Phase I/II Study of Recombinant Human Granulocyte-Macrophage Colony-Stimulating Factor in Aplastic Anemia and Myelodysplastic Syndrome

By Joseph H. Antin, Brian R. Smith, Wendy Holmes, and David S. Rosenthal

We performed a phase I/II study of the administration of recombinant human granulocyte-macrophage colony-stimulating factor (GM-CSF) to patients with aplastic anemia or myelodysplastic syndrome. Doses ranging from 15 to 480 μg/m² were administered as a one-hour or four-hour intravenous infusion daily for 7 days or as a 12-hour infusion for 14 days. Temporary improvements were seen in granulocyte counts, monocyte counts, and reticulocyte counts in six of eight patients with aplastic anemia and five of seven patients with myelodysplastic syndromes. The patients with myelodysplastic syndromes had larger increases in granulocyte, monocyte, and reticulocyte counts than did those with aplastic anemia, and they also had increases in the numbers of eosinophils (two of seven).

GRANULOCYTE-MACROPHAGE colony-stimulating factor (GM-CSF) is one of a family of glycoproteins that is important in the production, survival, and differentiation of bone marrow progenitors. Its activities include stimulation of myeloid and monocyte colony formation (CFU-G, CFU-M, CFU-GM), erythroid bursts (BFU-E), and mixed colonies containing erythroid and megakaryocytic elements in addition to monocytes and granulocytes (CFU-GEMM or CFU-Mix).13 Factors such as GM-CSF are also important functional activators of granulocytes and monocytes.14 Preliminary studies by Donahue et al demonstrated that the administration of GM-CSF to nonhuman primates consistently increases blood counts, even in animals with virally mediated marrow insufficiency.15 Administration of GM-CSF speeds the recovery of blood counts after marrow grafting in nonhuman primates.9 Furthermore, it increases the WBC counts in patients with acquired immunodeficiency syndrome (AIDS).10

Patients with aplastic anemia who have failed to respond to antithymocyte globulin (ATG) and patients with myelodysplastic syndromes (MDS) are particularly suited for studies of marrow growth factors. There are no effective therapies except marrow transplantation, and pancytopenia results in substantial morbidity and mortality. The ability of GM-CSF to stimulate granulocyte and monocyte function may be valuable since many patients with myelodysplasia have mature but dysfunctional leukocytes.11,12 Furthermore, it may not be coincidental that the long arm of chromosome 5 is the chromosomal locus of several hematopoietic growth factors including GM-CSF and that the loss of this genetic material (5q–) is associated with some cases of myelodysplasia.13,14

MATERIALS AND METHODS

Patients. All studies were performed after informed consent was obtained under the guidelines of the Committee for the Protection of Human Subjects from Research Risks of the Brigham and Women's Hospital. None of the patients was eligible for marrow transplantation by virtue of age or lack of a suitable donor. Seven patients with severe aplastic anemia (as defined by Camitta et al15) were studied. All patients had absolute granulocyte counts <10^9/μL, platelet counts <2 × 10^9/μL, and/or an RBC transfusion requirement. Their clinical characteristics at enrollment are shown in Tables 1 and 2. All patients required packed RBC transfusions to maintain the hematocrit value above 20%, and five of the six patients required platelet transfusions to maintain the platelet count ≥2 × 10^9/μL. Bone marrow biopsy demonstrated ≤10% cellularity in six patients and ≤25% cellularity in one patient with aplastic anemia due to paroxysmal nocturnal hemoglobinuria (PNH). The ages ranged from 18 to 77 years. The duration of marrow aplasia prior to GM-CSF treatment ranged from 30 days to 10 years. Prior therapy included ATG in four patients, androgens in three, thymosin in one, prednisone in two, and cyclophosphamide and hydroxyurea in one. An additional individual with idiopathic agranulocytosis (patient no. 13) was included in the aplastic anemia group. He had normal platelet counts and hemoglobin level but absolute neutrophil counts of <100/μL. He also had hypogammaglobulinemia, but no clonal expansion of T cells or natural killer (NK) cells could be demonstrated.

Seven patients with MDS were studied. The diagnosis was established by bone marrow aspirate and biopsy findings, and they were classified according to the French-American-British Cooperative Group (FAB) criteria.16 Their clinical characteristics are detailed in Tables 1 and 2. Six of seven patients had absolute granulocyte counts of <10^9/μL and six of seven were dependent on packed RBC transfusions. Three patients were thrombocytopenic as well. One patient had been treated with low-dose arabinosyl cytosine (Ara-C) 2 years before, one patient had received hydroxyurea, one patient...
had been treated with androgens followed by retinoic acid, and one patient received prednisone and cyclosporine. The others had not received therapy other than transfusions and supportive care. The ages of subjects in this group ranged from 39 to 67 years, and the marrow morphology demonstrated no evidence of dysplasia that would allow characterization of this disorder.

<table>
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<th>Number</th>
<th>Diagnosis</th>
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<th>Duration of Illness</th>
<th>Prior Therapy</th>
<th>Marrow Cytogenetics</th>
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<td>5</td>
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<td>31/F</td>
<td>10 yr</td>
<td>Androgens, ATG, thymosin</td>
<td>Monoclonal antibodies</td>
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<td>54/F</td>
<td>6 mo</td>
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<td>7</td>
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<tr>
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<tr>
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<td>2 yr</td>
<td>ATG</td>
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<tr>
<td>14</td>
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<td>38/M</td>
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<tr>
<td>13</td>
<td>Agran/Hypogam</td>
<td>36/M</td>
<td>5 yr</td>
<td>Prednisone, cyclophosphamide</td>
<td>Splenectomy</td>
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<tr>
<td>1</td>
<td>RAEB</td>
<td>55/F</td>
<td>60 d</td>
<td>None</td>
<td>-4,-17,-5q,-7q,inv(15),+r,dic t(15;16),t(13;20), (p11;cen), -del(13p,20q)</td>
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<tr>
<td>3</td>
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<td>RA</td>
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<tr>
<td>10</td>
<td>RAEB</td>
<td>67/F</td>
<td>9 mo</td>
<td>Hydroxyurea</td>
<td>del(5)(q12),del(13)(p13), -6,-12</td>
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<tr>
<td>11</td>
<td>RA</td>
<td>39/F</td>
<td>1 yr</td>
<td>Androgens, retinoic acid</td>
<td>5q-</td>
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<tr>
<td>12</td>
<td>RAEB</td>
<td>67/M</td>
<td>9 mo</td>
<td>None</td>
<td>Normal</td>
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<tr>
<td>15</td>
<td>MDS</td>
<td>69/M</td>
<td>18 mo</td>
<td>Prednisone, cyclophosphamide</td>
<td>del(9)(p23)</td>
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</table>

Abbreviations: AA, aplastic anemia; Agran, idiopathic agranulocytosis; Hypogam, hypogammaglobulinemia; RA, refractory anemia; RAEB, refractory anemia with excess blasts.

The diagnosis of MDS was made by FAB criteria and is listed in Table 1 as MDS.

**Table 1. Patient Characteristics**

**Table 2. Hematologic Characteristics at Enrollment and Peak Response to GM-CSF Infusion**

**Laboratory evaluation.** Complete blood counts, differential counts, reticulocyte counts, serum chemistry profile (SMA-20), urinalysis, prothrombin time/partial thromboplastin time, electrocardiogram, and chest x-ray were obtained through the clinical laboratories of the Brigham and Women’s Hospital. Data were obtained daily or every other day during the infusion and one or more times between days 14 and 28. Granulocytes are reported as the sum of the absolute neutrophil and band counts. Immature cells are defined as the sum of metamyelocytes, myelocytes and promyelocytes. Bone marrow biopsies and aspirates were obtained from the posterior iliac crests, and an aspirate was sent for cytogenetic analysis (Cytogenetics Laboratory, Dana-Farber Cancer Institute, Boston).

**Recombinant human GM-CSF.** Recombinant human GM-CSF was obtained from the Immunex Corp (Seattle). It was cloned from HUT-102 and expressed in yeast. It differs from native GM-CSF by substitution of a leucine for an arginine at position 23. It consists of three molecular weight species of 15,500, 16,800, and 19,500 due to differences in glycosylation; the three species are present in approximately equal proportions. The specific activity was approximately 5 x 10⁹ colony-forming units/mg of protein.

**Measurement of anti–GM-CSF antibodies.** Pre- and post-GM-CSF infusion sera were examined for the presence of antibodies by using an enzyme-linked immunosorbent assay (kindly performed by Dr Steven Gillis, Immunex Corp). One hundred nanograms of recombinant human GM-CSF was applied to 96-well polystyrene microtiter plates. Nonspecific binding sites on the plates were blocked by incubation with 5% nonfat dry milk. Patient sera were added to the plates, followed by goat antihuman immunoglobulin conjugated to horseradish peroxidase, the plates were developed with Bio-Rad peroxidase developing reagent, and the optical density (OD) was read at 405 nm. Normal rabbit sera, immune rabbit sera, and normal human volunteer donors served as controls. Samples from six patients (two with MDS and four with aplastic anemia) were obtained before infusion and monthly after subsequent treatments.
Three patients were studied after each of two consecutive treatments, two patients donated samples after each of three consecutive treatments, and one patient had antibodies measured after each of four consecutive treatments.

Study design. Patients were admitted to the Clinical Research Center of the Brigham and Women’s Hospital where they received GM-CSF by daily one-hour peripheral intravenous infusion for 7 days. Later in the course of the study the infusion duration was increased to four hours, and subsequently patients were given 12-hour infusions through a central line for 14 days. The 12-hour infusions were administered either as inpatients as just described or as outpatients. Patients received a 1:100 dilution of GM-CSF as an intradermal injection prior to the commencement of the infusion. The infusion was begun only if there was no evidence of immediate hypersensitivity. Dose levels were 15, 30, 60, 120, 240, and 480 μg/m²/d. Dosage increase occurred when three or more patients had been treated at the previous level. Patients were allowed repeated treatments at progressively higher dose levels if there was no observed toxicity. The highest dose infused was 480 μg/m². Increases in dose were instituted separately for the patients with aplastic anemia and MDS. The 15 patients received a total of 44 doses of GM-CSF. Individual patients received multiple cycles of therapy ranging from one to seven monthly treatments.

RESULTS

Aplastic anemia. The baseline and peak responses of granulocytes and reticulocytes are demonstrated in Figs 1 and 2. In the patients with aplastic anemia there was only modest improvement in counts, and there was substantial variability between patients. The median time to peak granulocyte counts was 4 days (range, one to 21 days) in the aplastic anemia group. On five occasions peak granulocyte counts occurred between days 14 and 21. Similarly, monocyte numbers peaked at a median time of 5 days (range, one to 21 days), and the reticulocyte counts peaked at a median of 13 days (range, one to 21 days). It is noteworthy that the GM-CSF infusion stopped on day 7 in all of the cases in which the peak effect occurred after day 7, which suggests that in many patients the stimulation of early progenitors resulted in improved counts after the growth factor was no longer present. This observation is shown for a representative individual in Fig 3.

It was difficult to demonstrate a dose-response relationship for the entire group because of the heterogeneity of the subjects’ initial hematologic status. We observed increases in granulocyte and monocyte counts at the lowest doses used, while reticulocyte responses were more frequently observed at the higher dose levels. There was substantial variability among patients in their response to GM-CSF (Figs 1 and 2), although for a single patient, either all three cell lines responded to the infusion, or none did. Overall, at least a 100% increase in counts was observed in granulocytes in 13 of 22 courses, in monocytes in 14 of 22 courses, and in reticulocytes in 13 of 16 evaluable courses. There were no increases in numbers of eosinophils or immature myeloid cells and no amelioration of RBC or platelet transfusion requirements. As demonstrated in Table 3, there appeared to
be a tendency for the first course of GM-CSF to give the maximal response; however, there was no significant trend toward decreasing response, and the response to subsequent cycles was generally similar. Two patients with aplastic anemia (patients 2 and 8) had substantially reduced responses at a higher dose. Neither of these patients made measurable anti-GM-CSF antibodies.

The patient with idiopathic agranulocytosis (patient no. 13) had no responses in any cell line to GM-CSF infusion. He received four-hour infusions for 7 days, and although he had an underlying monocytosis (monocytes ranged from 2,000 to 3,000 cells/μL), monocyte numbers remained stable throughout the infusion.

Myelodysplasia. The baseline and peak responses of the patients with myelodysplasia are shown in Figs 1 and 2. Compared with the patients with aplastic anemia, the myelodysplastic patients generally had more impressive responses of all three cell lines to GM-CSF infusion; however, in no case was there extreme leukocytosis or amelioration of platelet or RBC transfusion requirements. The granulocytes, monocytes, and reticulocytes responded in tandem in a fashion similar to the patients with marrow aplasia. The median time to peak granulocyte counts was 9 days (range, one to 14 days), and peak granulocyte counts occurred on or after day 7 on six occasions. Similarly, the median time to maximal monocyte counts was day 6 (range, days 2 to 14); however, the peak monocyte count occurred on day 14 only once. Reticulocytes had a median time to maximal count of 5 days (range, two to 21), and the peak response occurred on or after day 14 on eight occasions. At least a doubling of counts was observed in granulocytes in 18 of 21 courses, in monocytes in 18 of 23 courses, in reticulocytes in 18 of 23 evaluable courses, in myelocytes and metamyelocytes in ten of 23 courses, and in promyelocytes and myeloblasts in four of 23 courses.

As we observed in the patients with aplastic anemia, it was difficult to detect a dose-response relationship in the patients with myelodysplasia when the group was examined as a...
Table 3. Relationship Between Dose of GM-CSF and Peak Granulocyte and Reticulocyte Response

<table>
<thead>
<tr>
<th>Patient Number</th>
<th>Dose Level (μg/m²)†</th>
<th>Granulocytes (Cells/μL)</th>
<th>Reticulocytes (Cells/μL)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Pre-GM-CSF</td>
<td>Peak</td>
<td>Pre-GM-CSF</td>
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<tr>
<td>Aplastic Anemia</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>2</td>
<td>15, 30, 60, 240, 480</td>
<td>864</td>
<td>2,352</td>
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<tr>
<td>5</td>
<td>15, 30, 120</td>
<td>192</td>
<td>480</td>
</tr>
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<td>925</td>
<td>1,377</td>
</tr>
<tr>
<td>8</td>
<td>60, 240, 480</td>
<td>143</td>
<td>650</td>
</tr>
<tr>
<td>9</td>
<td>120</td>
<td>25</td>
<td>63</td>
</tr>
<tr>
<td>13</td>
<td>240</td>
<td>0</td>
<td>280</td>
</tr>
<tr>
<td>14</td>
<td>240*</td>
<td>324</td>
<td>820</td>
</tr>
</tbody>
</table>

| Myelodysplasia |                      |                         |                          |                         |
|----------------|----------------------|-------------------------|--------------------------|
| 1              | 15                   | 1,020                   | 1,638                    | 3,340                   | 12,520                  |
| 3              | 15, 30, 60, 120, 240, 240, * 480 | 558                     | 1,520                    | 1,890                   | 14,000                  |
| 4              | 15, 30, 60, 240, 240, * 240* | 840                     | 4,620                    | 2,560                   | 12,560                  |
| 10             | 30, 240              | 897                     | 2,337                    | 3,520                   | 16,800                  |
| 11             | 60, 240*             | 300                     | 1,014                    | 5,840                   | 15,450                  |
| 12             | 120, 240, * 240*     | 546                     | 3,484                    | 33,320                  | 73,440                  |
| 15             | 240*                | 557                     | 156                      | 10,880                  | 16,150                  |

*Doses marked with an asterisk indicate 12-hour infusion for 14 days. The unmarked doses are either one-hour or four-hour infusions daily for 7 days.
†Underlined values are the doses at which the largest responses (depicted to the right) occurred.
‡This was the only patient in whom the peak granulocyte and reticulocyte counts were not observed at the same dose level. The largest reticulocyte count response was at a dose of 480 μg/m²—the count rose from 48,750 to 159,600.
§The hemoglobin concentration was normal at baseline, and the reticulocyte response could not be differentiated from an effect of phlebotomy.

whole because of heterogeneity among patients and differences in dosing schedules. There were detectable increases in granulocyte, monocyte, and reticulocyte counts at the lowest dose level, but the largest responses occurred at the dose levels of 240 and 480 μg/m² (Fig 4). More individuals in the myelodysplasia group were treated with 12-hour infusions for 14 days, and the responses to GM-CSF administered in this fashion were generally the largest observed (Table 3). There was no statistical difference between the groups. Six of the seven individuals with myelodysplasia had some improvement in counts, although the improvements were minor in two patients.

In this small series of patients there was no obvious difference in response between patients with and without chromosome abnormalities. However, both the individuals with the weakest responses (patients no. 10 and 11) were missing the long arm of chromosome 5; one had 5q− in addition to other chromosomal abnormalities, and one had the 5q− syndrome.

In two individuals (patients no. 4 and 10) there was an increase in myeloblast numbers late in the infusion. Figure 5 shows an example of the relationship between a 14-day infusion of GM-CSF at 240 μg/m² and stimulation of blood cells in patient no. 4. It is noteworthy that, although the number of circulating blasts increased after day 10 of the infusion, a bone marrow biopsy specimen taken on day 13 showed increased overall cellularity but with maturation of all cell lines and no proportionate increase in myeloblasts (Fig 6). The blast count returned to baseline after discontinuation of GM-CSF treatment. Interestingly, when patient no. 4 was treated with 240 and 480 μg/m² of GM-CSF.
administered over four hours for 7 days, we did not observe
the appearance of immature cells in the blood. The increase
in blast numbers in patient no. 10 was apparent at 30 and at
120 µg/m² administered over four hours for 7 days but also
returned to baseline promptly with discontinuation of the
infusion. She had extensive marrow fibrosis, and no change
in marrow cellularity was observed after the infusion. Both
of these patients had concomitant increases in numbers of
circulating metamyelocytes, myelocytes, and promyelocytes.
An additional individual (patient no. 12) had an increase in
marrow cellularity and a reduction in the relative proportion
of myeloblasts in the marrow from 15% to 5%. In none of
the subjects did we observe an increase in lymphocyte counts.
Two patients had improved platelet counts on a single
occasion.

Toxicity was uniformly mild (Table 4). The predominant
complaints were low-back or rib discomfort, anorexia, myal-
gias/archalgias, and low-grade fever. The low-back discom-
fort was severe enough to require analgesia in two patients. It
was interesting that back, rib, and sternal discomfort were
observed primarily in patients with the more cellular mar-
rows, ie, MDS, which suggests that the pain was due in some
way to the stimulation of marrow cells. We did not observe a
difference in toxicity between the 1-, 4-, and 12-hour infu-
sions. Furthermore, patients who noted a side effect with one
administration of GM-CSF tended to have it again with
subsequent administrations. Only one of 44 courses was
abbreviated, and that was because of the development of
gram-negative sepsis in a nonresponding patient with aplas-
tic anemia. There was no evidence of leukemic conversion in
any of the patients, and although on two occasions an
increased number of myeloblasts was observed in patients
with myelodysplasia, in both cases it was transient and
resolved with discontinuation of the infusion.

We were unable to detect any anti–GM-CSF antibodies in
any of the six patients tested even though all of the patients
tested had multiple exposures to the protein, and no patient
developed hypersensitivity or skin test reactivity. Repeated
exposures to GM-CSF appear to be safe. Four individuals
(patients 2, 5, 6, and 7) received three or four monthly
infusions, and two individuals (patients no. 3 and 4) were
repeatedly treated for 7 months, with no apparent increase in
toxicity.

DISCUSSION

Details of the control and regulation of hematopoiesis are
still obscure; however, the discovery, cloning, and production
of purified hematopoietic growth factors may allow the
dissection of the role of these proteins in the generation of
blood cells. There is little evidence that circulating GM-CSF
is involved in the tonic control of hematopoiesis; however,
local interactions with stroma may be very important.1 It is
probable that a primary role of GM-CSF in vivo is the
stimulation of the marrow and the activation of mature cells
in response to infection.1

We took advantage of the known proliferative effects of
GM-CSF and administered recombinant human GM-CSF
to two pathophysiologically distinct groups of patients: those
with aplastic anemia and those with myelodysplasia. These
studies were undertaken primarily to assess the toxicity of
the growth factor when administered to humans but also to
determine whether the proliferative effects of GM-CSF on
the bone marrow would ameliorate the cytopenias of patients
with deficient numbers of stem cells or disordered matura-
tion of stem cells. We chose brief infusions of GM-CSF for
three reasons. Since GM-CSF inhibits neutrophil migration,6
we were concerned that a component of the leukocytosis
observed in animals and humans reflected restricted egress of
mature cells from the blood in addition to marrow stimula-
tion. Such confinement of granulocytes to the circulation
could be detrimental. Experimental evidence for this phe-
nomenon has been observed recently (W.P. Peters, Duke
University, personal communication). Second, if GM-CSF
were valuable in the treatment of bone marrow failure, we
surmised that it would need to be administered frequently;
bolus administrations would be more easily accommodated
by patients than continuous infusions. Third, we wanted to
avoid the extreme leukocytosis and possibility of extramedul-
ary hematopoiesis that have been observed with continuous
infusions.19

Patients with aplastic anemia and myelodysplasia are
similar only in the peripheral blood manifestations of the
diseases. Myelodysplasia is thought to arise from disordered
growth of marrow cells, and marrow aplasia may reflect a
variety of pathophysiologic processes such as stem cell defi-
ciency, disorders of humoral and cellular immunity, or
microenvironment abnormalities. Patients who have failed to
respond to immunosuppressive agents probably have a defi-
ciency of bone marrow stem cells. This view is supported by
observations of marrow transplantation in identical twins
where immunosuppression is not uniformly necessary to
allow engraftment.30 In the setting of reduced numbers of
marrow stem cells and progenitors, the administration of
GM-CSF would be expected to result in modest improve-
mants in counts. These improvements are transient, which
suggests that there is no increase in the stem cell compart-
ment responsible for self-renewal of the marrow. Improve-
Fig 6. Bone marrow biopsy material from patient no. 4 taken before (A, B) and after (C, D) 240 μg/m² of GM-CSF administered over 12 hours for 14 days. Comparison of the marrow demonstrates an overall increase in cellularity, primarily due to increases in numbers of myeloid cells. There was no change in proportion of myeloblasts between the two samples. Biopsy specimens were stained with Wright-Giemsa (left panels: original magnification x 100; right panels: original magnification x 1,000).
ments in blood counts and marrow cellularity are likely related to the stimulation of partially committed progenitors such as CFU-GM and CFU-Mix. The time to peak granulocyte counts may suggest the mechanism of the granulocytosis; an early peak may reflect the maturation of late, committed progenitors, while the increments in granulocytes that occurred later may reflect the stimulation and recruitment of earlier progenitors. The suggestion that early progenitors were stimulated is supported by the observation that increases in granulocyte, monocyte, and reticulocyte counts were often observed 7 to 14 days after the infusion had been discontinued.

The improvements in reticulocyte counts in addition to those of granulocytes and monocytes suggest that GM-CSF supplies burst-promoting activity. In the presence of the large amounts of erythropoietin, which is produced by anemic patients, the addition of exogenous GM-CSF results in the production or early release of reticulocytes. One individual (patient 2) had a large enough reticulocytosis to reasonably expect a contribution to the recovery of a normal hemoglobin concentration. Unfortunately, because of her PNH new RBCs were hemolyzed as rapidly as they were formed. We could detect no effects on platelets in our patients, thus suggesting that the stimulation of marrow progenitors did not include cells responsible for the production of megakaryocytes, and we observed no effects on eosinophils or immature myeloid cells in this group. It is unclear whether the putative multipoietin activity of GM-CSF is due to direct effects on the marrow or a combination of direct effects and indirect effects from the stimulation of stromal elements such as macrophages.

Two individuals (patients no. 2 and 8) had reductions in response with higher doses of GM-CSF. This reduction was not associated with the production of anti--GM-CSF antibodies, and it is not clear whether it represents tachyphylaxis, the natural history of the disease, or depletion of marrow stem cells. The latter is unlikely in patient no. 2 since her hematologic status remained unchanged for the subsequent 6 months. Patient no. 8 developed progressively worse granulocyte counts and platelet alloimmunization and subsequently died of hemorrhage. We cannot exclude the possibility of a detrimental effect of GM-CSF in this patient.

Patients with myelodysplasia generally had more impressive increases in numbers of myeloid and erythroid cells than did patients with aplastic anemia, perhaps because most of these individuals had more stem cells and progenitors available to stimulate. However, the responses appeared to be transient. The one exception was the individual (patient no. 4) depicted in Fig 5 in whom there did appear to be an increase in his baseline granulocyte counts. His reticulocyte counts did not demonstrate this effect. These patients were also more likely to have immature cells in the blood, including bands, myelocytes, metamyelocytes, promyelocytes, and blasts. We were concerned that the growth requirements of some leukemic cells for colony-stimulating factors such as GM-CSF would result in proliferation of myeloblasts and leukemic conversion. However, while we did observe increases in numbers of immature cells, these increases were not maintained, and no leukemia was observed. It is reasonable to assume that the stimulus to the growth of myeloblasts was not maintained beyond the presence of the GM-CSF and that the exposure to GM-CSF did not result in autonomous proliferation of myeloblasts with leukemic potential. Furthermore, leukemic cells that are already producing GM-CSF and responding to this abnormally produced growth factor might not be stimulated by the further addition of exogenous GM-CSF. This observation confirms similar results reported by Vadn-Raj et al with more prolonged infusions. It should be noted, however, that our study entrance criteria specifically excluded patients in transformation to acute nonlymphocytic leukemia (FAB classification, RAEB-IT) and that the number of patients was small, which precluded an accurate assessment of the risk of leukemic transformation. It is unlikely that the use of GM-CSF reduces the risk of leukemia conversion since previous studies demonstrated the persistence of clonal cytogenetic abnormalities in maturing cells. Thus, the beneficial effect on blood counts does not appear to be due to selective stimulation of residual cytogenetically normal cells.

In contrast to previous studies in animals and humans, we observed no increases in lymphocyte counts and much less impressive increases in eosinophil counts. Increases in platelet numbers were observed on a single occasion in two patients and are considered of questionable significance. It is likely that this discrepancy is in some way related to differences in duration of administration of the GM-CSF and species differences.

The toxicity of the infusions was generally mild. The bone discomfort appears to be a characteristic finding in patients receiving GM-CSF. The pathophysiology of this symptom is unclear. It generally occurred during the first day or two of treatment and then abated. Thus, it seems unlikely that the pain reflects marrow engorgement with cells. It may indicate the release of inflammatory mediators from stimulated mature cells; however, if so, the effect diminished rapidly. We observed similar responses with 1-, 4-, and 12-hour infusions. The mildness of the side effects generally allowed the administration of therapy with no or minimal analgesia, and toxicity did not result in the discontinuation of therapy in any patient. In addition, it is clear that, although this recombinant human GM-CSF is produced in yeast and has an amino acid difference from the native protein, the production of antibodies did not appear to be a problem.

In summary, brief infusions of recombinant human GM-CSF are capable of stimulating bone marrow precursors and

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<tr>
<th>Table 4. Toxicity of GM-CSF Infusions (Total of 44 Courses)</th>
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<tr>
<td>Side Effect</td>
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<tr>
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</tr>
<tr>
<td>Bone discomfort</td>
</tr>
<tr>
<td>Myalgia/arthritis</td>
</tr>
<tr>
<td>Fever</td>
</tr>
<tr>
<td>Headache</td>
</tr>
<tr>
<td>Anorexia</td>
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<td>Muscle twitching</td>
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<td>Fatigue</td>
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progenitors in vivo with acceptable toxicity. This stimulation results in increases in numbers of myeloid cells, monocytes, and reticulocytes. It is likely, based on data from other studies, that longer infusions of GM-CSF would produce more dramatic improvements in blood counts and perhaps reduce transfusion requirements in some patients, but criteria for the selection of patients who are likely to benefit and the overall value of this type of therapy remain to be established. In addition, although in limited numbers of treated patients there does not seem to be any stimulation of leukemic cells, more patients need to be studied to determine the safety of GM-CSF infusions in myelodysplastic patients.

REFERENCES

Phase I/II study of recombinant human granulocyte-macrophage colony-stimulating factor in aplastic anemia and myelodysplastic syndrome

JH Antin, BR Smith, W Holmes and DS Rosenthal

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