Effect of Granulocyte-Monocyte Colony-Stimulating Factor Therapy on Leukocyte Function and Clearance of Serious Infection in Nonneutropenic Patients*

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**Study objective:** Impaired leukocyte function in patients with serious infections may increase mortality. Granulocyte-monocyte colony-stimulating factor (GM-CSF) broadly activates peripheral monocytes and neutrophils. We performed a clinical trial of GM-CSF in septic, hemodynamically stable patients to see whether GM-CSF treatment improved leukocyte function and mortality.

**Design:** Randomized, unblinded, placebo-controlled, prospective study.

**Setting:** A 600-bed academic tertiary care center with a 120-bed ICU census with a high proportion of immunocompromised, solid-organ transplant recipients.

**Patients:** Forty adult patients with infections meeting the criteria for the systemic inflammatory response syndrome but without hemodynamic instability or shock.

**Interventions:** Patients with sepsis and a documented infection were randomized to a 72-h infusion of GM-CSF (125 µg/m²) or placebo.

**Measurements and main results:** GM-CSF infusion caused the up-regulation of the β₂-integrin adhesion molecule CD11b and the appearance of the activated (“sticky” or “avid”) form of the molecule on circulating neutrophils and monocytes. CD11b density and avidity increases in response to the administration of tumor necrosis factor-α were blunted prior to treatment in these patients with serious infection. GM-CSF partially repaired this blunted response on both monocytes and neutrophils. It also caused the down-regulation of the adhesion molecule L-selectin on neutrophils and the up-regulation of human leukocyte antigen on monocytes. These changes were consistent with a broad activation of the circulating leukocyte pool. Although mortality and organ failure scores were similar in both groups, infection resolved significantly more often in patients receiving GM-CSF.

**Conclusions:** GM-CSF infusion up-regulated the functional markers of inflammation on circulating neutrophils and monocytes and was associated with both the clinical and microbiological resolution of infection. There was no detectable exacerbation of sepsis-related organ failure or other deleterious side effects with the administration of this proinflammatory agent to patients with serious infections.

Key words: cell adhesion molecules; colony-stimulating factors; human study; granulocyte macrophage colony-stimulating factor; immune modulation; immune responsiveness; integrins; randomized controlled trial; sepsis; surface molecules; tumor necrosis factor-α

Abbreviations: G-CSF = granulocyte colony-stimulating factor; GM-CSF = granulocyte-monocytes colony-stimulating factor; HLA = human leukocyte antigen; RFI = relative fluorescence intensity; TNF = tumor necrosis factor; UPMC = University of Pittsburgh Medical Center

Severe infections in the critically ill patient population remain a major cause of morbidity and mortality. The vital role of neutrophils in combating infection has long been appreciated. Mortality is markedly increased in the neutropenic patient and is principally related to opportunistic infections. Even patients with normal bone marrow function and neutrophilia can be shown to have multiple deficiencies in leukocyte function with severe infections. Experience with granulocyte colony-stimulating factor (G-CSF) and its effectiveness in neutropenic patients with infection brought the hope that the stimulation of leukocytes by either G-CSF or granulocyte-monocyte colony-stimulating factor (GM-
CSF) may aid critically ill patients to clear infection and improve survival, even in patients with nonneutropenic sepsis.\textsuperscript{4,5} The direct administration of colony-stimulating factors to humans with nonneutropenic infection has been limited mostly to G-CSF due the perception of it as a safer agent with a narrower myeloid target and the absence of tumor necrosis factor (TNF)-\(\alpha\) up-regulation.\textsuperscript{9–8}

Compared to G-CSF, GM-CSF has a broader spectrum of leukocyte stimulation that includes not only neutrophils, but also monocytes, macrophages, and eosinophils. GM-CSF induces proinflammatory effects such as the increased production of TNF-\(\alpha\) and interleukin-1\(\beta\) in monocyctic cell lines.\textsuperscript{9} There has been very limited reporting on prior investigations of the utility of GM-CSF as a therapeutic adjunct in nonneutropenic adult human infection. Presnell and colleagues\textsuperscript{10} presented preliminary data from a 5-day continuous GM-CSF infusion (3 \(\mu\)g/kg/d) in patients with sepsis-induced respiratory failure. They observed some modest improvements in gas exchange and ARDS resolution compared to a placebo group.

However, like many other immune system-enhancing agents, GM-CSF may also be detrimental if its nonspecific activation of the proinflammatory cascade were to make the generalized inflammatory response worse. This contention is supported by an increased incidence of side effects of GM-CSF, as opposed to G-CSF, in cancer patients being treated with each agent,\textsuperscript{11} although it appears that the glycosylated yeast-derived form of GM-CSF (sargramostim; Immunex; Seattle, WA [which was used in this study]) is better tolerated than the bacteria-derived formulation (molgramostim) and that only higher GM-CSF doses (ie, > 15 \(\mu\)g/kg/d) are associated with more serious side effects.\textsuperscript{12,13} The use of G-CSF in some animal models of severe sepsis from one group was either not protective\textsuperscript{14} or was actually deleterious with some types of infecting organisms.\textsuperscript{15}

These findings point out the need for caution in the use of these agents in patients with severe sepsis. Based on these arguments, we report the effects of a continuous infusion of GM-CSF or placebo on cellular markers of immune response in septic patients.

**Methods and Materials**

**Study Site and Patient Population**

The study was conducted at the University of Pittsburgh Medical Center (UPMC) over a 36-month period (September 1996 to September 1999). UPMC is a 600-bed, academic, tertiary care center with a 120-bed ICU census with a high proportion of immunocompromised, solid-organ transplant recipients. The protocol was approved by the Institutional Review Board of Biomedical Experimentation and as an Investigational New Drug application by the Food and Drug Administration. All patients or their surrogates signed informed consent forms prior to participation in the study. The GM-CSF (sargramostim; Immunex) was supplied in a form of the drug that is a glycosylated recombinant human GM-CSF, which is produced in a yeast-derived (Saccharomyces cerevisiae) system. Forty patients (GM-CSF infusion, 20 patients; placebo, 20 patients) were studied. About one third of the study population was composed of solid-organ transplant recipients. These patients received standard immunosuppressive therapy as determined by the surgical teams. None of the patients were undergoing organ rejection at the time of the study.

Potentially eligible ICU patients were screened for the following inclusion criteria: age > 18 years; negative urine pregnancy test finding; admission to an ICU; at least three systemic inflammatory response syndrome criteria\textsuperscript{16} (temperature > 38°C or < 36°C, heart rate > 90 beats/min, respiratory rate > 20 breaths/min or receiving mechanical ventilatory support, and leukocyte count of > 12,000 or < 4,000 cells/\(\mu\)L, or > 10% band forms); and a defined focus of infection with intent-to-treat with antimicrobial agents.

Exclusion criteria consisted of suspected or confirmed pregnancy (drug is category C), prior bone marrow transplantation (to avoid those with other indications for GM-CSF therapy), prior solid-organ transplant within the last month (transplant surgery request to avoid possible increase in graft rejection), lymphoproliferative or myeloproliferative disease, neutropenia (absolute neutrophil count, < 1,000 cells/\(\mu\)L, leukocytosis (leukocyte count > 30,000 cells/\(\mu\)L), aplastic anemia or pancytopenia, uncontrolled hemorrhage, receiving G-CSF, moribund condition, prior toxic reaction to GM-CSF, catheter-related infection, recent myelosuppressive chemotherapy, septic shock (mean arterial pressure of < 60 mm Hg or pressor dependence), or active autoimmune disease.

**Randomization, Study Drug Administration, and Follow-up**

Patients were block-randomized (block size, 10 patients) to either the GM-CSF arm or the placebo arm in a 1:1 manner based on a randomization sequence maintained within the UPMC investigational drug pharmacy. After randomization, the study investigators and the patient and/or patient’s proxy were unblinded to the study arm assignment. Patients who were randomized to receive GM-CSF received a continuous peripheral or central infusion for 72 h at a dose of 125 \(\mu\)g/m\(^2\). This dose amounted to approximately 3 \(\mu\)g/kg/d. Patients to whom placebo was given received a continuous infusion of 5% dextrose in water.
Criteria for Discontinuation of Study Drug

Patients enrolled in the study were discontinued from the study during the 72-h infusion period as result of the following events: leukocyte count of >50,000 cells/μL; perceived serious adverse event from GM-CSF; or withdrawal of life support measures.

Clinical and Routine Laboratory Assessment

Patients were followed up to the termination of antimicrobial therapy for the assessment of their clinical response to the infecting infection, and to the 28-day follow-up and hospital discharge for the assessment of survival. Baseline demographic, organ failure, and microbiological data (ie, pathogens and sites of infection) were recorded for all patients. Routine laboratory measures included total leukocyte count and differential count, platelet count, serum creatinine level, liver-associated enzyme levels, bilirubin level, and prothrombin time. Follow-up clinical, microbiological, and routine radiologic studies (if indicated) were obtained at the end of antimicrobial treatment for the determination of a response. Assessments at the end of antimicrobial treatment were defined as cure (ie, resolution of all signs and symptoms of infection), improvement (ie, partial resolution of signs and symptoms of infection), persistence (ie, minimal or no change of signs and symptoms of infection), failure (ie, worsening signs and symptoms or patient death due to infection), and indeterminate (ie, insufficient clinical or microbiological data).

Analysis of Cell Surface Receptors

Fresh blood was collected for cell surface molecule analysis at time zero (ie, immediately prior to the start of the study infusion) and at 24 h after the initiation of the infusion. We chose the 24-h time point instead of a later one because of the well-known tendency for leukocyte activators to have progressively less effect over time. Blood samples were collected through plastic 16-gauge or 18-gauge central venous lines. Care was taken to handle all samples quickly and identically. Samples were injected into heparinized tubes, inverted once to mix, placed into a 37°C thermost, and quickly processed (ie, in <5 min). Blood from eight healthy volunteers was collected to serve as a normal control.

The activation of the β2-integrin adhesion molecule CD11b was tested with an ex vivo leukocyte stimulation assay that we have described previously.17 Briefly, freshly obtained whole blood was stimulated by TNF-α, a cytokine that is known to be important in leukocyte activation in inflammation and infection. We measured the coordinated changes of leukocytes under exogenous stimuli, aliquots of fresh blood, each containing 2.5 × 10^7 leukocytes, were placed into tubes containing 0, 1, 4, 10, 25, or 200 U/mL (equivalent to picograms per milliliter) TNF-α in a 37°C water bath. The activity of the recombinant TNF-α was 10^6 U/mg. After 15 min of incubation, the blood was removed and diluted into a larger volume of chilled (4°C) phosphate-buffered saline-azide-fetal calf serum for staining with monoclonal antibodies to L-selectin, and with both total CD11b and its avid form. All subsequent cell handling was performed at 4°C. All tubes, reagents, and labware were confirmed to be free of endotoxin (ie, <1 U/mL) by the Limulus test.

To test for interference with our ex vivo TNF-α assay by soluble TNF-α receptors, we looked for a correlation between soluble TNF-α receptor levels and the response of CD11b to exogenous TNF-α. We found only a very weak negative correlation of soluble TNF-α receptor type I level to CD11b response (r = -0.291; p = 0.025 [2-tailed Spearman correlation]). We concluded that soluble TNF-α receptors in the plasma would only very weakly interfere with the ex vivo TNF-α stimulation assay.

Flow Cytometry

Flow cytometry was performed (FACScan; Becton-Dickinson; Franklin Lakes, NJ) at a laser power of 14.8 to 15.0 mW and 5.2 to 5.3 mA. The following filters were used: FL1, dichroic 530 ± 30 nm; FL2, dichroic 585 ± 42 nm; and FL3, long-pass 650 nm. The following fluorochromes were used for staining: CD11b (phycoerythrin, direct conjugate); CBRM1/5 (phycoerythrin, indirect conjugate); and L-selectin (fluorescein isothiocyanate, direct conjugate). All samples were processed at the Immunologic Monitoring and Diagnostics Laboratory at the University of Pittsburgh Cancer center. The Immunologic Monitoring and Diagnostics Laboratory performs clinical and research flow cytometry at high volumes. Stringent control tests were run on each instrument every day including two-color compensation by CD4 and CD8 staining. The protocol that was used to prepare the samples was standardized throughout the course of this trial. The output of every sample (cell scatter plots) was inspected for appropriate staining and was found to be acceptable before the data were included in the study.

Aliquots of blood were prepared for standard flow cytometry using commercially available monoclonal antibodies to CD11b, L-selectin, GM-CSF, and TNF-α receptors. Forward and side scatter identified the neutrophil population. CD14 labeling showed good separation from monocytes. The monoclonal antibody to active CD11b (CBRM1/5) was a gift from T. Springer (Boston, MA). A different method was used to study the binding of CBRM1/5. Conventional flow cytometry is not sensitive enough to detect cell surface markers of low density. Even fully activated neutrophils display only 10 to 50% of total CD11b with the CBRM1/5 epitope. Thus, we used high-sensitivity flow cytometry to assess CBRM1/5 (+) CD11b on neutrophils as previously described by us.17 For high-sensitivity flow cytometry, cells were stained with three successive layers including the CBRM1/5 antibody, biotinylated anti-Ig, and then a streptavidin-bearing fluorochrome. The monoclonal antibody CBRM1/5 does not cross-react with inactive CD11b. It has been consistently shown to react only with activated CD11b on neutrophils and eosinophils. All flow cytometry samples, both conventional and high-sensitivity, included matched isotype controls to control for nonspecific staining. We tested our 15-min incubation conditions against the immediate chilling of blood to 4°C and determined that they did not cause any detectable up-regulation of active CD11b on neutrophils from healthy donors. Activated CD11b only up-regulated when blood was treated with activators of neutrophils. All dose-response curve data (see the “Results” section) are based on ex vivo treatment with TNF-α. For the HLA-DR analysis, the identity of monocytes was confirmed by staining for CD14.

Data Analysis

Cell Surface Data: The flow cytometry data were log-normal in distribution. The surface density of leukocyte adhesion molecules...
was estimated by calculating the relative fluorescence intensity (RFI). The RFI was obtained by converting the mean channel number to linear fluorescence intensity. The linear fluorescence intensity of the isotype controls was subtracted from the linear fluorescence intensity of the population of cells stained with the monoclonal antibody of interest. We used nonparametric statistics to avoid having to assume a normal distribution of our data. To test for significant increases of cell surface markers with GM-CSF treatment within the same patient, the Wilcoxon signed-rank test (the nonparametric equivalent of the paired t test) was performed. To test for differences between groups, the Mann-Whitney U test (nonparametric equivalent of the unpaired t test) was used. To test for correlations between markers and outcome, the Spearman correlation coefficient was calculated. A p value of < 0.05, two-tailed, was considered to be significant.

Clinical Data: Statistical comparisons of medians for leukocyte counts and molecular results were performed with nonparametric tests and are specifically identified in the “Results” section for each comparison. The Fisher exact test or the χ² test with Yates correction was used for the comparison of sample proportions of categoric variables. A p value of < 0.05, two-tailed, was considered to be significant.

Results

A total of 40 patients were equally randomized to receive GM-CSF infusion (n = 20) or placebo infusion (n = 20) in an unblinded fashion during the study period. Thirty-three of 40 patients (GM-CSF, 18 patients; placebo, 15 patients) enrolled in this clinical trial had leukocyte cell surface molecules measured by flow cytometry. The lack of inclusion of all 40 randomized patients was due to the unavailability of technicians at certain times (eg, nights and weekends).

Effect of GM-CSF Therapy on Clinical Outcome Parameters

Description of the Study Groups at Baseline: The major clinical and microbiological characteristics of both groups are summarized in Table 1. There were no significant differences in the demographic pattern, the illness severity as evidenced by the number and type of organ failures, or the etiologic pathogen and primary anatomic sites of infection. The median baseline total leukocyte count was somewhat higher in the GM-CSF cohort (13,800 vs 10,600 cells/μL; p = 0.20), although this difference was not statistically significant.

On the day of enrollment into the study, all patients were receiving treatment with at least one effective antibiotic. We did not confirm that the patients had previously been receiving the correct empiric antibiotic prior to study enrollment.

Comparison of Clinical Outcomes Between GM-CSF and Placebo Groups: The major outcome parameters are summarized in Table 2. The analysis of daily leukocyte counts within the 3-day infusion period demonstrated both significantly higher median absolute peak leukocyte counts (p < 0.0001) and leukocyte increments (baseline to peak) in the GM-CSF group (p < 0.0001). The rates of infection cure/improvement (after the exclusion of indeterminate cases) were significantly greater in the GM-CSF cohort than in the placebo cohort (14 of 16 vs 5 of 14 patients; p = 0.01 [χ² test with Yates correction]). However, there was no demonstrable difference in mortality at the observed time intervals of 14 days, 28 days, and hospital discharge.

Comparison of Clinical Outcomes Between Solid-Organ Transplant Patients and Nontransplanted Patients: There were seven solid-organ recipients in the GM-CSF group (patients 5, 7, 11, 15, 17, 37, and 39) and eight solid-organ recipients in the (patients 3, 13, 14, 20, 21, 25, 26, and 29) in the placebo group. The rates of organ failure (renal failure, mechanical

Table 1—Major Characteristics of the GM-CSF and Placebo Groups at Baseline

<table>
<thead>
<tr>
<th>Parameter</th>
<th>GM-CSF Treatment Group (n = 18)</th>
<th>Placebo Treatment Group (n = 15)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median age, yr</td>
<td>56</td>
<td>52</td>
</tr>
<tr>
<td>Male gender</td>
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<td>9</td>
</tr>
<tr>
<td>Median LOS, d</td>
<td>8</td>
<td>10</td>
</tr>
<tr>
<td>Prior to organ transplant</td>
<td>7</td>
<td>8</td>
</tr>
<tr>
<td>Hospital admission SIRS criteria</td>
<td>3</td>
<td>7</td>
</tr>
<tr>
<td>ICU</td>
<td>4</td>
<td>11</td>
</tr>
<tr>
<td>Renal replacement therapy</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Mechanical ventilation</td>
<td>18</td>
<td>12</td>
</tr>
<tr>
<td>Liver failure</td>
<td>7</td>
<td>8</td>
</tr>
<tr>
<td>Platelets &lt; 50,000 cells/μL</td>
<td>5</td>
<td>7</td>
</tr>
<tr>
<td>Site of infection</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pneumonia</td>
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<td>9</td>
</tr>
<tr>
<td>Intraabdominal</td>
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<td>6</td>
</tr>
<tr>
<td>CNS</td>
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<td>0</td>
</tr>
<tr>
<td>Bloodstream</td>
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<td>4</td>
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<tr>
<td>Microbial etiologies</td>
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<tr>
<td>Gram-positive</td>
<td>9</td>
<td>10</td>
</tr>
<tr>
<td>Gram-negative</td>
<td>11</td>
<td>10</td>
</tr>
<tr>
<td>Yeast</td>
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<td>1</td>
</tr>
<tr>
<td>Polymicrobial</td>
<td>4</td>
<td>6</td>
</tr>
<tr>
<td>Median leukocyte count, ×10³ cells/μL</td>
<td>13.8</td>
<td>10.6</td>
</tr>
</tbody>
</table>

*There were no significant differences between the GM-CSF and placebo groups. LOS = length of stay; SIRS = systemic inflammatory response syndrome; liver failure = rise in serum bilirubin level to > 5 mg/dL or a prothrombin time of > 20 s.
Withdrawal of Patients From the Study: One placebo patient was removed from the study on day 3 (withdrawal of support), and one GM-CSF patient was removed from the study on day 2 (due to leukocytosis).

Effects of GM-CSF Therapy on Leukocyte Cell Surface Molecules

Effects of GM-CSF on the Up-regulation and Activation of the Adhesion Molecule CD11b, and the Shedding of L-Selectin on Circulating Neutrophils: The density of CD11b and its activated (avid) form, and L-selectin on neutrophils were measured before and after 24 h of continuous infusion of GM-CSF or placebo.

Total CD11b: The baseline (i.e., before GM-CSF or placebo treatment) density of total CD11b on all patients was not significantly different from that of healthy control subjects (Fig 1). However, the TNF-α-stimulated increase in CD11b density in the ex vivo assay was significantly suppressed in all patients prior to treatment compared to healthy control subjects (all p values were < 0.01 for doses of

### Table 2—Clinical and Microbiological Outcomes of GM-CSF and Placebo Cohorts

<table>
<thead>
<tr>
<th>Parameters</th>
<th>GM-CSF (n = 18)</th>
<th>Placebo (n = 15)</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leukocyte count, ×10^3 cells/μL</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median peak*</td>
<td>35.6</td>
<td>10.6</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Median increment (baseline to peak)</td>
<td>8.7</td>
<td>1.2</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Infection outcome</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cure/improvement</td>
<td>14</td>
<td>5</td>
<td>0.01†</td>
</tr>
<tr>
<td>Failure/persistence</td>
<td>2</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>Indeterminate</td>
<td>2</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Survival</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14-d</td>
<td>14</td>
<td>10</td>
<td>0.10</td>
</tr>
<tr>
<td>28-d</td>
<td>14</td>
<td>9</td>
<td>0.53</td>
</tr>
<tr>
<td>Hospital discharge</td>
<td>12</td>
<td>8</td>
<td>0.18</td>
</tr>
</tbody>
</table>

*The highest leukocyte count during the 3-day infusion period.
†Comparing cure/improvement to failure/persistence with the exclusion of the indeterminate outcomes.

Table 2—Clinical and Microbiological Outcomes of GM-CSF and Placebo Cohorts

- **Leukocyte count, ×10^3 cells/μL**
  - Median peak: GM-CSF 35.6 vs. Placebo 10.6 (p < 0.0001)
  - Median increment: GM-CSF 8.7 vs. Placebo 1.2 (p < 0.0001)

- **Infection outcome**
  - Cure: GM-CSF 14 vs. Placebo 5 (p = 0.01)
  - Failure: GM-CSF 2 vs. Placebo 9
  - Indeterminate: GM-CSF 2 vs. Placebo 1

- **Survival**
  - 14-d: GM-CSF 14 vs. Placebo 10 (p = 0.10)
  - 28-d: GM-CSF 14 vs. Placebo 9 (p = 0.53)
  - Hospital discharge: GM-CSF 12 vs. Placebo 8 (p = 0.18)

Figure 1. The displays of PMN CD11b in the placebo groups and pretreatment (Pre-rx) GM-CSF group (1) were all significantly suppressed compared to healthy control subjects (2) [p < 0.01] and the posttreatment (Post-rx) GM-CSF group (3) [p < 0.01]. The GM-CSF posttreatment patients (3) were not significantly different than healthy control subjects (2). The pretreatment samples were obtained just after study enrollment prior to any drug being administered. The posttreatment samples were obtained after 24 h of continuous GM-CSF infusion. Normals = healthy control subjects; PMN = polymorphonuclear neutrophil leukocytes.
TNF-α of ≥ 4 pg/mL). After 24 h, treatment with GM-CSF significantly elevated the resting level of CD11b on neutrophils (all, p < 0.01). Treatment with GM-CSF also reversed the suppression of TNF-α-induced CD11b up-regulation (p < 0.01 for all doses) to a level that was not distinguishable from that of healthy control subjects. GM-CSF administration therefore produced a partial activation of circulating neutrophils and improved a suppressed TNF-α-induced CD11b response to a normal response.

_Avid CD11b:_ The baseline density of avid CD11b on circulating neutrophils was significantly elevated in GM-CSF patients compared to that of healthy control subjects (p = 0.015) prior to any treatment; however, the appearance of new avid receptors in response to TNF-α was suppressed in all patients (p < 0.02), and treatment with GM-CSF did not improve this finding (Fig 2). Thus, although GM-CSF improved the numeric up-regulation of CD11b, it did not repair the decreased activation of the receptor in response to TNF-α that was seen on the neutrophils of patients.

_L-Selectin:_ Treatment with GM-CSF caused a significantly decreased level of L-selectin display on neutrophils (p < 0.01) compared to pretreatment values (data not shown).

**Effects of GM-CSF on Up-Regulation and Activation of the Adhesion Molecule CD11b, and Shedding of L-Selectin on Circulating Monocytes**

The density of CD11b and its activated (avid) form, and L-selectin on monocytes was measured before and after 24 h of continuous infusion with GM-CSF or placebo.

_Total CD11b:_ The baseline (ie, before treatment) density of total CD11b on all patients was not significantly different from that of healthy control subjects, as was the case for neutrophils (Fig 3). Likewise, the TNF-α-stimulated increase in CD11b density was lower in all of the patients (p < 0.05), although not as profoundly depressed as that with the neutrophil cell line. Treatment with GM-CSF, but not placebo, elevated baseline total CD11b levels on monocytes (p < 0.01). Treatment also im-

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**Figure 2.** The placebo group, and the pretreatment and posttreatment GM-CSF groups (2) were all significantly immunosuppressed (p < 0.02) in terms of the display of avid CD11b in response to TNF-α compared to healthy control subjects (1). The pretreatment samples were obtained just after study enrollment prior to any drug being administered. The posttreatment samples were obtained after 24 h of continuous GM-CSF infusion. See the legend of Figure 1 for terms not used in the text. See Figure 1 legend for abbreviation.
proved the increase of total CD11b in response to TNF-α, but this was more variable on monocytes and did not reach statistical significance for all doses of TNF-α (1 pg/mL, \( p = 0.022 \); 4 pg/mL, \( p = 0.043 \); 10 pg/mL, \( p = 0.064 \); 25 pg/mL, \( p = 0.053 \); 200 pg/mL, \( p = 0.048 \)). Thus, there was a tendency for GM-CSF to repair the numerical response of total CD11b on monocytes in response to TNF-α, but it was less clear than on neutrophils.

**Avid CD11b:** The baseline (before treatment) density of avid CD11b in all patients was not significantly different from that in healthy control subjects (Fig 4). However, again, the appearance of new avid receptors in response to TNF-α was suppressed in all patients. Treatment with GM-CSF, but not with placebo, elevated baseline levels of avid CD11b (\( p = 0.01 \)). Treatment also tended to return the increases in avidity toward normal, but this was variable and did not reach statistical significance.

**L-Selectin:** In contrast to the situation in neutrophils, treatment with GM-CSF did not cause a significant decrease in L-selectin levels (data not shown).

![Figure 3](http://www.chestjournal.org/pdfaccess.ashx?url=/data/journals/chest/22026/)

**Effect of GM-CSF on HLA-DR Display on Circulating Monocytes**

HLA-DR surface display was decreased significantly to below normal levels (\( p < 0.01 \)) on the monocytes in all patients prior to treatment. The display of HLA-DR increased in every patient after GM-CSF treatment, but not after placebo treatment (Fig 5). The levels of HLA-DR increased to a level that was not significantly different from those of healthy control subjects (\( p = 0.27 \)). Thus, GM-CSF treatment restored significantly depressed levels of monocyte HLA-DR expression toward normal levels.

**Effect of GM-CSF on Leukocyte TNF-α Receptors upon Treatment With TNF-α**

As has been reported previously, the addition of TNF-α to cells reduces the display of TNF-α receptors I and II within 15 min. We observed the loss of leukocyte cell surface TNF-α receptors in all samples treated with TNF-α ex vivo. Treatment with GM-CSF had no effect on maintaining TNF-α receptors on cells. There was no significant difference in the amount of loss of either TNF-α receptor I or
II in the GM-CSF or placebo patients (all p > 0.20) when exogenous TNF-α was added. This was only tested at a single high dose (200 U/mL) of exogenous TNF-α.

Effect of GM-CSF on Leukocyte GM-CSF Receptors

There was no significant difference in the display of GM-CSF receptors on neutrophils between day 1 and day 2 in the placebo group. There was a tendency toward a lower display of GM-CSF receptors on neutrophils in the GM-CSF group after 1 day of therapy. This trend did not reach significance (p = 0.053 [Wilcoxon test]). There was no significant difference in the display of monocyte GM-CSF receptors on day 1 and day 2 in the placebo or treatment groups (Wilcoxon test) in this study.

Relationship of Enhanced Leukocyte Adhesion Molecule Display Caused By GM-CSF Therapy and Clinical Outcome

There was no relationship between changes in CD11b or its activation on neutrophils or monocytes and the clearance of infection or mortality. The only correlation of a cell surface marker to outcome was with monocyte HLA-DR. There was a positive correlation (r = 0.41; p = 0.02) between the amount of increase in HLA-DR expression and the clearance of infection.

**Discussion**

We undertook this study to determine whether the stimulation of leukocytes with GM-CSF in patients in the ICU with serious infections would, first, be safe and, second, aid in the clearance of infection. Due to the theoretical risk of exacerbating sepsis with a proinflammatory agent, we eliminated patients with severe sepsis or septic shock, and we performed a randomized but unblinded trial. To our knowledge, this is the largest study of the utility of GM-CSF in human adult nonneutropenic infection and the most detailed examination of its influence on circulating leukocyte surface markers. Although our unblinded study design is a major limitation when interpreting clinical outcomes, it should not have had an effect on baseline and 24-h molecular events.
The continuous infusion of GM-CSF for 24 h caused both leukocytosis and alterations in adhesion molecule display that were consistent with inflammatory activation. We studied the \( \beta_2 \)-integrin CD11b, which is a crucial adhesion molecule that is important in the transendothelial migration of neutrophils and monocytes. This molecule both increases its density on the cell surface and increases its avidity for counterreceptors in response to many inflammatory stimuli. As we have previously shown, the recruitment of CD11b on neutrophils in response to TNF-\( \alpha \) administration is depressed in patients with serious infections. In this work, we extend that observation to circulating monocytes.

Leukocyte hyporesponsiveness (ie, diminished CD11b expression) to \textit{ex vivo} TNF-\( \alpha \) was present at baseline and at 24 h in both cell lines. Therapy with GM-CSF (but not with placebo) enhanced CD11b expression at both time points. However, the GM-CSF response pattern of CD11b up-regulation and avidity change to TNF-\( \alpha \) was different across the two cell types. In neutrophils, there was significant improvement of the increase in CD11b surface density but no improvement in avidity change. In monocytes, the up-regulation in CD11b density was present but less dramatic, and there was a tendency for greater improvement of the avidity change.

These results are consistent with those of prior reports that demonstrated up-regulatory effects of GM-CSF on human leukocyte responsiveness in sepsis and a variety of other pathologic states. It should be noted that circulating leukocytes represent the most easily measurable subset of leukocytes that is not necessarily representative of all leukocytes (ie, those that have migrated outside of the vasculature). Our observations that leukocyte activation occurred after GM-CSF therapy is limited to only the 24-h interval examined. It is not known whether these signs persisted for the entire treatment period since no leukocyte flow cytometry was performed beyond the 24-h time point. In an examination of other leukocyte cell surface molecules, we showed, as have others, a significantly decreased HLA-DR expression in patients with serious infections. In our analysis, GM-CSF treatment was associated with a clear increase in monocyte HLA-DR expression. This increase was the only parameter derived from cell surface molecule measurements that correlated with an improved clearance of infection. GM-CSF did not prevent the loss of TNF-\( \alpha \) receptors on exposure to TNF-\( \alpha \). There was a tendency toward GM-CSF receptor loss on neutrophils, but not on monocytes, in patients who were treated with GM-CSF.

These data are not surprising, however, because GM-CSF has been used for infection prophylaxis of high-risk patients with cancer who were undergoing surgery, increasing the number of circulating neutrophils without obvious deleterious side effects. GM-CSF also up-regulated the expression of complement receptors 1 and 3 (a complex of CD11b and CD18) in chronically neutropenic patients. In a study of burn patients, GM-CSF normalized neutrophil oxidative function \textit{in vitro} postinjury. In trauma, post-cardiopulmonary bypass, and septic patients, GM-CSF reversed the hyporesponsiveness of whole blood to infection. The resistance of neutrophils in BAL fluid to apoptosis in patients with ARDS correlated with GM-CSF concentration.
GM-CSF generates a more inflammatory monocyte,\textsuperscript{25} restores respiratory burst activity in monocytes from septic patients,\textsuperscript{21} reverses the apoptotic tendency of monocytes in response to endotoxin exposure, and increases the display of HLA-DR antigens on the cell surface.\textsuperscript{22} Thus, GM-CSF augments not only nonspecific immunity but, with the activation of monocytes and macrophages, specific immunity and tissue defense.

Some natural antiinflammatory mechanisms were operative in our study patients and were not altered by the administration of GM-CSF. For example, the shedding of leukocyte TNF-α receptors in response to exogenous TNF-α given in the \textit{ex vivo} assay was not affected by the GM-CSF therapy. Soluble TNF-α receptors bind and inactivate circulating TNF-α, thus attenuating its proinflammatory effects. There was a trend toward the loss of GM-CSF receptors on neutrophils after treatment with GM-CSF, as has been seen with many other receptors after exposure to their ligand. However, we could not rule out the steric hindrance of antibody by bound GM-CSF.

GM-CSF has been associated with an increased production of proinflammatory substances (presumably due to monocyte activation) in \textit{ex vivo} studies of blood,\textsuperscript{26} and an increased mortality with GM-CSF therapy in a rat cecal ligation/puncture model and a lipopolysaccharide-challenged murine model.\textsuperscript{27,28} In \textit{vivo} elevations of serum TNF-α levels in human recipients of GM-CSF have also been reported.\textsuperscript{29} This has led some to conclude that the risk of using GM-CSF in patients with serious infections is too high. Despite these concerns, deleterious clinical effects of the proinflammatory actions of GM-CSF were not detected. We saw no increase in the incidence of progression to circulatory shock or other de novo organ failures in the GM-CSF group. This lack of harm may be due to the fact that the patient population was selected to have serious infections but not to have septic shock. This logic is consistent with a study of GM-CSF therapy in neutropenic patients wherein low-risk patients benefited while high-risk patients did not derive benefit.\textsuperscript{21} Furthermore, the detrimental effects GM-CSF therapy may be dose-dependent, as reported from an early experience with higher doses of GM-CSF in oncology patients. Thus, we chose a GM-CSF dose of 125 µg/m² in our study to limit potential dose-related deleterious effects.

Moreover, it has been shown that many critically ill patients with serious infections do not manifest immune activation but rather a net state of acquired immunodepression or “immunoparalysis.” This effect has been demonstrated by initially low TNF-α/interleukin-10 ratios or diminished monocyte HLA class II antigen expression, both of which have been shown to predict less favorable outcomes in serious infection.\textsuperscript{30–33} Thus, the main problem is not unregulated systemic inflammation but rather the inability to clear the inciting infection. Thus, animal models that focus on the effects of colony-stimulating factors using overwhelming infection (\textit{i.e.}, cecal ligation and puncture) may not be relevant to the majority of septic patients.

In this small study, there was no significant reduction of mortality in the treatment group. Establishing a survival benefit in such a complex population would obviously require a much larger number of study subjects. However, there was a statistically significant 2.5-fold increase in the proportions of evaluable patients whose infections were either cured or improved in the GM-CSF group (14 of 16 patients [88%] vs 5 of 14 patients [36%], respectively; \(p = 0.01\)). Clearly, other contributory factors that favored the higher rates of pathogen clearance in the GM-CSF group could have been present. However, the augmentation of pathogen clearance rates after GM-CSF treatment are concordant with GM-CSF-treated animal models of infection with \textit{Staphylococcus aureus}, \textit{Pseudomonas aeruginosa}, \textit{Salmonella typhimurium}, \textit{Leishmania tropica}, and \textit{Trypanosoma cruzi} that showed enhanced eradication or quantitative reduction of the number of organisms when compared to controls.\textsuperscript{34–38}

CONCLUSIONS

In conclusion, GM-CSF therapy resulted in significant changes of functional molecule expression on the cell surface of circulating leukocytes to a pattern that was consistent with increased inflammatory activation and restored responsiveness of the β-integrin adhesion molecules to TNF-α. The monocyte antigen-presentation molecule HLA-DR also increased from subnormal to normal cell surface density. Natural antiinflammatory mechanisms, such as the shedding of soluble receptors, did not appear to be down-regulated. In this population of infected patients who did not have shock, there was no evidence that the activation of circulating leukocytes by GM-CSF was deleterious. In fact, there was a significant increase in the number of patients whose conditions improved or infections cleared. Further studies to evaluate the clinical potential of GM-CSF in patients with sepsis but without shock appear to be warranted.

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