Aerosol Granulocyte Macrophage-Colony Stimulating Factor: A Low Toxicity, Lung-specific Biological Therapy in Patients with Lung Metastases

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ABSTRACT

The objective was to study the feasibility of granulocyte macrophage-colony stimulating factor (GM-CSF) delivery to the lung using an aerosol in humans. A Phase I dose escalation study provided GM-CSF at three dose levels as a twice-a-day (BID) × 7 days schedule. Pulmonary functions were monitored using a remote spirometry device. Blood counts were checked at the beginning and end of each week of GM-CSF nebulization. If no toxicity was encountered, patients rested for 7 days and then were treated at the next dose level. Six of seven patients were successfully dose escalated from 60 μg/dose BID × 7 days, to 120 μg/dose BID × 7 days, then 240 μg/dose BID × 7 days. No toxicity was seen. Comparison of day 0 and day 7 blood leukocyte counts showed no significant increases in either leukocyte numbers or percentage of neutrophils. Pulmonary functions test changes were minor. No significant change in forced vital capacity, FEV1, peak flow, or FEF 25-75 related to either time or dose level was observed. One patient’s lung metastases progressed. The other five patients received an additional 2–6 months of intermittent aerosol GM-CSF at dose level 3 without side effects. One patient with Ewing’s sarcoma has a complete response, and a patient with melanoma had a partial response; the other three had stabilization of pulmonary metastases for 2–6 months. Aerosol delivery of GM-CSF is feasible, safe, and possibly effective. Aerosol cytokine delivery may achieve effective immunological activation against cancer in the lung and is worthy of further study.

INTRODUCTION

GM-CSF stimulates proliferation and differentiation of hematopoietic progenitor cells and augments functional activities of neutrophils, monocytes, macrophages, and dendritic cells (1). Recombinant GM-CSF has been used primarily to enhance neutrophil recovery after cancer chemotherapy. New and emerging applications of GM-CSF include use in infectious disease, as a vaccine adjuvant, amelioration of diarrhea, stomatitis, and mucositis after chemotherapy, as a treatment for pulmonary alveolar proteinosis, and for antitumor therapy (1). Although in many studies GM-CSF has facilitated dose escalation of intensive chemotherapy and/or successfully reduced duration of neutropenia (2–13), survival benefit has been seen only in a minority of studies (14–20). These include two studies in acute myelogenous leukemia (18, 20) and one in small cell lung cancer (19). An intensive schedule of s.c. Escherichia coli-derived GM-CSF (i.e., 5 μg/kg BID for 2 weeks after chemotherapy) was associated with favorable event-free survival in sarcomas (14–17). Because toxic deaths were rare in the latter studies, it is possible that survival benefit could possibly be related to more effective immune activation against the cancer.

These reports of superior survival after GM-CSF regimens are especially intriguing in view of murine gene therapy studies with GM-CSF. Dranoff et al. (21) demonstrated that irradiated tumor cells expressing GM-CSF reliably stimulate potent, specific, and long-lasting antitumor immunity in three different tumor models. Of 10 different immunomodulatory proteins studied, GM-CSF most significantly protected mice from subsequent tumor challenge. In a recent study of GM-CSF-transformed melanoma cells, secretion of GM-CSF facilitated killing of nontransformed bystander tumor cells (22). GM-CSF in gelatin microspheres was as effective as genetically engineered cells (23).

Thus, local GM-CSF may achieve enhanced antitumor effects. Because local or regional effects of GM-CSF may contribute to antitumor immunity, nebulization may provide a means to deliver this protein specifically to the lung. Inhaled IL-2 has been reported to be a modestly effective treatment of pulmonary metastases of renal cell carcinoma (24, 25); dose-limiting toxicity was airway irritation and nonproductive cough (26). Because aerosol delivery of GM-CSF may achieve high lung concentrations of GM-CSF, a Phase I dose escalation study of aerosol GM-CSF was done to demonstrate feasibility. This

The abbreviations used are: GM-CSF, granulocyte macrophage-colony stimulating factor; IL, interleukin; BID, twice a day; FVC, forced vital capacity; PEF, peak expiratory flow; FPT, pulmonary function test; GEE, generalized estimating equation; WBC, white blood count; PBPC, peripheral blood progenitor cell; TNF, tumor necrosis factor.
Report is the first clinical trial of aerosol GM-CSF in humans; low toxicity and promising antitumor effects against lung metastases were seen.

**MATERIALS AND METHODS**

Although GM-CSF is an Food and Drug Administration-approved drug, the study was conducted under an investigator-initiated IND (#7389) because of the complete absence of information regarding the aerosol route and possible toxicity of aerosol GM-CSF in humans. The clinical design was peer reviewed within the Mayo Clinic Cancer Center (protocol 97-02-01) and approved by both the Pediatric Research Committee and the Mayo Clinic Institutional Review Board. All patients (or guardians, if the patient was <8 years of age) provided informed consent prior to study entry.

Patient characteristics are summarized in Table 1. Requirements for study entry included age >11 years, life expectancy >12 weeks, and histological proof of cancer with radiographic evidence of prior or active involvement of the lung, pleura, or mediastinum. Types of cancer were not limited to sarcomas but could include other varieties involving the lung with no known standard therapy for the patient’s disease that was potentially curative or definitely capable of extending life expectancy. Laboratory values required within 14 days preregistration included absolute neutrophil count >1,000/ml, hemoglobin >8.0, total bilirubin within two times the normal limits, aspartate aminotransferase less than three times the upper limit of normal, and creatinine less than 2.5 times the upper limit of normal. Exclusions were poor performance status (Eastern Cooperative Oncology Group performance status 3 or 4), uncontrolled infection, and any of the following: concurrent or prior chemotherapy <3 weeks, mitomycin C or nitrosoureas <6 weeks, other immunotherapy <2 weeks, a biological therapy <2 weeks, or radiation therapy <2 weeks. Patients with New York Heart Association classification III or IV or central nervous system metastases or seizure disorder were ineligible because of concern regarding possible pleural effusions and hyperventilation during nebulization worsening a preexisting seizure disorder, respectively.

**GM-CSF.** Yeast-derived glycosylated recombinant GM-CSF (Leukine; sargramostim) was obtained from Immunex (Seattle, WA). To facilitate correct dosing without manipulation of vials by the patients, Mayo Clinic Oncology pharmacy transferred liquid contents of GM-CSF vials into unit dose 1-ml TB syringes in a laminar flow hood. Patients were provided with 14 unit dose syringes on the first day of each dose level. Patients stored the syringes containing GM-CSF in their refrigerator at home.

**Nebulization.** Aerosol therapy was done by adding contents of a GM-CSF unit dose syringe and 2 ml of Bronchosaline (Blalrex Laboratories, Columbus OH) to the nebulizer bowl of a PARI LC PLUS nebulizer set (part no. 22F81 with tubing, part no. 2280 without tubing; Pari Respiratory Equipment, Inc., Richmond, VA). This nebulizer set was chosen because of its conical design (little leftover waste in the bowl), rapid rate of nebulization, correct droplet size, and valved mouthpiece to achieve maximal aerosol delivery. A standard air compressor was used to generate the aerosol mist (Pulmo-Aide; DeVilbiss) To achieve correct droplet characteristics, an air flow of 3.5–8 l/min is recommended. Patients inhaled the mist containing GM-CSF through the valved mouthpiece provided with the nebulizer. Aerosol GM-CSF treatments were typically completed in about 10–15 min. The first aerosol GM-CSF treatment was observed in the clinic to be certain that aerosol technique was mastered; all subsequent treatments were self administered at home.

**Dose Escalation Scheme.** Patients were treated using a BID schedule at each dose level for 7 days as indicated in Table 2. One week of rest was mandatory after completion of each dose level. Patients without toxicity were then permitted to begin aerosol GM-CSF at the next dose level.

**Monitoring of PFTs.** Patients were instructed on the use of remote spirometry at the time of nebulization training. The device used in this study was the Asthmalog (Datalog, Stillwater, MN). The Asthmalog reliably measures FVC, FEV1, peak flow, and FEF 25-75 and also provides preset feedback to the patient if there is significant change. The patient sees a green light after a good test; a red light was preset to go on if effort was insufficient or there was a change. We preset the device to indicate a change of >10% in FVC. PFT data were transmitted to Datalog and forwarded to Mayo Clinic Pediatrics by Fax.

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**Table 1** Characteristics of patients with lung metastases receiving aerosol GM-CSF

<table>
<thead>
<tr>
<th>Age</th>
<th>Diagnosis</th>
<th>Prior surgery</th>
<th>Measurable lung metastases</th>
<th>Response</th>
<th>TTP or F/U</th>
</tr>
</thead>
<tbody>
<tr>
<td>21</td>
<td>Leiomyosarcoma</td>
<td>×2</td>
<td>bilateral, &lt;3 mm</td>
<td>Stable</td>
<td>8 mo</td>
</tr>
<tr>
<td>52</td>
<td>Renal cell carcinoma</td>
<td>×1</td>
<td>bilateral, &gt;2 cm</td>
<td>Progress</td>
<td>2 mo</td>
</tr>
<tr>
<td>13</td>
<td>Ewing’s sarcoma</td>
<td>×1</td>
<td>bilateral, &lt;5 mm</td>
<td>CR</td>
<td>&gt;12 mo</td>
</tr>
<tr>
<td>19</td>
<td>Osteosarcoma</td>
<td>×1</td>
<td>TNTC*</td>
<td>bilateral, &lt;5 mm</td>
<td>Stable</td>
</tr>
<tr>
<td>20</td>
<td>Osteosarcoma</td>
<td>×2</td>
<td>bilateral, &gt;2 cm</td>
<td>Stable</td>
<td>&gt;11 mo</td>
</tr>
<tr>
<td>57</td>
<td>Melanoma</td>
<td>×1</td>
<td>bilateral, &lt;2 cm</td>
<td>PR</td>
<td>&gt;10 mo</td>
</tr>
<tr>
<td>67</td>
<td>Melanoma</td>
<td>×1</td>
<td>bilateral, &lt;2 cm</td>
<td>PR</td>
<td>&gt;9 mo</td>
</tr>
</tbody>
</table>

* TTP, time to progression; F/U, follow-up of responding patients; CR, complete response; PR, partial response; TNTC, too numerous to count.

**Table 2** Aerosol GM-CSF doses and schedule

<table>
<thead>
<tr>
<th>Level</th>
<th>GM-CSF/dose</th>
<th>Schedule</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>60 µg</td>
<td>BID × 7 days, then rest × 1 week</td>
</tr>
<tr>
<td>2</td>
<td>120 µg</td>
<td>BID × 7 days, then rest × 1 week</td>
</tr>
<tr>
<td>3</td>
<td>240 µg</td>
<td>BID × 7 days, then rest × 7 days</td>
</tr>
<tr>
<td>Continuation</td>
<td>240 µg</td>
<td>BID × 7 days, repeat after 7 days rest</td>
</tr>
</tbody>
</table>
modem within the device. The safety monitoring by remote spirometry was intended to facilitate evaluation of possible pulmonary side effects.

**Assessment of Subjective and Objective Toxicity.** This was done by interviews and exam of each patient after 7 days of therapy at each dose level. Blood counts (complete blood count with differential) were also obtained at the start and end of each week of nebulization of GM-CSF. Clinical responses were documented by chest X-ray for patients with lung metastases >2 cm in diameter and by computed tomography scan for those 2 cm or less in diameter.

**Statistical Methodology.** The study was monitored in accordance with policies and procedures developed specifically for Mayo Comprehensive Cancer Center Phase I studies. Routine summary reports were produced biweekly regarding accrual, toxicity, and efficacy data as part of a standardized series of descriptive and graphical summary measures constructed by the Mayo Comprehensive Cancer Center Statistics Unit.

Analysis of blood count data at the beginning and end of 7 days of nebulization was done using paired \( t \) tests for each dose level. Pulmonary function test data were analyzed by graphic analysis of individual parameters to observe for trends of dose and time effect and interpatient variability. Each parameter (FVC, FEV1, peak flow, and FEF 25-75) was then analyzed with respect to pairwise correlation between time and dose level to examine for a dose-response relationship. A repeated measures mixed model ANOVA was also undertaken to examine area under the curve for each PFT by dose level and time (27). This approach was cross-validated by the use of GEE models (28).

**RESULTS**

**Patient Entry and Completion.** Seven patients were entered in the study. One patient with pulmonary metastases too numerous to count developed plural effusions at dose level one, had pleuritic chest pain, and poor compliance with PFTs. No evaluable data were obtained, and this subject was not offered the opportunity for dose escalation. The remaining six patients completed all three dose levels and had excellent compliance with required testing and follow-up visits. All six had analyzable data concerning blood counts, PFTs, subjective and objective toxicity, and response. Four patients had stable or improving lung metastases at completion of dose level 3 and chose to continue to receive aerosol GM-CSF using a 1 week on/1 week off schedule for an additional 2–6 months.

**Blood Counts.** No effect on hemoglobin or platelet count was seen. Summary data concerning mean WBC and neutrophil percentage of the total WBC is presented in Table 3. No dose response was seen. Although minor WBC increases were seen after 14 doses compared with before nebulization at the two higher dose levels, neither increases in total WBC nor percentage of neutrophils was statistically significant.

Pulmonary function monitoring was done in a very reliable manner using the remote PFT device. No patient had to have early evaluation (i.e., earlier than day 7 of each dose level) because of concern about adverse pulmonary function changes. FVC and FEV1 had less variance than FEF 25-75 and peak flow. Seventeen of 18 data sets contained 10 or more FVC data points of a possible 14 data points obtained before each nebulization. Use of the remote PFT device in this study was designed to allow real-time evaluation of pulmonary side effects and to facilitate the collection of this data. A recent study evaluated the reliability of remote PFTs with responses compared with test results obtained using the same device in a laboratory setting (26). A correlation of 0.99 was noted between these two sets of test results, indicating that the remote device is reliable for use in evaluating pulmonary function.

Table 3  WBC counts and percentage of neutrophils after aerosol GM-CSF

<table>
<thead>
<tr>
<th>Unit dose</th>
<th>Day 0 WBC (SD)</th>
<th>Day 7 WBC (SD)</th>
<th>Day 0 % PMN (SD)</th>
<th>Day 7 % PMN (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>60 µg</td>
<td>7.1 (2.6)</td>
<td>6.7 (2.5)</td>
<td>62.8 (9.3)</td>
<td>62.3 (13.8)</td>
</tr>
<tr>
<td>120 µg</td>
<td>6.2 (2.0)</td>
<td>7.3 (3.1)</td>
<td>63.1 (6.1)</td>
<td>69.4 (9.0)</td>
</tr>
<tr>
<td>240 µg</td>
<td>6.2 (2.5)</td>
<td>7.0 (3.2)</td>
<td>62.3 (7.0)</td>
<td>67.4 (7.9)</td>
</tr>
</tbody>
</table>

\( ^{a} n = 6. \)

\( ^{b} \text{Paired } t \text{ test day 0 versus day 7, not significant. PMN, polymorphonuclear cells.} \)
lization. After 1 week of aerosol GM-CSF, a mean of 12.1 remote spirometry tests of a possible 14 was completed.

Fig. 1 shows FVC tests obtained sequentially for each subject at each dose level. There is strong evidence for the presence of interpatient variability and the absence of intrapatient variability over time. Interestingly, the patient with the worst FVC had not only remarkably consistent test results across dose levels but also showed some minor improvement with long-term use of aerosol GM-CSF. This was a 13-year-old girl with Ewing’s sarcoma (patient C on Fig. 1 and Table 4) and a prior history of radiation pneumonitis. She completed 13–15 tests at each dose level; means (SD) were 46.1 (1.3), 44.5 (1.6), and 43.2 (1.6) at dose level 1, 2, and 3, respectively. After 6 months of 240 mg/dose BID 1 week on/1 week off, FVC had increased from 43–46% predicted to 50% predicted. At 10 months (4 months off GM-CSF), FVC was increased to 55% predicted.

Table 4 summarizes mean values and SDs for all PFT data. Although small differences in PFT parameters were seen in some patients, PFT changes were in both directions: improvement and worsening. No obvious dose or time effect was observed when data were depicted graphically for not only FVC (Fig. 1) but also for FEV1, peak flow, and FEF 25-75. Fig. 2 collapses the data across the patient to present the median profile of each PFT parameter by dose level.

Pairwise correlations between time point values indicated that there is strong correlation between virtually all PFT time point data because all coefficients were 0.60, and the majority 0.80 (Table 5). In other words, the PFT parameters did not change much over time and would suggest a covariance structure of constant correlation. As a result, we computed a summary statistic for each PFT that uses the area under the curve expressed as an average score to retain the original units (i.e., % predicted PFT parameter). There was no significant dose level effect for any of the four tests of pulmonary function. Further confirmation of these findings was undertaken via repeated measures mixed model ANOVA (Table 6). Results were confirmed through GEE modeling. An autoregressive correlational structure was assumed for the GEE models because of

<p>| Table 4 Pulmonary function tests during aerosol GM-CSF |</p>
<table>
<thead>
<tr>
<th>Dose level</th>
<th>n</th>
<th>FVC (SD)</th>
<th>FEV1</th>
<th>PEF</th>
<th>FEF25-75</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient A</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. 60 µg BID × 7 days</td>
<td>11</td>
<td>99.5 (3.0)</td>
<td>82.5 (4.0)</td>
<td>89.4 (7.4)</td>
<td>55.4 (4.3)</td>
</tr>
<tr>
<td>2. 120 µg BID × 7 days</td>
<td>13</td>
<td>102.0 (4.8)</td>
<td>86.4 (4.9)</td>
<td>93.2 (6.9)</td>
<td>59.6 (6.7)</td>
</tr>
<tr>
<td>3. 240 µg BID × 7 days</td>
<td>12</td>
<td>105.0 (3.5)</td>
<td>88.0 (3.8)</td>
<td>88.7 (4.1)</td>
<td>60.3 (6.7)</td>
</tr>
<tr>
<td>Patient B</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. 60 µg BID × 7 days</td>
<td>10</td>
<td>119.4 (6.9)</td>
<td>75.2 (3.8)</td>
<td>82.2 (6.2)</td>
<td>21.9 (2.6)</td>
</tr>
<tr>
<td>2. 120 µg BID × 7 days</td>
<td>12</td>
<td>104.9 (7.8)</td>
<td>72.3 (5.7)</td>
<td>82.9 (7.0)</td>
<td>29.1 (5.7)</td>
</tr>
<tr>
<td>3. 240 µg BID × 7 days</td>
<td>11</td>
<td>110.8 (9.6)</td>
<td>71.2 (8.6)</td>
<td>80.4 (12.3)</td>
<td>24.8 (4.5)</td>
</tr>
<tr>
<td>Patient C</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. 60 µg BID × 7 days</td>
<td>13</td>
<td>46.1 (1.3)</td>
<td>48.2 (1.8)</td>
<td>63.6 (16.6)</td>
<td>78.3 (9.2)</td>
</tr>
<tr>
<td>2. 120 µg BID × 7 days</td>
<td>14</td>
<td>44.5 (1.6)</td>
<td>47.3 (1.9)</td>
<td>82.0 (13.9)</td>
<td>78.8 (10.6)</td>
</tr>
<tr>
<td>3. 240 µg BID × 7 days</td>
<td>15</td>
<td>43.2 (1.6)</td>
<td>46.0 (1.6)</td>
<td>90.4 (2.4)</td>
<td>82.6 (8.1)</td>
</tr>
<tr>
<td>Patient D</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. 60 µg BID × 7 days</td>
<td>15</td>
<td>113.9 (4.7)</td>
<td>98.2 (9.2)</td>
<td>83.2 (15.0)</td>
<td>80.5 (13.8)</td>
</tr>
<tr>
<td>2. 120 µg BID × 7 days</td>
<td>14</td>
<td>116.3 (2.5)</td>
<td>88.8 (6.1)</td>
<td>61.2 (7.0)</td>
<td>54.4 (8.4)</td>
</tr>
<tr>
<td>3. 240 µg BID × 7 days</td>
<td>14</td>
<td>119.4 (2.4)</td>
<td>86.9 (6.3)</td>
<td>61.3 (4.6)</td>
<td>50.6 (9.0)</td>
</tr>
<tr>
<td>Patient E</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. 60 µg BID × 7 days</td>
<td>3</td>
<td>96.2 (1.1)</td>
<td>91.2 (2.4)</td>
<td>97.8 (14.7)</td>
<td>74.6 (7.7)</td>
</tr>
<tr>
<td>2. 120 µg BID × 7 days</td>
<td>12</td>
<td>90.6 (4.1)</td>
<td>83.9 (3.5)</td>
<td>102.9 (9.7)</td>
<td>62.9 (8.5)</td>
</tr>
<tr>
<td>3A. 240 µg BID × 7 days</td>
<td>14</td>
<td>89.5 (4.1)</td>
<td>84.2 (3.2)</td>
<td>107.7 (4.1)</td>
<td>65.4 (6.3)</td>
</tr>
<tr>
<td>3B. 240 µg BID × 7 days</td>
<td>15</td>
<td>91.3 (4.6)</td>
<td>85.2 (3.5)</td>
<td>103.3 (3.1)</td>
<td>64.9 (4.9)</td>
</tr>
<tr>
<td>Patient F</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. 60 µg BID × 7 days</td>
<td>14</td>
<td>111.8 (2.4)</td>
<td>102.6 (2.5)</td>
<td>100.5 (16.1)</td>
<td>69.7 (6.4)</td>
</tr>
<tr>
<td>2. 120 µg BID × 7 days</td>
<td>11</td>
<td>102.2 (8.1)</td>
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<td>54.9 (7.6)</td>
</tr>
</tbody>
</table>

Fig. 2 Effect of dose level on FVC, FEV1, peak flow, and FEF 25-75. Columns, median PFT values (n = 6); bars, SE.
strong correlation of repeated PFT values, as described previously. Again, no dose level or time effect was seen.

In summary, during treatment with aerosol GM-CSF, there would seem to be neither a dose nor a time effect on four repeatable, sensitive, and reliable measures of pulmonary function obtained via remote spirometry. Despite the small sample size, the magnitude of the effect sizes for all of the descriptive and test procedures was impressively small; lung function remained at baseline.

**Toxicity.** Patients were interviewed and examined at the completion of each week of aerosol GM-CSF treatment at each dose level. One patient developed pleural effusions during administration of dose level 1 and was removed from the study. Whether this was related to GM-CSF toxicity or progressive disease is unknown. In the six patients who completed all three dose levels, no episodes of cough, dyspnea, tachypnea, chest pain, fatigue, fevers, myalgia, or bone pain were reported. Although some subjects were able to describe a vague feeling that there was a difference in their breathing pattern, these subjects reported no limitation in activity. Complete resolution of this subjective feeling occurred within a day or two after completion of 1 week of GM-CSF aerosol treatment.

**Tumor Response.** Because this was a Phase 1 dose escalation study that, by design, used a small number of high-risk patients to examine toxicity and feasibility, objective demonstration of clinical or immunological responses to cancer in the lungs were not aims of the study. Nevertheless, some patients with measurable disease were treated, and limited data were available. Table 1 summarizes the clinical situations of the six patients who completed the study. All of these patients were at high risk for recurrence and/or progression of lung metastases and would normally be expected to have new lung metastases or a measurable increase in size of metastases within 2–6 months. Apparent short-term (>9–12 month) stability in five of six patients was seen (Table 7). One patient with bilateral lung metastases of melanoma had a partial response after 9 months of 1 week on/1 week off aerosol GM-CSF. A 13-year-old with Ewing’s sarcoma had removal of pulmonary nodules after 6 months of aerosol GM-CSF. No viable tumor was found in the nodules. Although numbers are small and follow-up was very short, results are suggestive that local antitumor effects in the lung may be seen using aerosol delivery of GM-CSF.

**DISCUSSION**

The absence of toxicity of aerosolized yeast-derived recombinant human GM-CSF is one of the more important observations of this study. Toxicity of GM-CSF is not only dose and schedule related but also a function of the particular type of recombinant product. Yeast-derived glycosylated GM-CSF (sargramostim; Leukine) is much less toxic than the nonglycosylated E. coli-derived protein (29–32). Although yeast-derived recombinant human GM-CSF is generally well tolerated, more intensive use can be associated with toxicity. An intensive BID schedule in limited-stage small cell lung cancer was associated with increased toxicity (33). In that study, dose intensity could not account for increased thrombocytopenia, antibiotic use, hospitalization, and toxic deaths. i.v. high-dose GM-CSF infusions >1 week have been associated with side effects. In human volunteers, doses of >100 µg/kg (4000 µg of GM-CSF/m²/day or 16 times the recommended dose of 250 µg/m²) were given by continuous i.v. infusion for 7–18 days (34). Increases of WBC up to 200,000/mm³ were observed. Adverse events associated with these very high doses of GM-CSF included dyspnea, malaise, fever, rash, nausea, sinus tachycardia, headache, and chills. All events were reversible after discontinuation of the cytokine.

Chronic GM-CSF overproduction in genetically engineered mice was associated with accumulations of macrophages, blindness, and a fatal syndrome of muscle wasting (35). Lung damage was not a feature of pathological abnormalities in GM-CSF transgenic mice. However, unregulated chronic GM-CSF production in rat lung after intrapulmonary gene transfer was associated with eosinophilia, monocytosis, and fibrotic reactions in the lung (36–38). Because the intention of our study was to test the feasibility of an aerosol GM-CSF regimen that could possibly be used for months without tissue damage and minimal toxicity, if any, an intermittent 1 week on/1 week off schedule was chosen.

GM-CSF may play a more important role in homeostasis of the lung than hematopoiesis. Studies with GM-CSF knockout mice indicated that GM-CSF was absolutely required for normal lung homeostasis but not for hematopoiesis (39, 40). GM-CSF-deficient mice had normal blood counts and marrow function but had accumulations of lipid and protein material within the lungs in a pattern similar to alveolar proteinosis, a disease
associated with an attenuated hematopoietic response to GM-CSF (41). GM-CSF receptor-deficient mice also had alveolar proteinosis, which was incompletely corrected after bone marrow transplant (42, 43). Although it is probable that that endogenous GM-CSF is necessary for normal lung homeostasis, it has not been investigated whether aerosol GM-CSF can improve alveolar function.

Although not an obligatory requirement for hematopoiesis, GM-CSF has impressive effects on bone marrow hematopoiesis and mobilization of hematopoietic cells into the blood. Like G-CSF, GM-CSF promotes increased numbers of PBPCs capable of supporting long-term hematopoietic engraftment after myeloablative chemotherapy or chemo-radiotherapy preparative regimens. Mobilization of PBPCs is dose dependent and schedule dependent (44). Peak numbers of PBPCs are observed on days 4, 5, and 6 after initiation of daily G-CSF (45); by day 7, numbers of PBPCs are decreased. This observation also prompted us to use a “week-on, week-off schedule” to maximize intrapulmonary mobilization, if any, associated with aerosol GM-CSF. A recent randomized study of G-CSF versus GM-CSF versus G-CSF + GM-CSF showed that GM-CSF increases yields of dendritic cells in the PBPC collection (46).

Aerosol delivery provides a means to achieve local effects in the lung with minimal systemic drug exposure (47). Commercially available nebulizers are efficient and deposit 3–9% of output into mouth, 5–10% into conductive nonrespiratory bronchi (generations 0–16), and 12–20% into the bronchial generations 17–23, the transitory zones of airways leading to terminal alveolated lung (48, 49). Thus, an anticipated deposition of ~15–20% of the aerosolized protein is delivered specifically to the lung with each dose. At dose level 3, this would be ~100 μg of GM-CSF/day.

Proteins delivered to the lung by aerosol are absorbed by bronchial and pulmonary lymphatics, drain to pulmonary and mediastinal lymph nodes, and finally enter the circulation via the thoracic duct. However, if immune cells with high-affinity receptors are present in lymphatics or lymph nodes in the chest, absorbed cytokine will be stopped within the chest by this “immunological gauntlet” and may activate cells within the thorax but not the circulation. In other words, aerosol delivery of an immune-modulating protein to the lung may provide a significant dose to immune cells within the lung, but little systemically, if the dose is bound to receptor-bearing cells. This may account for our observations of not only lack of hematopoietic effect of aerosol GM-CSF on peripheral blood WBCs but also possible antitumor activity within the lung.

GM-CSF has been demonstrated to be a potent means to augment antitumor immunity in mice (21). In murine melanoma, fibrosarcoma, and colon cancer models, irradiated tumor cells expressing GM-CSF stimulated potent, specific, and long-lasting antitumor immunity (21). GM-CSF was superior to IL-2, IL-4, IL-5, IL-6, IFN-γ, intercellular adhesion molecule, and TNF-α in stimulation of immunity by genetically engineered cells (21). GM-CSF-transduced tumor cells were also effective in mediating cytotoxicity independent of NO, O₂⁻, H₂O₂, TNF-α, and matrix metalloproteinase in a manner that activated macrophages killed tumor cells expressing GM-CSF as well as tumor cells not expressing GM-CSF (22). Adipocytokine transferred cells exposed to GM-CSF-producing melanoma were also effective in mice (50). In an acute myelogenous leukemia murine model, GM-CSF-secreting cells were more potent than IL-4, TNF-α, or B7 in their ability to kill tumor cells; the effect was not T cell dependent (51, 52).

A number of possible mechanisms of action may account for lack of toxicity and antitumor effects of GM-CSF observed in our study. Aerosol delivery probably results in low systemic delivery (46) while achieving very high, biologically active concentrations of GM-CSF in the lung. Canine studies using free IL-2 and IL-2 liposomes indicate that the aerosol route was nontoxic and biologically effective (53). Dogs with osteosarcoma lung metastases have achieved complete remissions after 1 month of nebulization of IL-2 liposomes (54). Biodistribution of aerosol IL-2 preparations to lung tissue was radically different compared with i.v. or s.c. administration. High pulmonary uptake and prolonged retention of the cytokine were observed using radioscintigraphy of labeled IL-2 in dogs (55). Because GM-CSF is also an immunologically active protein, it may have a similar fate within the lung.

Additionally, GM-CSF has multiple effects on immune function, which may contribute toward immune recognition and/or tumor destruction in hosts previously unable to immunologically contain pulmonary metastases. These include promotion of increased numbers and cytotoxicity of activated macrophages (23), CD4 T cells (56), improved accessory cell function (57, 58), increased natural killer activity (59), and facilitation of immune responsiveness via dendritic cells (60). It is quite possible that effects may be observed only in individuals with minimal tumor burdens or immunogenic tumors, because all patients with numerous metastases or large metastases progressed.

To our knowledge, the only prior aerosol GM-CSF study used intubated, anesthetized, nonhuman primates (61). Monkeys were given 1 mg of aerosolized GM-CSF via a cuffed endotracheal tube to deliver an estimated ~80 μg human GM-CSF to the lung on one or two occasions without untoward effects. Aerosol GM-CSF produced a transient rise in circulating myeloid cells 3–5 days after primate inhalation. The lack of such an effect in our trial is possibly because the primate GM-CSF binding of human GM-CSF in the lung may be less efficient. Also, monkeys are very small compared with human subjects, and nebulization is at least twice as efficient via a cuffed tube (49), thus resulting in 10–20 times the dose given in our study. Although the benefits of local routes, including aerosol, for lung delivery have been advocated as a means to improve the therapeutic index of cytokines (62, 63), few aerosol studies of cytokines have been done.

In summary, our study is the first human aerosol GM-CSF study. Unexpectedly good results were obtained in cancer patients showing that: (a) chronic administration of aerosol GM-CSF appears to be feasible and without symptoms in most patients; (b) no pulmonary toxicity could be documented using sensitive tests of pulmonary function; and (c) clinical courses of some patients were better than expected, including >6 months of stable disease in a patient with melanoma lung metastases and an adolescent with Ewing’s sarcoma having an apparent complete response. Thus, aerosol GM-CSF has potential as a well-tolerated biological therapy.

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