</td>
<td>6.04</td>
<td>0.05</td>
</tr>
<tr>
<td>(1.1-4.7)</td>
<td>(4.5-96.5)</td>
<td>(0.09-1.43)</td>
<td>(0.51-62.73)</td>
<td>(0-0.24)</td>
<td>(0.03-1.93)</td>
</tr>
<tr>
<td>500/n = 5</td>
<td>1.5</td>
<td>16.7</td>
<td>0.34</td>
<td>7.01</td>
<td>0.05</td>
</tr>
<tr>
<td>(0.02-2.6)</td>
<td>(4.3-106.0)</td>
<td>(0.02-0.81)</td>
<td>(0.51-62.73)</td>
<td>(0-0.47)</td>
<td>(0.29-7.42)</td>
</tr>
</tbody>
</table>

*Median (range) values are shown.
†Post represents the maximal response in counts during first cycle of rhGM-CSF treatment.
after treatment. Preliminary results of radionuclide bone erythroid cells. The frequency of cycling progenitor cells cannot change was seen in the number of megakaryocytes and eosinophils in bone marrow (range, 5% to 19%). Peripherial blood eosinophilia had increased percentages of patients with MDS, one with AML) became independent of RBC transfusions for 17 to 37 weeks of follow-up, and the transfusion requirement of one patient (who had myelofibrosis) decreased from every 2-week to every 5- to 6-week intervals. Two patients (one with MDS and one with liposarcoma) who had previously required RBC transfusions at 2- to 4-week intervals had increases in reticulocyte counts of 3.5% and 8.5%, respectively. These two patients did not require transfusions during two treatment cycles (6 and 8 weeks, respectively), despite the frequent phlebotomies required for blood tests during the study.

Twelve of the 14 patients had thrombocytopenia before rhGM-CSF treatment. In response to rhGM-CSF, six of these patients (who also showed responses in RBCs) had twofold or greater increases in platelet counts up to a level of 100,000/µL. Three of these six patients did not require any platelet or RBC transfusions for 17 to 37 weeks follow-up. A transient decrease in platelet count was observed in two patients on treatment (from 95,000/µL and 30,000/µL to 50,000/µL and 8,000/µL, respectively).

Thus, a total of six patients (five with hematologic disease and one with solid tumor) had responses in multiple cell lineages (granulocytes, RBCs, and platelets). The responses of RBCs and platelets did not seem to be dose dependent.

**Effects on bone marrow.** Bone marrow examinations (aspiration and biopsy) were done both before and after treatment cycle in 23 patients. The specimens from 21 patients were adequate and evaluable. Treatment was associated with an increase in bone marrow cellularity from a baseline mean value of 45% (range, 10% to 100%) to a maximal mean value of 70% (range, 15% to 100%). Treatment also resulted in an increase in the myeloid:erythroid cell ratio (range, two- to 27-fold) in 19 patients. Patients with peripheral blood eosinophilia had increased percentages of eosinophils in bone marrow (range, 5% to 55%). No significant change was seen in the number of megakaryocytes and erythroid cells. The frequency of cycling progenitor cells (CFU-GM), as measured by the high-specific-activity tritiated thymidine kill technique, was significantly increased after treatment. Preliminary results of radionuclide bone marrow scans performed on some patients revealed increased activity in peripheral bones and lungs (S. Vadhan-Raj and L. Lamki, manuscript in preparation).

A total of 15 patients with MDS or smouldering AML with refractory cytopenias were treated in this study. Because of concern that GM-CSF might stimulate proliferation of leukemic cells, these patients were observed closely for disease progression; evidence of maturation was seen in several of these patients. The effects of rhGM-CSF treatment in eight patients with MDS have been described elsewhere. However, in three patients (two with AML and one with myelofibrosis evolving into AML), increases in blast counts were observed in the peripheral blood and bone marrow. Treatment with rhGM-CSF was discontinued in all three patients during the first cycle. Two patients were subsequently treated with chemotherapy, and in the third patient, blood counts decreased to baseline levels. The biologic activity of rhGM-CSF in patients with smouldering AML will be the subject of another separate report (S. Vadhan-Raj et al, manuscript in preparation).

**Antitumor activity.** No evidence of antitumor effect was seen in the six patients who had solid tumors. Five experienced progression of disease while on therapy, and one patient’s disease remained stable.

**Clinical course.** Of the 25 patients entered in the study, 21 received at least one cycle of treatment. As to the other four patients, treatment was discontinued in three because of disease progression, and one patient (who had refractory severe thrombocytopenia) died during the first week of treatment, probably from intracerebral bleeding. There were two other patient deaths during the study. One patient with multiple myeloma had received high-dose chemotherapy and total body irradiation followed by autologous bone marrow transplantation, 2 months before GM-CSF. Despite a response in granulocyte count during the first rhGM-CSF treatment cycle, this patient expired during the first off-treatment period from infectious and hemorrhagic complications of cytopenias. The other patient who had had myelodysplastic syndrome died of gram-negative sepsis. This patient also had shown a response in granulocyte count during treatment; rhGM-CSF had been discontinued two days before the episode of sepsis. There were two additional episodes of infection during the study (one gram-negative sepsis related to urinary tract infection and one catheter-related gram-positive infection), both of which responded to IV antibiotics.

At present, at least two patients at each dose level have received the prescribed two cycles of treatment, and 11 patients have undergone more than two cycles because of their favorable hematologic response. During maintenance therapy, rhGM-CSF dose was decreased to maintain their WBC count within a physiologic range. Despite this dose reduction, baseline granulocyte counts improved with repeated cycles of treatment in several patients with cytopenias.

**Side effects.** Twelve patients received a fixed dose of rhGM-CSF by IV bolus injection on day 1. Five of these 12 patients experienced lower backache in the lumbosacral area ten to 20 minutes after the injection; the pain lasted from

| Table 2. Hematologic Responses in 14 Patients* with Cytopenias |
|------------------|------------------|------------------|
| Lineage          | No. of Patients | No. of Patients |
|                  | With Cytopenia† | With Response‡ |
| Granulocyte      | 10              | 10              |
| Platelets        | 12              | 6§              |
| RBCs             | 14              | 6§              |

*Fourteen of 20 patients with cytopenias received at least two cycles of treatment.
†Cytopenia in granulocytes, platelets, and RBCs was defined, respectively, as granulocyte count <1,000/µL, platelet count <100,000/µL, and anemia requiring RBC transfusion.
‡Response in granulocytes, platelets, and RBCs was defined, respectively, as a more than twofold increase in granulocyte count to a level of >1,000/µL, more than doubling of platelet count to a level of >100,000, and a decrease in requirement for RBC transfusion.
§Three patients became independent of RBC and platelet transfusion for 17 to 37 weeks of follow-up.
seconds to about 30 minutes. The pain was severe in two patients (one with MDS [60 μg/m²] and one with metastatic melanoma [250 μg/m²]) requiring analgesics and narcotic pain medication. One of these two patients experienced nausea and vomiting during this episode. The pain did not recur in any of the five patients during treatment by continuous IV infusion. Other side effects observed after the bolus injection were fever up to 38.7°C in one patient treated with 250 μg/m².

The clinical side effects associated with continuous infusion of rhGM-CSF in the 21 patients who received at least one cycle of treatment are listed in Table 3. The most common side effects were pains in the rib, sternum, cervical spine, shoulder, and hip areas, pains that were generally mild to moderate and manageable with analgesics. Two patients with MDS, both of whom had marked leukocytosis (one had a WBC of 96,000/μL at a dose level of 250 μg/m², and the other 106,000/μL at 500 μg/m²), experienced severe hip and thigh pain after 1 week of treatment. Evaluation for the causes of pain, including x-rays and radionuclide scans, revealed no focal abnormalities. The bone pain resolved in both patients after rest and analgesics and was coincident with decreases in their WBC counts. Both patients tolerated treatment well at a reduced dose of 30 μg/m² without pain recurrence. As Table 3 shows, side effects such as fever, chills, myalgias, headache, decreased appetite, nausea, emesis, and diarrhea were observed in some patients. These side effects were generally mild to moderate in severity, tended to disappear during the first few days of treatment, and did not require dose reduction. No major changes in the patient’s serum chemistries were seen. Reversible, mild increases in serum lactate dehydrogenase and alkaline phosphatase were seen in a few patients with leukocytosis. No evidence of organ dysfunction that could be attributed to treatment have been seen.

**DISCUSSION**

To evaluate the biologic activity and tolerance of rhGM-CSF in humans, we administered this hormone to 25 patients with either bone marrow failure or malignancy. The continuous administration of rhGM-CSF produced dramatic hematologic effects. A prompt increase in the total WBC count was caused, in most cases, by rising numbers of neutrophilic granulocytes and bands, but dramatic increases in peripheral blood eosinophils were also seen in some patients, as were increases in blood monocytes.

Several interesting facets of hematologic response to rhGM-CSF were observed in these patients. The elevation in WBC count that resulted from rhGM-CSF administration was sustained throughout the entire treatment period and was associated with marked increases in bone marrow cellularity and frequency of cycling progenitor cells. This suggests that one effect of rhGM-CSF is stimulation of hematopoiesis at the stem/progenitor cell level. The elevation in WBC numbers also occurred rapidly, which suggested that rhGM-CSF may act also as a marrow release factor facilitating egress of cells from marrow. After the infusion was discontinued the WBC count returned to baseline levels, which may mean that continuous administration of hormone is necessary to maintain the WBC count elevation. Retreatment of patients with GM-CSF after a 2-week rest period, however, resulted in prompt and often pronounced elevations in WBC numbers, demonstrating that previous rhGM-CSF exposure resulted in improved bone marrow reserve that could be called upon with repeated treatment. The improved reserve may have resulted from increased cellularity of the bone marrow, stimulation of peripheral bone marrow, and possibly extramedullary hematopoiesis as suggested by our preliminary findings on bone marrow scans.

Several of the patients with cytopenia had responses in multiple lineages. These responses were characterized by increases in hematocrit values and platelet counts, with the result that three patients became independent of both RBC and platelet transfusions. These results are encouraging because a substantial proportion of patients with preleukemia and smouldering leukemia die of cytopenia-related infections and hemorrhagic complications. We cannot say, based on this study, whether the multipotential effects of rhGM-CSF in vivo are direct ones on the hematopoietic progenitor cells or are mediated through an action on accessory cells. Since GM-CSF has been shown to induce the expression of tumor necrosis factor (TNF) gene in monocytes, it may be that at least some of the biologic effects we noted are mediated by release of monokines such as interleukin-1 or TNF, which in turn may induce production of hematopoietic growth factors from human stromal cells.

GM-CSF has been shown to stimulate both proliferation and differentiation of myeloid leukemia cell lines. The ultimate in vivo effect of this hormone would depend on the balance between these two opposing actions on each patient's leukemic cells. One concern, therefore, with the use of rhGM-CSF in patients with myeloid diseases is that it might enhance leukemic progression. However, the patients treated in the present study had prolonged cytopenias and were at high risk for infection and hemorrhage. To reduce this risk, patients were treated with rhGM-CSF in an attempt to

<table>
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<tr>
<th>rhGM-CSF Dose (μg/m²)</th>
<th>No. of Patients*</th>
<th>Fever</th>
<th>Chills</th>
<th>Myalgias</th>
<th>Fatigue</th>
<th>Headache</th>
<th>Reduced</th>
<th>Appetite</th>
<th>Nausea/</th>
<th>Vomiting</th>
<th>Indigestion/</th>
<th>Diarrhea</th>
<th>Bone</th>
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<td>15-60</td>
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</tr>
<tr>
<td>250</td>
<td>4†</td>
<td>4</td>
<td>2</td>
<td>1</td>
<td>4 (1)‡</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>—</td>
<td>4 (1)‡</td>
<td>4 (1)‡</td>
<td>4 (1)‡</td>
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</tr>
<tr>
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<td>6†</td>
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<td>3 (1)‡</td>
<td>—</td>
<td>3 (1)‡</td>
<td>5</td>
<td>1</td>
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</tbody>
</table>

*Twenty-one patients received at least one cycle of treatment.
†One patient required dose reduction because of severe bone pain and fatigue.
‡Numbers in parentheses indicate number of patients with severe side effects.
STIMULATION OF HEMATOPOIESIS BY rhGM-CSF

restore hematopoiesis. Evidence of maturation and improvements in hematologic parameters were observed in patients with MDS and in four of the seven patients with smouldering AML. However, in a subset of patients with smouldering AML, the proliferative effect outweighed the differentiative effect, resulting in a greater increase in immature cells. Nevertheless, GM-CSF might be useful in this subset of patients as a recruiting agent, thus increasing the sensitivity of leukemic burden to subsequent chemotherapy, since most cytotoxic agents are more effective on rapidly proliferating cell populations.

In addition to stimulating the proliferation and differentiation of hematopoietic progenitors, GM-CSF is a neutrophil-activating factor. However, the hormone also inhibits neutrophil motility in vitro and is believed to be identical to the neutrophil migration-inhibition factor derived from T lymphocytes. We therefore examined the in vitro functional activities of neutrophils derived after in vivo rhGM-CSF treatment. Our preliminary studies with purified neutrophils from patients with solid tumors and hematologic diseases suggest that in vivo exposure to rhGM-CSF does not significantly alter these patient's neutrophil chemotaxis. In addition, their cells are capable of secreting H2O2 normally in response to PMA (S. Buescher and S. Vadhan-Raj, submitted manuscript).

Of the relatively few side effects, the most common was mild to moderate bone pain that was generally manageable with analgesics. In two patients with MDS, however, the bone pain was dose limiting when associated with a very high WBC count consisting mainly of neutrophilic granulocytes. In contrast, other patients treated at the same dose levels (250 to 500 μg/m2) had neither WBC elevations to this level nor severe bone pains. Thus the severity of bone pain seems to be related to the level of WBC count and granulocyte count rather than directly to the rhGM-CSF dose. The mechanism of bone pain is not well understood, but it could be related to the release of mediators from neutrophils since GM-CSF is a neutrophil activator or to the direct stimulation of bone marrow. There was no evidence of microthrombi or ischemic necrosis on histology of the bone marrow of these patients. Other side effects were constitutional symptoms similar to those associated with interferons and other cytokines such as TNF, but they were milder and tended to diminish after a few days. Some of these symptoms could be related to the monokines such as interleukin-1 and TNF that might be released from activated monocytes in response to rhGM-CSF.

Recently rhGM-CSF was shown to enhance tumoricidal activity of monocytes against melanoma and other tumor cell lines. Although we did not observe any antitumor activity in the six patients with solid tumors, increases were observed in the number of granulocytes, reticulocytes, and platelets in these patients who had been heavily pretreated with chemotherapy. This may be of future significance, because rhGM-CSF in combination with higher doses of chemotherapy may prove valuable in improving the therapeutic index of cytotoxic agents. At the same time it must be noted that some solid tumor cell lines have been shown to exhibit receptors for GM-CSF. In one study GM-CSF stimulated colony growth of small cell lung cancer cell line, whereas in the other study, GM-CSF had an antiproliferative effect. This suggests that caution should be exercised in the use of this hormone in multi-modality therapy.

The genes encoding several of the myeloid hematopoietic growth factors were recently cloned, and rhG-CSF and rhGM-CSF are now under clinical investigation. Several points will have to be considered when attempts are made to combine CSF with chemotherapy. Although the major cell important in host defense against microbial invasion is the neutrophil, other cell types such as monocytes, eosinophils, and lymphocytes play important roles against opportunistic fungal, mycobacterial, and parasitic infections. The multipotential effects of GM-CSF may prove useful in increasing various WBC types. In addition, its stimulative effects on thrombopoiesis and erythropoiesis may confer an advantage over rhG-CSF, which, in vivo in primates, predominantly affects neutrophilic granulocytes.

In summary, these results demonstrate that yeast-derived rhGM-CSF is well tolerated when administered by continuous IV infusion. The hormone has great biological activity, even at low doses. In light of these and other findings, rhGM-CSF might have a role in several clinical situations, including the following: as a stimulator of hematopoiesis in patients with cytopenia resulting from cytoreductive therapy or hematologic diseases; as a recruiting agent in association with chemotherapy in patients with leukemia; and as a stimulator of functional activities of mature effector cells in patients with infections. The introduction of this hematopoietic growth factor into therapeutics therefore, might have a significant impact on the management of patients with cancer and bone marrow failure.

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