Hematologic Effects of Recombinant Human Granulocyte Colony-Stimulating Factor in Patients With Malignancy

By Albrecht Lindemann, Friedhelm Herrmann, Wolfgang Oster, Gerd Haffner, Walter Meyenburg, Larry M. Souza, and Roland Mertelsmann

The effect of recombinant human granulocyte colony-stimulating factor (G-CSF) on hematologic parameters was evaluated in a phase I clinical study in 18 patients with advanced malignancy. G-CSF was administered once daily as a 30-minute infusion for 14 days; three patients each were treated at increasing dose levels of 1, 3, 10, 30, and 60 μg kg⁻¹ day⁻¹. A transient decrease in neutrophil and monocyte counts was observed immediately after the G-CSF infusion, followed by a dose-dependent increase of up to 15-fold. G-CSF–induced neutrophils exhibited an increased O²⁻ radical production, and serum levels of enzymes related to granulocyte turnover, including lypo-

zyme and elastase, were markedly elevated during therapy. A dose-dependent depression of platelet counts occurred in the second third of the treatment course, followed by a spontaneous recovery despite continuing therapy. G-CSF was well-tolerated; minor to moderate bone pain was the most common side effect. The primary course of the malignant diseases studied was not significantly altered. G-CSF appears to be an appropriate means to selectively increase the number of functionally competent polymorphonuclear phagocytes.

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phoresis and immunoprecipitation using specific antisera (provided by Bioscience, Ingelheim, FRG).

**Immunoctfluorescence analysis.** To investigate the in vivo effects of G-CSF on selected functionally relevant surface structures, immunofluorescence analysis was performed. Cells investigated included PB PMNs, monocytes, lymphocytes, and platelets. Cells to be analyzed were obtained before G-CSF administration, four times in the first 2 hours after initiation of the G-CSF infusion, and after termination of the G-CSF treatment. Mononuclear cells were separated by Ficoll-Hypaque gradient centrifugation (Pharmacia Laboratories, Uppsala, Sweden; density 1.077 g/dL). PMNs in the pellet were further enriched (greater than 99% pure) by sedimentation through dextran sulfate 0.4% vol/vol in Hank's balanced salt solution (HBSS; GIBCO, Grand Island, NY) as described previously. Cells were analyzed by one- or two-color fluorescence analysis using flow cytometry and fluoroexcinated or phycoerythrin-conjugated monoclonal antibodies (MoAbs) to the CD3 (T3), CD25 (IL-2R), CD14 (Mo2), CD11b (MO1) (Coulter Electronics, Hick- leah, FL), CD11a (LFA-1) (Janssen, Beerse, Belgium), CD11c (Leu-M5) (Becton Dickinson, Mountain View, CA), and 1D3 antigens as previously described. Specificity of MoAbs T3, IL-2R, Mo2, LFA-1, MO1, and Leu-M5 were described in detail previously. ID3 MoAb reacts with mature neutrophils only and was provided by J.D. Griffin, Dana Farber Cancer Center, Boston, MA. Platelet-rich plasma (PRP) was obtained by centrifugation of citrated blood samples at 200 g for 20 minutes. Platelets were washed with a HEPES-buffered modified Tyrode's solution (HBMT) as described, and were adjusted to 10 6/mL. Samples of 100 μL platelet sample were incubated for 30 minutes at 4°C with an anti-Gp2b3a-specific MoAb (Clone HPL), Seralab, Crawley Down, Sussex, England) or an isotype-identical control antibody. After three wash steps, cells were analyzed with a flow cytometer (FACS Scan, Becton Dickinson).

**Chemiluminescence (CL) assay.** PMNs were separated as described above and resuspended at 10 6/mL in Eagle's minimal essential medium without phenol red (D-MEM, Boehringer Ingel- him, Ingelheim, FRG). FMLP (10-7 mol/L)-induced O2- radical production was measured with luminol added (2 × 10-5 mol/L) in a luminescence analyzer (Biotram LB 9500; Bertold, Wodlbad, FRG), as described previously.

**RESULTS**

The clinical characteristics of the 18 patients who entered the study are shown in Table 1. In three patients, the G-CSF course was interrupted: One patient with colon carcinoma experienced a spastic ileus that had already occurred repetitively before G-CSF treatment; another patient with a marked leukocytosis receiving 60 μg/kg G-CSF experienced severe thrombocytopenia grade IV; the third patient was treated at the highest dose level, and treatment was discontinued when the total white blood cell (WBC) count reached 100,000/μL. Two patients with BM involvement by the underlying disease (multiple myeloma) were not considered for evaluation of hematologic response but were included in the toxicity analysis.

All patients responded to G-CSF with a rapid increase of PB PMN counts as early as 4 hours after treatment was started. As shown in Fig 1A, the response was dose-dependent, with a mean maximum "fold" increase (SD) as follows: 1.7-fold (±0.4) (1 μg/kg), 2.7-fold (±0.9) (3 μg/kg), 5.2-fold (±1.4) (10 μg/kg), 7.3-fold (±2.1) (30 μg/kg), and 11.5-fold (±2.5) (60 μg/kg). Although G-CSF was administered only once daily as a short-term infusion, no major decrease of WBC counts occurred within 24 hours after administration. Peak levels of total WBC counts were observed after 1 week of treatment, followed by a plateau or even minor decrease in WBC counts despite continuation of therapy. After discontinuation of treatment, cell counts returned to pretreatment values within 3 to 6 days.

Cells of the neutrophil lineage predominated in the differential count at all dose levels (Fig 1B). However, the percentage of band forms and immature neutrophilic cells increased at higher doses, representing about 20% of total WBCs at 10 μg/kg, and more than 35% at 60 μg/kg G-CSF. Myelocytes and promyelocytes appeared in the circulation at higher dose levels only. The PMNs displayed Dohle bodies, toxic granulation, and vacuolization. Eosinophil and reticulocyte counts were unchanged during G-CSF therapy, whereas lymphocyte counts increased slightly without demonstrating a strict dose relatedness: 1.5-fold (±0.2) (1 μg/kg); 2.9-fold (±1.6) (3 μg/kg); 1.3-fold (±0.3) (10 μg/kg); 2.2-fold (±0.8) (30 μg/kg); and 2.7-fold (±0.8) (60 μg/kg). Numbers of monocytes were elevated at higher dose levels: 2.4-fold (±1.2) (10 μg/kg); 3.2-fold (±0.7) (30 μg/kg); 5.1-fold (±1.8) (60 μg/kg).

Unexpectedly, platelet counts decreased over time (Fig 2) with a nadir occurring at about day 10 of G-CSF administration, whereas a spontaneous recovery was observed thereafter despite continuation of therapy. Concomitant changes in the coagulation profile were not observed, however. In particular, no increase in fibrinogen cleavage products and no decrease of AT III serum levels were detectable. The number of BM megakaryocytes remained unchanged or increased slightly in patients in whom BM aspiration and biopsy

**Table 1. Patient Characteristics**

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Total number of patients</th>
<th>Age (Median yr)</th>
<th>Range (yr)</th>
<th>Sex (M/F)</th>
<th>Performance status (Karnofsky scale)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colon cancer</td>
<td>6</td>
<td>56</td>
<td>26-65</td>
<td>11/7</td>
<td>&gt;70%</td>
</tr>
<tr>
<td>Mesothelioma</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Breast cancer</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lung cancer</td>
<td>1</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Renal cell carcinoma</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hodgkin’s disease</td>
<td>3</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Non-Hodgkin’s lymphoma</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Multiple myeloma</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chemotherapy</td>
<td>15/18</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Radiotherapy</td>
<td>7/18</td>
<td></td>
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<td></td>
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<tr>
<td>Hematologic pretreatment values (× 10 12/L)*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WBC</td>
<td>7.48 ± 1.86</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Neutrophils</td>
<td>5.16 ± 1.78</td>
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<tr>
<td>Lymphocytes</td>
<td>1.41 ± 0.67</td>
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<td></td>
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<td></td>
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<tr>
<td>Platelets</td>
<td>355 ± 156</td>
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<td></td>
<td></td>
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<tr>
<td>Reticulocytes</td>
<td>4.82 ± 0.92</td>
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</tr>
</tbody>
</table>

*Mean values of patients evaluated for hematologic response.
material could be examined \((n=8)\). The mean platelet volume was increased at day 10, and serum levels of platelet factor IV were elevated, arguing for an enhanced consumption of platelets and a relative increase of newly synthesized platelets. The moderate rebound of platelet counts after discontinuation of therapy (Fig 2) is in accord with this interpretation.

As shown in Fig 3 for three patients at dose level IV, the initial response to intravenous (IV) G-CSF administration is characterized by an immediate decrease in circulating PMNs within 10 minutes, paralleled by a similar though less pronounced decrease of monocytes. Absolute counts of eosinophils, lymphocytes, and platelets remained unchanged during this period. Determination of surface expression of adhesion molecules (CD11a-c) by neutrophils and monocytes did not reveal concomitant changes. A modulation of adhesion structures, however, was detected when platelets were analyzed. Fluorescence analysis of platelet gp2b3a expression revealed a marked decrease as early as 60 minutes after G-CSF administration, followed by a slow reexpression to baseline values in the following days (Fig 4).

To investigate the effect of G-CSF on granulocyte function in vivo, \(O_2^\text{radical} production was analyzed in a CL assay. PB PMNs obtained before administration of G-CSF and 1 hour after termination of the infusion were separated as described in the Materials and Methods section. As shown in Fig 5, an increased and more sustained \(O_2^\text{radical} production could be observed in response to FMLP.

As shown in Table 2, several laboratory variables changed during G-CSF therapy: LAP, as measured at day 14 of therapy, was increased in all patients, but significant dose relationship was not demonstrated. In parallel with elevated
neutrophil counts, an increase of serum lysozyme and elastase levels was observed, most likely reflecting the cellular turnover and transmembrane leakage of these enzymes. An increase of sIL-2R levels paralleled total WBC counts. Because we did not detect significant p55 IL-2R surface expression by T lymphocytes (not shown), we analyzed other PBC populations. 1D3-positive neutrophils of patients receiving G-CSF weakly expressed p55 IL-2Rs in three of eight cases investigated (Fig 6). Serum levels of TNF-α and IL-1β were not affected by G-CSF administration (not shown). Serum levels of AP and LDH were highly elevated in parallel to the increases in WBC counts. Further specification of the isoenzymes accounting for these changes led to identification of the bone-specific AP-isoenzyme and the isoenzymes 4 and 5 of LDH, respectively (not shown). A marked increase of uric acid serum levels required prophylactic administration of allopurinol in four of seven patients treated at dose levels IV and V who experienced high WBC counts.

The therapy was generally well-tolerated, and bone pain of mild to moderate degree was the major complaint. Bone pain was usually localized in the lower back, sternum, and ribs and was mostly of short duration and related to administration of G-CSF. In other cases, bone pain also occurred later during the course of therapy (days 4 through 6), 4 to 8 hours after G-CSF administration, and lasted for several hours; analgetic treatment was required only sporadically. Sweating and fatigue were observed in some patients at higher dose levels, closely associated with markedly elevated WBC counts; the latter was the most important parameter correlating to subjective discomfort. According to tumor measurements performed pre- and posttreatment and spanning a
Fig 5. Superoxide anion production of PB PMNs in response to FMLP (10^{-7} mol/L), measured by CL assay (mean of three patients) pretreatment and 60 minutes after start of G-CSF infusion (10 μg/kg) at days 1 and 5 of the treatment cycle. Quantity of O_{2}^{-} radical production is expressed in cpm.

3-week interval, no significant change in the course of the disease could be detected.

**DISCUSSION**

To evaluate drug tolerance and biologic in vivo activity of G-CSF, we conducted a phase I clinical trial in patients with solid tumors and selected hematologic malignancies. In line with other studies,^{8,10} single daily IV administration of G-CSF resulted in a dose-dependent increase in numbers of circulating segmented neutrophils and band forms, whereas monocyte counts were elevated at higher dose levels only. Numbers of eosinophils and reticulocytes remained unchanged as compared with pretreatment values. A slight increase in lymphocyte counts was not strictly dose related as reported previously.^{20} Because some increase in total WBC counts was detectable as early as 4 hours after treatment was started, G-CSF might also act on the BM reserve pool to release mature and immature neutrophils. The sustained increase of WBC counts for more than 24 hours, as induced by a single IV dose of G-CSF, may be of major importance for clinical practice. In view of the short serum half-life of G-CSF,^{9} this may be due to an accumulation of G-CSF in the hematopoietic microenvironment or may be caused by a hit-and-run effect at the progenitor cell level.^{21,22}

A transient decrease in WBC counts that is an early effect of IV G-CSF administration^{9} was not restricted to neutrophils but involved monocytes as well, whereas numbers of eosinophils and lymphocytes, which do not express G-CSF receptors,^{1} remained unaffected. A modulation of adhesion structures may be involved in aggregation and adhesion to endothelial cells, thus leading to a depletion of PMNs and monocytes from the circulation.^{23,24} However, we did not
HEMATOLOGIC EFFECTS OF rhG-CSF

Table 2. Changes in Laboratory Variables During G-CSF Treatment

<table>
<thead>
<tr>
<th>Laboratory Variable</th>
<th>Dose (µg/kg)</th>
<th>Off Therapy (Range)</th>
<th>On Therapy (Range of Maxima)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LAP (10-100)</td>
<td>10</td>
<td>14-75</td>
<td>190-242</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>18-62</td>
<td>213-386</td>
</tr>
<tr>
<td>Lysozyme (3-9 mg/L)</td>
<td>10</td>
<td>2.7-5.0</td>
<td>7.2-9.5</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>3.2-4.8</td>
<td>11.3-18.3</td>
</tr>
<tr>
<td>Elastase (100-300 µg/L)</td>
<td>10</td>
<td>186-245</td>
<td>485-650</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>140-235</td>
<td>593-2307</td>
</tr>
<tr>
<td>sIL-2R (50-500 U/mL)</td>
<td>10</td>
<td>270-520</td>
<td>540-1520</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>220-610</td>
<td>1610-3150</td>
</tr>
<tr>
<td>Serum AP (50-200 U/mL)</td>
<td>10</td>
<td>126-172</td>
<td>585-1052</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>136-225</td>
<td>240-560</td>
</tr>
<tr>
<td>LDH (50-240 U/mL)</td>
<td>10</td>
<td>132-164</td>
<td>340-1320</td>
</tr>
<tr>
<td>Uric acid (2-8 mg/dL)</td>
<td>10</td>
<td>2.1-6.9</td>
<td>5.7-9.0</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>4.9-7.9</td>
<td>9.8-11.0*</td>
</tr>
</tbody>
</table>

Values of three patients each at dose levels I and V are shown.

*Allopurinol was administered when uric acid values were more than 10 mg/dL.

detect significant changes of membrane expression of the CD11 clustered leukocyte adhesion molecules on circulating cells. Whether cells with an enhanced expression of CD11 no longer circulate or if different adhesion structures are involved in margination remains to be determined. In this period, we never observed acute side effects other than bone pain as did other investigators, although transient dyspnea

and pulmonary embolism was previously reported in patients receiving GM-CSF. Downmodulation of gp2b3a expression on platelets is of unknown significance. However, capping and internalization of gp2b3a has been observed as a consequence of anti-gp2b3a MoAb binding. Loss of gp2b3a from the membrane has also been observed in the presence of protease activation, eg, by plasmin. Whether any relationship exists between these immediate changes on platelet membranes and the transient decrease of platelet counts after several days of treatment remains to be determined. BM biopsies and laboratory parameters suggested an enhanced consumption of platelets. A similar course of platelet counts, including decreases at higher G-CSF doses and spontaneous recovery despite continuation of therapy, has been reported in hamsters. This phenomenon was not observed in previously reported clinical trials using G-CSF, probably because treatment lasted no more than 5 days in those studies. A significant decrease in platelet counts has also been observed in several GM-CSF studies. In a dose range of 1 to 10 µg/kg/d G-CSF, useful for future phase II studies, the transient decrease in platelet counts was not more than 30% and was thus clinically negligible in patients with platelet counts of >50,000/µL.

Changes in laboratory values as shown in this study, and extending previous observations, may relate to an increased turnover in WBCs rather than being a specific effect of G-CSF. This notion is supported by the absence of comparable changes in patients receiving similar doses of

![Fig 6. Two-color fluorescence analysis of p55 IL-2R expression on PMNs after in vivo treatment with G-CSF. Neutrophil-specific 1D3 antigen was detected with fluoresceinated anti-1D3 (g, x-axis), and p55 IL-2R was detected with phycoerythrin (PE)-conjugated IL-2R1 MoAb (r, y-axis). NEG: Control staining with isotype- and concentration-matched irrelevant antibodies. POS: Two-color staining with 1D3-FITC and IL-2R1-PE MoAbs. Horizontal panels show surface marker analysis off (A) and on (B) therapy with G-CSF.](http://www.bloodjournal.org/content/108/5/2649/F6)

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G-CSF without experiencing a major increase in WBCs.\textsuperscript{8} Soluble IL-2R levels were also correlated to total WBC counts in patients receiving G-CSF, similar to patients receiving GM-CSF (Lindemann et al, unpublished observations). Surface marker studies on 1D3-positive myeloid cells and previous reports in the literature suggest that granulocyte lineage cells may be a source of soluble IL-2R.\textsuperscript{13,30,31}

An enhanced potential of O2\textsuperscript{-} radical release suggested functional activation of G-CSF-induced PMNs, in accordance with previous findings demonstrating an enhancement of ADCC, chemotaxis, and expression of FMLP- and surface Fc receptors for immunoglobulin A.\textsuperscript{4,7} However, the increased availability of functional PMNs did not result in a measurable antitumor effect in our patients. Thus, the major therapeutic role of this cytokine may be reduction of degree and duration of chemotherapy-induced myelosuppression in cancer patients. Detection of subtle effects of G-CSF on tumor cell growth was beyond the scope of this study, however. Therefore, considering recent in vitro experiments demonstrating stimulatory effects of CSFs on nonhematopoietic tumor cell lines,\textsuperscript{32,33} further studies are needed to address this question specifically.

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Hematologic effects of recombinant human granulocyte colony-stimulating factor in patients with malignancy

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