The Effect of Recombinant Human Granulocyte Macrophage Colony-Stimulating Factor on Neutrophil Activation in Patients with Refractory Carcinoma

By S.S. Kaplan, R.E. Basford, E.J. Wing, and R.K. Shadduck

Patients with refractory carcinoma were treated with recombinant human granulocyte-macrophage colony-stimulating factor (rhGM-CSF) by intravenous (IV) infusion. During the period of treatment, studies of polymorphonuclear leukocyte superoxide (O$_2^-$) release in response to formylmethionylleucylphenylalanine (fMLP) and phorbol myristate acetate (PMA) and studies of chemotaxis in response to fMLP and C5a were performed. We observed that patients receiving rhGM-CSF in vivo exhibited primed O$_2^-$ release after stimulation both with fMLP and PMA. Chemotaxis, however, was not enhanced by the treatment. These data suggest that host defenses may be enhanced by this treatment and that rhGM-CSF may be a useful therapeutic adjunct in compromised patients.

POLYMORPHONUCLEAR (PMN) leukocytes are essential for host defense against microbial infection, and serious depletion of their number or compromise of their function may lead to severe or fatal infections. A substance, therefore, that could stimulate the production of PMNs and also augment their functional capacity would be an important therapeutic modality in patients with neoplasia. It was of considerable interest, then, when the colony-stimulating factors, a family of glycoproteins that stimulate hematopoiesis, were found to enhance PMN phagocytosis, microbial killing, chemotaxis, and the production of activated oxygen species in vitro in response to surface membrane-mediated stimulation. This body of work suggested that the administration of these factors might enhance resistance to infection. We report, herein, the effect on PMN function of administering human recombinant granulocyte-macrophage colony-stimulating factor (rhGM-CSF) to a group of patients with refractory carcinomas. The results indicate that PMN oxidative metabolism is enhanced while chemotaxis appears unchanged.

MATERIALS AND METHODS

Patients. Ten ambulatory patients with refractory neoplastic disease participated in the study. No chemotherapy or radiation therapy was given for at least 6 weeks prior to study. They were given continuous intravenous (IV) infusions of rhGM-CSF (Immunex Corp, Seattle, WA; specific activity of 5 x 10$^6$ colony forming units/mg protein, purified, sterile, endotoxin and pyrogen free) at dosage levels of 100 g/m2/24 h or 500 g/m2/24 h. Each patient received 5% of the total dose by bolus injection on day 0. Six days later a continuous IV infusion was begun and continued for 14 days. Blood samples for evaluation of PMN function were obtained twice prior to the bolus injection to determine pretreatment activity. Samples were taken two days after the bolus, prior to starting the continuous infusion, and then on days 3 and 10 of the continuous infusion. Leukocyte counts and 200 cell differentials were done at intervals throughout the study.

Isolation of PMNs. PMNs were separated from whole blood with a modification of Boyum's technique, and were suspended in Krebs Ringer phosphate buffer pH 7.4 with 4.4 mmol/L glucose added (KRPG) for studies of O$_2^-$ production or in medium 199 with 1% bovine serum albumin (BSA) for studies of chemotaxis. The separated cells were at least 98% viable as determined by Trypan blue dye exclusion.

Function studies. Superoxide anion (O$_2^-$) was determined as described previously measuring superoxide dismutase (SOD) inhibitable reduction of ferricytochrome c from resting cells and after stimulation with 10$^{-7}$ or 10$^{-8}$ mol/L fMLP or 1 g/mL phorbol myristate acetate (PMA). The data were obtained as nmol O$_2^-$/10$^6$ PMNs and are expressed as a percent of the pretreatment O$_2^-$ release.

Chemotaxis was evaluated by using a modification of Falk's method with a 48-well microchemotaxis chamber and a 5-$\mu$m pore-size polycarbonate filter. Zymosan-activated plasma (ZAP) as a source of C5a and fMLP were the chemoattractants, and incubation was 15 minutes at 37°C, as described. Cells on the lower surface of the filter were counted, and the data are expressed as a percent of pretreatment chemotaxis.

Significance of results was determined using the paired t test.

RESULTS

A marked increase in the number of peripheral blood neutrophils was seen during the continuous infusion of rhGM-CSF. The pretreatment neutrophil count and the counts after the bolus and before the continuous infusion ranged from 5.9 x 10$^9$/L to 6.9 x 10$^9$/L. Infusion of 100 g/m2/m2 was associated with a neutrophil count of 13.9 ± 2.8 x 10$^9$/L and 14.7 ± 2.7 x 10$^9$/L on days 3 and 10 respectively. At the dosage of 500 g/m2 these three- and ten-day counts were 18.8 ± 4.5 x 10$^9$/L and 34.6 ± 9.3 x 10$^9$/L.

The effect of each of the dose levels of rhGM-CSF given by IV infusion is shown in Table 1. The results shown are the mean ± SEM of data from four patients in each group. In the absence of additional stimulation (resting PMNs), the four patients given 100 g/m2 had no consistent effect on O$_2^-$ release at any of the time points. PMNs stimulated with 10$^{-7}$ mol/L fMLP, however, exhibited significant enhancement of O$_2^-$ release both on day 3, 146% ± 15% of control (P < .05), and on day 10, 204% ± 14% of control (P < .01), of the continuous IV infusion but not after the bolus injection.
Table 1. Effect of Infused rhGM-CSF on Superoxide Release by Neutrophils*

<table>
<thead>
<tr>
<th>Conditions†</th>
<th>Bolus + Two Days</th>
<th>Continuous Day 3</th>
<th>Infusion Day 10</th>
</tr>
</thead>
<tbody>
<tr>
<td>No Stimulus</td>
<td>100 μg/m²</td>
<td>129 ± 9</td>
<td>175 ± 87</td>
</tr>
<tr>
<td></td>
<td>500 μg/m²</td>
<td>152 ± 20</td>
<td>126 ± 44</td>
</tr>
<tr>
<td>10⁻⁷ mol/L fMLP</td>
<td>100 μg/m²</td>
<td>146 ± 15†</td>
<td>204 ± 14§</td>
</tr>
<tr>
<td></td>
<td>500 μg/m²</td>
<td>143 ± 46</td>
<td>106 ± 9</td>
</tr>
<tr>
<td>10⁻⁸ mol/L fMLP</td>
<td>100 μg/m²</td>
<td>164 ± 31</td>
<td>339 ± 77†</td>
</tr>
<tr>
<td></td>
<td>500 μg/m²</td>
<td>180 ± 26§</td>
<td>133 ± 28</td>
</tr>
<tr>
<td>PMA</td>
<td>100 μg/m²</td>
<td>111 ± 11</td>
<td>172 ± 9§</td>
</tr>
<tr>
<td></td>
<td>500 μg/m²</td>
<td>143 ± 11§</td>
<td>151 ± 29</td>
</tr>
</tbody>
</table>

*Data shown as percent of baseline O₂⁻ release and are the mean ± SEM of four observations.
†The presence and type of O₂⁻ stimulant and the dose of GM-CSF are shown.
‡P = <.05.
§P = <.01.

PMNs stimulated with suboptimal (10⁻⁸ mol/L) fMLP showed significantly augmented reactivity only on day 10 of the infusion (339% ± 77% of control [P = <.05]). Although three of the four patients also showed a primed response on day 3 (145% to 239% of control), the mean did not differ statistically from the baseline observation. Using PMA to induce superoxide release, no increase was seen in any of the patients until day 10, when all four patients exhibited enhanced production of O₂⁻.

Infusion of the larger dose of rhGM-CSF was associated with less enhancement of O₂⁻ production under most conditions. Unstimulated PMNs and PMNs incubated with 10⁻⁷ mol/L fMLP showed no consistent alteration of O₂⁻ production. PMNs incubated with 10⁻⁸ mol/L fMLP showed significant enhancement of O₂⁻ production on day 3 of the infusion (180% ± 26% of control) and two of the four patients continued to show increased O₂⁻ release on day 10. Stimulation with PMA was also associated with primed O₂⁻ release on day 3 (143% ± 11% of control) with three of the four patients continuing to show priming on day 10.

The effect of infusing rhGM-CSF on chemotaxis is shown in Table 2. No enhancement was seen, and chemotaxis often was below the pretreatment level. The bolus injection at the higher dosage level was associated with significantly depressed chemotaxis when a suboptimal concentration of fMLP (10⁻⁸ mol/L) rather than suboptimal (10⁻⁷ mol/L) concentrations of fMLP. The priming effect of rhGM-CSF on PMN superoxide release has been well documented by in vitro studies. The finding that the in vivo use of this cytokine also results in priming of PMNs is indicative of an in vivo effect of this factor.

Several studies using colony-stimulating factors suggest a slight lessening of priming and colony-stimulating effectiveness at the very highest concentrations used, thus supporting our observation that the infusion of more of this cytokine does not result in greater degrees of priming. In addition, the conditions surrounding the in vivo use of rhGM-CSF, in contrast to in vitro studies, are necessarily more complex, since the cytokine may interact with cells that may themselves stimulate the release of other cytokines with regulatory activity. Mayer et al., however, studying the effect of IV and subcutaneous (SC) rhGM-CSF in primates, also demonstrated primed responses for O₂⁻ release and for whole-blood microbial killing. Baldwin et al also studied PMN host defense activity during and after infusion of rhGM-CSF. They failed to show enhanced fMLP induced O₂⁻ release during the 60-minute initial infusion except at one of the dosage levels, and they did not see a primed response 2 weeks following the 14 days of continuous infusion. These results are not surprising, since in vitro studies have shown that two hours of incubation are needed for priming of O₂⁻ release. The absence of a primed response at the later examination may be explained by the presence of a PMN population that developed after the period of exposure to the cytokine. Their results should not be directly compared to ours, since the doses of cytokine and the times of PMN study with respect to rhGM-CSF infusion are different.

Our studies also demonstrate a primed response for stimulation with PMA. This was seen after ten days of IV infusion of the lower dose and after three days of infusion of the higher concentration. These data differ from in vitro studies published by Weisbart et al, who found priming for fMLP, C5α and LTB₄, but not for PMA. They used much smaller stimulating concentrations of PMA, however, and the data may not be directly comparable. In addition, Wing et al.
studying macrophage colony stimulating factor (M-CSF) in a murine model, found enhanced release of reactive oxygen species of peritoneal macrophages stimulated with PMA and opsonized zymosan.

Our data did not show any stimulation of chemotactic activity at either dosage level of rhGM-CSF. Although early work with this cytokine indicated that it inhibited spontaneous PMN movement (thus the name neutrophil migration inhibition factor), subsequent study has shown that it also has an effect on chemotaxis and that the effect on cell movement is highly dependent upon the time of incubation. Short periods of exposure result in the increased expression of high-affinity fMLP receptors that mediate chemotaxis, while prolonged exposure to the cytokine results in expression of the low-affinity receptors that mediate the metabolic response produced by fMLP.8 Our data are consistent with this observation, since the process of infusion with later sampling carries a long incubation period. Although enhanced chemotaxis was not seen after infusion of rhGM-CSF, neither was the chemotaxis impaired. This suggests that the PMNs produced in response to rhGM-CSF are capable of at least normal reactivity.

Our results support and extend the results of in vitro studies on rhGM-CSF showing enhanced PMN reactivity to mediators of oxidative metabolism. These findings, together with the marked stimulation of myelopoiesis9 suggest that this cytokine may be a useful adjunct in the therapy of patients with cancer, both to increase the numbers of circulating PMNs and to increase the host defense capability of those PMNs.

REFERENCES

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