Expression of the M-CSF (CSF-1) Gene by Human Monocytes

By Alessandro Rambaldi, Diane C. Young, and James D. Griffin

Monocyte colony-stimulating factor (M-CSF, CSF-1) is a macrophage lineage-specific growth factor. Northern blot analysis using a human M-CSF cDNA probe and a specific bioassay for human M-CSF were used to investigate the cellular sources of M-CSF. Expression of the M-CSF gene was induced in blood mononuclear cells stimulated by phorbol myristate acetate (PMA) or γ-interferon. When mononuclear cells were fractionated into highly purified populations of T cells and monocytes, M-CSF transcripts were detected predominantly in monocytes. Further, monocytes stimulated with PMA released an M-CSF-like activity. These results demonstrate that M-CSF can be produced by activated cells of the macrophage lineage.

COLONY STIMULATING FACTORS (CSFs) are a group of glycoproteins that stimulate proliferation of hematopoietic precursor cells. Four murine and human CSFs have been identified on the basis of different physical properties and target cell specificities. Multi-CSF or interleukin-3 (IL-3) has the capacity to stimulate proliferation of a broad range of progenitor cells including erythroid, granulocytic, mast cell, megakaryocytic, eosinophil, and multipotent progenitor cells. GM-CSF stimulates granulocyte, monocyte, eosinophil, megakaryocyte, and erythroid colony formation. G-CSF stimulates the formation of granulocytic colonies, and M-CSF (CSF-1) selectively stimulates the formation of monocyte colonies. In addition, these factors may activate mature granulocytes and monocytes.

Despite the importance of these factors in the regulation of hematopoiesis, little is known about the cell types that supply CSFs or the control of CSF gene expression. In this study, we describe the use of a cDNA probe for human M-CSF to investigate the expression of the M-CSF gene by highly purified populations of hematopoietic cells. A specific bioassay was used to detect human M-CSF activity separately from GM-CSF and G-CSF activity. The results indicate that unstimulated T lymphocytes and monocytes neither secrete M-CSF nor have detectable M-CSF transcripts. Exposure of monocytes but not T lymphocytes to γ-interferon (IFN) or phorbol myristate acetate (PMA) induces expression of the M-CSF gene and release of biologically active M-CSF. This suggests that monocytes, like T cells and B cells, can be induced to release growth factors specific for their own lineage.

MATERIALS AND METHODS

Human cells and cell lines. Peripheral blood cells were obtained from volunteer donors and mononuclear cell suspensions prepared by Ficoll-Hyphaque density gradient centrifugation. T lymphocytes were prepared by E rosetting and were further purified by removal of any residual plastic-adhering cells. Monocytes were prepared by two different methods. In the first method, monocytes were purified from E cells by two plastic-adhering steps as previously described. In the second method, B cells, T cells, and natural killer cells were removed from E cell fractions by immune rosettes using the lineage-specific monoclonal antibodies anti-μ, anti-HLA-DR, -T11, -T3, -B1, and -NKH1. All monocyte preparations used in this study contained less than 1% detectable T11+ or T3+ cells. All T cell preparations contained less than 1% detectable Mo2+ cells. Purified cells were cultured for various times at a concentration of 1 x 10⁶/mL in RPMI 1640 with 10% fetal bovine serum (FBS), glutamine, and antibiotics (GIBCO, Grand Island, NY) at 37°C. The concentration of endotoxin in this medium was less than 0.5 ng/mL as determined by the limulus amebocyte assay. Where indicated, 500 U/mL recombinant γ-IFN (kindly provided by Dr Steven Wright, Biogen Research Corp., Cambridge, MA), PMA (10⁻¹⁰ mol/L; Sigma Chemical Co, St Louis), or recombinant GM-CSF (final dilution, 1:1000 of COS cell supernatant; kindly provided by Dr Steven Clark, Genetics Institute, Cambridge, MA) were added to the culture media. The U937² and HL-60³ cell lines were obtained from the American Type Culture Collection, Rockville, MD, and cultured in RPMI 1640 medium supplemented with 10% FBS.

Preparation of total cellular RNA and Northern blot analysis. Total cellular RNA was isolated as previously described by lysing cells in guanidium isothiocyanate followed by recovery of RNA by centrifugation through cesium chloride. Fifteen-microgram samples were then fractionated on a 1.2% agarose gel with 6% formaldehyde and blotted onto synthetic membranes (Gene Screen Plus, New England Nuclear, Boston). M-CSF mRNA was detected by using a 3.5-kb segment of the human M-CSF cDNA (CSF-1) inserted in the Xho site of the pXMT 2 vector (Gordon Wong, manuscript in preparation). This M-CSF probe was generously provided by Drs Gordon Wong and Steven Clark, Genetics Institute. The probe was labeled to a specific activity of 10⁹ cpm/μg by using hexanucleotide primers and [³²P]-dCTP. Hybridization was performed at 60°C in a solution containing 1 mol/L NaCl, 1% sodium dodecyl sulfate (SDS), 10% dextan sulfate, 100 μg/mL salmon sperm DNA, and 1 x 10⁶ cpm/mL labeled probe. The membrane was washed with 2x SSC/1% SDS for one hour at 65°C and 0.1x SSC at room temperature for one hour (1x SSC = 0.15 mol/L NaCl, 15 mmol/L sodium citrate, pH 7.0). The blots were then dried and used to expose Kodak Xomat x-ray film with intensifying screens. In one experiment, GM-CSF transcripts were detected with a cDNA probe for GM-CSF that was previously described and supplied by Drs Wong and Clark. Reanalysis of a blot for the second probe was done by washing the membrane in boiling water followed by rehybridization as described above.

Bioassay for human M-CSF. Supernatants were prepared by

From the Division of Tumor Immunology, Dana-Farber Cancer Institute, Boston.

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Dr Griffin is a Scholar of the Leukemia Society of America. Address reprint requests to James D. Griffin, MD, Division of Tumor Immunology, Dana-Farber Cancer Institute, 44 Binney St, Boston, MA 02115.

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incubating purified cell populations in RPMI 1640 medium with 10% FBS at 1 x 10⁶ cells/mL for 48 hours. Human M-CSF activity was detected by its ability to stimulate the growth of murine macrophage colonies in agar. Murine cells were used as targets for two reasons: (1) human M-CSF stimulated much larger macrophage colonies from mouse bone marrow than it did from human bone marrow; and (2) human GM-CSF does not stimulate the growth of any mouse colonies, whereas human G-CSF stimulates the growth only of granulocyte colonies (see Results). Thus, stimulation of murine macrophage colony growth distinguishes human GM-, G-, and M-CSFs. Colonies were grown in a double-layer agar culture system previously described in detail. Recombinant human CSFs were obtained from Dr Steven Clark, Genetics Institute, and were in the form of media conditioned by COS cells transfected with a vector containing a complete cDNA for GM-CSF, G-CSF, or M-CSF. The titers were determined by a CFU-GM assay using human or mouse (BALB/c) light-density, nonadherent marrow cells. Control supernatant from COS cells conditioned by mock (vector-only) transfection contained neither stimulatory nor inhibitory activity for mouse or human marrow CFU-GM.

RESULTS

Induction of the M-CSF gene in human monocytes. Expression of M-CSF mRNA was investigated in peripheral blood cells (Fig 1). In an initial experiment, M-CSF transcripts were detected in peripheral blood mononuclear cells stimulated with PMA (10⁻¹⁰ mol/L, 24 hours) but not in unstimulated mononuclear cells (Fig 1). To determine which cell type was expressing the M-CSF message, blood mononuclear cells were carefully fractionated into T lymphocytes (greater than 99% T11+ cells) and monocytes (greater than 95% Mo2+ cells). The cells were then cultured with control medium, γ-IFN (500 U/mL), or PMA (10⁻¹⁰ mol/L). M-CSF transcripts were induced in monocytes by either γ-IFN or PMA but not in T cells by either treatment. In order to be certain that stimulation of the purified T cells was sufficient, the blots shown in Fig 1 were washed and rehybridized with a cDNA probe for GM-CSF (a factor known to be secreted by activated T cells). In contrast to M-CSF, expression of the GM-CSF gene was readily detected following treatment of T cells with PMA (data not shown). To define the kinetics of induction of the GM-CSF message with γ-IFN, purified adherent monocytes were cultured with γ-IFN (500 U/mL) for 2, 5, 12, or 18 hours. Detection of M-CSF transcripts was maximal at five to 12 hours (Fig 2). These results suggest that expression of the M-CSF gene is inducible in human monocytes by exposure to the T cell lymphokine γ-IFN or by activation of protein kinase C by PMA.

To further demonstrate that cells of the monocytic lineage are able to express the M-CSF gene, we studied the U937 and HL-60 cell lines after induction of monocytic differentiation with several stimuli. As shown in Fig 3, PMA-stimulated HL-60 cells expressed the M-CSF message.

Secretion of biologically active M-CSF by highly purified human monocytes. To determine whether the increased levels of M-CSF mRNA detected in monocytes following treatment with γ-IFN or PMA were accompanied by
secretion of biologically active CSF activity, we cultured purified populations of monocytes and T cells either in control medium or medium containing γ-IFN (500 U/mL) or PMA (10^{-10} mol/L). In pilot experiments using recombinant human CSFs it was shown that rM-CSF stimulated the formation of only very small clusters of monocytes in agar culture of light-density human marrow cells. In contrast, GM-CSF stimulated the formation of large granulocyte and monocyte clusters and colonies, whereas G-CSF stimulated the formation of only granulocyte colonies. In mouse marrow, however, human rM-CSF stimulated the formation of large colonies of monocytes; rGM-CSF was inactive, and rG-CSF stimulated the formation of only granulocyte colonies (Table 1). Therefore, the activity of M-CSF could be distinguished from that of GM-CSF and G-CSF by using mouse marrow cells as targets and enumerating only monocyte colonies. Supernatants collected from unstimulated monocytes cultured for 48 hours contained a small amount of M-CSF activity (Table 1). Treatment of monocytes with γ-IFN or PMA induced the release of approximately twofold and tenfold more M-CSF activity, respectively. M-CSF activity was not detectable in stimulated or unstimulated cultures of T cells. PMA also induced the release of a G-CSF-like activity from monocytes (Table 1).

DISCUSSION

Human M-CSF (CSF-1) was the first of the human CSFs to be purified to homogeneity. M-CSF from human urine is a glycoprotein of molecular weight 45,000 that has been shown to stimulate the growth of murine and human macrophage colonies. A cDNA for human M-CSF has been isolated by using oligonucleotide probes based on the amino-terminus sequence for urinary CSF. A full-length cDNA

### Table 1. Secretion of M-CSF by Human Monocytes

<table>
<thead>
<tr>
<th>Source of Human CSF</th>
<th>Murine CFU-GM/5 × 10^4</th>
<th>Marrow Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Monocyte</td>
<td>Granulocyte</td>
</tr>
<tr>
<td></td>
<td>Exp 1</td>
<td>Exp 2</td>
</tr>
<tr>
<td>Recombinant human CSFs*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>rM-CSF</td>
<td>145 ± 6</td>
<td>141 ± 5</td>
</tr>
<tr>
<td>rG-CSF</td>
<td>21 ± 6</td>
<td>4 ± 1</td>
</tr>
<tr>
<td>rGM-CSF</td>
<td>14 ± 2</td>
<td>2 ± 2</td>
</tr>
<tr>
<td>Mock-CSF</td>
<td>16 ± 5</td>
<td>1 ± 1</td>
</tr>
<tr>
<td>T cell (T)/monocyte (Mono) conditioned media</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>19 ± 5</td>
<td>0 ± 0</td>
</tr>
<tr>
<td>Mono-CM</td>
<td>37 ± 2</td>
<td>5 ± 3</td>
</tr>
<tr>
<td>Mono + PMA-CM</td>
<td>136 ± 6</td>
<td>35 ± 1</td>
</tr>
<tr>
<td>Mono + IFN-CM</td>
<td>63 ± 5</td>
<td>23 ± 1</td>
</tr>
<tr>
<td>T-CM</td>
<td>16 ± 3</td>
<td>0 ± 0</td>
</tr>
<tr>
<td>T + PMA-CM</td>
<td>19 ± 5</td>
<td>0 ± 0</td>
</tr>
<tr>
<td>T + IFN-CM</td>
<td>17 ± 2</td>
<td>0 ± 0</td>
</tr>
<tr>
<td>PMA (10^{-10} mol/L)</td>
<td>22 ± 4</td>
<td>4 ± 2</td>
</tr>
<tr>
<td>IFN (50 U/mL)</td>
<td>16 ± 1</td>
<td>0 ± 0</td>
</tr>
</tbody>
</table>

Abbreviations: Exp, experiment; NT, not tested.
*Each CSF has used as a 1:500 final dilution of medium conditioned by COS cells transfected with a CSF cDNA. These supernatants were previously titered on human (GM-, G-CSF) or mouse (M-CSF) marrow CFU-GM, and a concentration more than twice the maximum was used in these experiments.
*Murine CFU-GM were assayed in a double-layer agar system (see Materials and Methods).
†Purified cell populations were cultured at 1 × 10^6/mL for 48 hours and cell-free medium used as a stimulus for murine CFU-GM at a final concentration of 10% (data shown) and 1% (data not shown, revealing similar results in lesser magnitude).
has been obtained from a human pancreatic cancer cell line known to be able to secrete M-CSF.

Cells capable of producing M-CSF in the human and mouse have not been extensively investigated. A number of murine tissues have been shown to be capable of making M-CSF including fibroblasts26 and embryonic yoke sac.29 Adherent, stromal cell lines established from murine long-term marrow cultures have been shown to secrete M-CSF.31 Also, murine bone marrow cells transformed by the human R-myc oncogene have been shown to secrete M-CSF.34

The cellular sources of human M-CSF have not been investigated. This is at least partly due to the lack of highly specific assays for human M-CSF activity. In this study, we have used a human M-CSF cDNA to investigate the expression of the M-CSF gene in highly purified populations of T lymphocytes and monocytes. These cells were selected for study because preliminary experiments indicated that M-CSF transcripts could be detected following PMA stimulation of blood mononuclear cells and also because T lymphocytes and monocytes have been shown in previous studies to have potentially important effects in regulating many aspects of hematopoiesis and immune function. Although M-CSF transcripts were undetectable in unstimulated fresh monocytes, "activation" of these cells with PMA or γ-IFN induced M-CSF gene expression. With the conditions used in these experiments, M-CSF transcripts were not detected in unstimulated or stimulated purified T lymphocytes. It is possible that M-CSF expression can be induced by other types of T cell activation and further possible that monokines such as IL-1 may be required. Additional experiments will be required to determine whether the M-CSF gene can be expressed by normal T cells during other conditions of activation.

Although the exact role of M-CSF in human hematopoiesis is uncertain, the finding that M-CSF can be secreted by macrophage lineage cells in a regulated manner is of considerable interest. Human macrophage precursors proliferate in response to M-CSF alone, and it is possible that M-CSF acts synergistically with other CSFs in the production of human macrophage colonies. The recently described hemopoietin-1 isolated from the human bladder carcinoma cell line 5637 may be a factor of particular interest.35

The results presented here further suggest that the role of M-CSF in the immune response should be investigated. Recent evidence in the mouse has indicated that exposure of macrophages to M-CSF markedly influences the basal level of Fe receptor capacity and expression of the Mac-1 antigen and promotes subsequent activation by lymphokines such as γ-IFN.18 Thus, M-CSF may stimulate selected monocyte functions in an autostimulatory manner. Finally, other cells of the immune system also secrete autostimulatory factors. T cells produce IL-2 in response to specific stimuli,31,16 and normal and neoplastic B cells may produce B cell growth factor 1.31,16 Thus, release of autostimulatory factors appears to be a common mechanism whereby immunocompetent cells augment certain aspects of the immune response.

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A Rambaldi, DC Young and JD Griffin