A Phase I/II Trial of Recombinant Granulocyte-Macrophage Colony-Stimulating Factor for Children With Aplastic Anemia

By Eva C. Guinan, Colin A. Sieff, Dagmar H. Oette, and David G. Nathan

Nine pediatric patients (median age, 8 years; range, 0.7 to 19 years), eight with refractory aplastic anemia and one with newly diagnosed aplasia, were enrolled in a phase I/II trial of recombinant human granulocyte-macrophage colony-stimulating factor (rhGM-CSF) administered via continuous intravenous infusion. Doses ranged from 8 to 32 μg/kg/d. Six of eight evaluable patients responded with a significant rise in neutrophil count (median fourfold increase; range, 2.5- to 31-fold) during the 28-day induction period. Five patients completed 2 further months of therapy (maintenance) with persistent or improved neutrophil responses. Three patients had bone marrow aspirates suggestive of increased erythropoiesis, although only one patient had improvement in peripheral hematocrit and platelet count. In the five patients completing maintenance, three experienced a rapid return to baseline counts after rhGM-CSF administration. These results demonstrate that rhGM-CSF increases peripheral neutrophil counts in children with refractory and newly diagnosed aplastic anemia and may be able to stimulate a multilineage response in a more limited number. Randomized, prospective trials are necessary to determine if rhGM-CSF administration will impact favorably on the morbidity and mortality of severe aplastic anemia.

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MATERIALS AND METHODS

Patients’ characteristics. Patients were eligible for inclusion on study if they were younger than 19 years of age, did not have a histocompatible family donor, were 12 or more weeks posttreatment with ATG without durable response, and had less than 1.0 × 10^9/L neutrophils, less than 50 × 10^9/L platelets, and less than 60 × 10^9 reticulocytes in the setting of a bone marrow biopsy demonstrating less than 20% cellularity. Patients with aplasia in the setting of paroxysmal nocturnal hemoglobinuria, Fanconi’s anemia, or clonal cytogenetic abnormalities were excluded from the study. Nine children were enrolled (Table 1), eight with idiopathic aplastic anemia and one (UPN 01) whose aplasia occurred in the setting of non-A, non-B hepatitis. The median age of the patients was 8 years and the male to female ratio was 4:5. Median disease duration was 7 months (range, 1 to 31 months). Seven of nine patients had received treatment with ATG; two had received more than one course and four patients had a history of ATG response followed by relapse (UPN 01, 02, 03, 07). The median time since the last round of ATG therapy was 6.5 months (range, 3.0 to 24.0 months). The remaining two patients had been unable to receive ATG due to drug unavailability. Most patients (seven of nine) had received cyclosporin A corticosteroids, and five of the nine had been treated with cyclosporin, all with minimal or no response. All patients had a history of transfusion. Eight of the nine patients were red blood cell (RBC)

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transfusion dependent on entering study; one patient (UPN 01) maintained a hemoglobin of 6 to 7 g/dL without transfusion. Seven of nine were platelet transfusion dependent; patients 01 and 07 maintained platelet counts of 15 and 2 \times 10^9/L, respectively, without transfusion and without significant hemorrhage. Marrow cellularity was less than 5% in eight of nine patients and 10% to 15% in one patient (UPN 01).

All patients were without clinical evidence of infection on entering the study. Patients were required to have normal cardiac, pulmonary, renal (creatinine <1.8 mg/mL), and hepatic (liver function tests <1.5 x normal) function. The studies were approved by the Committee for the Protection of Human Subjects, The Children's Hospital (Boston, MA). Informed consent was obtained from all patients and/or their parents.

Recombinant human granulocyte-macrophage colony-stimulating factor. The rhGM-CSF used in this study was provided by Sandoz Corporation (East Hanover, NJ). The GM-CSF cDNA clone was expressed in Chinese hamster ovary cells, producing a single-chain, glycosylated polypeptide with a molecular weight of 14 to 34 Kd. The purified product had a specific activity of 5.4 \times 10^9 U/mg of glycoprotein.

Study design. The drug was administered by constant infusion through a central venous catheter for 28 days (defined as induction); patients failing to have a complete hematologic response after the first 14 days had their dose doubled for a second 14 day period. Four patients initiated therapy at 8 \mu g/kg/d; their dose was escalated to 16 \mu g/kg/d. Five patients initiated therapy at 16 \mu g/kg/d; four of these subsequently had their dose increased to 32 \mu g/kg/d. Drug was interrupted or discontinued for worse than grade 2 toxicity.

RESULTS

Eight of the nine patients completed the induction period and are evaluable both for response and toxicity. Five patients completed their induction course without interruption (within 28 days), while three patients required additional time to receive 28 days of treatment (see below). A single patient (UPN 04) failed to tolerate induction therapy and is evaluable for toxicity but not for hematologic response. Six patients responded and were placed on maintenance, with five of the six completing 3 months of drug therapy according to the study design. The remaining patient was removed from the study because of noncompliance.

Hematologic response during induction. All eight evaluable patients were evaluable for hematologic response.

Table 1. Patient Characteristics

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age (yr)/Sex</th>
<th>Disease Duration* (mo)</th>
<th>Prior Therapy</th>
<th>Current Status/Survival†</th>
</tr>
</thead>
<tbody>
<tr>
<td>01</td>
<td>5/M</td>
<td>24.5</td>
<td>ATG x 1, CSA, steroids, gammaglobulin, splenectomy</td>
<td>Alive/20† mo</td>
</tr>
<tr>
<td>02</td>
<td>16/F</td>
<td>24.0</td>
<td>ATG x 2, steroids</td>
<td>Dead/9 mo</td>
</tr>
<tr>
<td>03</td>
<td>9/F</td>
<td>8.0</td>
<td>ATG x 1, steroids</td>
<td>Dead/10 mo</td>
</tr>
<tr>
<td>04</td>
<td>19/M</td>
<td>1.5</td>
<td>CSA</td>
<td>Dead/10 mo</td>
</tr>
<tr>
<td>05</td>
<td>0.7/F</td>
<td>5.0</td>
<td>ATG x 1, steroids, CSA</td>
<td>Alive/11† mo</td>
</tr>
<tr>
<td>06</td>
<td>8/M</td>
<td>4.0</td>
<td>ATG x 1, steroids</td>
<td>Alive/11† mo</td>
</tr>
<tr>
<td>07</td>
<td>10/M</td>
<td>31.0</td>
<td>ATG x 3, CSA, steroids</td>
<td>Alive/8† mo</td>
</tr>
<tr>
<td>08</td>
<td>8/F</td>
<td>1.0</td>
<td>None</td>
<td>Alive/7† mo</td>
</tr>
<tr>
<td>09</td>
<td>2.8/F</td>
<td>7.0</td>
<td>ATG x 1, steroids, gammaglobulin</td>
<td>Alive/5† mo</td>
</tr>
</tbody>
</table>

Abbreviation: CSA, cyclosporin A.
*Calculated from diagnosis until rhGM-CSF initiation.
†Calculated from time of rhGM-CSF initiation.

The interface mononuclear cells were washed and resuspended at 1 x 10^9/mL (bone marrow) or rhGM-CSF and IL-3 had specific activities of 1 to 4 \times 10^6 U/mg, respectively, in the chronic myelogenous leukemia blast proliferation assay. The factors were kindly provided by Genetics Institute. Erythroid burst-forming units, granulocyte and/or monocyte colony-forming units (CFU-G, CFU-M, and CFU-GM) were counted on day 14.

Laboratory tests. Before and during drug administration, patients were evaluated by complete history and physical and laboratory examinations, which consisted of chest radiograph, electrocardiogram, complete blood cell count with differential, reticulocyte count, serologic biochemical profile, coagulation profile, and peripheral blood immunophenotyping. Bone marrow aspirates and biopsies were also obtained at predetermined intervals.

In vitro progenitor assays. Bone marrow and blood samples were separated over Ficoll-Hypaque (Pharmacia, Piscataway, NJ). The interface mononuclear cells were washed and incubated at 37°C in 5% CO₂ in Iscove's modified Dulbecco's medium/20% fetal calf serum on 100-mm tissue culture dishes for 1 hour. The nonadherent cells were washed and resuspended at 1 x 10^7/mL (bone marrow) or 2 x 10^7/mL (blood) in Iscove's modified Dulbecco's medium/30% fetal calf serum, 1% deionized bovine serum albumin (Cohn fraction V, Sigma Chemical Co, St Louis, MO), 10⁻⁴ mol/L B-mercaptoethanol (Sigma Chemical Co), and 0.9% methylcellulose. The cultures were stimulated with 2 U/mL erythropoietin (human recombinant, 300,000 U/mg, kindly provided by T. Gesner and S. Clark, Genetics Institute, Cambridge, MA) and rhGM-CSF (1 nmol/L), interleukin-3 (IL-3; 1 nmol/L), or a combination of both factors. The rhGM-CSF and IL-3 were isolated from medium conditioned by Chinese hamster ovary cells and from Escherichia coli, respectively, engineered to express high levels of the factors. The homogeneous rhGM-CSF and IL-3 had specific activities of 1 to 4 x 10^6 U/mg and 2 x 10^6 U/mg, respectively, in the chronic myelogenous leukemia blast proliferation assay. The factors were kindly provided by Genetics Institute. Erythroid burst-forming units, granulocyte and/or monocyte colony-forming units (CFU-G, CFU-M, and CFU-GM) were counted on day 14.

RESULTS

Eight of the nine patients completed the induction period and are evaluable both for response and toxicity. Five patients completed their induction course without interruption (within 28 days), while three patients required additional time to receive 28 days of treatment (see below). A single patient (UPN 04) failed to tolerate induction therapy and is evaluable for toxicity but not for hematologic response. Six patients responded and were placed on maintenance, with five of the six completing 3 months of drug therapy according to the study design. The remaining patient was removed from the study because of noncompliance.

Hematologic response during induction. All eight evalu-
able patients had an increase in peripheral white blood cell (WBC) count during the 28-day induction (from median initial counts of $1.78 \times 10^9/L$ [range, 0.78 to $4.0 \times 10^9/L$] to median end of induction counts of $2.89 \times 10^9/L$ [range, 1.58 to $58.57 \times 10^9/L$]). Six of the eight patients met the criteria for a partial hematologic response, as measured by increments of greater than $0.5 \times 10^9/L$ in the absolute neutrophil count (ANC), by 4 weeks of treatment (Fig 1). The median ANC increased almost fourfold in these responding patients (from median initial ANCs of $0.375 \times 10^9/L$ [range, 0.08 to $0.68 \times 10^9/L$] to median end induction ANCs of $1.41 \times 10^9/L$ [range, 0.70 to $12.30 \times 10^9/L$]). In both dose groups, some patients did not attain their maximal ANC until 6 to 8 weeks of rhGM-CSF administration. Of particular note, the frequency and degree of ANC responses were virtually the same in both dose cohorts (Fig 1).

An increase in absolute eosinophil count (AEC) was seen in all eight patients (median maximal AEC, $1.16 \times 10^9/L$; range, 0.05 to $29.29 \times 10^9/L$). The increase in AEC was generally proportional to the degree of neutrophil response but was transient, decreasing after the first month of treatment in four of the five patients receiving 3 months of drug therapy. The AEC also decreased in the fifth such patient, but somewhat later in her course. The majority of patients had an increase in monocytes proportional to the increase in WBC count, but only one (UPN 08) developed a monocytosis. Lymphocyte counts increased variably with the increase in WBC count.

**Hematologic response during maintenance and after study cessation.** Of the eight patients who completed the first month of therapy, six were eligible for the maintenance phase. The other two (UPN 05 and 09) were ineligible for continuation because of inadequate response during induction. Five of the six patients placed on continuation completed 3 months of therapy. The sixth patient (UPN 02) was removed from study at 6 weeks because of lack of compliance.

Four of five patients (UPN 01, 06, 07, and 08) sustained their neutrophil response throughout the maintenance course. Patient 01 has maintained a normal WBC count and ANC for over a year off therapy. A second patient (UPN 08) maintained improved neutrophil counts (ANC 0.7 to $1.5 \times 10^9/L$) for 2 months posttreatment. Her ANC then decreased sharply, and she has just been initiated on extended therapy with subcutaneous rhGM-CSF at $2 \mu g/kg/d$. The ANC decreased sharply within 10 to 20 days of drug cessation in the two other patients (06 and 07). Both elected a trial of extended subcutaneous rhGM-CSF treatment at 2 $\mu g/kg/d$ and again demonstrated a granulocyte response. The remaining patient (UPN 03) that completed maintenance developed a viral syndrome 1 month into her course. Her clinical picture, shared by her family, was characterized by diarrhea and was accompanied by an increase in atypical lymphocytes on blood smear and a decrease in ANC to zero despite continued rhGM-CSF administration. Her ANC increased again as her clinical picture improved, but decreased on study termination. She was not rechallenged.

In addition to his dramatic increase in ANC (Fig 1A), patient 01 (who was transfusion independent at study inception with hemoglobin 6 to 7 g/dL and platelets $15 \times 10^9/L$) experienced a steady increase in hemoglobin after 2 months of therapy and subsequently also demonstrated an increase in platelet count (Fig 2). This response has been maintained for over a year without further rhGM-CSF administration. Although three other patients (UPN 06, 07, and 08) had intermittent, modest ($\leq 5\%$) increases in reticulocyte count, no effect on RBC or platelet transfusion requirement was discerned in these or other patients during the period of the study. Interestingly, patient 08, who achieved a peak reticulocyte count of 5.0% at the end of the study, required only one RBC transfusion in the 2 months after drug cessation, in
have *Staphylococcus aureus* bactiuria and positive beta-hemolytic streptococcus throat culture (both cultures obtained before drug initiation) 2 days after drug treatment was started. These infections were treated successfully. The streptococcal throat infection recurred and was retreated later in the patient’s rhGM-CSF treatment course. No other infections were documented in any patient while on treatment. Patient 02 (removed from study because of noncompliance) developed *Pseudomonas aeruginosa* cellulitis very shortly after rhGM-CSF was discontinued.

Table 1 indicates the current status of all of the patients. Five of the nine patients enrolled in the study are alive; four of these patients are responders who completed maintenance and either sustained their improved hematologic function off rhGM-CSF (UPN 01 and, for 2 months, UPN 08) or elected to receive extended treatment (UPN 06 and 07, with patient 08 just initiating further therapy). Patient 09, the only nonresponder now alive, has received steroid and cyclosporin A therapy with intermittent periods of response. Four of the patients have died of hemorrhagic and infectious complications. Of these, patient 05 was a treatment failure, patient 04 failed to tolerate the drug, patient 02 responded but was removed because of noncompliance shortly after completing induction, and patient 03 both responded and completed maintenance, but did not elect to receive further rhGM-CSF.

**Toxicity.** Drug therapy was generally well tolerated. The patients treated at 8 or 16 µg/kg/d all completed 14 days at each dose level without interruption and without dose reduction. Similarly, all four patients completing induction at the next dose level received the first 2 weeks at 16 µg/kg/d without interruption. However, three of the four received abbreviated courses of the 32 µg/kg/d dose (drug held at 4, 5, and 7 days because of fever, fever, and rash, respectively). In these patients, the rhGM-CSF was held (for 1 to 3 days), and symptomatic treatment with acetaminophen and/or diphenhydramine was initiated. When the symptom(s) resolved, rhGM-CSF was restarted at a lower dose.

Only one episode of fever (<38.5°C) in a single patient and geographically limited, rapidly resolving rashes in three patients were observed in the cohort of patients initiated at 8 and escalated to 16 µg/kg/d. Two of these patients also had a single measured elevation of liver enzymes to two times normal, which, like the fever and rashes, resolved without cessation or alteration of rhGM-CSF delivery. The only patient intolerant of induction was in the group treated at 16 and then 32 µg/kg/d. He developed unremitting fever and a diffuse urticarial rash during the first 2 weeks of therapy and was removed from the study. Three of the remaining four patients in this cohort experienced some fever during the first 2 weeks of therapy. These episodes resolved spontaneously. Fever recurred in two patients when the dose was escalated. Limited and rapidly resolving rashes were seen in three patients, while one patient had a more pronounced urticarial rash occurring with his dose increase. Later in the maintenance course, a more limited urticarial rash recurred in this patient and intermittent low-grade fever (<38.5°C) and rash recurred in another; the former patient’s rash was treated successfully with oral diphenhydramine and the fever and rash of the second patient (08) resolved without symptomatic
therapy. Patient 08 complained of malaise in addition to fever and rash.

Patient 08, with a chronic requirement for high-volume blood product replacement, also developed cough and tachypnea after 5 weeks of treatment (in the higher dose group). She was found to have moderate hypoxemia, bilateral pleural effusions, and a modest pericardial effusion with normal ventricular function. The rhGM-CSF (24 μg/kg/d) was stopped, diuresis was initiated, and she normalized her clinical and laboratory status within several days. After 1 week, the rhGM-CSF was reinitiated at 8 μg/kg/d and continued for another 2 months without evident cardiopulmonary toxicity.

**DISCUSSION**

This trial was designed to evaluate the effects of rhGM-CSF administration to children with aplastic anemia, with particular attention to the effects of dose on efficacy and toxicity. Drug was delivered in escalating doses via continuous central infusion and continued for weeks to months without interruption. All evaluable patients demonstrated an increase in WBC count and the majority achieved a neutrophil count generally regarded as "protective" in terms of susceptibility to serious infection. Bone marrow aspirates and biopsies appeared to demonstrate increased erythropoiesis in three patients, but only one demonstrated a significant increase in hemoglobin concentration. With one exception, no measurable effect on platelet number was observed. While our patients did have an increase in AEC, this did not appear to be as marked or long-lived as that reported by others.15,17

Although the patient number was limited, it was striking that in the fourfold dose range examined here, dose did not appear to influence the degree or frequency of response. There was one nonresponder in each dose cohort. Although both WBC count and ANC generally increased during the period of dose escalation (days 14 through 28), this was probably attributable more to duration of therapy than to the dose increase. This interpretation is supported by the continued increase in ANC of patients 04, 07, and 08 despite their limited number of treatment days at the increased dose. In addition, four of the five patients continued on maintenance did not attain their maximal ANC until substantially later in their course, and at less than their maximal rhGM-CSF dose. No evidence for tachyphylaxis or stem cell exhaustion was observed. All five patients eligible for maintenance therapy appeared to remain sensitive to rhGM-CSF administration for the duration of the 3-month study. In addition, two patients were rechallenged after study completion and again responded to drug administration with significant improvement in neutrophil counts.

There was a trend to better response in patients with higher initial ANC (Fig 1). If our patients’ responses are analyzed according to the criteria used by Champlin et al.,17 the four patients with an initial ANC less than 0.28 \( \times 10^9 \) /L (range, 0 to 0.23) had a median increment of 0.37 \( \times 10^9 \) /L (range, 0.05 to 1.21) at day 28, while the four patients with higher initial ANC (range, 0.35 to 0.68) had a median increment of 1.17 \( \times 10^9 \) /L (range, 0.73 to 11.9) at the same time point. However, poor initial peripheral counts did not always predict poor response. For example, patient 06 had a prestudy ANC of 0.08 \( \times 10^9 \) /L, which rose substantially to 1.21 \( \times 10^9 \) /L by day 28 and to nearly twice that later in his course. Residual bone marrow cellularity was also not predictive in the sense that seven of eight patients had equivalently hypocellular marrows yet different degrees of response. However, the single patient (UPN 01) to have somewhat greater (10% to 15%) cellularity did have the greatest improvement. Marrow culture results, unlike those reported by Champlin et al.,15 but consistent with the report of Vadhan-Raj et al.,13 neither predicted response nor improved with drug administration.

In view of the responsiveness of these children to the lower doses of rhGM-CSF and the apparent lack of a dose-response relationship in the studied dose range, the experience with toxicity was particularly important. Although some toxicity was seen, it appeared to be substantially less at each dose level than that observed in adults.12,15,17 While multiple constitutional complaints have commonly been reported by adults receiving rhGM-CSF,12,15,17 only a single pediatric patient complained of myalgia. No patient experienced bony pain nor a "flare" of inflammation at a previously involved site, both problems also reported in adults.12,15,17 Indeed, patients treated in the 8 or 16 μg/kg/d group were free from significant toxicity. Those treated at 16 or 32 μg/kg/d did experience more frequent fever and rash at the higher dose; in general, these symptoms subsided rapidly and patients tolerated further therapy at the lower dose (16 μg/kg). The one patient to experience what may have been the "vascular leak" syndrome reported previously in patients treated with high doses of rhGM-CSF16,17 recovered uneventfully and went on to tolerate and respond to drug administered at a lower dose.

The second measure by which our patients differed from those in previously reported studies is in the striking absence of infectious episodes. Although all the children had central venous access sites (eight central lines and one Portacath), we saw no episodes of gram-positive bacteremia nor did we observe gram-negative bacteremia or soft-tissue infections. Such events were reported in adult studies,13,15,17 including that of Champlin et al.17 in which the source of drug, route of administration, and dose were most analogous to our own. However, the patient numbers in all of the reported series are relatively small, and a prospective randomized trial of rhGM-CSF administration would be necessary to answer definitively questions relating to infection rate and infectious morbidity.

It is noteworthy, however, that all four patients who completed induction and maintenance followed by either a sustained response or placement onto extended rhGM-CSF therapy are still alive. This is in marked contradistinction to patients who for one reason or another received more abbreviated treatment. Of course, the present study includes too few patients for statistical analysis of benefit, and it may well be that response to and tolerance of rhGM-CSF are measures of, rather than contributors to, a more resilient patient. Therefore, the role of rhGM-CSF in a palliative approach to aplastic anemia in childhood, especially with regard to the potential of an increased ANC to temper the...
eliminate infectious episodes, will only be fully elucidated by a randomized, prospective study. The results presented here, in which a high rate of response was observed to occur without toxicity at the lowest dose used, suggest that daily treatment at a "conservative" dose may be the therapeutic study design of choice. The largely benign clinical course of these patients suggests that hematopoietic growth factor administration may be of value in children with aplastic anemia.

REFERENCES


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