Characterization of the Clinical Effects After the First Dose of Bacterially Synthesized Recombinant Human Granulocyte-Macrophage Colony-Stimulating Factor

By Graham J. Lieschke, Jonathan Cebon, and George Morstyn

Bacterially synthesized recombinant human granulocyte-macrophage colony-stimulating factor (rhGM-CSF) is an agent with therapeutic potential for neutropenic states, but even at doses below the maximal tolerated dose adverse effects occur during short courses of administration. We have recognized a syndrome of hypoxia and hypotension that follows the first but not subsequent doses of rhGM-CSF. Thirteen of 42 patients receiving rhGM-CSF in phase I studies and 4 of 6 patients in a phase II study developed a reaction that occurred after the first dose of 24 of 74 cycles of rhGM-CSF therapy. The reaction was characterized by flushing (16 of 24), tachycardia (16 of 24), hypotension (14 of 24), musculoskeletal pain (13 of 24), dyspnea (12 of 24), nausea and vomiting (11 of 24), rigors (5 of 24), involuntary leg spasms (3 of 24), and syncope (3 of 24). The reaction did not occur after any of more than 600 second and subsequent consecutive rhGM-CSF doses. Oxygen saturation decreased during first-dose reactions by 8% ± 4% as compared with 3% ± 1% on first days without reactions (P < .001) and 2% ± 1% on subsequent days (P < .001). Pulmonary dysfunction was characterized by hypoxemia (59 ± 9 mm Hg, mean ± SD) that was fully correctable with supplementary oxygen, decreased single-breath carbon monoxide diffusion capacity, and increased alveolar-arterial oxygen gradients (25 ± 6 to 60 ± 4 mm Hg, mean ± SD), but no significant abnormalities on chest roentgenogram or lung perfusion scan. Factors predisposing to reactions were rhGM-CSF dose ≥ 3 μg/kg (P < .01), intravenous (IV) rather than subcutaneous (SC) administration (P < .05), occurrence of a reaction after the first dose of a previous cycle of rhGM-CSF therapy (P < .01), and for patients receiving 15 μg/kg/d by SC bolus, the presence of lung cancer (P < .05). Administration of 15 μg/kg/d rhGM-CSF by 24-hour SC infusion rather than SC bolus resulted in a delayed onset of reaction from 30 ± 8 minutes to 240 ± 190 minutes (mean ± SD, P < .001), and a slower rate of initial transient decrease in neutrophil levels and a more prolonged duration of transient hypotension. The time of onset of reactions correlated with the rate of rise of rhGM-CSF levels. After rhGM-CSF there was no change in serum levels of total hemolytic complement, the third and fourth components of complement, histamine, and tumor necrosis factor-α (TNF-α). We conclude that transient hypoxia and hypotension are significant complications of the first dose of rhGM-CSF that are not solely explained by the transient leukopenia or pulmonary sequestration of leukocytes. The cardiovascular changes and rise in alveolar-arterial oxygen gradient suggest that regional intrapulmonary ventilation/perfusion mismatching owing to release of a vasodepressor mediator is a significant contributing factor.

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MATERIALS AND METHODS

Patients. As of December 29, 1988, we had administered 78 cycles of bacterially synthesized rhGM-CSF to 48 patients as part of our previously reported phase I dose escalation studies24 and ongoing phase II study of rhGM-CSF after chemotherapy for small cell carcinoma of the lung.14 In the phase I studies, groups of 2 to 4 patients received single daily doses of 0.3 to 30 μg/kg/d rhGM-CSF.
by either IV bolus, IV 2-hour infusion, or SC bolus administration for 10 consecutive days, followed by a nontreatment interval of 10 days, followed by a second 10-day treatment period. Selected patients continued to receive the same dose of rhGM-CSF by the same route on alternate days for 10 more doses. In the phase II study, patients with newly diagnosed small cell carcinoma of the lung received chemotherapy with etoposide (120 mg/m^2/d) and carboplatinum (100 mg/m^2/d) for 3 consecutive days followed by rhGM-CSF (15 μg/kg/d SC) for up to 3 weeks beginning on either the first or fifth day after chemotherapy according to five different schedules of rhGM-CSF administration. The protocols were approved by the Board of Medical Research and the Ethics Committee of The Royal Melbourne Hospital. All patients met the eligibility criteria for the studies as previously reported.5 All patients received the first two doses of rhGM-CSF as inpatients. Observations of vital signs were recorded at least hourly for 6 hours after the first and second doses of rhGM-CSF. At least one patient at each dose level by each route of administration had frequent blood tests performed during the first 6 hours after the first rhGM-CSF dose for blood cell counts and measurement of levels of rhGM-CSF and other factors (see below). After it was recognized that hypoxia was occurring, patients were routinely monitored after the first two doses of rhGM-CSF by finger pulse oximetry using a Criticare 502-US finger pulse oximeter (Criticare Systems Inc., Kent, England) with measurements made every 15 minutes for 2 hours. The alveolar-arterial O_2 gradient was calculated from arterial blood gas analysis using the standard alveolar gas equation:

\[ \text{P}_{A02} - \frac{\text{P}_{A02} - \text{P}_{ACO2}}{R} \left( \frac{1}{R} - \frac{R}{\text{P}_{ACO2}} \right) \]

and alveolar-arterial O_2 gradient \( \text{P}_{A02} - \frac{\text{P}_{A02} - \text{P}_{ACO2}}{R} \) (assuming that for patients breathing air \( \text{P}_{ACO2} = 40 \text{ mm Hg} \), \( \text{F}_{iO2} = 0.21 \), and the respiratory quotient \( R = 0.8 \). Abbreviations: a, arterial; A, alveolar; P, partial pressure; O_2, oxygen; CO_2, carbon dioxide; F_{iO2}, inspired fractional oxygen concentration; P_{iO2}, partial pressure of oxygen (inspired gas). We preferred to use \( \text{O}_2 \) saturation measurements rather than arterial blood gas measurements after we had observed that desaturations were produced in patients breathing air of \( \approx 70 \text{ mm Hg} \). These definitions were used because the normal \( \text{O}_2 \) saturation for adults is 97%, and an \( \text{O}_2 \) saturation of 93% corresponds to an arterial \( \text{O}_2 \) tension of 70 mm Hg.5

Biochemical methods. For the biochemical studies, blood collected from patients was allowed to clot at room temperature and was then held at 4°C. Serum was separated and stored at –70°C within 12 hours of collection for assaying for tumor necrosis factor and total hemolytic complement levels. Serum was assayed for tumor necrosis factor (TNF) using a commercially available enzyme-linked immunosorbent assay (ELISA) kit (Bioline TNF Test Kit, T-Cell Sciences, Cambridge, MA), with a calculated sensitivity of 10 pg/mL. Total hemolytic complement was measured by a commercially available radial diffusion method (Quantiplate Total Complement Test kit, Kallestad Laboratories, Austin, TX) standardized against Kallestad standards with a coefficient of variation of 15% in our laboratory. The third and fourth components of complement (C3 and C4) in sera were measured by immunonephelometry on a Behring nephelometer using commercially available antisera (Hoechsr-Behring, Marburg, FRG) and a Kallestad calibrator. In our laboratory, the coefficient of variation for C3 was 5% at 0.88 g/L and 3.4% at 2.6 g/L, and the coefficient of variation for C4 was 9% at 0.16 g/L and 6.4% at 0.50 g/L. C3 and C4 levels were also measured in sera from selected patients that had been stored at –20°C for rhGM-CSF pharmacokinetic studies. To standardize for the variability in baseline complement levels, sequential complement levels were expressed as a percentage of baseline values. Tissue was assayed for plasma immediately separated from blood samples collected on ice and assayed within 7 days with a commercially available radionuclide assay (Histamine RIA50, Pharmacia, Uppsala, Sweden). rhGM-CSF in sera was measured on specimens stored at –20°C with a modification of a previously described sensitive ELISA assay.

RhGM-CSF. Nonglycosylated, bacterially synthesized rhGM-CSF was supplied by Schering-Plough (Kenilworth, NJ) as a lyophilized powder. Five different batches of rhGM-CSF were used. Vials containing 50, 100, 400, 500, or 700 μg rhGM-CSF (specific activity 10^8 U/mg) were reconstituted with 1 mL sterile water for injection (Delta West, Perth, Western Australia); rhGM-CSF was prepared immediately before use. Subcutaneous bolus injections were prepared as more concentrated solutions when necessary to limit volume of injected fluid to a maximum of 1.1 mL. For IV bolus injections, no further dilution was made. For 2-hour IV infusions, rhGM-CSF was further diluted in 100 mL normal saline (Delta West). rhGM-CSF contained less than 12 U endotoxin per vial by the limulus amoebocyte lysate assay. Because no DNA was detectable in the bulk product, each dose contained <30 pg. Escherichia coli proteins were present in insignificant levels, and there was no evidence of any toxins (E. Bonnew, personal communication, June 1989).

Statistics. Unless otherwise stated, results are expressed as mean ± SD. Significance values were calculated by the chi-square test or two-sample t test.
the phase I study of IV rhGM-CSF, we initially used bolus IV injections. Symptomatic hypotension occurred 120 minutes after 1 μg/kg rhGM-CSF in one patient and 20 minutes after 3 μg/kg rhGM-CSF in another patient. This latter patient had transient syncope, followed by vomiting, sweating, and rigor. Arterial blood gases taken during the episode showed an O2 tension of 69 mm Hg while breathing air, CO2 tension of 38 mm Hg, and pH of 7.42. In view of the unexplained hypoxia, we routinely monitored the O2 saturation of subsequent patients and documented clinical observations after the first and second doses of rhGM-CSF in 18 other patients who received 32 more cycles of therapy.

Table 1 shows the frequency of adverse clinical observations after the first dose of rhGM-CSF. In the phase I studies, 16 of 66 cycles of rhGM-CSF were characterized by first doses followed either by symptomatic hypotension or hypoxia or both, with at least two of the other common symptoms: flushing sensation; muscular or bony aches; throbbing lumbar back pain; dyspnea, nausea, and vomiting; rigor or fever; and involuntary leg spasms. For the purpose of this analysis, this definition was used to distinguish patients who had reactions from those who did not. Flushing was the most common symptom, occurring in 67% of reactions and usually heralded onset of a reaction. Bone pain was also common, and in six instances was associated with throbbing lumbar discomfort that two patients described as synchro-

<p>| Table 1. Adverse Clinical Observations Occurring During First-Dose Reactions to rhGM-CSF |
|-------------------------------------|-------------------------------------|-------------------------------------|-------------------------------------|</p>
<table>
<thead>
<tr>
<th>Clinical Observation</th>
<th>No. (%) of First-Dose Reactions</th>
<th>No. (%) of First-Dose Reactions</th>
<th>No. (%) of First-Dose Reactions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Symptoms</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flushing sensation</td>
<td>10(65)</td>
<td>6(75)</td>
<td>16(67)</td>
</tr>
<tr>
<td>Musculoskeletal pain</td>
<td>9(56)</td>
<td>4(50)</td>
<td>13(54)</td>
</tr>
<tr>
<td>Throbbing lumbar pain</td>
<td>6(38)</td>
<td>0(—)</td>
<td>6(25)</td>
</tr>
<tr>
<td>Dyspnea</td>
<td>8(50)</td>
<td>4(50)</td>
<td>12(50)</td>
</tr>
<tr>
<td>Nausea and vomiting</td>
<td>6(38)</td>
<td>5(63)</td>
<td>11(46)</td>
</tr>
<tr>
<td>Rigor*</td>
<td>5(31)</td>
<td>0(—)</td>
<td>5(21)</td>
</tr>
<tr>
<td>Involuntary leg spasms</td>
<td>3(19)</td>
<td>0(—)</td>
<td>3(13)</td>
</tr>
<tr>
<td>Signs</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood pressure</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Increase ≥ 20 mm Hg</td>
<td>4(25)</td>
<td>1(13)</td>
<td>5(21)</td>
</tr>
<tr>
<td>Decrease ≥ 20 mm Hg</td>
<td>9(56)</td>
<td>5(63)</td>
<td>14(58)</td>
</tr>
<tr>
<td>Heart rate</td>
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<tr>
<td>Increase ≥ 20/min</td>
<td>10(63)</td>
<td>6(75)</td>
<td>16(67)</td>
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<tr>
<td>Decrease ≥ 20/min</td>
<td>3(19)</td>
<td>0(—)</td>
<td>3(13)</td>
</tr>
<tr>
<td>Syncope</td>
<td>2(13)</td>
<td>1(13)</td>
<td>3(13)</td>
</tr>
<tr>
<td>Fever ≥ 37.5°C</td>
<td>2(13)</td>
<td>0(—)</td>
<td>2(8)</td>
</tr>
<tr>
<td>Hypoxia</td>
<td>12(75)</td>
<td>7(88)</td>
<td>19(79)</td>
</tr>
</tbody>
</table>

Observations were limited to those occurring within 6 hours of rhGM-CSF administration.

*Defined as involuntary shaking with or without fever.
†In 12 of 16 episodes, hypoxia was documented by arterial blood gas measurement (P_{O2} ≤ 70 mm Hg breathing air) or O2 saturation measurement by finger pulse oximetry (O2 saturation, decrease ≥ 4% sustained for at least three consecutive readings in 5 minutes). In 4 of 16 episodes, O2 levels were not monitored.
‡In 7 of 8 episodes, O2 levels were monitored as above. In 1 of 8 episodes, the patient was not monitored.

nous with the pulse recording. Dyspnea was always accompanied by hypoxia. During reactions, three patients complained of involuntary muscular contractions in the legs without paresthesias or spasms of the hands. During two of these reactions with spasms, we documented that patients had normal arterial CO2 tensions and were not alkalotic on arterial blood gas analysis. During one such reaction, a patient was shown to have normal plasma sodium, potassium, magnesium, calcium, and phosphate levels during the spasms.

Blood pressure (BP) decreased ≥20 mm Hg in 14 of 24 (58%) reactions and increased ≥20 mm Hg in 5 of 24 (21%) reactions. Of the 10 patients who experienced reactions on day 1 of the phase I studies and who had comparable hourly BP readings on both the first and second study days, 5 of 10 had BP decreases ≥20 mm Hg on day 1 but none had decreases of this magnitude with the subsequent rhGM-CSF dose (chi square = 6.1, P < .05). Tachycardia occurred in 16 of 24 (67%) reactions and bradycardia occurred in 3 of 24 (13%) reactions. Hypoxia was documented in all reactions for which blood gas measurements or oxygen saturation readings were available (19 of the 24 episodes); for the other events, documentation of hypoxia was not available (described later).

In the phase I studies, the reaction occurred at 60 ± 40 minutes after rhGM-CSF was injected or after the infusion was started (range 20 to 180 minutes). For patients in the phase II study, all of whom received 15 μg/kg rhGM-CSF by bolus SC injection, the reaction occurred at 30 ± 8 minutes (range 20 to 45 minutes). Eleven of 16 reactions had completely resolved by 2 hours after rhGM-CSF, and all had resolved by 4 hours after rhGM-CSF.

The occurrence of a reaction at the start of the first cycle of rhGM-CSF therapy increased the likelihood of a reaction at the beginning of subsequent rhGM-CSF treatment cycles. Of 30 patients who received more than one cycle of rhGM-CSF, 19 of 23 patients who did not experience a reaction in the first cycle had a second treatment cycle without a reaction, whereas 6 of 7 patients who had a reaction after the first dose of the first cycle had a reaction at the start of the subsequent cycle (chi square = 11.3, P < .001).

None of the 17 patients who had first-dose reactions in either phase I study had similar symptoms or signs with any of the following consecutive daily doses of rhGM-CSF in the same treatment cycle, which totaled more than 600 doses. Alternate-day dosing with rhGM-CSF immediately after consecutive daily rhGM-CSF was not associated with further reactions in patients who had previously experienced them at the start of a treatment cycle, but stopping rhGM-CSF for ≥10 days was associated with recurrence of the reaction in six of seven patients.

**Influence of dose and route of rhGM-CSF.** Table 2 shows the incidence of the first-dose reactions as defined according to dose and route of administration of rhGM-CSF. There were significantly more reactions with first doses in cycles of ≥3 μg/kg rhGM-CSF than in cycles ≤1 μg/kg rhGM-CSF (chi square = 5.4, P < .01), but not in terms of numbers of patients at these dose levels (chi square = 3.3, P < .1). In the phase I study, 2 of 21 patients receiving SC
rhGM-CSF had first-dose reactions as compared with 11 of 21 patients receiving IV rhGM-CSF (chi square = 9.0, P < .01). There were significantly more reactions with IV rhGM-CSF even when the four patients in the phase II study who received SC rhGM-CSF who experienced first-dose reactions are included in the comparison (chi square = 4.7, P < .05). Reactions occurred at an earlier time after doses ≥15 μg/kg, (46 ± 21 minutes) than doses of ≤10 μg/kg (65 ± 32 minutes). Reactions occurred at a similar time after both IV and SC administration. The severity of the reactions as measured by percentage of decrease in O₂ saturation or in BP was not affected by the dose in patients who had a reaction.

In an attempt to avoid occurrence of a reaction in phase II study patients who had experienced reactions previously, we administered the first dose of rhGM-CSF by SC continuous infusion in 24 hours rather than by SC bolus to three patients who had reactions at the start of two or three previous cycles of rhGM-CSF (Table 3). In two of three patients, the severity of symptoms was markedly reduced as compared with the previous reactions, but the third patient who had previously had symptomatic hypotension with syncope experienced syncope again. The time of onset of the reaction was significantly delayed from 30 ± 8 minutes for SC bolus administration to 240 ± 190 minutes for SC infusions (t = 3.3, P < .001).

**Influence of patient characteristics.** There was no difference in the proportion of patients on the phase I study who had first-dose reactions as compared with those who did not in terms of age, sex, performance status, tumor type including proportion with carcinoma of the lung, past history of respiratory disease including asthma, and past smoking history. For the 10 patients who received 15 μg/kg by SC bolus on either the phase I or II studies, the presence of lung cancer increased the likelihood of occurrence of a reaction. The four patients who had reactions all had lung cancer, but only 2 of 6 who did not have reactions had lung cancer (chi square = 4.4, P < .05). The proportion of patients with other adverse effects attributed to rhGM-CSF was the same in patients with and without first-dose reactions.

Pretreatment neutrophil level did not influence occurrence of reactions. The mean neutrophil count before treatment for patients who had reactions was 4.7 ± 2.3 as compared with

<table>
<thead>
<tr>
<th>Table 2. Incidence of First-Dose Reactions to rhGM-CSF by Dose and Route of Administration</th>
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<tbody>
<tr>
<td>Dose of rhGM-CSF (μg/kg)</td>
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<tr>
<td>---------------------------</td>
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<tr>
<td></td>
</tr>
<tr>
<td>Phase I studies</td>
</tr>
<tr>
<td>0.3</td>
</tr>
<tr>
<td>1</td>
</tr>
<tr>
<td>3</td>
</tr>
<tr>
<td>10</td>
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<tr>
<td>15</td>
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<tr>
<td>20</td>
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<tr>
<td>30</td>
</tr>
<tr>
<td>Phase II study</td>
</tr>
<tr>
<td>15</td>
</tr>
<tr>
<td>Total (%)</td>
</tr>
</tbody>
</table>

Numerator shows number of patients or treatment cycles in which first-dose reactions occurred and denominator shows total number of patients or treatment cycles for each dose and route of administration.

*One patient experiencing a first-dose reaction to 15 μg/kg IV infusion rhGM-CSF in cycle 1 was treated with 10 μg/kg in cycle 2 and again experienced a first-dose reaction.

<table>
<thead>
<tr>
<th>Table 3. Comparison of Clinical Features of First-Dose Reaction With rhGM-CSF in Patients Receiving rhGM-CSF by SC Bolus Injection or 24-Hour Infusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient</td>
</tr>
<tr>
<td>---------</td>
</tr>
<tr>
<td>Time of Onset (min)</td>
</tr>
<tr>
<td>C.M.</td>
</tr>
<tr>
<td>30</td>
</tr>
<tr>
<td>20</td>
</tr>
<tr>
<td>A.A.</td>
</tr>
<tr>
<td>30</td>
</tr>
<tr>
<td>P.E.</td>
</tr>
<tr>
<td>30</td>
</tr>
</tbody>
</table>

Abbreviations: NR, not recorded; SC, subcutaneous; BP, blood pressure.

Patients with small cell lung cancer received SC rhGM-CSF (15 μg/kg) by bolus injection or 24-hour infusion on the day after 3 days of chemotherapy with carboplatin 100 mg/m²/d and etoposide 120 mg/m²/d.
4.3 ± 2.3 x 10^9/L for those who did not have reactions (t = 0.55, P > .5). Neutropenia did not protect against the reaction, because one of five neutropenic patients (neutrophils < 1.5 x 10^9/L) who received doses of 1 to 20 μg/kg had a reaction. However, of the four patients who received 20 μg/kg rhGM-CSF by IV infusion, the only patient who did not have a reaction was neutropenic.

Correlation with hematologic effects. Because a transient leukopenia (affecting neutrophils, monocytes, and eosinophils, but not lymphocytes) immediately follows a dose of rhGM-CSF, and blood counts monitored in parallel, the onset of the transient leukopenia and hypoxia. In the eight patients who had both frequent O₂ saturation measurements and blood counts monitored in parallel, the onset of the leukopenia preceded the decrease in oxygen saturation (Fig 1A). Transient leukopenia was documented to occur for 12 first doses not accompanied by first-dose reactions. Furthermore, three patients who had documented hypoxia and leukopenia with the first dose of rhGM-CSF had transient leukopenia on subsequent days of rhGM-CSF administration without hypoxia, during which larger absolute numbers of neutrophils temporarily left the circulating pool. For the patient shown in Fig 1B, the circulating leukocyte level decreased by 6.3 x 10^9/L on the first day of the second cycle of rhGM-CSF administration (day 21) and by 9.6 x 10^9/L on the next day (day 22) on which hypoxia did not occur. We conclude that the transient leukopenia alone did not account for the occurrence of hypoxia.

The transient leukopenia showed a different temporal profile when the same dose of rhGM-CSF was administered by SC bolus and 24-hour SC infusion (Fig 2). After bolus injection, the initial rate of decrease in leukocyte levels was greater and the duration of leukopenia was shorter than for 24-hour SC infusions. For neutrophils, the rate of decrease after SC bolus injection was 0.17 ± 0.05 x 10⁹/L/min, and after start of the SC infusion it was 0.06 ± 0.04 x 10⁹/L/min (t = 3, P < .01). One patient receiving a SC infusion had a maximal initial decrease in neutrophils from baseline values of 4 to 2.8 x 10⁹/L at 60 minutes after start of the infusion, but 45 minutes before onset of a delayed first-dose reaction, the granulocytes had decreased to 1.3 x 10⁹/L.

Characterization of pulmonary dysfunction. Because hypoxia was a significant component of the sequence of events after the first dose of rhGM-CSF, we conducted further studies to characterize the pulmonary dysfunction in these patients. Figure 1 shows the results of studies of the effect of rhGM-CSF on O₂ saturation and arterial O₂ tension on different days of rhGM-CSF administration. Figure 1C shows that for a patient experiencing a first-dose reaction a decrease in O₂ saturation from 98% to 87% correlated with a decrease in arterial O₂ tension from 91 to 53 mm Hg. The baseline arterial CO₂ tension was 41 mm Hg and at the time of maximal oxygen desaturation was unchanged at 38 mm Hg. Supplementary O₂ administration fully corrected the hypoxia. Arterial O₂ tensions during the reaction were measured in six phase I patients and averaged 39 ± 9 mm Hg (range 47 to 69 mm Hg). Figure 1B shows that O₂ desaturation occurred only on the first day of each of two cycles of rhGM-CSF but did not occur on subsequent days (data shown only for the second day of cycle 2; this patient was also monitored on the third and fifth days of cycle 2, and a decrease in O₂ saturation did not occur). Patients who experienced reactions were monitored on a subsequent day during seven cycles of rhGM-CSF; for these patients, the mean maximal decrease in O₂ saturation was 7% ± 3% on the first day and 2% ± 1% on subsequent days (t = 4.3, P < .001). Six patients who did not have symptomatic reactions on day 1 were monitored by oximetry, and their mean maximal O₂ desaturation was 3% ± 2% (range 0% to 6%) as compared with 8% ± 4% (range 3% to 14%) for the nine monitored day 1 reactions (t = 3.3, P < .001). Only two patients who were asymptomatic on day 1 had O₂ desaturation of more than 4%.

One patient experiencing a first-dose reaction was studied radiologically. This patient had chronic lymphocytic leukemia and received 20 μg/kg/d rhGM-CSF by 2-hour IV infusion. The patient developed transient granulocytopenia at 30 minutes, followed by O₂ desaturation of 4% 40 minutes after the first dose of rhGM-CSF, which corresponded to
and on the final treatment day; no new pulmonary infiltrates were observed.

To monitor for bronchospasm, we performed chest auscultation during reactions on all patients and sequential peak expiratory flow rate recordings during five reactions. In three episodes, there were reductions in peak expiratory flow rates (from $570 \pm 60$ to $300 \pm 80, 630 \pm 30$ to $590 \pm 10$ and $410 \pm 10$ to $360 \pm 10$ L/min); in two episodes, there was no change.

One neutropenic patient with marked expiratory obstruction on baseline pulmonary function tests (forced expiratory volume [1 second] to vital capacity ratio of 54%, peak flow rate $120 \pm 30$ L/min) did not develop hypoxia after $20$ $\mu$g/kg/d rhGM-CSF by IV infusion. Chest auscultation during episodes showed no increased wheeze as compared with pretreatment in any patient.

Because margination or embolization of small aggregates of leukocytes in pulmonary capillaries has been suggested as a mechanism for the transient leukopenia, we measured sequential single-breath lung diffusion capacities in a patient during the period of leukopenia on day 1 of a treatment cycle and subsequently on day 6 when transient leukopenia also occurred. Although hypoxia occurred only on the first day, the single-breath lung diffusion capacity for carbon monoxide (sDLCO) transiently decreased from 23 to 17 mL/min/mm Hg on both days. This paralleled the transient decrease in leucocyte levels on both days. A similar transient decrease in sDLCO was documented in another patient during the rhGM-CSF–induced leukopenia although other features of the first-dose reaction did not occur.

To assess the degree of ventilation/perfusion inequality, the alveolar-arterial $O_2$ gradient was calculated from arterial blood gas measurements for patients breathing air. In the three patients studied, the alveolar-arterial $O_2$ gradients increased from 22 to 63, 32 to 56, and 20 to 62 mm Hg.

**Biochemical studies.** We studied serum rhGM-CSF levels after the first dose of rhGM-CSF to determine if a correlation existed between rhGM-CSF levels and the occurrence of first-dose reactions. In view of the delay in onset of the first-dose reaction, we wished to compare serum rhGM-CSF levels in the same three patients on days of SC bolus and SC infusion administration (Fig 2, bottom). In all patients, $15$ $\mu$g/kg rhGM-CSF by SC bolus achieved serum levels of more than $1$ ng/mL within 15 minutes of injection, and serum rhGM-CSF levels continued to increase rapidly in the first 2 hours to levels of 13.5 to 46 ng/mL. Serum rhGM-CSF levels increased much more slowly after the 24-hour SC infusion was started, and levels of more than $1$ ng/mL were achieved 4 to 8 hours after the infusion was started. None of the three SC infusion patients achieved a peak rhGM-CSF level of more than $7$ ng/mL.

We studied complement levels in patients receiving rhGM-CSF. There was no change in total hemolytic complement or C3 or C4 levels over the first 6 hours after rhGM-CSF, which varied in the ranges 91% to 109%, 97% to 112%, and 102% to 111% of baseline values, respectively.

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Because hypoxia occurred only on the first day, the single-breath lung diffusion capacity for carbon monoxide (sDLCO) transiently decreased from 23 to 17 mL/min/mm Hg on both days. This paralleled the transient decrease in leucocyte levels on both days. A similar transient decrease in sDLCO was documented in another patient during the rhGM-CSF–induced leukopenia although other features of the first-dose reaction did not occur.

To assess the degree of ventilation/perfusion inequality, the alveolar-arterial $O_2$ gradient was calculated from arterial blood gas measurements for patients breathing air. In the three patients studied, the alveolar-arterial $O_2$ gradients increased from 22 to 63, 32 to 56, and 20 to 62 mm Hg.

**Biochemical studies.** We studied serum rhGM-CSF levels after the first dose of rhGM-CSF to determine if a correlation existed between rhGM-CSF levels and the occurrence of first-dose reactions. In view of the delay in onset of the first-dose reaction, we wished to compare serum rhGM-CSF levels in the same three patients on days of SC bolus and SC infusion administration (Fig 2, bottom). In all patients, $15$ $\mu$g/kg rhGM-CSF by SC bolus achieved serum levels of more than $1$ ng/mL within 15 minutes of injection, and serum rhGM-CSF levels continued to increase rapidly in the first 2 hours to levels of 13.5 to 46 ng/mL. Serum rhGM-CSF levels increased much more slowly after the 24-hour SC infusion was started, and levels of more than $1$ ng/mL were achieved 4 to 8 hours after the infusion was started. None of the three SC infusion patients achieved a peak rhGM-CSF level of more than $7$ ng/mL.

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Plasma histamine levels were measured in two patients with flushing, hypoxia, and hypotension and in one patient who did not experience the reaction. Histamine levels did not vary significantly from baseline levels of 11.2 to 19.5 ng/mL during the 6 hours after rhGM-CSF. Serum TNF-α levels were measured during first-dose reactions in five patients; TNF-α levels did not alter from baseline levels of less than 50 pg/mL during 6 hours after administration of rhGM-CSF.

Management and prevention of first-dose reactions. Symptoms of the first-dose reaction were managed empirically. Oxygen was administered to eight phase I and all phase II study patients. On three occasions, IV fluids were administered for symptomatic hypotension. Pain was managed with acetaminophen, indomethacin, or ibuprofen. Two patients receiving 20 μg/kg/d by IV infusion also received morphine for severe throbbing lumbar back pain. For patients receiving IV rhGM-CSF, 4 of 14 first days with reactions occurred despite premedications as described below, whereas a premedication was administered on 13 of 22 first days without reactions (chi square = 3.2, P < .1). Overall, reactions were not prevented by premedication with acetaminophen (n = 5), indomethacin (n = 1), ibuprofen (n = 3), H1 and H2 receptor antagonists (n = 2), pseudoephedrine (n = 2), cyproheptadine (n = 1), and concomitant steroid therapy (n = 1). Prophylactic O2 administration reduced the degree of O2 desaturation but did not prevent symptoms and hypotension.

Administration of rhGM-CSF by 24-hour SC infusion was the modification that most influenced the clinical features of the reaction. Although onset of transient hypoxia was delayed in 3 of 3 patients, the severity of symptoms was subjectively reduced in 2 of 3 patients. One patient who previously experienced flushing, sweating, pain, and dyspnea developed only mild headache during the SC infusion; the other patient who previously experienced chest tightness, nausea, and dyspnea with SC bolus rhGM-CSF experienced mild chest heaviness and brief sweating during the SC infusion.

DISCUSSION

Although bacterially synthesized nonglycosylated rhGM-CSF can be administered at tolerable doses that achieve elevated levels of leukocytes,6,8 even at these doses it is associated with adverse effects. Most commonly, these symptoms have been fever,4,6,8,10,13,16,25-29 bone pain,4,10,16,26,28,29 myalgia or arthralgia,4,6,8,9,13,15 gastrointestinal disturbances,4,8,9,28,29 rash,6,4,15 and elevated serum enzymes.6,8,29 The dose-limiting toxicities with courses of administration of up to 14 days have been pericarditis and pleural or pericardial effusions,5,13,15 fluid retention,13 gastrointestinal disturbance,28 and thrombosis around central venous catheters.13 We describe a different type of reaction characteristic of the first but not subsequent doses of rhGM-CSF in a treatment cycle, which is uncomfortable for patients but is not dose limiting. Because the reaction is infrequent after SC rhGM-CSF, we did not clearly define it in our initial study, although dyspnea was noted in two patients.5 We recognized it during our study of IV bolus or short infusions of rhGM-CSF when it was more frequent.6 The reaction is characterized by both symptomatic hypoxia and hypotension, and it is important that other investigators using rhGM-CSF be aware of the syndrome so that appropriate treatment can be promptly instituted should it occur.

Except for the presence of lung cancer, no patient characteristic was identified that increased the likelihood of occurrence of a reaction. In particular, preexisting neutropenia which is protective against pulmonary dysfunction after cellophane membrane hemodialysis30 was not protective. For unselected patients receiving 15 μg/kg/d rhGM-CSF by SC bolus, the presence of small cell lung cancer appeared to correlate with the occurrence of reactions. Patients with lung cancer have usually been smokers and have impaired baseline respiratory functional reserve, which may predispose them to this reaction. The patient experiencing the most severe reactions, in which hypotension and hypoxia was associated with syncope, also had the Eaton-Lambert syndrome. This neuromuscular defect may have impaired the ability to compensate for rhGM-CSF-induced hemodynamic changes. Some small cell lung cancers have been shown to possess functional GM-CSF receptors.31

We used bacterially synthesized nonglycosylated rhGM-CSF, which in vitro appears to have a higher specific activity than glycosylated rhGM-CSFs synthesized in mammalian cell and yeast expression systems.10,31 An important question is whether the effect we observed is peculiar to the bacterially synthesized nonglycosylated form of rhGM-CSF used. Other investigators using yeast- and mammalian cell-derived rhGM-CSFs have not described the complete reaction. There have been occasional reports of symptoms specifically following the first dose of other forms of rhGM-CSF: facial flushing (n = 3),4 lumbosacral backache at 20 minutes postinjection (n = 5),4 nausea and vomiting (n = 1),4 fever,8,28 and bilateral shoulder pain (n = 1).18 Two groups of investigators specifically stated that pulmonary dysfunction was not observed.25,24 One study reported “slight dyspnea” 2 to 6 hours after rhGM-CSF in 2 of 24 patients.29 These observations indicate that the reaction we have described is probably not unique to nonglycosylated bacterially synthesized rhGM-CSF and may occur with yeast- and mammalian cell-derived rhGM-CSF as well. It may be less common in some of these studies because of use of more prolonged IV infusions, use of lower rhGM-CSF doses, or the presence of glycosylation. However, no conclusions can be drawn until careful monitoring with oximetry is undertaken in patients receiving other forms of rhGM-CSF and a comparative study is performed.

Because the rhGM-CSF used in these studies was prepared from an E coli expression system, we considered that the reaction might be mediated by endotoxin. Each vial of rhGM-CSF contained less than 12 U endotoxin by the limulus amebocyte lysate assay, <30 pg per dose DNA, and insignificant levels of E coli proteins (E. Bonnem, personal communication, June 1989). For the higher doses of rhGM-CSF, it was necessary to prepare the dose from a maximum of three vials of rhGM-CSF. Endotoxin administration (4 ng/kg, 20 μg/kg) to normal human volunteers caused constitutional symptoms, fever, and tachycardia with elevated
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serum levels of TNF-α that peaked at 90 minutes after endotoxin injection.35 The effect was reduced by premedication with ibuprofen. These doses of endotoxin are 40 times greater than the maximum we could have administered to our patients, and the syndrome in our patients was not prevented by ibuprofen.

We performed various studies to determine whether the reaction is directly mediated by rhGM-CSF or if it is mediated indirectly by the effects of rhGM-CSF on leukocytes or by a mediator released during rhGM-CSF therapy. We have observed leukoaggregation in blood films of patients with high leukocyte levels owing to rhGM-CSF administration in previous days, but not in the first 6 hours immediately after the first dose of rhGM-CSF (D. Maher, unpublished observations). Patients undergoing cellophane membrane hemodialysis develop pulmonary dysfunction with hypoxemia and reduced lung diffusion capacity,38 which is a result of leukoaggregation and pulmonary sequestration of granulocytes during a period of transient leukopenia28 and which is mediated by products of complement activation.30,37 Superficially this reaction resembles that observed after the first dose of rhGM-CSF. During the rhGM-CSF–induced transient leukopenia, radiolabeled leukocytes sequester in the lungs.31 In patients receiving rhGM-CSF, there is upregulation of the expression of the leukocyte surface adhesion molecule CD11b (Mo-I),29,34 and variable effects on CD11a (LFA-1)36 and CD11c (Leu-M5).29,34 The increased expression of CD11b was evident on circulating granulocytes 60 minutes after administration of rhGM-CSF in two patients and at 12 hours in one patient,34 indicating that the effect is rapid in onset in vivo, as is also observed in vitro.38 However, these studies do not provide direct information about the cells which at this time have transiently left the circulation, although it is inferred that they have left the circulation by margination or sequestration in the lungs.32,34,35 Although effects on leukocyte aggregation may be the cause of the transient O₂ desaturation in our patients, we do not believe this is true because we showed that (a) transient leukopenia occurs on every day of rhGM-CSF administration but hypoxia occurred only on the first day; (b) transient leukopenia occurred with all first doses regardless of whether a reaction occurred; and (c) the pretreatment leukocyte count was not related to the occurrence of the reaction or the degree of hypoxia. However, a reduction in lung diffusion capacity occurred on both the first and a subsequent day of rhGM-CSF administration, suggesting that this may be related to the transient reduction in circulating leukocytes but that the degree of diffusion impairment was not alone sufficient to cause the degree of hypoxia observed.

Hypoventilation did not contribute to hypoxia in our patients because respiratory drive was not impaired and the patients did not develop hypercapnia. The patients were not known to have right-to-left shunts. Ventilation/perfusion mismatching therefore must be a major factor contributing to the hypoxemia.39 The severity of ventilation/perfusion inequality was indicated by the threefold increase in alveolar-arterial oxygen gradient observed in our patients. A reduction in cardiac output will also exaggerate the effects of ventilation/perfusion inequality by reducing the O₂ tension of mixed venous blood.39 These interacting processes would therefore be expected to exaggerate the effects of ventilation/perfusion inequality transiently. We have not measured cardiac output or venous return during this reaction.

Flushing and hypotension suggest that vasodilatation is an important component of the reaction. We therefore determined whether these patients had increased levels of circulating vasoactive compounds during reactions. rhGM-CSF has been reported to cause mast cell degranulation in vitro.40 Three patients had reductions in peak expiratory flow rates, suggesting bronchoconstriction, and two patients complained of nasal stuffiness, but we observed no other symptoms associated with histamine release such as urticaria or pruritus. Neither did we detect increased levels of circulating histamine or its methylated metabolite,41 in the patients we tested. We have not excluded the possibility that local release of histamine from rhGM-CSF–activated mast cells contributed to the reaction.

Complement activation is a potential etiologic mechanism for some of the immediate and later adverse effects of rhGM-CSF because complement activation produces activated molecules such as C₃a and C₅a, which induce leukocyte expression of CD11b adhesion molecules,33 cause leukocyte aggregation and margination42,47 and mast cell degranulation,43 and increase vascular permeability.44 Our studies of complement levels have shown no significant changes in complement components and activity after rhGM-CSF administration. Because serum complement components have a rapid metabolic turnover46 and are synthesized by macrophages and monocytes susceptible to a variety of external influences,46 further metabolic studies may provide more information about this phenomenon. The effect may be mediated by TNF-α because in vitro rhGM-CSF induces expression of mRNA for TNF-α48 and TNF-α production,49 and TNF-α stimulates adhesion of neutrophils to endothelial cell surfaces.46 Administration of TNF-α to cancer patients is complicated by hypotension and tachypnea and a reduction in DLCO.50 We therefore measured serum TNF-α levels in five of our patients during the first 6 hours after rhGM-CSF administration but observed no detectable increase from low baseline levels, even during the course of typical reactions. rhGM-CSF also has direct effects on endothelial cells.47 The role of this effect in mediation of this reaction is unknown.

In an attempt to modify or abrogate the first-dose reaction, we slowed the rate of rhGM-CSF administration. Although continuous SC infusion reduced the severity of the reaction in two patients with a proven propensity to experience it, in one patient the reaction was severe and occurred later. This is a disadvantage because a longer period of monitoring would be required. We previously showed that SC bolus dosing is more effective at inducing a leukocytosis than are 2-hour IV infusions, and is less frequently associated with the reaction.6,51 It also appears from a comparison between studies that 24-hour continuous IV infusions are more potent than intermittent shorter IV infusions.52,53 We therefore suggest that SC bolus administration or prolonged IV infusion may prove to be the optimal ways of administering rhGM-CSF, but this needs further examination.
We conclude that further studies of the potential clinical benefits of bacterially synthesized rhGM-CSF can be undertaken using doses in the tolerated dose range defined by the phase I studies (up to 15 μg/kg/d). However, we alert other investigators to a significant first-dose reaction to bacterially synthesized rhGM-CSF occurring even within the tolerated dose range characterized by hypoxia and hypotension. Until methods to prevent the reaction can be elucidated, we recommend that patients who are beginning treatment with rhGM-CSF be monitored carefully for respiratory and cardiovascular dysfunction and that those who treat such patients be prepared to administer O₂ therapy and fluid replacement if the reaction occurs.

REFERENCES

Characterization of the clinical effects after the first dose of bacterially synthesized recombinant human granulocyte-macrophage colony-stimulating factor

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