CLINICAL TRIALS with hematopoietic growth factors are proceeding rapidly with encouraging results to date. Both recombinant human GM colony-stimulating factor (GM-CSF) and G-CSF have produced leukocyte increases in patients with hematopoietic disorders. A beneficial effect of rhGM-CSF on bone marrow (BM) function has been reported in patients with aplastic anemia. The agent stimulates not only the progenitors of the neutrophils and monocytes but also those of RBCs and megakaryocytes. In contrast, rhG-CSF stimulates only the production of neutrophilic granulocytes. In a phase I/II clinical trial, administration of rhG-CSF increased the neutrophil count in patients with congenital neutropenia and myelodysplastic syndrome. We report the results of administering rhG-CSF to 20 children with aplastic anemia. The findings indicate that rhG-CSF increased the neutrophil count and was effective as adjunctive treatment of infection in these patients.

MATERIALS AND METHODS

Twenty patients with aplastic anemia ranging in age from 1 to 17 years entered the study from June 1988 to August 1989. Their clinical and laboratory data are summarized in Table 1. All but three had severe disease according to currently accepted criteria. Aplasia was secondary to acute hepatitis in two patients, and the cause was unknown in 18. The interval between diagnosis and initiation of therapy ranged from 1 to 240 weeks. Eight patients had acute aplastic anemia (diagnosed within 12 weeks of the study); 12 had chronic disease. All but five patients had received previous treatment for the aplastic anemia which had included antilymphocyte globulin, high-dose methylprednisolone, androgen, and cyclosporine. While receiving rhG-CSF, four patients had active infection, two had sepsisemia, and two had phlegmon. Except for three patients with moderate aplastic anemia, all patients received transfusions of platelets and RBCs every 4 to 2 weeks. 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.

The rhG-CSF used in this study was provided by Kirin Brewery (Tokyo, Japan). The recombinant protein was expressed in Escherichia coli and had an activity of 1 × 10^6 U/mg protein. rhG-CSF was administered at a dose of 400 μg/m^2 on a 30-minute intravenous (IV) infusion for two weeks. Higher dose levels (800 or 1,200 μg/m^2 per day) were used for five patients who did not respond to the initial dose. Patients were evaluated by a complete blood count and BM aspirates before and after the end of each treatment course. Response to treatment was defined as a twofold or greater increase and >1.0 × 10^9/L in neutrophil counts.

RESULTS

Hematologic effects. The hematologic response of the patients before and after the course of rhG-CSF treatment is shown in Table 2. Of the 20 patients, 12 (60%) had a marked neutrophil response after the initial course of treatment. The responses occurred within 48 hours of the start of the infusion in 9 of the 12 responders. Three patients (patients 2, 3, and 15) responded to treatment at 6, 8, and 12 days, respectively. In the 12 responders, treatment led to a marked increase (2.7- to 28.0-fold) in the neutrophil count, and nine also showed at least a twofold increase in the peripheral monocytes. There was no significant change in either the lymphocyte or eosinophil count. The number of circulating RBCs and platelets and the patient's transfusion requirements were unaffected by treatment. The response was transient, with the blood count returning to baseline within 2 to 10 days after treatment was discontinued.

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BM morphology was evaluated before and after administration of rhG-CSF. In 10 of the 12 patients who responded to initial rhG-CSF therapy, the myeloid elements increased, leading to an increase in the myeloid/erythroid cell ratio (from a baseline median value of 2.5 to a maximum of 10.0). Neither BM cellularity nor the number of committed myeloid progenitors changed significantly, however (Table 2).

Two variables, a short interval between time of diagnosis to initiation of rhG-CSF therapy and a neutrophil count <0.1 × 10^9/L, were significantly correlated with the poor response to rhG-CSF in univariate analyses (P = .004, P = .001, respectively). Multivariate logistic regression analysis showed that only the interval between diagnosis and administration of rhG-CSF was significantly correlated with a poor response (Table 3). Patients treated with rhG-CSF...
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Table 3. Factors Related to Response: Multivariate Logistic Regression

<table>
<thead>
<tr>
<th>Factor*</th>
<th>No. of Patients</th>
<th>Response</th>
<th>No Response</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time from diagnosis to rhG-CSF therapy</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 ~ 12 wk</td>
<td>1</td>
<td>8</td>
<td></td>
<td>.008</td>
</tr>
<tr>
<td>&gt; 12 wk</td>
<td>11</td>
<td>1</td>
<td></td>
<td></td>
</tr>
</tbody>
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*Other factors evaluated that were not significantly associated with response included age, sex, infections occurring during rhG-CSF therapy, previous therapy for aplastic anemia, neutrophil count, platelet count, reticulocyte count, and hemoglobin level.

within 12 weeks of diagnosis had a lower response rate than those treated thereafter (P = .008). Not significantly associated with a poor response were age, sex, infection occurring during rhG-CSF therapy, previous therapy for aplastic anemia, neutrophil count, platelet count, reticulocyte count, and hemoglobin level.

Higher doses of 800 or 1,200 \( \mu g/m^2 \) per day were administered to the five patients who had not responded to 400 \( \mu g/m^2 \) per day (Fig 1). In two of the five, a dose of 800 \( \mu g/m^2 \) per day increased the neutrophil count and the level was further increased by administration of 1,200 \( \mu g/m^2 \) per day to the three nonresponders. The higher doses were well tolerated. An increase in neutrophils was noted in one of three patients.

Clinical outcome. After the trial, five patients who had not received any treatment for aplastic anemia other than rhG-CSF received a combination of immunosuppressive therapy with antilymphocyte globulin and high-dose methylprednisolone and four who had an HLA-compatible donor received a BM transplant (BMT). During rhG-CSF therapy, four patients had bacterial infection for which they received treatment with IV antibiotics. None of them had responded to the initial rhG-CSF dose of 400 \( \mu g/m^2 \) per day for 2 weeks, their neutrophil counts remained critically low, and high fever persisted during the treatment. Additional courses of rhG-CSF administered at an increased dose were administered to three of these four patients. While they were receiving the higher dose, their neutrophil counts increased, their general clinical condition improved, and the infection cleared. One patient who did not receive any further courses of rhG-CSF after the trial deteriorated progressively and died of septicemia 1 month after discontinuance of rhG-CSF.

Two other patients died of infectious complications. In patient 12, an increase in the dose to 1,200 \( \mu g/m^2 \) per day did not increase the neutrophil count. After combined immunosuppressive therapy with antilymphocyte globulin and high-dose methylprednisolone, she developed bilateral pneumonia. A second course of rhG-CSF, 1,200 \( \mu g/m^2 \) per day for 4 weeks, was administered when she had no circulating neutrophils. She failed to respond and died of fungal pneumonia.

Patient 11 did not respond to the initial dose of 400 \( \mu g/m^2 \) per day, but at a dose of 1,200 \( \mu g/m^2 \) per day, her neutrophil count increased. Nevertheless, she required transfusion of RBCs and platelets. She received combined immunosuppressive therapy with antilymphocyte globulin and high-dose methylprednisolone after discontinuing rhG-CSF therapy without improvement. Because her neutrophil count was persistently low, she then received a second course of rhG-CSF, 1,200 \( \mu g/m^2 \) per day for 8 weeks, without improvement. She then developed disseminated aspergillosis. No anti-G-CSF antibody was detected in her serum. This patient became critically ill and died during rhG-CSF administration, 10 months after it was initiated. The remaining 17 patients have survived for a median of 12 months since initiation of rhG-CSF.

Side effects. Therapy with rhG-CSF was well tolerated. We did not observe any toxicity attributable to rhG-CSF, such as fever, chills, myalgia, or bone pain. All six patients who did not respond to the initial course of treatment and who subsequently received rhG-CSF showed an increase in the serum alkaline phosphatase level > 1.5-fold. No other significant changes in serum chemistry were noted.

Fig 1. Neutrophil counts in five patients with aplastic anemia who did not initially respond to 400 \( \mu g/m^2 \) per day rhG-CSF and who subsequently received an increased dose of 800 or 1,200 \( \mu g/m^2 \) per day.
DISCUSSION

We investigated the safety and efficacy of rhG-CSF administered to 20 young patients with aplastic anemia at a dose of 400 μg/m² per day for 2 weeks. Twelve patients responded with an increase in neutrophils during the initial course of treatment. Of the five patients who did not respond to the initial dose and who then received a higher dose (800 or 1,200 μg/m² per day), the treatment increased the neutrophil count in three. Thus, our experience suggests that an optimum dose of rhG-CSF may increase the neutrophil count in more than 80% of patients with aplastic anemia.

Several phase I/II studies have been conducted with rhGM-CSF in aplastic anemia, and some positive effects have been observed. Vadhan-Raj et al demonstrated that rhGM-CSF increased the neutrophil count in 10 patients with severe or moderate aplastic anemia. In another study, however, only one of four patients with very severe neutropenia responded to rhGM-CSF.

We attempted to identify factors that would predict the therapeutic response of patients with aplastic anemia to rhG-CSF. Nissen et al suggested that rhGM-CSF cannot reestablish myelopoiesis in patients who have long-standing complete aplasia and who are critically ill and recommended that rhGM-CSF be administered at an earlier stage to such patients with minimal myelopoiesis. In contrast, our study demonstrates that newly diagnosed younger patients with aplastic anemia had a lower response rate than those diagnosed earlier. Of five newly diagnosed patients, three of whom had pretreatment neutrophils >0.3 × 10⁹/L and residual myelopoiesis in BM, none responded to the initial rhG-CSF therapy. After combined immunosuppressive therapy with antilymphocyte globulin and high-dose methylprednisolone, three responded to a subsequent course of rhG-CSF. Because such newly diagnosed patients may have ongoing myelopoietic suppression, this suppression may need to be abrogated when neutrophil production is stimulated with rhG-CSF.

Patients with very severe neutropenia tended to be refractory to the initial course of rhG-CSF in that none of the five patients with pretreatment neutrophils <0.1 × 10⁹/L showed improvement in neutrophil counts after the initial course. One patient who received only an initial course of rhG-CSF had no improvement in neutrophil count and died of septicemia. In the remaining four patients, the dose was increased to 800 or 1,200 μg/m² per day and all responded, showing a substantial increase in circulating neutrophils that allowed clearance of infection. Because patients with very severe neutropenia may improve only slowly, the response observed at the higher dose may actually be attributed to the extended duration of treatment, not necessarily to the higher dose. Accordingly, the optimum dose schedule remains to be established for patients with very severe neutropenia.

In addition, the relative indications for rhG-CSF and rhGM-CSF in aplastic anemia remain to be established. Concerning hematologic effects, rhG-CSF administration caused no significant change in eosinophils and marrow cellularity, in contrast to findings in patients given rhGM-CSF. The patients tolerated rhG-CSF well, and none exhibited any significant toxicity attributable to this agent. In contrast, in patients treated with a higher dose of rhGM-CSF, fatigue and myalgia were common and some developed pulmonary infiltrates. Thus, rhGM-CSF appears to be more toxic than rhG-CSF, perhaps owing to the rhGM-CSF-induced priming of monocytes, which would enhance the secondary release of such inflammatory mediators as tumor necrosis factor and interleukin-1.

Administration of rhG-CSF may be potentially useful in treating aplastic anemia in several settings. Six of the 20 patients had episodes of bacterial or fungal infection during the study, and the three who failed to respond to rhG-CSF died of infection within 10 months of diagnosis of aplastic anemia. The other three patients with infection responded to rhG-CSF, and their infections cleared. They are alive with a median survival of 12 months after diagnosis. Thus, rhG-CSF is a promising adjuvant to the conventional treatment of infections in these patients.

Because severe neutropenia indicates a poor prognosis in patients with aplastic anemia treated with either immunosuppressive therapy or BMT, an attempt to increase the circulating neutrophils by administering rhG-CSF appears to be warranted. Use of rhG-CSF to treat severe aplastic anemia may reduce the mortality from infections during the interval between initial diagnosis and BMT or between the initiation of immunosuppressive therapy and the response to such agents.

REFERENCES

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Treatment of aplastic anemia in children with recombinant human granulocyte colony-stimulating factor

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