Stimulation of Granulopoiesis by High-Dose Recombinant Human Granulocyte Colony-Stimulating Factor in Children With Aplastic Anemia and Very Severe Neutropenia

By Seiji Kojima and Takaharu Matsuyama

We investigated the efficacy and safety of high-dose recombinant human granulocyte colony-stimulating factor (rhG-CSF) in treating 10 children with severe aplastic anemia and fewer than 0.05 × 10^9/L neutrophils. Doses of rhG-CSF ranging from 400 to 2,000 μg/m²/d were administered as a 30-minute intravenous infusion daily for 4 weeks. In 6 of the 10 children, treatment increased the neutrophil count by 10-fold to greater than 60-fold (range, 0.21 to 1.8 × 10^9/L). Bacterial or fungal infections that were present at study entry resolved in all 6 responders, who are still alive with a median survival of more than 27 months (range, 15 to 54 months) since the initiation of treatment. Three of 4 nonresponders died of infection, whereas 1 nonresponder received a bone marrow transplant and is alive. No serious toxicity was attributable to rhG-CSF. It was well tolerated at doses up to 2,000 μg/m²/d and effectively stimulated granulopoiesis. This agent thus offers promise as adjuvant treatment for severe infections in children with aplastic anemia and very severe neutropenia.

MATERIALS AND METHODS

Patients. Forty-nine children (aged 1 to 17 years) with severe or moderate acquired AA were treated with rhG-CSF from June 1988 to December 1992. Among them, children with very severe AA who had fewer than 0.1 × 10^9/L neutrophils for more than 2 weeks were eligible for inclusion in the study. The clinical characteristics are shown in Table 1. Their median age was 6 years (range, 1 to 13 years). The male-to-female ratio was 64. The median interval between diagnosis and initiation of rhG-CSF treatment was 9 weeks (range, 6 to 26 weeks). All had received previous treatment for AA such as ALG, high-dose methylprednisolone, or cyclosporine, but none had received any of these therapies for at least 4 weeks before study entry. AA was secondary to non-A, non-B, non-C hepatitis in 3 patients, but was of unknown cause in the other 7. Eight patients exhibited clinical evidence of bacterial or fungal infection at study entry; pneumonia in 4, cellulitis in 3, and septicemia in 1. The absolute neutrophil count was less than 0.05 × 10^9/L in all 10 patients; 5 lacked circulating neutrophils on entering the study. All 10 patients had essentially no reticuloocytes in the peripheral blood and were dependent on transfusions of red blood cells and platelets. Although none had an HLA-matched sibling donor, 2 later received a BMT from HLA-genotypically mismatched related donors, the first from a one HLA-antigen/mismatched sibling and the second from an HLA-phenotypically matched cousin. This study was approved by the Committee on Clinical Investigation at the Japanese Red Cross Nagoya First Hospital. Informed consent for the patient's participation was obtained from the parents in each case.

rhG-CSF. The rhG-CSF used in the study was provided by Kirin Brewery (Tokyo, Japan). The recombinant protein was expressed in Escherichia coli and had a molecular weight of 10.8 kD. The purified protein had a specific activity of approximately 1 × 10^7 U/mg and is greater than 95% pure.

Study design. The treatment schedule included four dose levels of rhG-CSF (400, 800, 1,200, and 2,000 μg/m²/d). The drug was administered daily via a 30-minute intravenous infusion for 28 days. Patients who failed to show a hematologic response after the first 14 days received a higher dose of rhG-CSF for a second 14-day period. Of the 3 patients (nos. 1 through 3) initially administered 400 μg/m²/d, 2 subsequently had the dose increased to 800 μg/m²/d. All 3 patients initially receiving rhG-CSF at 800 μg/m²/d (nos. 4 through 6) then received a dose of 1,200 μg/m²/d. Patients no. 7 and 8 initially received rhG-CSF at 1,200 μg/m²/d and then received a dose of 2,000 μg/m²/d. The dose of rhG-CSF for patients no. 9 and
10 was 2,000 μg/m²/d. A positive response to treatment was defined as an increase of neutrophil count greater than 0.2 × 10⁹/L.

Both before and during the study, the patients were monitored by means of a complete medical history, physical examination, and laboratory tests, including complete blood counts, differential and reticulocyte counts, chemistry profiles, coagulation profiles, and urinalysis. BM was aspirated before and after each course of treatment for cytologic and cyogenetic analysis.

Statistical calculations were performed using the χ² test with Yates correction. A level of P < .05 was accepted as statistically significant.

RESULTS

The hematologic status of the 10 patients before and after treatment with rhG-CSF is shown in Table 2 and Fig 1. Six of the 10 had an increase in absolute neutrophil counts (10-fold to >60-fold) during the 28 days of treatment. The median absolute neutrophil count increased from 0.01 × 10⁹/L (range, 0 to 0.05 × 10⁹/L) to 0.44 × 10⁹/L (range, 0.21 to 1.8 × 10⁹/L) in 6 responders. In 2 of the 6 responders, the response occurred within 2 weeks of initiating therapy. Other patients did not respond until after 3 to 4 weeks of rhG-CSF administration. The pretreatment neutrophil count was significantly correlated with the clinical response to rhG-CSF administration (P = .05). Only 1 of the 5 patients with an absence of circulating neutrophils before treatment responded to therapy. All 5 patients with minimal residual myelopoiesis (neutrophil counts, 0.01 to 0.05 × 10⁹/L) showed an increase in neutrophil count after rhG-CSF therapy. In the responders, treatment led to at least a twofold increase in the peripheral monocytes. There was no increase in the number of circulating erythrocytes and platelets and the need to supply them by transfusion was unaffected by treatment. Because neutrophil counts returned to baseline levels 10 days after the treatment had been discontinued in patient no. 1, 5 patients later received maintenance therapy with rhG-CSF for 9 to 52 months. The maintenance dose was 200 μg/m²/d and was administered by subcutaneous injections three times a week. The maintenance therapy produced sustained increases in the number of neutrophils and monocytes in all 5 patients.

BM morphology was evaluated before and after rhG-CSF therapy in 8 of 10 patients (Table 2). There were improve-

Table 2. Effects of High-Dose rhG-CSF on Hematologic Indexes of Patients Studied

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Dose of G-CSF (μg/m²/d)</th>
<th>Peripheral Blood (×10⁹/L)</th>
<th>BM (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>WBC</td>
<td>Neutrophils</td>
<td>Monocytes</td>
</tr>
<tr>
<td>1</td>
<td>400</td>
<td>1.1 2.1</td>
<td>0.02 0.44</td>
</tr>
<tr>
<td>2</td>
<td>400/800</td>
<td>1.4 2.6</td>
<td>0.06 0.21</td>
</tr>
<tr>
<td>3</td>
<td>400/800</td>
<td>0.3 1.7</td>
<td>0.05 0.50</td>
</tr>
<tr>
<td>4</td>
<td>800/1,200</td>
<td>2.8 3.1</td>
<td>0 0 0 0</td>
</tr>
<tr>
<td>5</td>
<td>800/1,200</td>
<td>1.5 3.9</td>
<td>0 0 0 0</td>
</tr>
<tr>
<td>6</td>
<td>800/1,200</td>
<td>1.9 1.6</td>
<td>0 0 0 0</td>
</tr>
<tr>
<td>7</td>
<td>1,200/2,000</td>
<td>0.1 0.6</td>
<td>0.01 0.24</td>
</tr>
<tr>
<td>8</td>
<td>1,200/2,000</td>
<td>1.0 1.0</td>
<td>0 0 0 0</td>
</tr>
<tr>
<td>9</td>
<td>2,000</td>
<td>1.7 3.1</td>
<td>0.03 1.8</td>
</tr>
<tr>
<td>10</td>
<td>2,000</td>
<td>3.1 4.9</td>
<td>0 1.5 0.28</td>
</tr>
</tbody>
</table>

Abbreviation: ND, not done.

* The patient developed myelodysplasia, which progressed to acute myeloid leukemia at 23 months since the initiation of rhG-CSF therapy.
† The patients received a BMT from HLA-genotypically mismatched related donors.
ments in marrow cellularity and in myeloid maturation in 6 patients who responded. Before rhG-CSF treatment, BM cellularity was less than 1% in all evaluable patients; after treatment, cellularity increased to levels of 5% to 10% in all responders. In the responding patients, myeloid elements in BM increased from a baseline median level of 6.0% (range, 1.0% to 18.0%) to 31.5% (range, 11.0% to 70.5%), leading to an increase in the myeloid:erythroid cell ratio.

Marrow cytogenetic studies were performed for 9 patients before treatment, and for 5 patients during the maintenance period. Adequate material for cytogenetic analysis was obtained from patients no. 3 and 6 before treatment and showed normal karyotypes. Cytogenetic studies could not be performed because of the low mitotic rate in 7 patients. In patient no. 7, chromosomal analysis was performed 6 months after the initiation of rhG-CSF treatment, showing 20 of 20 cells with a normal male karyotype. Subsequent examination of BM cells at 10 months showed 7 of 20 with a monosomy 7 karyotype. Twelve months later, when he had developed an acute myeloid leukemia, a monosomy 7 karyotype was found in 100% of the metaphase obtained from his leukemic BM. Repeat cytogenetic examinations showed normal karyotypes during the maintenance period in 4 of 5 patients.

Eight patients had bacterial or fungal infections upon entry into the study. Except for patient no. 8, who received a BMT, the other 3 nonresponders died from infectious complications (Patients no. 4 and 5, disseminated aspergillosis; patient no. 6, pneumonia caused by unknown organism) between 2 and 6 months after initiating treatment. All responders were cleared of their infections and are still alive between 15 and 54 months since the initiation of treatment. Immunosuppression with cyclosporine was started when the number of neutrophils exceeded $1 \times 10^9/L$. Cyclosporine was administered orally at a dose of 10 mg/kg/d in two divided doses. The dose was subsequently modified according to serum levels of cyclosporine measured by radioimmunoassay to obtain a level of 100 to 200 ng/mL. Urea and creatinine were measured weekly and the dose was modified according to the results. In 4 of 6 responders, there was an increase in the number of reticulocytes and platelets after 2 to 3 months of maintenance therapy with cyclosporine and rhG-CSF; these patients became transfusion independent and their condition has remained stable. Patient no. 9 showed an increase in neutrophil count but depended on red blood cell and platelet transfusions. He received a marrow graft from his sibling who was haploidentical to the patient and mismatched for one HLA DR locus antigen on the nonidentical haplotype.

The administration of high-dose rhG-CSF was well tolerated. We observed no signs of toxicity attributable to rhG-CSF, such as fever, chills, myalgia, or bone pain, even at a dose of 2,000 µg/m²/d. No patients had increase in the size of spleen during the observation period. All responders showed a greater than 1.5-fold increase in the serum alkaline phosphatase level, but no other significant change in serum chemistry was noted.

**DISCUSSION**

Four different recombinant human colony-stimulating factors (G-CSF, GM-CSF, IL-3, IL-1α, and IL-1β) were used in treating patients with AA in an attempt to increase the production of mature neutrophils from the remaining small pool of precursor cells. The results suggest...
that, although all these factors except IL-1 can elevate neutrophil levels in some patients with SAA, those with very severe neutropenia generally do not respond to these agents.

Nissen et al.²⁹ treated 4 patients with very severe AA whose pretreatment neutrophil count was less than 0.05 x 10⁹/L; the dose of rhGM-CSF was 4 to 32 µg/kg/d. One patient with minimal residual myelopoiesis (pretreatment neutrophil count, 0.03 x 10⁹/L) responded transiently to this agent. The other 3 patients, who showed no evidence of residual myelopoiesis (pretreatment neutrophil count, 0 to 0.01 x 10⁹/L) failed to respond. All 4 patients died within 6 months after the initiation of rhGM-CSF therapy. The investigators concluded that rhGM-CSF could not induce hematopoiesis in long-standing SAA with complete myelopoietic failure.

The conventional doses of rhG-CSF previously used were in the range of 50 to 400 µg/m²/d or 2 to 10 µg/kg/d. At these doses, the majority of cytopenic patients had evidenced augmented granulopoiesis,¹⁸,¹⁹ whereas patients with very severe AA were refractory. Asano et al.²¹ reported the results of treatment with rhG-CSF for 30 patients with AA. Although 70% responded to the conventional doses of rhG-CSF, there was no elevation of neutrophil count in 7 patients whose neutrophil counts were initially less than 0.1 x 10⁹/L. In our previous study,⁴ none of the 5 patients with pretreatment neutrophil counts less than 0.1 x 10⁹/L showed any improvement in neutrophil count after the administration of rhG-CSF at a dose of 400 µg/m²/d. When increasing doses (800 or 1,200 µg/m²/d) were administered to the nonresponders, 3 of them showed an increase in neutrophil count.

Based on these findings in this phase I/II study, we investigated the optimal dosage schedule for rhG-CSF in children with very severe AA whose pretreatment neutrophil counts were less than 0.05 x 10⁹/L. Six of 10 patients responded with an increase in neutrophil counts during the 28 days of treatment. It was difficult to demonstrate a dose-response relationship for the entire group because of the heterogeneity of the initial hematologic status of the patients. All 3 patients receiving the lowest doses (400/800 µg/m²/d) showed a good response, but none of the 3 patients receiving 800/1,200 µg/m²/d responded. Two patients showed a prompt response after the administration of the highest dose (2,000 µg/m²/d) of rhG-CSF. The response to rhG-CSF appeared to be correlated with the pretreatment neutrophil count rather than with the dose level of rhG-CSF. Only 1 of the 5 patients who had absolutely no circulating neutrophils responded to therapy, even after receiving 2,000 µg/m²/d. Accordingly, it remains unclear whether a very high dose of rhG-CSF, eg, 2,000 µg/m²/d, can restore granulopoiesis in patients who lack circulating neutrophils.

It is noteworthy that patients with very severe AA took longer to respond than the patients with less severe disease. In our previous study,⁴ a response occurred within 48 hours of initiating the rhG-CSF infusion in 9 of the 12 responders whose initial neutrophil counts ranged from 0.15 to 0.68 x 10⁹/L. In the present study, the responses occurred between 2 and 4 weeks after the start of rhG-CSF infusion. The rapid increases in neutrophil count observed in patients with less severe AA probably resulted from the ability of G-CSF to induce the mobilization of mature neutrophils into the blood stream from storage pool in the marrow.²⁰ In patients with very severe AA, the increase in neutrophil count would be attributable to an increase in granulopoiesis, as indicated by the increased cellularity and myeloid-erythroid ratio in BM.

It is not known whether colony-stimulating factors influence the natural history of AA or whether their long-term administration would be effective in preventing the morbidity and mortality caused by severe bacterial or fungal infection. Although rhGM-CSF was able to elevate the neutrophil counts of patients with AA, frequent episodes of severe infection occurred during treatment in previous studies.¹⁰,¹¹ In the present investigation, 8 of the 10 patients had episodes of bacterial or fungal infection upon entry into the study. The infection resolved in all 4 infected responders; all 6 of the responders are still alive, with a median survival time of 35 months. They had no episodes of severe infection during maintenance therapy with rhG-CSF. This is in marked contrast to the findings in the 4 nonresponders, 3 of whom died from infection within 6 months of the start of rhG-CSF therapy. Only 1 nonresponder is still alive; she received a BMT from an HLA-phenotypically matched cousin. These data indicate that high-dose rhG-CSF therapy can decrease the mortality from severe infection and affect the natural history of patients with very severe AA.

In patients previously treated with higher doses of rhGM-CSF, fatigue and myalgia were common and some developed pulmonary infiltrates.¹¹ Such toxicity limited the dose escalation of rhGM-CSF. In contrast, no major toxicity occurred in the children receiving higher doses of rhG-CSF in the present study. Administration of very high doses of rhG-CSF (100 to 2,500 µg/kg/d) to mice resulted in a decrease in BM cellularity, a decrease in marrow colony-forming cells (CFCs), and an increase in spleen weight and splenic CFCs.¹² Mild splenomegaly probably caused by myeloid hyperplasia has also been noted in some patients with congenital agranulocytosis treated with rhG-CSF.²² These findings suggest that G-CSF functions to redistribute hematopoietic progenitor cells from the marrow compartment to extramedullary sites. Because few hematopoietic progenitors remain in the BM that redistribute to the spleen, patients with very severe AA probably do not develop symptoms such as splenomegaly even after receiving very high doses of rhG-CSF.

From the present study, we concluded that rhG-CSF administered in doses up to 2,000 µg/m²/d is well tolerated, restores granulopoiesis, and may provide useful adjuvant therapy for severe infections in children with AA and very severe neutropenia.

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