Interleukin-1, Tumor Necrosis Factor, and the Production of Colony-Stimulating Factors by Cultured Mesenchymal Cells

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Although the genes for four hematopoietic colony-stimulating factors (CSFs) have been cloned, neither the mechanism of the regulation of their production nor their cellular origins have been established with certainty. Monocytes are known to produce colony-stimulating and burst-promoting activities, as well as several monokines such as interleukin-1 (IL-1) and tumor necrosis factor (TNF). These monokines indirectly stimulate other mesenchymal cells to produce certain colony-stimulating factors such as granulocyte-macrophage CSF (GM-CSF). To determine whether monocytes produce other CSFs and if so, to compare the mechanism of regulation of production with that of endothelial cells and fibroblasts, we investigated the synthesis of CSFs by monocytes, human umbilical vein endothelial cells, and fibroblasts. We used total cellular RNA blot analysis to determine interleukin-3 (IL-3), GM-CSF, granulocyte CSF (G-CSF), and monocyte CSF (M-CSF) messenger RNA (mRNA) content and immunoprecipitation or bioassay to confirm the presence of the specific secreted proteins. The results indicate that M-CSF mRNA and protein are produced constitutively by all three cell types and their level of expression does not increase after induction. In contrast, GM-CSF and G-CSF mRNAs are barely detectable in uninduced monocytes and show an increase in expression after lipopolysaccharide treatment. Retrovirus-immortalized endothelial cells, unlike primary endothelial cells or both primary and immortalized fibroblasts, produce IL-1 constitutively; this correlates with their constitutive production of GM-CSF and G-CSF. IL-3 mRNA was not detectable in any of these cells either before or after induction. The results indicate that these mesenchymal cells can produce three CSFs: GM-CSF, G-CSF, and M-CSF; furthermore, the data suggest that the mechanism of regulation of M-CSF production is different from that of GM-CSF and G-CSF, and that the latter two inducible CSFs are regulated by different factors in monocytes compared with the other mesenchymal cells.

MONOCYTE MACROPHAGES, endothelial cells, and fibroblastoid reticular cells are constituents of the bone marrow microenvironment, within which hematopoiesis occurs.\(^1\) Cultured human monocytes,\(^2-4\) endothelial cells,\(^5-7\) and fibroblasts\(^8\) have the capacity to produce colony-stimulating activity (CSA) and burst-promoting activity (BPA). In semisolid media, CSAs are capable of inducing the formation of colonies derived from granulocyte-macrophage, granulocyte, or macrophage colony-forming units (CFU-GM, CFU-G, CFU-M), while BPA induces the initial proliferation of erythroid burst-forming units (BFU-E) and mixed erythroid-myeloid colony-forming units (CFU-MIX).

The genes for four human colony-stimulating factors (CSFs), granulocyte-macrophage CSF (GM-CSF),\(^9\) granulocyte CSF (G-CSF),\(^10\) macrophage CSF (M-CSF),\(^11\) and interleukin-3 (IL-3) or multi-CSF\(^12\) have been isolated. The biologic activities of the purified recombinant CSFs have now been established, but because of considerable overlap in the target cell range, human progenitor bioassays are of limited use in identifying the CSF or CSFs produced by a given cell type. For example, all four factors have colony-stimulating activity,\(^13\) while GM-CSF\(^14-17\) and IL-3\(^18\) also have burst-promoting activity. The availability of molecular probes specific for the four CSFs has made possible the identification and study of individual CSF gene expression at the RNA level, and in this report the terms CSA and BPA are restricted to bioassays in which the precise factors present have not been identified.

Neither the cellular sources of the CSFs nor the mechanism by which their production is regulated are known with certainty. We\(^19\) and others\(^20-24\) recently showed that primary human umbilical vein endothelial cells and fibroblasts can produce CSA or BPA in response to induction with interleukin-1 (IL-1) or tumor necrosis factor (TNF): GM-CSF and recently G-CSF messenger RNA (mRNA) production was identified by RNA analysis.\(^19,22-27\) Since GM-CSF acts as a multipotenti,\(^13-17\) these observations were consistent with the increase in CSA and BPA noted after induction.

In this investigation, we wished to determine whether monocytes could produce the other CSFs and if so, whether their production was regulated in the same way as endothelial cells and fibroblasts. The data show that monocytes, endothelial cells, and fibroblasts produce M-CSF "constitutively." Monocytes respond to lipopolysaccharide induction by producing both GM-CSF and G-CSF. Furthermore, certain retrovirus-immortalized endothelial cells produce both IL-1 and the inducible CSFs and do not require exogenous IL-1 for CSF induction.

METHODS

Bone marrow samples. Normal human bone marrow was obtained by aspiration from adult volunteers. The procedure and attendant risks were discussed with all volunteers before bone marrow aspiration, in accordance with institutional guidelines, and...
all gave informed consent. The marrow suspensions were separated over Ficoll-Paque (1.077 g/cm^3) (Pharmacia Fine Chemicals, Piscataway, NJ) at 400 × g for 40 minutes at 20°C, and the interface mononuclear cells were collected, washed three times, and resuspended in Iscove's modified Dulbecco's medium (IMDM) containing 20% fetal calf serum (FCS). The cells were incubated overnight at 37°C and the nonadherent cells removed with two gentle washes and centrifuged. The progenitors in the nonadherent cell fraction were enriched by immunoadsorption to Ig-coated plates (panning), as previously described.28 Briefly, bone marrow cells were incubated at 4°C for 30 minutes with optimal concentrations of a panel of eight monoclonal antibodies directed against myeloid (Mo),29 Mye,30 TG1,31 and Leu M1 (Becton-Dickinson & Co, Mountain View, CA), erythroid (glycoprotein A),32 and lymphoid (Leu 1, 5, and 12 [Becton-Dickinson & Co, Mountain View, CA]). Antibody-negative cells were removed and washed twice by gently swirling, tilting, and decanting. A second incubation on another antibody-coated plate was carried out to ensure removal of all antibody-labeled cells.

Culture procedures. The bone marrow cells were cultured in a mixture containing 30% FCS, 1% bovine serum albumin (BSA; Sigma Chemical Co, St Louis), 10^-4 M/L mercaptopoethanol (Sigma Chemical Co), penicillin and streptomycin, and 0.9% methylcellulose. Varying concentrations of the conditioned media to be tested or Mo lymphoblast cell line-conditioned medium (Mo-CM) were added to culture plates. IL-1 (10 U/mL) was added to one of the duplicate plates. IL-1 (10 U/mL) was added to one of the duplicate plates. Purified monocytes were suspended in the same medium to each plate. IL-1 (10 U/mL) was added to one of the duplicate plates. The supernatants were harvested and 10 μL phenylmethylsulfonyl fluoride (Sigma) (100 mmol/L), IL-1 (10 U/mL) or TNF (1,000 U/mL). Northern analysis was carried out by electrophoresis of 15 to 20 μg total RNA per lane in 1% agarose gels, transfer to nitrocellulose filters, and hybridization to full length 32P-labeled GM-CSF, G-CSF, M-CSF, IL-3, IL-5, IL-6, IL-10, and Leu M1 (Becton-Dickinson & Co, Mountain View, CA) cDNA probes. The G-CSF cDNA was isolated from the TPA-30-I trophoblast cell line cDNA library by oligonucleotide hybridization. The IL-1 cDNA was isolated by similar methods from the 5637 bladder carcinoma cell line cDNA library (Wong GG and Clark SC, unpublished). The T-cell α-chain was a gift from T. Mak and all the other probes were gifts of S. Clark and G. Wong.

Antibodies. Rabbit antiserum to M-CSF and sheep antiserum to GM-CSF were provided by S. Clark. The antibodies were tested for specificity by immunoprecipitation of 35S methionine-labeled COS cell supernatants transfected with expression vectors containing GM-CSF, M-CSF, and G-CSF inserts. The appropriate size protein bands were immunoprecipitated, and no crossreactivity was observed.

Immunoprecipitation of GM-CSF. HUVE grown to confluence on 100-mm tissue culture plates were washed and 3 ml methionine-free medium (MEM; Gibco, Grand Island, NY) containing 10% dialyzed FCS, penicillin and streptomycin, and glucose was added to each plate. IL-1 (10 U/mL) was added to one of the duplicate plates. Purified monocytes were suspended in the same medium without or with LPS (15 μg/mL). One mCi of 35S-methionine (New England Nuclear, Boston) was added to each plate, and the plates were incubated for 12 to 16 hours at 37°C in a 5% CO2 incubator. The supernatants were harvested and 10 μL phenylmethylsulfonyl fluoride (Sigma) (100 mmol/L), 10 μL protamine (1 mg/mL) (Sigma), and 100 μL 10x protein lysis buffer (1x ~ 1% Triton X100, 100 mmol/L NaCl, 10 mmol/L Na phosphate buffer [pH 7.5], 0.5% deoxycholate, 0.1% sodium dodecyl sulphate) were added to each milliliter. Preclearing was carried out by incubation of the supernatants with 20 μL protein A sepharose per milliliter (Pharmacia) for one hour at 4°C on a shaker, centrifugation (18,000 rpm, 15 minutes), and transfer to 1.5-ml tubes. For immunoprecipitation, 5 μL of polyclonal sheep anti-GM-CSF or rabbit anti-M-CSF antiserum were added to the appropriate tubes. Supernatants from monocyte COS cells transfected with the GM-CSF, M-CSF, or G-CSF cDNAs and labeled with 35S-methionine were used as controls. After overnight incubation at 4°C, 20 μL of protein A sepharose were added to each tube, and the tubes were incubated at 4°C for one hour on a shaker. The protein A sepharose was washed three times in protein lysis buffer and resuspended in 12 μL water, 3 μL diethiothreitol (Sigma Chemical Co), and 15 μL 2x protein
loading buffer (1 x 10 mmol/L Tris, pH 6.8, 10% glycerol, 0.5% sodium dodecyl sulfate (SDS)). The tubes were boiled for ten minutes at 100°C, centrifuged, and held on ice until being loaded. The proteins and 3H-labeled molecular weight (mol wt) markers were electrophoresed on standard discontinuous 12% polyacrylamide gels and fixed in 50% methanol and 6% acetic acid for 30 minutes. The pattern of labeled proteins was visualized by fluorography (Amplify, Amersham, MA) with Kodak XAR film that was exposed at room temperature.

Bioassay for IL-1 production. The murine T-cell line D10.G4 is strictly dependent on IL-1 and a T-cell antigen receptor signal for proliferation. The 3H-thymidine incorporation of D10.G4 was used as an indicator of the presence of IL-1 in medium, as previously described. Briefly, DNA synthesis assays were performed by culturing 2 x 10⁶ D10.G4 cells in 200 μL RPM1 1640 (Gibco) plus 10% fetal bovine serum and the appropriate stimuli for 48 hours at 37°C in humidified 5% CO₂. Each well was labeled with 3H-thymidine (New England Nuclear) at 1 μCi per well for the final six hours of culture. The cells were harvested onto glass fiber filters, using an automated cell harvester (Cambridge Technologies, Cambridge, MA), and incorporated radio-activity was determined by liquid scintillation counting. All assays were done in quadruplicate. Concanavalin A (Sigma Chemical Co) at a final concentration of 4 mg/mL, or IL-1 (recombinant) at a final concentration of 1 U/mL, were added to some of the wells prior to the 48-hour incubation. Conditioned medium from HUVE or HUVE-KSV was harvested after 24 hours and clarified by ultracentrifugation (57,000 x g for one hour) before to addition to the D10.G4 cultures.

RESULTS

Enriched bone marrow progenitor cells, depleted of known accessory “helper” cells such as monocytes and T lymphocytes, were totally dependent on exogenous BPA and CSA for progenitor proliferation in vitro. Four to five percent of the final fraction formed colonies, and progenitor recovery ranged from 20% to 100%.

Primary and immortalized dermal and synovial fibroblasts release CSA/BPA after induction with IL-1. Figure 1 shows the response of primary and retrovirus-immortalized fibroblasts to IL-1. Conditioned medium (CM) from both uninduced primary dermal fibroblasts (DF) and immortalized dermal fibroblasts (DF-KSV) and synovial fibroblasts (SF-KSV) showed no detectable BPA (A, open symbols) or CSA (B, open symbols). However, incubation of the cells with IL-1 induced the release of BPA and CSA from both the primary and immortalized cells (closed symbols). Since immortalized fibroblasts responded to IL-1 induction in a similar fashion to their primary counterparts, we found it convenient to use the immortalized DF in the expression experiments described below so that any possible effects caused by aging of fibroblasts in culture did not complicate the results.

Monocytes, endothelial cells, and fibroblasts produce M-CSF mRNA constitutively. Monocytes, induced by LPS or phorbol myristate acetate (PMA), but not TNF, released BPA and CSA into the medium (not shown). RNA extracted from monocytes without and with exposure to LPS showed similar levels of M-CSF mRNA (not shown). RNA was extracted from HUVE without and with induction by TNF, and from DF-KSV without or with induction by TNF or IL-1. After electrophoresis and transfer to nitrocellulose,

Fig 1. IL-1 induces primary and immortalized fibroblasts to produce burst-promoting and colony-stimulating activities. Enriched human bone marrow progenitors were cultured with increasing concentrations of medium conditioned by incubating primary dermal fibroblasts (A — A), immortalized dermal fibroblasts (B — B), or immortalized synovial fibroblasts (C — C) in IL-1 at 10 U/mL for 48 hours. (A) BFU-E and (B) CFU-GM were counted on day 14. Bone marrow progenitors were also incubated with medium conditioned by fibroblasts that had been incubated without IL-1 for 48 hours. The fibroblast source for these CM is indicated by open symbols that correspond to the closed symbols above. A similar lack of BPA/CSA production was observed when fibroblast growth medium alone was supplemented with IL-1 at 10 U/mL.

The blots were probed with a cDNA probe to M-CSF. Figure 2 shows that the uninduced and TNF-induced HUVE contained approximately equal amounts of M-CSF RNA. Likewise, DF-KSV, whether uninduced or induced with TNF or IL-1, contained approximately equal amounts of M-CSF.
mRNA. In contrast, GM-CSF and G-CSF mRNA transcripts were only detected in IL-1 or TNF-induced HUVE and DF-KSV, while IL-3 mRNA was undetectable in all three cell types, without or with exposure to inducing agents (not shown).

**Monocytes increase GM-CSF and G-CSF expression after exposure to LPS.** Figure 3 shows the results of a Northern analysis before and after exposure of monocytes to LPS. While the GM-CSF and G-CSF message was undetectable in monocytes incubated in medium alone, cells exposed for four hours to LPS were strongly positive for GM-CSF and G-CSF mRNA. Simultaneous hybridization using an actin probe showed that similar amounts of mRNA were present in the LPS-treated and untreated lanes. Simultaneous hybridization for transcripts from the β-chain of the T-cell receptor gene was negative in the monocytes but positive in the MLA-144 T-lymphoblast control, indicating that there was no significant T-lymphocyte contamination of the monocyte preparation.

**Immunoprecipitation of GM-CSF from endothelial cells and monocytes.** Endothelial cells were incubated without or with IL-1 for 16 hours in medium containing 35S-methionine. The supernatants (CM) or cell lysates were collected and proteins immunoprecipitated with a sheep antiserum to GM-CSF. The results are shown in Fig 4. HUVE incubated in medium alone released a 30-Kd immunoprecipitable protein (lane 6), and this was increased after exposure to IL-1 (lane 8). As controls, COS cells transfected with expression vectors containing G-CSF, GM-CSF, and M-CSF cDNAs were similarly labeled with 35S-methionine. Lanes 1, 3, 5, 7 (negative controls) show no immunoprecipitable protein species when CM from COS cells producing G-CSF (COS (G)), GM-CSF (COS (GM)), and HUVE without or with IL-1 were incubated with pre-immune normal rabbit serum. Similarly, no bands were observed when supernatants from COS cells producing G-CSF (lane 2) or M-CSF (not shown) were incubated with antiserum to GM-CSF, while COS cells producing GM-CSF showed a typically diffuse ~20-30 Kd band immunoprecipitable with the antiserum to GM-CSF.

**Uninduced and IL-1–induced HUVE secrete a CSA that stimulates murine CFU-M.** We wished to confirm the results of the M-CSF mRNA analysis at the protein level but were unable to consistently demonstrate M-CSF by immunoprecipitation. Human M-CSF acts on murine progenitor cells and induces macrophage colonies. We collected conditioned media from HUVE that had been incubated for 18 hours without or with IL-1 and tested the ability of these to stimulate murine CFU-M. The results are shown in Table 1.
Table 1. Effect of HUVE-Conditioned Medium on Murine Bone Marrow Colony Formation

<table>
<thead>
<tr>
<th>Addition</th>
<th>CFU-G</th>
<th>CFU-M</th>
<th>CFU-GM</th>
</tr>
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<tbody>
<tr>
<td>Medium alone</td>
<td>6.4</td>
<td>2.4</td>
<td>0.1</td>
</tr>
<tr>
<td>PWM-SCM (8%)</td>
<td>37.3</td>
<td>37.3</td>
<td>13.9</td>
</tr>
<tr>
<td>rh M-CSF 120 U</td>
<td>6.3</td>
<td>61.57</td>
<td>5.3</td>
</tr>
<tr>
<td>rh M-CSF 120 U + α-M-CSF†</td>
<td>2.0</td>
<td>51.49</td>
<td>1.0</td>
</tr>
<tr>
<td>rh M-CSF 12 U</td>
<td>16.7</td>
<td>40.49</td>
<td>4.4</td>
</tr>
<tr>
<td>rh M-CSF 12 U + α-M-CSF†</td>
<td>0.0</td>
<td>1.0</td>
<td>0.0</td>
</tr>
<tr>
<td>HUVE CM 10%</td>
<td>23.27</td>
<td>17.20</td>
<td>2.3</td>
</tr>
<tr>
<td>HUVE CM 10% + α-M-CSF†</td>
<td>24.30</td>
<td>3.3</td>
<td>2.0</td>
</tr>
<tr>
<td>HUVE/IL-1 CM 10%</td>
<td>52.44</td>
<td>16.10</td>
<td>0.0</td>
</tr>
<tr>
<td>HUVE/IL-1 CM 10% + α-M-CSF†</td>
<td>61.41</td>
<td>2.3</td>
<td>1.0</td>
</tr>
</tbody>
</table>

*HUVE growth medium.
†α-M-CSF: 2.5 μL rabbit polyclonal antiserum to M-CSF.

Both uninduced and IL-1-induced HUVE stimulated the formation of CFU-G and CFU-M. The CFU-M were inhibited by the antiserum to M-CSF. Controls included cultures established in the presence of either mouse pokeweed mitogen-treated spleen-conditioned medium (PWM-SCM) or human recombinant M-CSF, without and with the rabbit antiserum to M-CSF. CFU-M were inhibited by antiserum when stimulated by 12 units but not 120 units of recombinant human M-CSF. These data suggest that both uninduced and induced HUVE constitutively produce M-CSF.

Immortalized HUVE (HUVE-KSV) contain IL-1 messenger RNA. We previously demonstrated that HUVE-KSV, in contrast to primary HUVE and to DF and DF-KSV, did not require exposure to IL-1 or TNF for expression of GM-CSF and G-CSF messenger RNA (mRNA), but rather expressed them constitutively. In order to investigate this difference in monokine responsiveness, we extracted RNA from HUVE, HUVE-KSV, HUVE-MSV, DF, and DF-KSV. After electrophoresis and transfer to nitrocellulose, the blot was hybridized to a 32P-labeled IL-1 cDNA probe and exposed to Kodak XAR film for 14 hours. While HUVE, DF, and DF-KSV contain no detectable IL-1, both HUVE-KSV and HUVE-MSV are strongly positive for IL-1 mRNA.

DISCUSSION

It is established that monocytes respond to endotoxin by releasing several monokines, including IL-1 and TNF. We show here that endotoxin-treated human monocytes also express GM-CSF and G-CSF mRNA. It is also known that both endothelial cells and fibroblasts can respond to the monokines IL-1 and/or TNF by synthesis of GM-CSF mRNA. Recently it has been shown that IL-1 or TNF-α

Table 2. Immortalized Endothelial Cells Produce Interleukin-1

<table>
<thead>
<tr>
<th>Added Factor</th>
<th>Incorporated Thymidine (cpm) + SD*</th>
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<tbody>
<tr>
<td>0</td>
<td>230</td>
</tr>
<tr>
<td>Con A†</td>
<td>1,151</td>
</tr>
<tr>
<td>IL-1†</td>
<td>1,314</td>
</tr>
<tr>
<td>Con A + IL-1</td>
<td>7,820</td>
</tr>
<tr>
<td>HUVE</td>
<td></td>
</tr>
<tr>
<td>25% CM</td>
<td>301</td>
</tr>
<tr>
<td>50% CM</td>
<td>362</td>
</tr>
<tr>
<td>HUVE + Con A</td>
<td></td>
</tr>
<tr>
<td>25% CM</td>
<td>2,544</td>
</tr>
<tr>
<td>50% CM</td>
<td>2,080</td>
</tr>
<tr>
<td>HUVE-KSV</td>
<td></td>
</tr>
<tr>
<td>25% CM</td>
<td>537</td>
</tr>
<tr>
<td>50% CM</td>
<td>398</td>
</tr>
<tr>
<td>HUVE-KSV + Con A</td>
<td></td>
</tr>
<tr>
<td>25% CM</td>
<td>19,786</td>
</tr>
<tr>
<td>50% CM</td>
<td>18,403</td>
</tr>
</tbody>
</table>

*D10.G4 murine T-cell line is dependent on IL-1 and a T-cell antigen receptor signal (Con A) for proliferation. Results are expressed as counts per minute of 3H-thymidine incorporated over 24 hr +/− SD.
†Con A, at a concentration of 4 mg/mL.
‡Interleukin 1, at a concentration of 10 U/mL.
induce G-CSF production in lung fibroblasts and GM-CSF, G-CSF, and M-CSF production in endothelial cells as well. In contrast to the endothelial cell results of Seelentag et al and monocyte results of Oster et al, we did not observe induction of M-CSF in any cell type. Rather, M-CSF was produced constitutively by monocytes, endothelial cells, and immortalized fibroblasts, and its expression at the RNA or the protein level was not increased by incubation of monocytes in LPS or endothelial cells and fibroblasts in IL-1 or TNF. These results are consistent with studies of dermal fibroblasts reported recently. In contrast, all three cell types showed barely detectable GM-CSF or G-CSF mRNA expression when uninduced, but increased mRNA expression after incubation with appropriate inducers. At the protein level, immunoprecipitable GM-CSF and murine-active granulocyte CSA, presumably G-CSF, were detectable in conditioned media from uninduced HUVE. These data suggest that the human bioassay may be insufficiently sensitive to detect these levels of expression. Furthermore, stabilization of GM-CSF mRNA and possibly G-CSF mRNA after induction may be necessary for detection by Northern blotting. IL-3 mRNA was never detected in these cells. The presence of M-CSF protein in medium conditioned by uninduced and IL-1-induced HUVE was confirmed by a murine bioassay of CFU-M carried out in the absence or presence of antisera to M-CSF. In contrast, HUVE supernatants showed an increase of immunoprecipitable GM-CSF after induction with IL-1.

The results provide further support for the importance of IL-1 and/or TNF in the regulation of hematopoiesis. Both monokines have similar actions on responsive cells and induce the release of G-CSF and GM-CSF. Although we have not examined bone marrow mesenchymal cells directly in this report, our results indicate that fibroblasts from a variety of sources are capable of the synthesis and release of several CSFs, and bone marrow "fibroblasts" have been shown to produce CSA/BPA. G-CSF and GM-CSF have been postulated to play a role in the inflammatory response. It is possible that endotoxin released during infections stimulates the production of IL-1/TNF and GM-CSF/G-CSF by monocytes. The monokines in turn release the inducible GM-CSF and G-CSF locally from fibroblasts and endothelial cells, and at a distance from bone marrow fibroblastoid reticular cells and endothelial cells. GM-CSF inhibits neutrophil migration and stimulates the function of mature neutrophils, eosinophils, and monocytes, while G-CSF activates neutrophil cytotoxic function as well. IL-1/TNF-mediated release of these two CSFs may therefore also play an important role locally at the site of tissue inflammation.

M-CSF mRNA synthesis does not appear to be as tightly regulated as G-CSF or GM-CSF, since this factor is produced "constitutively" by both endothelial cells and the fibroblasts. It is possible that unknown factors present in the culture media could induce the production of M-CSF by cells in culture, and this could explain the differences in our results from those of Seelentag et al and Oster et al. However, M-CSF does appear to be necessary for the survival of macrophages, and its continuous production by ubiquitous cells such as fibroblasts, endothelial cells, or, indeed, monocytes themselves, could provide sufficient M-CSF protein to fulfill this role.

Retrovirus-immortalized endothelial cells produce both GM-CSF and G-CSF constitutively, ie, without exposure to IL-1 or TNF (not shown). This contrasts sharply with the IL-1 or TNF requirement of primary HUVE and both primary and immortalized fibroblasts for the production of GM-CSF and GM-CSF. It is intriguing that, in contrast to the latter three cell types, the immortalized HUVE contain IL-1 mRNA and secrete IL-1 protein. This suggests that IL-1 may act by an autocrine mechanism in these virus-infected cells and continuously induces the production of these CSFs. The fact that both KSV- and MSV-immortalized HUVE contain IL-1 mRNA suggests that this is not a unique event associated with the KSV immortalization, but is rather a characteristic of endothelial cells infected with murine sarcoma viruses.

GM-CSF has already been subjected to clinical trial and clinical assessment of the other CSFs is being planned. In conclusion, it is hoped that these studies of the cellular sources and mechanism of regulation of CSF production will begin to establish a basis for assessing impaired production in disease states. It is only by these means and by the measurement of circulating CSF levels that CSF therapy can be established on rational grounds.

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REFERENCES


43. Weisbart RH, Golde DW, Clark SC, Wong GG, Gasson


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CA Sieff, CM Niemeyer, SJ Mentzer and DV Faller