**Contrasting Effects of Recombinant Human Granulocyte-Macrophage Colony-Stimulating Factor (CSF) and Granulocyte CSF Treatment on the Cycling of Blood Elements in Childhood-Onset Cyclic Neutropenia**

By Daniel G. Wright, Richard F. Kenney, Dagmar H. Oette, Vincent F. LaRussa, Laurence A. Boxer, and Harry L. Malech

Recombinant human granulocyte colony-stimulating factor (G-CSF) treatment has been shown to increase average neutrophil counts substantially in patients with childhood-onset cyclic neutropenia (or "cyclic hematopoiesis"), but not to eliminate the cyclic oscillations of neutrophil counts or those of other blood elements (monocytes, platelets, eosinophils, and reticulocytes) that are characteristic of this hematopoietic disorder. Indeed, oscillations of neutrophil counts are amplified during G-CSF treatment. We have compared the effects of recombinant granulocyte-macrophage-CSF (GM-CSF) with those of G-CSF in three patients with this disease (2 men and 1 woman, 17, 30, and 32 years of age). These patients were treated with GM-CSF (2.1 μg/kg/day, subcutaneously) for 6 weeks, preceded and followed by 6 to 13 weeks of detailed observation to document changes in the cycling of blood neutrophils and other blood elements; two of the patients were subsequently treated with G-CSF (5.0 μg/kg/day, subcutaneously) and observed for comparable periods of time. Unlike G-CSF treatment, which increased average neutrophil counts more than 20-fold, GM-CSF increased neutrophil counts only modestly, from 1.6- to 3.9-fold, although eosinophilia of varying prominence was induced in each patient. However, at the same time, GM-CSF treatment dampened or eliminated the multilinear oscillations of circulating blood elements (neutrophils, monocytes, platelets, and/or reticulocytes) in each of the patients. In contrast, G-CSF treatment of the same patients markedly amplified the oscillations of neutrophil counts and caused the cycling of other blood elements (monocytes in particular) to become more distinct. These findings support the conclusion that the distinctive cycling of blood cell production in childhood-onset cyclic neutropenia results from abnormalities in the coordinate regulation of both GM-CSF-responsive, multipotential progenitor cells and G-CSF-responsive, lineage-restricted, neutrophil progenitors.

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Human cyclic neutropenia (or "cyclic hematopoiesis") is a rare but distinctive hematologic disorder characterized by regular oscillations in the numbers of circulating neutrophils and monocytes, as well as other blood elements. Neutrophil counts decrease to profoundly low levels at regular 3-week intervals, followed by a recovery phase during which neutrophils return to the circulation. Monocyte counts also cycle with the same periodicity but do so out of phase with neutrophils. Moreover, oscillations of monocyte counts, unlike the neutrophil cycles but like those of other blood elements (eg, platelets and reticulocytes), generally fluctuate between normal and above-normal levels. Patients with cyclic neutropenia typically experience recurrent malaise, fever, aphthous stomatitis, and, occasionally, serious cutaneous and subcutaneous infections during periods of profound neutropenia.

Cyclic neutropenia occurs both as a childhood-onset and an adult-onset disease. The latter form of the disease has been associated with a clonal proliferation of CD56" large granular lymphocytes, and treatment of this form of the disease with low-dose, alternate-day corticosteroids or with cyclosporine has been found to cause sustained remissions of the neutropenia and associated hematologic abnormalities. Childhood-onset cyclic neutropenia, on the other hand, has not been found to respond to these agents, or to a variety of other therapeutic interventions, including splenectomy, androgens, lithium, and plasmapheresis. This form of the disease appears to be a congenital condition in most if not all cases, is not associated with a lymphoproliferative disorder, and often occurs in a familial pattern suggestive of autosomal dominant inheritance.

Oscillations in the numbers of circulating neutrophils and other blood elements in both forms of cyclic neutropenia have been shown to result from abnormalities in the regulation of blood cell production from hematopoietic precursor and progenitor cells. Furthermore, findings from recent studies of the in vitro clonal growth of myeloid progenitor cells (colony-forming units granulocyte-macrophage [CFU-GM]) recovered from patients with childhood-onset cyclic neutropenia indicate that these cells express a relative insensitivity to two recombinant myelopoietic growth factors, granulocyte-macrophage colony-stimulating factor (GM-CSF) and granulocyte-CSF (G-CSF). These findings prompted us to investigate the comparative in vivo effects of exogenously administered G-CSF and GM-CSF in patients with childhood-onset cyclic neutropenia, as did our interest in the relative clinical usefulness of these recombinant human growth factors in treating patients with this disorder.

In 1989, Hammond et al reported the results of G-CSF treatment in five patients with childhood-onset cyclic neutropenia and one patient with the adult-onset form of the disease.

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Submitted September 20, 1993; accepted April 5, 1994.

Supported in part by National Institutes of Health research awards (RO1AI20065 and MO1RR0042) to the Clinical Research Center at the University of Michigan.

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ease. They found that G-CSF, both when administered intravenously (IV) at doses of 3 to 10 µg/kg/day and subcutaneously (SC) at 3 to 5 µg/kg/d, greatly increased mean blood neutrophil counts. However, in the patients with childhood-onset disease, cyclic oscillations of neutrophil counts not only persisted but were amplified. The cycling of other blood elements also continued during G-CSF treatment and in some cases appeared to become more distinct. Three subsequent case reports have described effects of GM-CSF treatment in patients with cyclic neutropenia. However, these reports describe conflicting results with GM-CSF treatment. In two of these reports, GM-CSF was administered only briefly, and it is unclear in the third report whether the case described had the childhood-onset or adult-onset form of the disease. Furthermore, none of these reports is informative with respect to the effects of GM-CSF on the cycling of neutrophils and other blood elements.

In the present report, we describe in detail contrasting effects of GM-CSF and G-CSF treatment in three patients with childhood-onset cyclic neutropenia. Although GM-CSF, as administered, proved to be relatively ineffective compared with G-CSF in increasing average neutrophil counts, GM-CSF treatment was found to dampen or eliminate the cyclic oscillations in the numbers of circulating neutrophils and other blood elements, whereas G-CSF, as previously reported, amplified these oscillations.

### MATERIALS AND METHODS

*Study subjects.* Three patients with well-characterized childhood-onset cyclic neutropenia took part in these studies, after informed consent was obtained and in accordance with protocols approved by the human studies review board at the Clinical Center, National Institutes of Health (NIH; Bethesda, MD), and also, in the case of patient no. 2, at the Mott Children’s Hospital, University of Michigan Medical Center (Ann Arbor, MI). Clinical features of these patients are summarized in Table 1. Each of the patients had been observed clinically by one or more of the investigators for 2 to 14 years before these studies, and two had been subjects of previous reports. At the beginning of these studies, each patient underwent a complete physical exam, review of medical history, chest x-ray, and routine screening blood tests. During periods of observation off therapy and during most of the time while receiving G-CSF, patients were observed as outpatients. However, during periods of treatment with GM-CSF, each patient was observed as an inpatient at the Clinical Center, NIH.

*GM-CSF and G-CSF treatment.* After an initial 6 to 13 weeks of observation during which they received supportive care only, patients were treated with recombinant human GM-CSF for 6 weeks (2.1 µg/kg/d, administered three times daily [TID] in equally divided doses every 8 hours by SC injection). Treatment was initiated during the recovery phase of neutrophil cycles in each patient. After 6 weeks of treatment, patients were observed for an additional 6 to 10 weeks off therapy. Purified, high specific activity, recombinant human GM-CSF, produced from Chinese Hamster Ovary cells, was provided by the Sandoz Research Institute (East Hanover, NJ). Patients no. 1 and 2 were subsequently treated with recombinant human G-CSF (filgrastim) for up to 10 weeks (5.0 µg/kg/d, administered SC in a single daily dose) after additional periods of observation off therapy. Purified, high specific activity, recombinant G-CSF, produced from *Escherichia coli*, was obtained from Amgen Inc (Thousand Oaks, CA) in its commercially available form (Neupogen [filgrastim]) and used off-label in patient no. 1 under an approved protocol at the Clinical Center, NIH, or in patient no. 2 at the University of Michigan Medical Center as part of a multicenter trial of G-CSF in severe congenital neutropenias sponsored by Amgen Inc.

*Hematologic assessment.* Complete blood counts, including white blood cell (WBC) differentials, were based on at least three times per week throughout these studies and daily during periods of GM-CSF treatment. Except where noted, all WBC counts were based on 200 cell differentials determined by a single observer (D.G.W.). Bone marrow aspirate and biopsy specimens were obtained for assessment of marrow cell morphology and for myeloid progenitor cell (CFU-GM) culture immediately before beginning and stopping GM-CSF treatment as well as before and during G-CSF treatment. Hematopoietic progenitor cell clonogenic assays were performed in 0.8% methylcellulose cultures with gradient-separated, light-density, adherent cell-depleted marrow cells using standard methodologies reported previously.

*Clinical assessment.* Each patient was instructed in maintaining daily records of symptoms and signs relevant to the morbidity typically encountered with cyclic neutropenia, eg, the occurrence of malaise, fever (oral temperature ≥99.5°F), aphthous stomatitis or pharyngitis, a change in routine activities because of illness, a clinically evident infection of the skin or other site, and use of an antibiotic upon instruction by patient’s physician. These records were maintained during all periods of observation both off and on growth factor therapy. Together with frequent clinical assessments by investigators, these records were used as measures of neutropenia-related morbidity and to calculate morbidity scores, similar to those described previously, before, during, and after treatment.

*Statistical analysis.* To quantify changes in the oscillations of blood cell counts during periods when patients were receiving or not receiving GM-CSF or G-CSF, coefficients of variation for blood cell counts recorded during the different periods of observation were calculated and compared. In addition, harmonic regression analysis, as described by Tong, was used to fit cyclical curves to the sequential blood cell counts observed for each patient during the various periods of observation before and during treatment with recombinant CSFs. This analysis assumes that data for blood cell counts can be fit to a model harmonic function described by the following equation:

\[ y_i = M + A \cos(2\pi \frac{d}{C_i} - \tau_i) \]

where \( i \) indicates the individual patient and period of observation, \( y_i \) is the cell count at any given day \( d \), \( C_i \) is the cycle length in days, \( M \) is the mean cell count, \( A \) is the amplitude of the harmonic curve, and \( \tau_i \) is the time at which the harmonic curve reaches its

### Table 1. Clinical Features of Patients With Cyclic Neutropenia

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Age/Sex</th>
<th>Age at Onset of Clinical Disease</th>
<th>Familial Occurrence</th>
<th>Morbidity at Time of Study</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>32/F</td>
<td>1-2 yr</td>
<td>+</td>
<td>Moderate to severe recurrent malaise, aphthous stomatitis, gingivitis, skin infections, cellulitis</td>
</tr>
<tr>
<td>2</td>
<td>17/M</td>
<td>&lt;1 yr</td>
<td>+</td>
<td>Moderate to severe recurrent malaise, aphthous stomatitis, gingivitis, skin infections</td>
</tr>
<tr>
<td>3</td>
<td>30/M</td>
<td>2-3 yr</td>
<td>-</td>
<td>Mild recurrent malaise, aphthous stomatitis</td>
</tr>
</tbody>
</table>
peak. $M_1$, $A_1$, and $T_1$ were calculated using this equation for varying cycle lengths ($C_1$) using least-squares regression. The value of $C_1$ that gave the largest value of $R^2$ (or closest fit) for data obtained during the before treatment period with each patient was selected as the cycle length time that best described each patient’s basic blood cell cycling pattern.

RESULTS

Hematologic effects of GM-CSF treatment. Serial blood neutrophil, monocyte, platelet, eosinophil, and (for patient no. 3) reticulocyte counts of the study patients before, during, and after GM-CSF treatment are shown in Figs 1, 2, and 3. Means, standard deviations, and coefficients of variation for these blood cell counts during study periods on and off GM-CSF therapy are shown in Table 2. As is apparent in the figures, each patient demonstrated an approximately 3-week cycling of neutrophil, monocyte, and platelet counts characteristic of cyclic neutropenia. Patient no. 3, for whom reticulocyte counts were also obtained, demonstrated distinct cycling of reticulocytes as well. During GM-CSF treatment,
Fig 2. Serial neutrophil, monocyte, platelet, and eosinophil counts from patient no. 2, before, during, and after 6 weeks of GM-CSF treatment (blood cell counts per microliter).

Fig 3. Serial neutrophil, monocyte, platelet, eosinophil, and reticulocyte counts from patient no. 3, before, during, and after 6 weeks of GM-CSF treatment (blood cell counts per microliter).
the cyclic oscillations of blood cell counts became generally less apparent, but promptly returned once GM-CSF was discontinued. The dampening of blood cell cycles induced by this growth factor was most readily apparent with monocytes, platelets, and, in patient no. 3 reticulocytes, but also with the cycling of neutrophil counts in patient no. 1. This dampening of blood cell cycles was reflected by consistent decreases in the coefficients of variation for blood cell counts recorded during GM-CSF treatment in all hematopoietic lineages examined, compared with periods off therapy both before and after GM-CSF treatment (Table 2). It should be noted that differences in the numbers (n) of observations during periods on and off treatment could independently influence coefficients of variation, although the independent effects of n would be small, given the large number of blood count measurements that were made. However, the numbers of observations off treatment were greater than those during treatment. Hence, the observed decreases in coefficients of variation during GM-CSF treatment are, if anything, underestimates of the true decreases in the variation of blood cell counts that occurred with this treatment.

The dampening of blood cell cycling during GM-CSF treatment was also apparent from harmonic curve fit analysis, illustrated in Figs 4 and 5. The oscillations of blood cell counts observed with patients no. 1, 2, and 3 before treatment could be fit best to curves generated by a simple harmonic function equation for cycle times of 18.5, 22.0, and 21.5 days, respectively. For all blood cell counts measured with patients no. 1 and 2 (Fig 4 and 5), and for the monocyte, platelet, and reticulocyte counts measured with patient no. 3 (data not shown), a dampening or elimination of detectable blood cell cycling was reflected by a decrease in the amplitude of the harmonic curve that fit the data and/or a decrease in the degree of fit (eg, decreased R² value). Statistically significant degrees of fit to cyclic, harmonic function curves persisted during GM-CSF treatment for the neutrophil counts in patient no. 2 (albeit with a diminished amplitude) and for neutrophil and platelet counts in patient no. 3 (albeit, again, with a diminished amplitude of the harmonic curve that fit the platelet counts in this patient).

Despite the apparent effects of GM-CSF on blood cell cycling, this growth factor had only modest effects on average neutrophil counts. Although these counts were increased on average in all patients during GM-CSF treatment, only in patient no. 3 was profound neutropenia (eg, neutrophil counts < 500/µL) clearly abated. Average monocyte counts were doubled in patients no. 2 and 3 from levels that had been elevated to begin with, whereas average platelet counts decreased slightly in patient no. 3 but were essentially unchanged in the other patients. Eosinophil counts increased during GM-CSF treatment in all patients. This effect of GM-CSF, similar to that described in a previous case report, was particularly prominent in patients no. 2 and 3, who developed eosinophilias as high as 22,700 and 50,400/µL, respectively, by 4 weeks of GM-CSF treatment. It should be noted that patient no. 1 was the most cytopenic of the patient cohort and that, whereas the absolute increase in average eosinophil counts during GM-CSF treatment was less in this patient than in the others, the proportional increase (35-fold) was similar (25-fold, patient no. 2; 51-fold, patient no. 3).

Bone marrow cellularity, assessed morphologically in biopsy specimens, was increased in each patient at the end of 6 weeks of GM-CSF treatment, and this change was reflected by increases in the numbers of nucleated marrow cells recovered from 10-mL marrow aspirate specimens obtained after treatment, compared with specimens obtained before treatment (Table 3). Consistent with the changes in circulating leukocytes observed in these patients, marrow cell morphology was dominated by eosinophil precursors in each patient following GM-CSF treatment. Total numbers of myeloid (CFU-GM), erythroid (burst-forming units-erythroid [BFU-E]), and multipotential (CFU-Mix) clonogenic progenitors recovered from marrow aspirates were also increased after GM-CSF treatment in each patient (Table 3).

Contrasting hematologic effects of G-CSF. After periods of treatment with GM-CSF, patients no. 1 and 2 were subsequently treated with G-CSF (5.0 µg/kg/d, SC). As reported by Hammond et al and in distinct contrast to GM-CSF treatment, G-CSF rapidly induced large increases in blood neutrophil counts (Figs 6 and 7, and Table 4). However, the cycling of neutrophil counts clearly persisted and was
Fig 4. Best fit harmonic function curves derived from the data for serial neutrophil, monocyte, and platelet counts from patient no. 1 before and during GM-CSF treatment, as well as during the first 6 weeks of G-CSF treatment. Best fit curves for the before treatment period were obtained for a cycle time of 18.5 days. The degrees of fit for these curves were highly significant \( P < .0001 \), neutrophils and monocytes; \( P < .0005 \), platelets. Harmonic curves generated from GM-CSF treatment period data for a cycle time of 18.5 days were of diminished amplitude and had a degree of fit that was significant only for platelets \( (P < .05) \). Best fit curves for data during G-CSF treatment were obtained for a cycle time of 15.5 days, had greatly increased amplitudes (note changes in scale), and had significant degrees of fit \( (P < .02 \) neutrophils; \( P < .0004 \), monocytes).

amplified. The cycling of monocyte counts and (in patient no. 2) platelet and reticulocyte counts also persisted and in general appeared to become more distinct, and this finding was reflected both by consistent increases in coefficients of variation (Table 4) and by harmonic curve fit analysis (Figs 4 and 5). Unlike GM-CSF treatment, G-CSF treatment was not associated with increases in eosinophil counts.

Marrow aspirate specimens obtained during G-CSF treatment showed substantial increases in the proportions of both mature and immature neutrophil precursors among morpho-
GM-CSF v G-CSF TREATMENT OF CYCLIN NEUTROPENIA

**Fig 5.** Best fit harmonic function curves derived from the data for serial neutrophil, monocyte, and platelet counts from patient no. 2 before and during GM-CSF treatment, as well as during the first 6 weeks of G-CSF treatment. Best fit curves for the before treatment period were obtained for a cycle time of 22.0 days. The degrees of fit for these curves were highly significant ($P < .0001$, neutrophils and monocytes; $P < .0004$, platelets). Harmonic curves generated for the GM-CSF treatment period with a cycle time of 22.0 days were of diminished amplitude and had a degree of fit that was significant only for neutrophils ($P < .01$). Best fit curves for data during G-CSF treatment were obtained for a cycle time of 14.0 days, had increased amplitudes (note changes in scale), and had highly significant degrees of fit for neutrophils and monocytes, but not for platelets ($P < .006$, $P < .005$, and $P = .08$, respectively).

Logically recognizable nucleated marrow cells, compared with marrow specimens obtained before treatment, as reported by Hammond et al. There were also clear increases in marrow cellularity that were similar to those observed with GM-CSF treatment (data not shown).

**Clinical effects of GM-CSF and G-CSF treatment.** Despite the relatively modest effects of GM-CSF treatment on neutrophil counts in these patients, each patient experienced a cessation or clear decrease in neutropenia-related morbidity while receiving GM-CSF. Decreases in the occurrence of episodic malaise, aphthous ulcers, low-grade fever, and/or skin infections were reflected by decreases in neutropenia-related morbidity scores (Table 5) that were computed in a manner similar to the clinical assessment scores reported by Hammond et al. Although no serious clinical side effects attributable to GM-CSF were encountered, each patient experienced mild bone pain at some time during treatment. Each patient while receiving GM-CSF also experienced pru-
ritic, erythematous, maculonodular lesions at rotating injection sites. These lesions were 2 to 5 cm in diameter, appeared within several days of using a particular skin site for repeated SC injections, and disappeared within 1 to 3 days after rotation of injections to an alternative site. One patient (no. 1) developed mild, palpable splenomegaly with intermittent left upper quadrant discomfort at the end of 6 weeks of treatment with GM-CSF, which resolved after treatment was discontinued.

Decreases in neutropenia-related morbidity were also experienced by patients no. 1 and 2 during G-CSF treatment (Table 5), and this degree of symptomatic improvement was similar to that observed with GM-CSF. These patients also experienced transient bone pain, similar to that noted during GM-CSF treatment, and mild palpable splenomegaly again occurred in patient no. 1. Erythematous lesions at injection sites did not occur with G-CSF. However, patient no. 1 developed an erythematous, papular rash on her lower legs after about 6 weeks of G-CSF treatment, which by biopsy was consistent with a leukocytoclastic vasculitis. This rash was distant from injection sites but was clearly related to the G-CSF therapy, for it resolved when G-CSF was temporarily withdrawn, returned when G-CSF was reinstituted, and then resolved again when the dose of G-CSF was reduced. A similar side effect has been reported in at least one other patient treated with G-CSF.12

**DISCUSSION**

The first clinical trials of recombinant, human myelopoeitic growth factors, GM-CSF and G-CSF, in patients with neutropenia were reported in 1987 and 1988, and there is now substantial experience with the short-term use of these agents to reduce the duration and severity of neutropenia in cancer patients after myelosuppressive chemotherapy and in patients with advanced human immunodeficiency virus (HIV) disease.10,11 Reported experience with these cytokines to treat chronic congenital neutropenias is more limited. Nonetheless, G-CSF has been shown to increase circulating neutrophil counts substantially and to reduce neutropenia-associated morbidity in some children with Kostmann syndrome,14,18,19 as well as in both children and adults with cyclic neutropenia.10,15 There is less information concerning the use of GM-CSF in such patients.

In studies reported by Welte et al16 of five children with severe congenital neutropenia (Kostmann syndrome), treated first with GM-CSF (3 to 30 μg/kg/d, administered by 30 to 60 minutes of daily IV infusions for >6 weeks) and subsequently with G-CSF (3 to 15 μg/kg/d administered SC for >20 weeks), it was found that increases in circulating neutrophil numbers occurred in only one of the five patients during treatment with GM-CSF, whereas a normalization of neutrophil counts was observed in all patients during G-CSF treatment, as also reported by Bonilla et al.19 Long-term G-CSF treatment of patients with childhood-onset cyclic neutropenia,10,14 on the other hand, although increasing average neutrophil counts substantially, was not found to eliminate the cyclic oscillations of neutrophil counts or those of other blood elements (eg, monocytes, platelets, and reticulocytes), which are characteristic of this disorder.1,2 These latter findings were confirmed in our present studies.

To date, there have been three case reports that describe

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<tr>
<th>Assessment</th>
<th>Patient No.</th>
<th>Off Treatment</th>
<th>After GM-CSF*</th>
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<tr>
<td>Marrow cellularity†</td>
<td>1</td>
<td>40%</td>
<td>80%</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>75%</td>
<td>&gt;90%</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>75%</td>
<td>&gt;90%</td>
</tr>
<tr>
<td>Nucleated cells per 10 mL marrow aspirate‡</td>
<td>1</td>
<td>17</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>42</td>
<td>117</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>38</td>
<td>65</td>
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<td>CFU-GM§</td>
<td>1</td>
<td>31</td>
<td>70</td>
</tr>
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<td>78</td>
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<td>103</td>
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<td>49</td>
</tr>
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<td></td>
<td>2</td>
<td>48</td>
<td>128</td>
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<tr>
<td></td>
<td>3</td>
<td>32</td>
<td>103</td>
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<tr>
<td>CFU-Mix§</td>
<td>1</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>7</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>1</td>
<td>6</td>
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* Evaluations were performed immediately before and at the end of 6-week treatment periods.
† Marrow cellularity expressed as a percentage estimated from biopsy specimens.
‡ Numbers of light-density, nucleated marrow cells (×10⁶)/10 mL marrow aspirate.
§ Numbers of clonogenic progenitors (×10⁶)/10 mL marrow aspirate.
experiences with GM-CSF treatment in cyclic neutropenia. However, the information provided by these reports is limited and contradictory.11,15 GM-CSF (6 μg/kg/d, SC) treatment of a 35-year-old man with cyclic neutropenia was described briefly in a letter by Freund et al11 and contrasted with subsequent treatment with G-CSF (1 to 3 μg/kg/d, SC). Although GM-CSF (administered for 9 days) did not increase neutrophil numbers in the patient, G-CSF treatment markedly increased the patient’s average neutrophil counts while at the same time amplifying neutrophil cycling, as described by others.16 In contrast, Locatelli et al12 found that brief periods of GM-CSF treatment (4 μg/kg/d by continuous IV infusion for 7 days) increased neutrophil counts in a child with cyclic neutropenia during periods of expected neutropenic nadirs with apparent therapeutic benefit. A third case report by Kurzrock et al13 described the normalization of neutrophil counts in a 30-year-old woman with cyclic neutropenia during treatment with low doses of GM-CSF (0.3 μg/kg/d, SC); however, the infrequency with which blood counts were obtained (≤1/week) renders this latter case report somewhat difficult to interpret with respect to effects of GM-CSF on neutrophil cycling, an issue that was not addressed in the other case reports. Moreover, none of these case reports provide information of any kind concerning the effects of GM-CSF on the cycling of blood elements other than neutrophils. In addition, it is not clear whether all patients described in these reports11-13 had the childhood-onset form of the disease. This issue is meaningful because the childhood- and adult-onset forms of cyclic neutropenia may well respond differently to GM-CSF treatment, as appears to be the case with G-CSF treatment.10

In our present studies, which compared in detail the effects of GM-CSF and G-CSF in three patients with childhood-onset cyclic neutropenia, GM-CSF treatment resulted in variable but generally modest increases in circulating neutrophil numbers, whereas G-CSF treatment increased average neutrophil counts more than 20-fold, as observed by others.15,16 However, G-CSF treatment also greatly amplified the oscillations of neutrophil counts and had similar effects on the oscillations of monocyte, platelet, and reticulocyte counts, as indicated by increased coefficients of variation for these counts during treatment and also by the increased amplitudes of harmonic curves that best fit the blood cell count data. In contrast, GM-CSF dampened or eliminated the oscillations of neutrophil, monocyte, platelet, and/or reticulocyte counts. This effect was apparent both in longitudinal graphs of blood cell counts and in harmonic curve fit analysis, as well as in the consistently decreased coefficients of variation for blood cell counts during treatment compared with those recorded during periods off treatment. These findings suggest that hematopoietic cycling in childhood-onset cyclic neutropenia originates from abnormalities in the numbers or function of GM-CSF-responsive, multipotential progenitor cells that

Fig 7. Serial neutrophil, monocyte, platelet, and reticulocyte counts from patient no. 2, before and during 10 weeks of G-CSF treatment (blood cell counts per microliter).
have not yet progressed developmentally to a stage of G-CSF-responsive neutrophil commitment.

The comparative effects of GM-CSF and G-CSF treatment in vivo in patients with childhood-onset cyclic neutropenia are of particular interest because recent in vitro studies of the clonal growth of myeloid progenitor cells (CFU-GM) recovered from the marrow of patients with this disorder have shown that CFU-GM in cyclic neutropenia express a relative insensitivity to both myelopoietic growth factors. Although pharmacologic administration of neither growth factor alone has been found to fully correct the intrinsic hematopoietic defects in patients, each cytokine appears to affect distinct aspects of the abnormally regulated blood cell production that results from these defects. These findings suggest the possibility that combined treatment with GM-CSF and G-CSF might more completely correct the hematologic abnormalities of childhood-onset cyclic neutropenia through coordinate activities.

Four separate mathematical models of the regulation of myelopoiesis have been based on the distinctive hematologic findings in cyclic neutropenia (or "cyclic hematopoiesis"). Each of these models incorporates features of a feedback regulation system in which there are obligatory time delays in the response to feedback signals (eg, the development of mature neutrophils from progenitor cells that are controlled by feedback signals). These models variously emphasize the size of the marrow storage pool of postmitotic neutrophilic cells and rate of egress from this pool, the strength of feedback signals (or of the sensitivity of responding progenitor cells to the signal), and the numbers of progenitors or stem cells available to the neutrophil developmental pathway, as variables that influence whether an intrinsic tendency of the myelopoietic system to cycle is expressed or not. These models of the regulation of myelopoiesis, which were formulated before GM-CSF, G-CSF, or other cytokines that influence hematopoiesis had been identified or well characterized, deserve to be reconsidered both in light of the distinct effects of GM-CSF and G-CSF on hematopoietic cycling in childhood-onset cyclic neutropenia, as observed in the present study, and in light of the distinct effects of G-CSF on cycling in the childhood-versus adult-onset forms of this disease described by Hammond et al.

Although the effects of GM-CSF treatment on neutrophil counts in patients who participated in our study were variable and generally modest, recurrent neutropenia-related signs and symptoms improved in each patient during treatment. Indeed, clinical improvements were similar to those observed with G-CSF treatment, which induced much greater increases in average neutrophil counts. This result is consistent with previous studies of patients with chronic, severe neutropenia that found that minor increases in neutrophil supplies can substantially reduce the morbidity experienced by these patients.

<table>
<thead>
<tr>
<th>Blood Element</th>
<th>Patient No.</th>
<th>Mean</th>
<th>SD</th>
<th>Coefficient of Variation*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neutrophils</td>
<td>1</td>
<td>124</td>
<td>2,781</td>
<td>112</td>
</tr>
<tr>
<td>Monocytes</td>
<td>1</td>
<td>83</td>
<td>632</td>
<td>45</td>
</tr>
<tr>
<td>Platelets</td>
<td>1</td>
<td>183</td>
<td>115</td>
<td>26</td>
</tr>
<tr>
<td>Reticulocytes</td>
<td>1</td>
<td>1.2</td>
<td>0.7</td>
<td>0.7</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Neutrophenia-Related Morbidity*</th>
<th>No Treatment</th>
<th>GM-CSF</th>
<th>G-CSF</th>
<th>No Treatment</th>
<th>GM-CSF</th>
<th>G-CSF</th>
<th>No Treatment</th>
<th>GM-CSF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Malaise</td>
<td>2.3</td>
<td>1.0</td>
<td>1.2</td>
<td>2.0</td>
<td>0.8</td>
<td>1.0</td>
<td>1.6</td>
<td>0</td>
</tr>
<tr>
<td>Fever</td>
<td>0.7</td>
<td>0.3</td>
<td>0</td>
<td>1.1</td>
<td>0.3</td>
<td>0.4</td>
<td>0.7</td>
<td>0</td>
</tr>
<tr>
<td>Aphthous stomatitis/pharyngitis</td>
<td>2.8</td>
<td>0.7</td>
<td>0.3</td>
<td>1.5</td>
<td>0.7</td>
<td>0.5</td>
<td>1.2</td>
<td>0</td>
</tr>
<tr>
<td>Change in activity due to illness</td>
<td>1.0</td>
<td>0</td>
<td>0.3</td>
<td>0.8</td>
<td>0</td>
<td>0.3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Infection of skin or other site</td>
<td>3.2</td>
<td>0.3</td>
<td>0</td>
<td>1.0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Antibiotic use</td>
<td>1.8</td>
<td>0.3</td>
<td>0</td>
<td>1.2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Morbidity score†</td>
<td>11.8</td>
<td>2.6</td>
<td>1.8</td>
<td>7.6</td>
<td>1.8</td>
<td>2.2</td>
<td>3.5</td>
<td>0</td>
</tr>
<tr>
<td>Weeks of observation</td>
<td>12</td>
<td>6</td>
<td>10</td>
<td>20</td>
<td>6</td>
<td>10</td>
<td>12</td>
<td>6</td>
</tr>
</tbody>
</table>

* Average number of days with symptom or sign per week of observation.
† Sum of the average symptom/sign days per week of observation.
At the same time, the prominent eosinophilic effect of GM-CSF in these patients raises the concern that hyperesinophilic toxicities might occur if treatment with this agent, as used in the present study, were to be prolonged. A similarly prompt and impressive eosinophilic effect of GM-CSF (administered at a 3-fold higher dose than that used in the present study) was described in the case report by Freund et al. Administration of comparable doses of GM-CSF to patients with cancer, HIV disease, and myelodysplastic disorders has been shown to cause moderate increases in eosinophil counts; however, the very marked, eosinophilic response to exogenous GM-CSF at these doses appears to be a distinctive feature of both childhood-onset cyclic neutropenia and Kostmann syndrome. It has been suggested that the relative neutrophilic versus eosinophilic effects of GM-CSF treatment may be dose related and that doses of this growth factor that are substantially lower (eg, 0.1 to 0.5 μg/kg/d) than those typically used clinically (2.0 to 5.0 μg/kg/d) might favor a neutrophilic rather than eosinophilic response. This possibility deserves further study.

ACKNOWLEDGMENT

The authors are grateful to Elizabeth C. Wright, PhD, for assistance and advice in performing harmonic function regression analysis of serial blood cell count data described in this report.

REFERENCES

Contrasting effects of recombinant human granulocyte-macrophage colony-stimulating factor (CSF) and granulocyte CSF treatment on the cycling of blood elements in childhood-onset cyclic neutropenia

DG Wright, RF Kenney, DH Oette, VF LaRussa, LA Boxer and HL Malech