Recombinant Human Granulocyte/Macrophage Colony-Stimulating Factor Enhances Monocyte Cytotoxicity and Secretion of Tumor Necrosis Factor α and Interferon in Cancer Patients

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The colony-stimulating factors (CSFs) promote the proliferation and differentiation of hematopoietic precursors and more recently have been shown to amplify the functions of mature phagocytes in vitro. In this study recombinant human granulocyte/macrophage colony-stimulating factor (rGM-CSF) was administered to cancer patients to determine whether the cytotoxic and secretory activity of their blood monocytes could be enhanced. Patients with refractory neoplastic disease were treated with rGM-CSF either as a single bolus or as a constant infusion for 14 days at either 100 or 500 μg/m² per day. As has been reported by others, the number of peripheral blood monocytes and granulocytes rose markedly in a dose-response fashion during infusion with rGM-CSF. The functional capacity of monocytes was increased by rGM-CSF, since the cytotoxicity of monocytes against antibody-coated xenogenic cells was increased during the constant infusion compared to baseline. In addition, monocytes harvested during the constant infusion and stimulated with lipopolysaccharide (LPS) in vitro secreted increased quantities of tumor necrosis factor α (TNF-α) and interferon (IFN). These data indicate that rGM-CSF can enhance both the number and function of peripheral blood monocytes in vivo.

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Fig 1. The number of blood monocytes and neutrophils (band and segmented cells) expressed as the mean of all patients treated with continuous rGM-CSF. Patients were infused with either 100 or 500 µg/m²/24 h GM-CSF IV for 14 days beginning on day 6.

Neither activated nor nonactivated monocytes lysed CRBC without antibody. Antibody alone did not lyse CRBC.

TNFα. Monocytes were incubated in 24-well tissue culture plates at a concentration of 2 x 10⁶ cells with Escherichia coli LPS (10 µg/mL; Sigma, St. Louis) for 48 hours at 37°C. Supernatants were then collected and tested for TNFα activity by an enzyme-linked immunosorbent assay (ELISA) developed by one of the authors (Theresa L. Whiteside). To perform the assay, microtiter plates were coated with monoclonal antibodies (MoAbs) to different epitopes on human recombinant TNFα, obtained from Dr Achim Moeller, BASF, A.G. Ludwigshafen, West Germany. Test samples as well as standards of recombinant TNFα (generously provided by Knoll Pharmaceuticals, Whippany, NJ) were added to the plates in twofold dilutions and incubated for two hours. Plates were washed, and biotin-conjugated monoclonal mouse antihuman TNFα antibody was added for two hours. Afterwards streptavidin-peroxidase complex was added for 30 minutes followed by 3-ethylbenzthiazol-6-sulfonic acid. Absorption was measured after 30 minutes and compared to a standard curve obtained with human recombinant TNFα. The assay detected > 0.1 ng/mL of TNFα. Data are expressed as the difference between the experimental value and the average of the baseline values.

Statistics. Wilcoxon’s signed-rank test was used to compare experimental values with the average of baseline values.¹²

RESULTS

Neutrophil and monocyte counts. Blood counts were performed during and after the constant infusion. During the constant infusion, neutrophil and monocyte counts began to rise by 24 hours and increased over the 14 days (Fig 1). At 100 µg/m²/24 h, neutrophil and monocyte counts increased more than twofold and at 500 µg/m² more than sixfold. At the termination of the constant infusion, the counts fell rapidly to baseline levels.

Monocyte ADCC. Monocyte ADCC activity against antibody-coated CRBC was measured two days after the bolus injection and at days 3 and 10 after the start of the constant infusion period. Results were compared to baseline. ADCC activity was increased at days 3 (nine out of ten patients) and 10 (eight out of eight patients) of the constant infusion period compared to baseline (P < .01; Fig 2; significant by Wilcoxon’s signed-rank test at the 1% level). Monocytes of patients treated with both doses of rGM-CSF gave similar results at each time period. ADCC activity was increased in eight of ten patients two days after the bolus injection, but these results were not significant at the 5% level.

Secretion of TNFα. The secretion of TNFα by monocytes stimulated for 48 hours with LPS was measured by an ELISA technique. No secretion was noted in the absence of LPS. Monocytes harvested from patients after ten days of continuous infusion secreted increased amounts of TNFα compared to baseline (P < .01; Fig 3). Values three days after the start of the continuous infusion were also higher than baseline but did not reach statistical significance. The
bolus injection had little effect on the capacity of monocytes to secrete TNFα.

**Secretion of interferon.** Total interferon levels were also measured in the supernatants of LPS-stimulated monocytes by a modification of a viral plaque inhibition assay. No secretion was noted in the absence of LPS. Similar to TNFα, interferon levels were increased significantly ten days after the start of the constant infusion but not at other times ($P < .05$; Figure 4).

**DISCUSSION**

These data provide strong evidence for the first time that constant parenteral administration of rGM-CSF activates the antitumor potential of peripheral blood monocytes. ADCC and the secretion of TNFα and interferon were increased significantly in the majority of patients during infusion of rGM-CSF. Unlike administration by constant
infusion, however, bolus injection at the doses used were insufficient to enhance cellular cytotoxicity.

These data are consistent with in vitro experiments that showed the induction of potential antitumor mechanisms in monocytes by rGM-CSF. GM-CSF has been shown to increase the number of Fc receptors on monocytes/macrophages in both the murine and human systems. Specifically, rGM-CSF increased the number of FcR II receptors on the U-937 monocyte-like cell line, whereas Interleukin-2 (IL-2), TNFα, IFN-γ, and CSF-1 did not. Increased numbers of Fc receptors may have been one mechanism for the increased ADCC activity observed in our studies. Human rGM-CSF also enhances the triggered respiratory burst pathway of human neutrophils and monocytes, making available toxic products such as H₂O₂ and oxygen radicals for antimicrobial and antitumor activity.6,7 GM-CSF also appears to induce TNFα-dependent antitumor mechanisms. rGM-CSF induced the accumulation of cytoplasmic TNFα messenger RNA (mRNA) in human monocytes and induced antibody-independent cytotoxicity of actinomycin D treated WEHI 164 target cells.8 Neutralizing antibody against TNFα eliminated the antitumor effects in these experiments. In a separate model, rGM-CSF and IFN-γ were shown to stimulate monocytes directly to kill A375 malignant melanoma target cells.9 Taken together, these in vitro data provide strong evidence that rGM-CSF has the potential to stimulate effector functions, particularly antitumor mechanisms, of human monocytes.

In addition, our study confirmed the observation that the infusion of rGM-CSF markedly increased the number of monocytes and neutrophils in peripheral blood. The details of this hematopoietic response will be published elsewhere.10 These findings corroborate recently published trials in which individuals with various causes of myelosuppression, including acquired immunodeficiency syndrome (AIDS),11 myelodysplastic syndromes,2 bone marrow transplantation3 have all had striking increases in leukocyte counts in response to rGM-CSF. Our results therefore confirm the possibility that GM-CSF may be useful not only as a hematopoietic agent but also as an immunostimulator. Indeed, the unique dual activities of the CSFs may augment host defense mechanisms at two discrete levels, potentially optimizing activity against both malignancies and microbial pathogens.

In the patients studied, no demonstrable effect on the tumor load was evident during the time of the infusion. This is not surprising, since only patients with widely metastatic and refractory solid tumors were enrolled, the number of patients was limited, and the course of treatment was short. The appropriate role for rGM-CSF in the treatment of malignancy will only be defined by expanded clinical trials including patients with a variety of tumors.

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REFERENCES


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