Treatment of Refractory Aplastic Anemia With Recombinant Human Granulocyte-Macrophage-Colony-Stimulating Factor

By R.E. Champlin, S.D. Nimer, P. Ireland, D. H. Oette, and D.W. Golde

Fifteen patients with refractory aplastic anemia or agranulocytosis received treatment with recombinant human granulocyte-macrophage-colony-stimulating factor (rhGM-CSF) in doses from 4 to 64 μg/kg/d by continuous intravenous (IV) infusion. Ten of 11 evaluable patients with aplastic anemia had substantial increments in granulocytes, monocytes, and eosinophils associated with myeloid and eosinophilic hyperplasia in the bone marrow. Patients with pretreatment granulocytes >0.3 x 10⁹/L had greater increments in circulating myeloid cells than patients with more severe granulocytopenia. Only one patient had improvement in erythrocytes and platelets. Blood counts fell to baseline after rhGM-CSF treatment was discontinued. Doses up to 16 μg/kg/d were relatively well tolerated in the absence of extreme leukocytosis. Fatigue and myalgia were common. Three patients developed pulmonary infiltrates that resolved with discontinuation of treatment. Patients tended to have recurrent inflammation in previously diseased tissues. These data indicate that rhGM-CSF will increase circulating granulocytes, monocytes, and eosinophils in patients with refractory aplastic anemia. Further studies are necessary to determine if rhGM-CSF treatment will reduce morbidity or improve survival.

Aplastic anemia is a life-threatening hematologic disorder characterized by pancytopenia and a hypocellular bone marrow. Patients with severe aplastic anemia have a poor prognosis unless recovery of hematopoiesis occurs. Bone marrow transplantation (BMT) is an effective treatment but carries substantial risk, and a histocompatible sibling donor can be identified for only a minority of patients. Antithymocyte globulin treatment is effective in improving hematopoiesis for approximately half of patients, but many patients either fail to respond or relapse after a transient response.

A variety of hematopoietic growth factors have been identified that stimulate proliferation of myeloid precursor cells in vitro. Granulocyte-macrophage-colony-stimulating factor (GM-CSF) is a glycoprotein hormone that induces hematopoietic progenitors to form granulocyte, macrophage, and eosinophil colonies in vitro. Stimulatory activity on erythroid and megakaryocytic colony formation has been demonstrated under some circumstances. In addition, GM-CSF has profound effects on mature neutrophil function, including augmentation of oxidative metabolism, enhanced chemotaxis in response to physiologic stimuli, random migration inhibition, increased phagocytosis, and stimulation of neutrophil antibody-dependent–cell-mediated cytotoxicity. GM-CSF is secreted by activated T lymphocytes, fibroblasts, and endothelial cells responding to cytokines and other cellular activators.

Advances in molecular biological techniques have made it possible to produce sufficient quantities of recombinant hematopoietic growth factors to conduct in vivo therapeutic trials. Recent studies have demonstrated that recombinant human granulocyte-macrophage-colony-stimulating factor (rhGM-CSF) stimulates hematopoiesis in vivo in primates and in humans with acquired immunodeficiency syndrome (AIDS), myelodysplasia, and following myelotoxic chemotherapy with autologous BMT. We evaluated rhGM-CSF as treatment for patients with refractory aplastic anemia that had previously failed to respond to antithymocyte globulin treatment. The results indicate considerable promise for hematopoietic hormone therapy in these patients.

METHODS

Patients less than 75 years of age with acquired aplastic anemia who had previously failed to durably respond to antithymocyte globulin treatment were eligible if the bone marrow contained >20% cellularity and two or more of the following were present: granulocytes >1.0 x 10⁹/L, reticulocytes <60 x 10⁹/L, or platelets <20 x 10⁹/L. Patients with agranulocytosis with granulocytes <1.0 x 10⁹/L were also eligible. Patients were required to have no evidence of spontaneous hematologic recovery over the preceding 8 weeks. The study design was approved by the UCLA Human Subject Protection Committee. Informed consent was obtained from all patients or their legal guardians.

rhGM-CSF was supplied by the Sandoz Corporation and had an activity of 5.4 x 10¹² U/mg glycoprotein. rhGM-CSF was administered to groups of three or more patients at a given dose level by constant intravenous (IV) infusion in normal saline through a central venous catheter for 14 days; if a complete response was not achieved and grade ≥2 toxicity did not occur, the dose was escalated to the next dose level for an additional 14 days. Patients with complete or partial responses continued on maintenance treatment at the same dose level by constant IV infusion for 2 additional months. Treatment was interrupted or discontinued for signs of toxicity. Patients with only a transient increment or less than a partial response after 1 month of therapy did not continue rhGM-CSF therapy.

If three consecutive patients received a given dose level for 14 days without toxicity, the succeeding group of patients was started at the next higher dose level. The dose levels employed were 4 μg/kg/d, 8 μg/kg/d, 16 μg/kg/d, 32 μg/kg/d, and 64 μg/kg/d. During the course of the study, it became apparent that doses of ≥32 μg/kg/d were poorly tolerated, and subsequent patients received a maximum of 16 μg/kg/d, with the dose adjusted downward if extreme leukocytosis occurred. Patients did not receive concomitant treatment with androgens. Corticosteroids were given...
only as needed for premedication for platelet transfusions. No patient received antithymocyte globulin within the preceding 2 months. Patients with active infections or major coexisting diseases were not eligible.

Response criteria were similar to those used in previous therapeutic studies in aplastic anemia. Complete response was defined as a sustained return to normal peripheral blood counts (granulocytes >2.5 x 10⁹/L, monocytes >0.3 x 10⁹/L, platelets >150 x 10⁹/L, Hb >12 g/dL). Partial response was defined as any of the following measured on three or more determinations: increment in granulocytes >0.5 x 10⁹/L above baseline; increment in monocytes >0.2 x 10⁹/L above baseline, increment in platelets >30 x 10⁹/L above baseline; or resolution of RBC transfusion requirements. Comparisons were made for peripheral blood counts taken pretreatment and at day 28 using the Wilcoxon signed-rank test. Each value was the average of three successive determinations, generally obtained within 1 week. The term granulocytes is defined herein as the sum of segmented neutrophils plus band forms.

Progenitor assays for colony-forming units–granulocyte, macrophage (CFU-GM) and burst-forming units–erythropoiesis (BFU-E) on Ficol-Hypaque (FH)-separated bone marrow and peripheral blood mononuclear cells were performed in triplicate by standard techniques.15,16

**RESULTS**

Fifteen patients were entered, 13 with aplastic anemia and two with agranulocytosis. Median age was 45 years (range 16 to 75 years). The etiology of aplastic anemia was unknown in ten, related to drugs in two and to hepatitis in one. Median interval from diagnosis and previous antithymocyte globulin treatment to rhGM-CSF therapy was 333 (range 60 to 2,120) days and 292 (57 to 2,091) days, respectively. Ten patients had previously failed to achieve any hematologic improvement following antithymocyte globulin treatment. Three patients previously had a transient response to antithymocyte globulin but relapsed; two had then failed to respond to a second antithymocyte globulin course. For the aplastic anemia patients, median (range) peripheral blood counts x 10⁹/L were leukocytes 1.7 (0.9 to 2.4), granulocytes 0.43 (0.03 to 0.77), lymphocytes 1.0 (0.46 to 2.2), monocytes 0.09 (0.02 to .27), and platelets 5 (5 to 25). Nine met the criteria for severe aplastic anemia of the International Aplastic Anemia Study Group, and four had moderate aplastic anemia as previously defined.7 All required erythrocyte transfusions, and eight of the 11 required ongoing platelet transfusions. Median bone-marrow cellularity was 3%, with a range of 2% to 15%. Agranulocytosis was associated with the T-gamma syndrome in one patient and was idiopathic in the other case.

Eleven patients with aplastic anemia received rhGM-CSF for more than 1 week and were evaluable for hematologic response. Peripheral blood counts taken pretreatment and after 1 month treatment with rhGM-CSF are summarized in Table 1. Two patients with aplastic anemia and both patients with agranulocytosis could not tolerate rhGM-CSF therapy and were evaluable for toxicity but not for hematologic response.

Ten of the 11 evaluable patients had a partial or complete

<table>
<thead>
<tr>
<th>Patient</th>
<th>Dose µ/kg/d</th>
<th>Marrow Cellularity %</th>
<th>WBC</th>
<th>Granulocytes x 10⁹/L</th>
<th>Monocytes x 10⁹/L</th>
<th>Lymphocytes x 10⁹/L</th>
<th>Eosinophils</th>
</tr>
</thead>
<tbody>
<tr>
<td>DM</td>
<td>4, 8</td>
<td>2</td>
<td>Pre</td>
<td>1.8</td>
<td>.53</td>
<td>.02</td>
<td>1.1</td>
</tr>
<tr>
<td>DB</td>
<td>4, 8</td>
<td>15</td>
<td>Pre</td>
<td>1.7</td>
<td>.51</td>
<td>.07</td>
<td>1.1</td>
</tr>
<tr>
<td>YB</td>
<td>4, 8</td>
<td>3</td>
<td>Pre</td>
<td>1.0</td>
<td>.43</td>
<td>.09</td>
<td>0.4</td>
</tr>
<tr>
<td>CR</td>
<td>8</td>
<td>15</td>
<td>Pre</td>
<td>2.1</td>
<td>.77</td>
<td>.27</td>
<td>1.0</td>
</tr>
<tr>
<td>OA</td>
<td>8, 16</td>
<td>3</td>
<td>Pre</td>
<td>1.0</td>
<td>.03</td>
<td>.04</td>
<td>.93</td>
</tr>
<tr>
<td>KM</td>
<td>16</td>
<td>3</td>
<td>Pre</td>
<td>2.3</td>
<td>.30</td>
<td>.02</td>
<td>1.9</td>
</tr>
<tr>
<td>SC</td>
<td>16</td>
<td>3</td>
<td>Pre</td>
<td>1.6</td>
<td>.34</td>
<td>.16</td>
<td>.93</td>
</tr>
<tr>
<td>RS</td>
<td>16, 32</td>
<td>2</td>
<td>Pre</td>
<td>2.4</td>
<td>.08</td>
<td>.06</td>
<td>2.2</td>
</tr>
<tr>
<td>TW</td>
<td>16, 32</td>
<td>10</td>
<td>Pre</td>
<td>1.6</td>
<td>.52</td>
<td>.13</td>
<td>0.9</td>
</tr>
<tr>
<td>CT</td>
<td>16, 32</td>
<td>3</td>
<td>Pre</td>
<td>0.9</td>
<td>.25</td>
<td>.07</td>
<td>0.6</td>
</tr>
<tr>
<td>RJ</td>
<td>32, 64</td>
<td>5</td>
<td>Pre</td>
<td>1.8</td>
<td>.29</td>
<td>.04</td>
<td>1.4</td>
</tr>
<tr>
<td>Mean</td>
<td>6</td>
<td></td>
<td>Pre</td>
<td>1.7</td>
<td>.37</td>
<td>.10</td>
<td>1.1</td>
</tr>
</tbody>
</table>

Post-treatment values taken on day 28 except for SC and OA, who stopped therapy because of hemorrhage on day 25.

*Difference pretreatment vs post-treatment value significant \( P = 0.01 \).

†\( P = 0.36 \).
response in granulocytes and monocytes. Representative changes in leukocytes over time are indicated in Fig 1. Seven patients had complete normalization of circulating granulocytes, and three had partial responses in granulocytes. The median granulocyte increment at 28 days was $1.8 \times 10^9/L$ above baseline with a range of 0.2 to $9.0 \times 10^9/L$. The difference in pre-treatment and post-treatment granulocytes was significant ($P = .01$). The greatest increments occurred in patients with higher pretreatment granulocyte counts, more cellular bone marrows, and greater number of pretreatment CFU-GM (Table 1). For seven patients with initial granulocyte counts 0.33 to $0.77 \times 10^9/L$ the median increment was 3.1 (range 1.4 to 9.0) $\times 10^9/L$. For four patients with granulocytes 0.08 to $0.28 \times 10^9/L$ the median increment was 0.9 (range 0.2 to 1.9). One of these patients with severe aplasia had no response, and in another the response was transient; granulocyte counts fell in patients when the rhGM-CSF dose was reduced and fell to baseline levels after discontinuation of the drug.

All patients were monocytopenic at study entry. Monocytes increased from a baseline median (range) of 0.07 (0.02 to 0.15) $\times 10^9/L$ to 0.34 (0.19 to 2.9) $\times 10^9/L$ ($P = 0.01$). Eight of the 11 evaluable patients achieved monocyte increases into the normal range, and two had partial responses. Eosinophilia occurred in each patient. Eosinophil counts increased after approximately 2 weeks of therapy and typically equalled or exceeded granulocytes after 4 to 6 weeks of therapy. Median peak eosinophil count was $5.8 \times 10^9/L$ with a range of 0.6 to $35.6 \times 10^9/L$. Lymphocyte counts fluctuated variably with no consistent changes observed.

Circulating erythrocytes, platelets, and their transfusion requirements were unaffected, except for one patient who had an increase in hemoglobin concentration from 7 to 11 g/dL and in platelets from 20 to $45 \times 10^9/L$; these returned to baseline over 3 weeks after rhGM-CSF was discontinued and increased again following retreatment with rhGM-CSF.

Bone marrow cellularity increased in all responding patients with patchy areas of granulocytic and eosinophilic hyperplasia. Median (range) cellularity increased from 3% (2% to 15%) to 36% (10% to 55%; $P = 0.01$). No changes were apparent in erythroid precursors and megakaryocytes. The marked increase in bone marrow cellularity for patient DB is shown in Fig 2.

Committed myeloid progenitors, CFU-GM, were markedly reduced at study entry, with a baseline median (range) of 8 (1 to 28) per $2 \times 10^9$ bone marrow cells and 13 (0 to 156)/mL in the peripheral blood. After 28 days of rhGM-
CSF treatment, CFU-GM increased to 13 (1 to 41) per 2 x 10⁷ cells in the bone marrow and 21 (12 to 639)/mL in peripheral blood. The difference between pretreatment and post-treatment values is significant (P = .02). There was no consistent change in peripheral blood BFU-E.

None of the patients developed gram-negative bacteremia while on therapy. Two patients had gram-positive bacteremia associated with cutaneous infections at their central venous catheter insertions site. One patient was noted to have bacteriuria with *Serratia marcescens* two days after starting rhGM-CSF prior to any change in peripheral blood counts. Another patient had *Klebsiella pneumoniae* bacteriuria after 1 month of therapy. One patient described below had *Herpes simplex* isolated from respiratory secretions. One patient developed a perirectal abscess associated with *Citrobacter freundii* infection that recovered while continuing rhGM-CSF therapy.

**TOXICITY**

No major toxicity occurred in five patients receiving 4 to 8 μg/kg/d. All patients complained of mild fatigue and myalgia. These symptoms were more prominent in patients receiving higher doses. Eight patients received 16 μg/kg/d; this dose was generally well tolerated but adverse effects did occur in patients who developed marked leukocytosis. One patient with past history of lower extremity thrombophlebitis developed thrombosis of her central venous catheter when her leukocyte count had increased 59.4 × 10⁹/L with 29% granulocytes and 60% eosinophils. She resumed treatment at 8 μg/kg/d by subcutaneous (SC) injections without adverse effects (see Fig 1B). Another patient developed dyspnea and pulmonary infiltrates after 5 weeks of rhGM-CSF therapy when her leukocyte count was 27.9 × 10⁹/L with 29% granulocytes and 52% eosinophils. One patient with agranulocytosis related to the T-gamma syndrome developed a leukocyte count of 41 × 10⁹/L with 50% eosinophils and 8% granulocytes after ten days of rhGM-CSF therapy. She developed fever, pulmonary infiltrates, and respiratory failure, requiring intubation; *Herpes simplex* was cultured from her tracheal secretions, and she recovered with discontinuation of rhGM-CSF and treatment with acyclovir.

Six patients received doses ≥ 32 μg/kg. Fatigue and myalgia were most prominent in this dosage group. Patients frequently developed symptoms in areas of prior inflammation. One patient with a history of non-A, non-B hepatitis had recurrence of jaundice that improved after discontinuing rhGM-CSF treatment. One patient with a history of pericarditis 3 years earlier had recurrence of pericarditis after five days of rhGM-CSF treatment at 32 μg/kg/d. Another patient with agranulocytosis developed transient fever and abdominal pain when treated with rhGM-CSF at 32 μg/kg/d. She had a previous abdominal infection that required placement of a permanent colostomy. One patient tolerated 32 μg/kg/d well for 2 weeks but developed pleuritis and pulmonary infiltrates after five days while receiving 64 μg/kg/d. All adverse effects resolved promptly when the rhGM-CSF was discontinued.

Treatment was discontinued in three patients because of bleeding complications. Each patient was receiving rhGM-CSF at a dose of 16 μg/kg/d. One patient developed a fatal CNS hemorrhage after three days of rhGM-CSF therapy. Two other patients had gastrointestinal (GI) or CNS bleeding occur after 25 days of rhGM-CSF therapy that resolved without sequelae. Each of these patients had severe thrombocytopenia, refractory to platelet transfusions at study entry. Platelet counts did not substantially change during their rhGM-CSF therapy. These bleeding complications were probably unrelated to rhGM-CSF treatment. The possibility that rhGM-CSF impairs hemostasis cannot be excluded.

**DISCUSSION**

This trial of rhGM-CSF was a dose escalation study to assess its toxicity and biological effects in selected patients with refractory aplastic anemia. We found that rhGM-CSF significantly increased peripheral blood granulocytes, monocytes, and eosinophils. Responses were seen promptly in patients with granulocytes counts >0.3 × 10⁹/L with an increase in band forms in the blood within one to three days and a progressive increase in granulocytic cells within the first 2 to 6 weeks of treatment. The bone marrow became increasingly cellular with granulocytic and eosinophilic hyperplasia. The prompt initial increase in circulating granulocytes probably resulted from mobilization and redistribu-
tion of cells. The sustained increase was primarily due to proliferation of myeloid precursors as reflected by bone marrow hyperplasia. Altered egress of granulocytes from the vasculature or prolongation of granulocyte survival may also have contributed to elevation of the granulocyte count. The eosinophilia that occurred was probably due to proliferation of eosinophils in the bone marrow as well as prolongation of eosinophil survival. Patients with more severe granulocytopenia or eosinophilia in the bone marrow as well as prolongation of eosinophil survival. Patients with more severe granulocytopenia had smaller increments in peripheral blood leukocytes and a slower tempo of response. Granulocytes, monocytes, and eosinophils fell to baseline in all patients following discontinuation of rhGM-CSF treatment.

Patients generally failed to have improvement in erythrocytes and platelets. These findings coincide with results in animals and in human GM-CSF studies for other diseases. An exception to this principle may be in GM-CSF treatment for patients with myelodysplasia where increased erythrocyte and platelet counts have been reported. Two recent reports using intermittent courses of recombinant GM-CSF in patients with aplastic anemia reported similar hematologic responses.

The toxicity of rhGM-CSF was relatively mild and tolerable in doses up to 16 μg/kg/d during the 4- to 12-week observation period unless extreme leukocytosis occurred. Three patients with marked leukocytosis became symptomatic with severe myalgia or pulmonary infiltrates, possibly related to the elevated levels of activated granulocytes and eosinophils. At higher doses, more severe myalgia, serositis (pericarditis or pleuritis), hepatitis, or abdominal pain occurred in the absence of major changes in leukocyte counts. Patients tended to have manifestations of inflammation in previously diseased tissues that resolved promptly with discontinuation of rhGM-CSF.

The optimal manner of administration for rhGM-CSF is uncertain. We used rhGM-CSF by constant IV infusion based upon preliminary studies in animals. Two patients were successfully maintained with intermittent SC therapy, and other studies have reported hematologic responses with IV infusions over three to six hours. It is unknown whether toxicity, granulocyte functional capabilities, or extravascular migration differs for any schedule or route of administration.

These data indicate that rhGM-CSF induces granulocytosis, monocytois, and eosinophilia in most patients with refractory aplastic anemia. Continued treatment was necessary in this study to maintain the hematologic response. Further study is necessary to determine if lifelong maintenance is necessary and effective and also if chronic treatment with rhGM-CSF is tolerable. One patient had a transient granulocyte response followed by recurrent granulocytopenia despite continued therapy, suggesting that stem-cell unresponsiveness may occur in some patients. This study focused on selected patients with long-standing aplastic anemia. This treatment must be studied in newly diagnosed patients, and controlled trials are necessary to determine whether rhGM-CSF will reduce morbidity and improve survival.

Short-term treatment with rhGM-CSF may be potentially useful in several settings for the management of aplastic anemia. Antithymocyte globulin is an effective treatment for approximately half of newly diagnosed patients with aplastic anemia. Typically there is a period of 1 to 3 months before peripheral blood counts increase. A short course of rhGM-CSF treatment following antithymocyte globulin may possibly induce a more prompt granulocytosis or enhance recovery. This approach requires study in controlled trials.

BMT is considered the preferred treatment for young patients with aplastic anemia. Only one third of otherwise appropriate patients have an HLA-identical sibling donor. Recently registries of potential HLA-matched unrelated donors have been formed in various countries, but several months is generally required to identify a histocompatible individual and to procure bone marrow for transplantation. rhGM-CSF therapy may be useful to support myelopoiesis during this period. rhGM-CSF has been reported to enhance granulocytic recovery after autologous marrow transplantation and may also be useful to accelerate hematopoietic recovery after allogeneic BMT in patients with aplastic anemia. It is necessary, however, to determine if rhGM-CSF will adversely affect graft rejection, graft-vs-host disease (GVHD), or other complications.

Hematopoietic growth factor therapies may ultimately be effective treatment for aplastic anemia. The hematologic responses observed in this study indicate that myeloid progenitors are usually present that are capable of proliferating in response to stimulatory agents. Since rhGM-CSF does not appear to have substantial effects on pluripotent stem cells or erythroid and megakaryocytic lineages, therapy of aplastic anemia will likely require concomitant treatment with other hematopoietic growth factors. Treatment with multilineage stimulatory factors such as interleukin-3 (IL-3) may be potentially more effective. Recent data indicate that rhGM-CSF has synergistic effects with IL-3, IL-1 and IL-6 also act synergistically with IL-3 and GM-CSF. Ultimately combinations of these and possibly other factors such as erythropoietin and/or thrombopoietic factors may be effective treatment for aplastic anemia.

REFERENCES

16. Golde DW, Bersch N, Quan SG, Lusis AJ: Production of erythroid-potentiating activity by a human T-lymphoblast cell line. Proc Natl Acad Sci USA 77:593, 1980
Treatment of refractory aplastic anemia with recombinant human granulocyte-macrophage-colony-stimulating factor

RE Champlin, SD Nimer, P Ireland, DH Oette and DW Golde