ABSTRACT

The administration of recombinant human macrophage colony-stimulating factor (M-CSF) i.p. in doses of 25 or 100 µg twice daily for 5 consecutive days to non-tumor-bearing C57BL/6 mice resulted in a dose-dependent infiltration of mononuclear cells in the livers but not the lungs of these treated animals. Immunohistochemical examination of fixed liver tissue with the murine macrophage-specific monoclonal antibody, F4/80, revealed a >5-fold increase in the number of hepatic macrophages. Quantification of F4/80-positive cells in a mononuclear single cell suspension derived from liver revealed a >25-fold expansion in the number of hepatic macrophages compared to control mice. These cells were then tested in 18-h 51Cr release assays for tumoricidal activity, after an 18-h incubation with or without γ-interferon, against cultured P815 targets. Significant tumor cell lysis by these liver-associated mononuclear cells occurred, which was enhanced by γ-interferon preincubation. The systemic administration of M-CSF alone at high dose had no antitumor impact in vivo against 3-day pulmonary metastases from the MCA-203 sarcoma and B16 melanoma or hepatic metastases from the B16 melanoma or MCA-105, -203, or -207 sarcomas. Although the systemic administration of M-CSF in combination with tumor-specific monoclonal antibody had no effect on 3-day pulmonary metastases from the B16 melanoma, significant reductions in liver metastases were seen. These murine studies demonstrate the biological activity of recombinant human M-CSF in vivo and suggest that the administration of this cytokine in combination with specific monoclonal antibody may be useful in the treatment of patients with metastatic disease at sites of monocyte/macrophage accumulation.

INTRODUCTION

Mononuclear phagocytes represent a cell lineage widely distributed throughout most tissues. Macrophages process antigen and present a modified protein antigen in conjunction with Class II major histocompatibility antigen molecules to the T-cell. Macrophages are also involved in the regulation of lymphocytes by the release of stimulatory or inhibitory cytokines and can participate as an important effector in cell-mediated immunity. Their responsiveness to cytokines can result in an amplification of immune responses initiated by the T-cell, mediated through this nonspecific effector. The development of macrophage tumoricidal and cytotoxic activity requires a sequence of reactions (1, 2). Amplification is followed by interaction between lymphokines which prime the macrophage to develop cytotoxic potential while other lymphokines trigger the primed cell to express cytotoxicity.

The role of macrophage colony-stimulating factor in these complex interactions is being defined. M-CSF is a glycoprotein that acts on both progenitor and mature cells of the macrophage lineage, via a specific cell surface receptor (3, 4). M-CSF has direct stimulating effects on the mature monocyte and macrophage. For example, M-CSF stimulates murine macrophages to secrete a variety of agents and cytokines important in immunity, inflammation, and tissue repair (5–12), and induces enhanced expression of Fc receptors (13). Incubation of murine bone marrow-derived macrophages in M-CSF stimulates pinocytosis by these cells (14) and is chemotactic (15). Moreover, murine peritoneal exudate cells incubated in M-CSF stimulated resistance to viral infection and killing of Candida albicans (16, 17).

In both murine and human in vitro macrophage/tumor models, M-CSF resulted in tumoricidal activity when used either alone or in conjunction with other biological agents (18–21). M-CSF has also been shown to enhance macrophage ADCC induced by lymphokines or to induce ADCC independent of other factors (19, 22). Thus, these studies demonstrated that M-CSF plays a role in the priming and activation of macrophages to express cytotoxicity against tumor cells. Because of these in vitro properties, we have now investigated the effects of the in vivo administration of recombinant M-CSF on organ-associated macrophages and the ability of this cytokine to mediate antitumor effects either alone or in combination with specific monoclonal antibody in mice.

MATERIALS AND METHODS

Mice. C57BL/6 and female BALB/c × C57BL/6 F1 mice were obtained from the Animal Production Colonies of the National Cancer Institute, Frederick Cancer Research Facility, NIH, Frederick, MD, and from the Charles River Laboratories, Wilmington, MA. They were maintained in filtered air cages and were used at age 9 weeks or older.

Tumors. MCA-induced fibrosarcomas MCA-105, -203, and -207 were generated in our laboratory in C57BL/6 mice and were passaged s.c. for seven transplant generations, at which time a cryopreserved vial from the first generation was thawed and transplanted. A weakly immunogenic B16 melanoma syngeneic to C57BL/6 mice was obtained from Dr. A. Ovejera of the Frederick Cancer Research Facility, and was serially transplanted s.c. in syngeneic mice. Single cell suspensions of freshly excised B16 melanoma or MCA sarcoma were prepared as previously described (24). The P815 mastocytoma was obtained from Dr. Judith L. Pace (University of Kansas Medical Center) and was maintained in continuous in vitro culture.

Cytokines. Highly purified, Escherichia coli-derived recombinant human macrophage colony-stimulating factor (25, 26) was kindly provided by the Cetus Corp. (Emeryville, CA) and had a specific activity of 7 x 10^7 units/mg protein, with purity of >95% by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and <0.04 mg/ml endotoxin. Murine γ-interferon purified to a specific activity of 3.8 x 10^4 lytic units/mg protein was obtained from Dr. Sidney E. Grossberg (Medical College of Wisconsin).

Anti-B16 Melanoma Antibody. Anti-B16 melanoma mAb of the IgG2b isotype was produced in ascites form from mouse hybridoma cells provided by Dr. Tsuyoshi Takami (Department of Pathology, Sapporo Medical College, Sapporo, Japan) and was purified on a Protein A column. The specificity of this mAb to the B16 melanoma was confirmed by its inability to react with a variety of different cells, including other fresh tumors, cultured cell lines, and cells from various normal tissues (27).
Isolation of Hepatic and Pulmonary Mononuclear Cells. Liver and lungs were excised, minced into 1-mm fragments, and stirred in a triple enzyme mixture of hyaluronidase, DNase, and collagenase (Sigma Chemical Co., St. Louis, MO) for 2- 3 h as described previously (28). The cells were then transferred to Ficol-Hypaque gradients (Lympholyte M; Cedarlane Laboratories Ltd., Hornby, Ontario, Canada) and were centrifuged at room temperature for 20 min. The interface, containing mononuclear cells, was collected and pelleted. The contaminating erythrocytes were lysed by resuspending the cell pellet in 10% buffered ammonium chloride solution (Scott/B & B Research, Fiskeville, RI) for 1 to 2 min at room temperature. The cells were washed 3 times in HBSS (Biofluids, Rockville, MD) and resuspended in CM. CM is composed of 500 ml of RPMI 1640 (Biofluids) with 10% heat-inactivated fetal calf serum (Biofluids), 0.5 mM minimal essential medium nonessential amino acid solution (Biofluids), 0.03% l-glutamine (NIH Media Unit, Bethesda, MD), 100 units/ml penicillin (NIH Media Unit), 100 /xg/ml streptomycin (NIH Media Unit), 50 /xg/ml of gentamicin sulfate (GIBCO, Grand Island, NY), 0.5 /xg/ml amphoteri
cin (Fungizone; Flow Laboratories, McLean, VA), and 5 x 10^-5 M 2- mercaptoethanol (Sigma).

Harvesting of Peritoneal Exudate Cells. C57BL/6 mice were given injections i.p. of 3 ml of thioglycollate (NIH Media Unit). Three days later, PEC were harvested by washing the peritoneum twice with 12 ml of cold RPMI containing 10% fetal calf serum. Washing of the peritoneum was repeated two times with HBSS and resuspended in CM.

In Vitro Cytotoxicity Assay. In vivo M-CSF-stimulated hepatic mononuclear cells as well as thioglycollate-stimulated PEC were harvested as described above. Cells were suspended in CM at 5 x 10^5 cells/ml, 1 x 10^6 cells/ml, 2 x 10^6 cells/ml, and 4 x 10^6 cells/ml. Two hundred /xl of each effector dilution were then plated in triplicate into two rows of a 96-well flat bottom plate (Costar, Cambridge, MA). Plates were then incubated for 2 h at 37°C and the nonadherent cells were removed by washing twice with 200 /xl of CM. Each row of cells containing each effector dilution in triplicate was then resuspended in 100 /xl CM with or without 20 units/ml of 7-interferon. The plates were then incubated at 37°C for 18 h. Cultured P815 plasmacytoma cells were washed and suspended at 10^5 cells/ml, and then incubated with 300 MCiof 51Cr for 90 min at 37°C. The labeled cells were washed three times in CM and then suspended at 10^3 /xl/mL; 100 /xl were added to each well. Targets and effectors were centrifuged at 500 rpm for 5 min, then incubated for an additional 18 h at 37°C. The supernatant samples were harvested by the Skatron apparatus (Skatron, Sterling, VA) and counted in a gamma counter. Spontaneous release of 51Cr was measured after incubation of target cells with CM only, and maximal release was measured after incubation of target cells in 0.1 N HCl. Percentage of lysis was calculated as follows:

\[
\text{Percentage of lysis} = \frac{\text{Experimental cpm} - \text{spontaneous cpm}}{\text{Maximal cpm} - \text{spontaneous cpm}} \times 100
\]

One lytic unit is defined as the number of effectors needed to cause 30% lysis of 10^5 fresh tumor target cells and is determined from the dose response curves.

Immunohistochemistry. Hepatic mononuclear cells were suspended at 10^6 cells/ml and were applied to glass slides by using a Cytospin (Shandon, Pittsburgh, PA). The Cytospin slides were placed in 1% methanol/hydrogen peroxide for 30 min to block endogenous peroxidase activity. The slides were hydrated in sequential alcohols and xylene; coverslips were applied with Permount.

Slides were incubated in ABC for 30 min. Primary antibodies F4/80 antibody (1:100, Vector Laboratories, Burlingame, CA) was then applied for 30 min to the slides incubated in F4/80 antibody. These slides were then washed in 2 changes of Tris buffer, 2 min each, and in 10% horse serum in Tris buffer for 2 min. ABC was prepared by combining 20 /xl avidin solution and 20 /xl biotinylated peroxidase enzyme with 2 ml of Tris buffer by using a Vectastain Elite ABC kit (Vector Laboratories). Slides were incubated in ABC for 30 minutes, then washed in 2 changes of Tris buffer for 2 min each. Chromogen solution was prepared by combining 100 mg diaminobenzidine, 50 /xg 30% hydrogen peroxide, and 200 ml Tris buffer. Slides were incubated in the chromogen solution for 5 min, counterstained with hematoxylin, dehydrated in sequential alcoholso and xylene; coverslips were applied with Permount.

The percentage of positive staining cells was determined by counting 200 cells for each primary antibody used in each treatment group: control (HBSS), low-dose, and high-dose M-CSF treated mice. The percentages were converted to the number of positive cells by multiplying the percentage of positive cells by the absolute number of cells obtained following mincing, enzymatic treatment, and suspension of the mouse livers (see above). Portions of liver were placed in 10% formalin solution. They were embedded in paraffin, sectioned, and immunostained with primary antibody F4/80 according to the method outlined above. Light microscopy was used to determine the number of positive staining cells per oil immersion field by counting 5 oil immersion fields/slide for control (HBSS), low- and high-dose M-CSF treatment groups in both tumor-bearing and non-tumor-bearing mice. All slides were read in a coded, blinded fashion by a pathologist (P. K.).

Induction of Liver and Lung Metastases. Visceral metastases were induced as described previously (24). Briefly, to induce liver metastases, 5 to 7.5 x 10^5 freshly prepared B16 melanoma or MCA sarcoma cells were injected under the splenic capsule of anesthetized mice. After 1 min tumor cells were flushed into the portal circulation and the spleen was excised. Lung metastases were induced by i.v. injection of 1 x 10^5 B16 melanoma cells or 5 x 10^4 MCA sarcoma cells via the lateral tail vein.

Adaptive Immunotherapy with M-CSF and Monoclonal Antibody. On day 3 after the induction of either pulmonary or hepatic metastases, mice were randomized to the following treatment groups: HBSS i.p. twice daily from day 3–7; 25 /xg M-CSF i.p. twice daily from day 3–7; 100 /xg M-CSF i.p. twice daily from day 3–7; 600 /xg–2 mg mAb i.v. for one dose on day 3; combination 25 /xg M-CSF twice daily for 5 days plus mAb; combination 100 /xg M-CSF twice daily for 5 days plus mAb. Groups contained 4 to 6 mice each. On day 14–18 mice were ear tagged, randomized, and then sacrificed; visceral metastases were enumerated as described previously (24, 29).

Statistical Analysis. The statistical significance of visceral metastases was analyzed with a normalized Kruskal-Wallis test. When 2-tailed P values for the Kruskal-Wallis test were significant, individual groups were compared by the Wilcoxon rank sum test. Two-tailed P values are presented.

RESULTS

Effects of M-CSF on Organ Cellularity. Normal C57BL/6 mice were treated i.p. with 50 or 200 /xg of M-CSF daily in 2 or 3 divided doses for 5 consecutive days. Livers and lungs were harvested on day 5 and enzymatically dispersed into single cells. Table 1 presents the results of 5 representative experiments. Hepatic cellularity was greatly increased with M-CSF administration, while only modest effects were seen on pulmonary cellularity. Similarly, M-CSF administration resulted in a significant increase in the wet weights of the treated livers but not lungs (data not shown). The changes in hepatic weight only partially explain the increased cellularity as demonstrated in Fig. 1, which corrects total cell number to g of hepatic tissue. The graph is representative of the 5 experiments listed in Table 1.

The phenotype of cells responsible for increased cellularity in the liver of M-CSF-treated mice was examined. Cytospins were prepared from enzymatically dispersed livers and the cells were stained with antibody to F4/80, Thy-1.2, or IgG. Differ-
Table 1 In vivo effect of M-CSF administration on organ cellularity

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Experiment 1</th>
<th>Experiment 2</th>
<th>Experiment 3</th>
<th>Experiment 4</th>
<th>Experiment 5</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Liver</td>
<td>Lungs</td>
<td>Liver</td>
<td>Lungs</td>
<td>Liver</td>
</tr>
<tr>
<td>HBSS</td>
<td>0.6</td>
<td>1.1</td>
<td>1.1</td>
<td>1.3</td>
<td>2.5</td>
</tr>
<tr>
<td>M-CSF</td>
<td>50 µg</td>
<td>4.2</td>
<td>1.7</td>
<td>6.0</td>
<td>1.7</td>
</tr>
<tr>
<td></td>
<td>200 µg</td>
<td>11.0</td>
<td>4.5</td>
<td>22.4</td>
<td>1.1</td>
</tr>
</tbody>
</table>

* Total daily dose of M-CSF/mouse given i.p. in 2 (Experiments 1–3) or 3 (Experiments 4 and 5) divided doses for 5 consecutive days.

ND, not done.

Table 2 In vivo effect of M-CSF administration on hepatic cellular infiltrate

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Experiment 1</th>
<th>Experiment 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>F4/80</td>
<td>Thy-1.2</td>
</tr>
<tr>
<td>HBSS</td>
<td>0.5a</td>
<td>0.3</td>
</tr>
<tr>
<td>M-CSF</td>
<td>50 µg</td>
<td>8.8</td>
</tr>
<tr>
<td></td>
<td>200 µg</td>
<td>42.2</td>
</tr>
</tbody>
</table>

a Total daily dose of M-CSF/mouse given i.p. in 2 divided doses for 5 consecutive days.

In Vitro Cytotoxicity by Hepatic Macrophages Stimulated by M-CSF in Vivo. The tumoricidal activity of hepatic macrophages from M-CSF-treated mice was examined in 18-hour 51Cr release assays against the P815 target. In 4 separate experiments hepatic macrophages were isolated from mice treated with 200 µg M-CSF daily for 4 to 5 consecutive days. Cells from the HBSS-treated control group were not included because of insufficient yields to perform the assay. M-CSF-stimulated macrophages were incubated with or without 20 units/ml of γ-interferon for 18 h before addition of target cells. Hepatic macrophages stimulated by M-CSF in vivo demonstrated lysis of P815 in 3 of 4 experiments. This lysis was enhanced by preincubation with γ-interferon in 3 of 4 experiments (Table 3). Concurrently in 2 experiments peritoneal exudate cells induced by thioglycolate showed significant lysis of P815 only when preincubated with γ-interferon (data not shown).

In Vivo Antitumor Effect of M-CSF Alone and in Combination with Tumor-specific Monoclonal Antibody. We next examined the therapeutic efficacy of the systemic administration of M-CSF either alone or in combination with anti-B16 melanoma mAb in mice bearing 3-day pulmonary or hepatic metastases. M-CSF alone (50 or 200 µg given daily in 2 divided doses) displayed no antitumor effect on 3-day pulmonary or hepatic metastases from the MCA-105, -203, or -207 sarcomas in 5 separate in vivo experiments (data not shown). Similarly, M-CSF with or without anti-B16 melanoma mAb showed no antitumor efficacy against 3-day B16 melanoma pulmonary metastases (Table 4). Although M-CSF alone had no significant effect on the number of 3-day B16 melanoma hepatic metastases, a significant antitumor effect was obtained when this cytokine was combined with anti-B16 melanoma mAb (Table 4; Fig. 4). Overall, the results of 9 separate experiments showed that the combination of M-CSF (50–200 µg/day) plus mAb (600 µg-2.0 mg) resulted in significant (P < 0.05) reductions of B16 melanoma liver metastases in all 9 experiments compared to HBSS alone, in 6 of 9 experiments compared to M-CSF alone, and in 5 of 9 experiments compared to mAb alone. In 2 of these experiments, a daily M-CSF dose of 50 µg was not sufficient to achieve a significant antitumor effect when combined with anti-B16 melanoma mAb, necessitating a higher daily dose of 200 µg (Fig. 4). (In a 10th experiment, greater than 50% mortality occurred during treatment of mice that resulted in no significant differences between groups). When the combination of M-CSF and specific anti-B16 melanoma mAb was used against an irrelevant tumor, MCA-207 sarcoma, no therapeutic benefit was seen (data not shown). Thus, anti-
Table 3 Tumoricidal activity of M-CSF-stimulated hepatic macrophages

<table>
<thead>
<tr>
<th>Experiment</th>
<th>- IFN-γ</th>
<th>+ IFN-γ</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>22.7*</td>
<td>26.3</td>
</tr>
<tr>
<td>2</td>
<td>11.0</td>
<td>22.0</td>
</tr>
<tr>
<td>3</td>
<td>13.5</td>
<td>32.8</td>
</tr>
<tr>
<td>4</td>
<td>&lt;1.0</td>
<td>29.4</td>
</tr>
</tbody>
</table>

* One LU (lytic unit) is defined as the number of effector cells mediating 30% specific lysis of 10⁶ target cells.

Table 4 In vivo treatment of 3-day pulmonary and hepatic metastases with M-CSF and anti-B16 melanoma mAb

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Experiment 1</th>
<th>Experiment 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Lungs</td>
<td>Liver</td>
</tr>
<tr>
<td>HBSS</td>
<td>233 (30)</td>
<td>455 (99)</td>
</tr>
<tr>
<td>M-CSF</td>
<td>243 (16)</td>
<td>203 (187)</td>
</tr>
<tr>
<td>mAb</td>
<td>246 (8)</td>
<td>212 (189)</td>
</tr>
<tr>
<td>M-CSF + mAb</td>
<td>194 (43)</td>
<td>7 (6)</td>
</tr>
</tbody>
</table>

* Six animals/group.

* Therapy was instituted on day 3 after tumor injection with HBSS (0.5 ml i.p. 3 times daily for 5 days); M-CSF (50 µg/day i.p. in 3 divided doses for 5 days); specific anti-B16 melanoma mAb (a single dose of 0.6-1.0 mg i.v. on day 3), or a combination of M-CSF and mAb.

* Metastases were generated by the i.v. injection of 1-3 × 10⁵ B16 melanoma cells as described in “Materials and Methods.”

* Metastases were generated by the intrasplenic injection of 5-7.5 × 10⁷ B16 melanoma cells as described in “Materials and Methods.”

* P < 0.05 versus M-CSF and mAb alone.

Fig. 3. Immunohistochemical staining of fixed portions of liver with F4/80 mAb. Mice were non-tumor-bearing and were treated with: HBSS (top) or 100 µg M-CSF (bottom) i.p. twice daily for 5 consecutive days.

Fig. 4. In vivo effect of M-CSF and anti-B16 melanoma mAb on 3-day B16 melanoma hepatic metastases. Mice were treated with therapy noted in figure. HBSS and M-CSF were given i.p. twice daily for 5 consecutive days (days 3 through 7). Anti-B16 melanoma mAb (1 mg) was given i.v. on day 3.

tumor effects could be generated by M-CSF in the liver, the site where macrophage infiltration/expansion was elicited, when it was administered in conjunction with specific antitumor mAb.

DISCUSSION

Tumor lysis in vitro by M-CSF-stimulated human macrophages has been demonstrated in conjunction with other cytokines, with mAb, or alone (18–22). The effect of in vivo administration of M-CSF on bone marrow progenitor cells and circulating blood monocytes has also been examined (23, 30–34). Our current study evaluated the effect of in vivo administration of M-CSF on organ cellularity of treated C57BL/6 mice, as well as its potential in antitumor responses when used alone or in combination with tumor-specific mAb. M-CSF treatment caused a marked increase in F4/80-positive macrophages in the livers of normal mice. In contrast, little to very modest effects on cellularity were observed in lungs (Table 1), as well as in spleen, PEC, and peripheral blood (data not shown). Spleno-
megaly was visually apparent despite very similar absolute splenocyte counts; differential cell counts revealed a predominance of mononuclear cells in this organ (not shown). The hematopoietic potential of the various organs appeared to correlate with the magnitude of their response to M-CSF administration and may account for this pattern of differential organ responses. Another potential explanation for the marked liver effect involves increased local delivery with elevated portal blood levels of cytokine due to peritoneal absorption; however, the virtual absence of effect on the absolute PEC count renders this explanation less likely. Further studies of lungs, liver, and spleen histology confirmed an increase in organ cellularity in liver (and to a substantially lesser extent in lungs) with a multicellular infiltrate as well as a dose-dependent increase in extramedullary hematopoiesis occurring in the liver and spleen (not shown). These findings confirm and extend similar observations reported earlier by others (35).

Due to the marked effect of M-CSF on the liver and the very modest effect on the lungs, we then investigated the potential antitumor effects of M-CSF with and without concomitant anti-B16 melanoma mAb administration on B16 melanoma hepatic and pulmonary metastases. While no direct antitumor effects were noted with M-CSF when given alone i.p. at high dose, combined immunotherapy with M-CSF and specific anti-B16 melanoma mAb significantly reduced the number of liver, but not lung, metastases. This finding is in contrast to the study of Hume et al. (36) in which the i.v. administration of M-CSF alone demonstrated antitumor efficacy against spontaneous B16 pulmonary metastases. In our study, the selective organ antitumor effect of the combination treatment may involve a differential capacity of anti-B16 melanoma mAb to bind to tumor at various sites, differing inherent organ cell populations, or increased liver effect due to high portal vein levels of locally absorbed M-CSF.

Macrophages isolated from the livers of M-CSF-treated mice exhibited direct tumor cytotoxicity in vitro. This tumoricidal activity was augmented by incubating these cells with IFN-γ (3 of 4 experiments). Mononuclear cell yields obtained from the livers of control mice receiving HBSS were insufficient for testing in this assay. Thus, M-CSF alone in vivo caused hepatic macrophage recruitment/expansion; in vitro treatment with IFN-γ appeared to enhance their state of activation. However, the lack of antitumor effect of M-CSF alone in vivo would argue against direct macrophage tumoricidal activity as the principal mechanism. The ability of macrophages activated in vitro by M-CSF to mediate enhanced ADCC of tumor cells has recently been reported by others (21), and may be responsible for reduction of B16 melanoma hepatic metastases observed following combination treatment with M-CSF and tumor-specific mAb.

Another possible mechanism of M-CSF-induced antitumor activity involves the induction of other cytokines that may mediate significant antitumor activity through direct effects, an increase in the number or cytolytic ability of effector cells, or an increase in the number of Fc receptors or other cell surface markers. Previously, M-CSF has been shown to cause the release of ADCC-enhancing lymphokines, such as tumor necrosis factor α and the interferons, from human monocytes (37). As we have recently shown, IFN-α can induce ADCC activity and, when combined with tumor-specific mAb, mediate the regression of established B16 melanoma liver metastases (38). These murine studies demonstrate the biological activity of M-CSF in vivo and suggest the potential utility of this cytokine in conjunction with other biological agents such as tumor-specific mAb or γ-interferon. Further laboratory and clinical investigations are being conducted to elucidate the mechanisms of M-CSF mediated recruitment, triggering, and activation of macrophages and its potential use in patients with metastatic disease in organ sites of monocyte/macrophage accumulation.

REFERENCES
BIOLOGICAL AND ANTITUMOR EFFECTS OF M-CSF IN MICE


Biological and Antitumor Effects of Recombinant Human Macrophage Colony-stimulating Factor in Mice

Steven N. Bock, Robert B. Cameron, Peter Kragel, et al.


Updated version

Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/51/10/2649

E-mail alerts

Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions

To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions

To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.