Differential Effect of Recombinant Granulocyte Macrophage Colony-stimulating Factor on Human Monocytes and Alveolar Macrophages

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ABSTRACT

The effect of granulocyte-macrophage colony-stimulating factor (GM-CSF), a pluripotent cytokine, on tumoricidal activity of alveolar macrophages and monocytes from nonsmoking normal volunteers was compared using [3H]thymidine-labeled human tumor cells (SK-MEL-28, melanoma) as targets. A dose-response study (500–5000 units/ml) of recombinant GM-CSF indicated dramatic differences between cytotoxicity of alveolar macrophages and blood monocytes. Macrophages exhibited significant (P < 0.01) tumoricidal activity at all GM-CSF doses tested. In contrast, monocytes showed no significant tumoricidal activity at 500 units/ml and significantly (P < 0.01) less activity than alveolar macrophages at doses of 1000–5000 units/ml. Maximal activity in alveolar macrophages occurred 72–96 h after exposure to 1000–5000 units/ml GM-CSF. Tumoricidal activity may be related to the state of maturation, because monocytes matured in vitro for 7 days displayed enhanced tumoricidal activity after GM-CSF exposure. Tumor necrosis factor α and interleukin 1β were measured in supernatant fluids of 24-h GM-CSF-treated cells. No significant increase in either cytokine was detected after GM-CSF treatment of alveolar macrophages. Monocyte interleukin 1β secretion was not enhanced by GM-CSF; however, tumor necrosis factor α secretion was enhanced in some donors (three of five). Superoxide anion production of alveolar macrophages was not enhanced by GM-CSF. These data suggest that alveolar macrophage tumoricidal activity is induced by GM-CSF and is not dependent on oxidative metabolism or secreted forms of interleukin 1β or tumor necrosis factor α.

INTRODUCTION

The gene for human GM-CSF has been cloned and the recombinant form has been shown to be similar to the natural form in its activities on hemopoietic cells (1, 2). In addition to promoting proliferation and differentiation of stem cells, GM-CSF affects mature cell functions. In granulocytes, GM-CSF has been shown to augment oxidative metabolism, cytotoxicity, and chemotaxis (3–6). The effects of GM-CSF on macrophages are less clear. Enzymic microbial killing by GM-CSF-treated monocyte-derived macrophages has been reported (7), but controversy exists concerning the effect of GM-CSF on monocyte tumoricidal activity. Kleinerman et al. (8) reported that both in vivo and in vitro exposure to GM-CSF failed to enhance monocyte tumoricidal activity, while others (9) report increased in vitro tumoricidal activity. The effect of GM-CSF on human alveolar macrophage tumoricidal activity has not been investigated. The purpose of the present study was to compare the effects of GM-CSF on human alveolar macrophage and monocyte activities.
calculated as follows:

\[
\% \text{ cytotoxicity} = \left( \frac{\text{dpm in target cells cultured with untreated macrophages/monocytes} - \text{dpm in target cells cultured with treated macrophages/monocytes}}{\text{dpm in target cells cultured with untreated macrophages/monocytes}} \times 100 \right)
\]

Endogenous (spontaneous) % cytotoxicity

\[
\% \text{ cytotoxicity} = \left( \frac{\text{dpm in target cells in medium alone} - \text{dpm in target cells cultured with untreated macrophages/monocytes}}{\text{dpm in target cells in medium alone}} \times 100 \right)
\]

The coefficient of variation for the tumoricidal assay was 14.1% and therefore cytotoxic values ≤14% were considered negative.

Target Cells. Target cells included SK-MEL-28 (melanoma) and CCD-11 Lu (nonneoplastic lung fibroblasts), obtained from the American Type Culture Collection (Rockville, MD). SK-MEL-28 is resistant to natural killer cell-mediated killing and is IL-1 resistant and TNF-α sensitive. Target cells were cultivated in RPMI 1640 medium that was supplemented with 10% fetal calf serum and were free of Mycoplasma and other microbial contaminants.

 Supernatants for Product Analyses. Monocytes or alveolar macrophages were cultured overnight in microtiter plates in the presence of 1000 units/ml γ-interferon, 1000 units/ml GM-CSF, 0.5 μg/ml LPS or in medium alone. Culture supernatants were collected and assayed for TNF-α, IL-1β, and PGE₂. TNF-α was measured with an enzyme-linked immunosorbent assay kit (T Cell Sciences, Cambridge, MA). Sensitivity of this assay is 35.0 pg/ml TNF-α. IL-1β was measured with an enzyme-linked immunosorbent assay kit (Cistron, Pine Brook, NJ). The sensitivity of the assay is 20.0 pg/ml IL-1β. PGE₂ was measured with a radioimmunoassay kit (Amersham, Chicago, IL). The sensitivity of the assay is 43 pg/ml PGE₂. Comparisons of cytokine levels in treated versus untreated cells were considered significantly different if treated cells showed a 2-fold increase over baseline.

 Superoxide Anion Assay. Superoxide anion production was measured in an enzyme immunoassay microplate reader, as previously described (15, 16). Microtiter plates with adherent macrophages were incubated for 4 days in medium alone or with Salmonella typhimurium LPS, γ-interferon, or GM-CSF. After incubation, the plates were gently washed with phenol red-free HBSS and each well received 100 μl HBSS containing 16 nmol ferricytochrome c, with 1 nmol phosphoryl myristate acetate to trigger the release of reactive oxygen species. Superoxide dismutase was included in all assays to demonstrate the specificity of the cytochrome c reduction. Superoxide anion production was totally inhibited by 600 units superoxide dismutase well. Production of accumulated superoxide anion was detected by measuring the absorbance of reduced ferricytochrome c at 550 nm after 4 h.

 Statistical Analysis. Statistical significance of data was determined by the two-tailed Student's t test for paired data in comparisons of monocytes and alveolar macrophages from the same donor and for unpaired data for all other comparisons.

RESULTS

Effect of GM-CSF on Tumoricidal Activity. Monocytes from all donors demonstrated cytotoxicity after exposure to LPS (51.7 ± 8.3%; mean cytotoxicity ± SE) or γ-interferon (45.8 ± 7.4%). Similarly, tumoricidal activity of alveolar macrophages from all donors was induced with LPS (58.2 ± 4.8% or recombinant γ-interferon (53.6 ± 4.9%). As shown in Fig. 1, 500 units/ml GM-CSF had no effect on monocyte tumoricidal activity, and at 1000–5000 units/ml GM-CSF monocyte tumoricidal activity was significantly lower than alveolar macrophage activity (P ≤ 0.001). In alveolar macrophages, GM-CSF enhanced tumoricidal activity in a dose-dependent manner, with peak activity at 1000 units/ml. Neither macrophages nor monocytes exhibited spontaneous tumoricidal activity when incubated in medium alone. GM-CSF-treated macrophages did not demonstrate cytotoxicity (4.5 ± 4.5%; mean cytotoxicity; three experiments) against a nonneoplastic lung fibroblast cell line (CCD-11 Lu).

Effect of Monocyte Maturation on Response to GM-CSF. In vitro matured monocytes were treated with GM-CSF and tumoricidal activity was determined and compared to results with freshly isolated monocytes (Fig. 2). Endogenous tumoricidal activity did not increase in any monocyte cultures, while response to GM-CSF increased (>40%) in three of four cultures. Response to γ-interferon increased significantly in only one of four cultures.

Monocyte/Macrophage Products. To investigate the nature of the differential cytotoxicity, the cytokines IL-1β and TNF-α were measured in supernatant fluids. Under certain conditions, macrophage activation and tumoricidal activity have been shown to correlate with these cytokines (17–19). Alveolar macrophages showed higher basal levels of TNF than monocytes...
and tumoricidal activity (22, 23). We examined alveolar macrophages to correlate with macrophage activation was detected in supernatants from untreated alveolar macrophages (three experiments) and 139 ± 25 pg/ml in those from monocytes (four experiments) incubated in medium alone or not detectable (<43 pg/ml PGE2) in 24-h supernatants from macrophages. Because of monocytes demonstrated enhanced IL-10 secretion (>625 pg/ml) and from all monocytes secretion by LPS-treated alveolar macrophages was generally higher than secretion by LPS-treated monocytes. Recombinant γ-interferon enhanced TNF-α secretion by monocytes but not macrophages. Basal levels of IL-1β secretion from all macrophages (five experiments) were ≤35 pg/ml IL-1β and from all monocytes (five experiments) were ≤130 pg/ml. IL-1β secretion from monocytes and macrophages was not significantly enhanced by GM-CSF or γ-interferon. All LPS-treated macrophages and monocytes demonstrated enhanced IL-1β secretion (≥625 pg/ml in macrophages; ≥1745 pg/ml in monocytes). Because of the regulatory role of prostaglandins in numerous immunological processes (20, 21), PGE2 levels were determined. PGE2 was not detectable (<43 pg/ml PGE2) in 24-h supernatants from monocytes (four experiments) incubated in medium alone or treated with GM-CSF. A mean level of 149 ± 33 pg/ml PGE2 was detected in supernatants from untreated alveolar macrophages (three experiments) and 139 ± 25 pg/ml in those from GM-CSF-treated macrophages.

Superoxide Anion Production. Production of oxygen metabolites has been shown to correlate with macrophage activation and tumoricidal activity (22, 23). We examined alveolar macrophage superoxide anion production in response to GM-CSF (Fig. 3). In contrast to LPS and γ-interferon, GM-CSF did not induce superoxide anion production in alveolar macrophages.

DISCUSSION

The present study demonstrates that tumoricidal activity is induced by GM-CSF in both alveolar macrophages and in vitro matured monocytes. However, in freshly isolated monocytes, GM-CSF-induced tumoricidal activity is significantly less. As in previous studies (14), alveolar macrophage and monocyte responses to LPS and γ-interferon were comparable. Maximal activity in alveolar macrophages occurred 72–96 h after exposure to GM-CSF and over a dose range of 1000 to 5000 units/ml GM-CSF. Others (8, 24) have also reported that GM-CSF-treated monocytes display minimal or no tumoricidal activity. In contrast, Grabstein et al. (9) reported that GM-CSF induced significant monocyte tumoricidal activity. Kleinerman et al. (8) suggest that one explanation for the discrepancy between studies may be the use of fetal calf serum versus human serum in the activation phase. We used human serum, as did Kleinerman et al., and our results are similar.

The exact mechanisms by which activated macrophages destroy susceptible tumor cells are unclear (25). Macrophage-mediated tumor lysis can occur by both direct and indirect processes. The direct process involves the binding of activated macrophages to the target cells, whereas the indirect mechanism can involve the release of diffusible cytotoxic molecules such as oxygen metabolites, neutral proteinases, complement products, arginase, TNF and IL-1 (20). Our results suggest that secreted forms of IL-1β and TNF-α are not involved in GM-CSF-induced alveolar macrophage tumoricidal activity, since no increase in levels of these cytokines were found in 24-h supernatants. Further, alveolar macrophages secreted high basal levels of TNF-α but did not display tumoricidal activity. However, these studies do not rule out the involvement of membrane-bound cytokines in tumoricidal activity. Membrane-bound IL-1 is induced earlier and persists longer than IL-1 secretion (26) and IL-1 and/or TNF associated with the plasma membrane of activated macrophages can produce lysis of sensitive target cells (27, 28). In the present studies, IL-1β is probably not a mechanism of cytotoxicity because the target cell line (SK-MEL-28) is IL-1β resistant. Further studies are under way to investigate membrane-associated tumoricidal activity induced by GM-CSF.

We have also shown that oxygen metabolites are probably not involved in GM-CSF-induced tumoricidal activity. Superoxide anion was not produced by normal alveolar macrophages in response to GM-CSF.

Prostaglandins of the E series have been shown to suppress certain immune responses in vitro as well as in vivo (29). PGE2 may be produced by activated monocytes and macrophages (20, 21). However, in the current study elevated PGE2 was not detected in 24-h supernatants from GM-CSF-treated macrophages or monocytes, suggesting that PGE2-mediated suppression of immune responses would be unlikely with GM-CSF therapy.

Our results showing increased tumoricidal activity in matured monocytes suggest that the low level tumoricidal activity of fresh monocytes is related to the state of maturation. Alternatively, extended culture may act as a priming signal. The necessity of two signals for full monocyte tumoricidal activity with GM-CSF is supported by the work of Cannistra et al. (24). These investigators demonstrated that, although GM-CSF alone failed to activate antibody-dependent cytotoxicity, subsequent treatment with endotoxin (second signal) resulted in enhanced cytotoxicity.

Several Phase I clinical trials with GM-CSF have demon-
strated a dose-dependent increase in circulating monocytes (8, 30, 31). Furthermore, studies with murine alveolar macrophages have demonstrated proliferation in vitro in the presence of murine GM-CSF (32), and administration of human GM-CSF to primates has resulted in increased numbers of alveolar macrophage in bronchoalveolar lavage fluid (33). These observations suggest that GM-CSF may be an effective agent in the therapy of lung cancer or metastatic pulmonary disease because of two possible major effects: (a) the increase of the macrophage effector cell population within the lung and (b) the increase of macrophage antitumor activity.

ACKNOWLEDGMENTS

The authors wish to thank Joyce Antal for expert technical assistance and Sue Simms for statistical analysis. We would also like to thank Dr. Marc Garnick of the Genetics Institute for generously providing the GM-CSF used in these studies.

REFERENCES


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