Interleukin 1 Stimulates Fibroblasts to Synthesize Granulocyte–Macrophage and Granulocyte Colony-stimulating Factors

Mechanism for the Hematopoietic Response to Inflammation

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Abstract

IL-1 is a family of polypeptides which play a critical role in the inflammatory response. Characteristics of this response include an enhanced release of bone marrow neutrophils, activation of circulating and tissue-phase phagocytes, and enhanced production of neutrophils and monocytes. We have sought to understand the hematopoietic response to acute and chronic inflammatory states on a cellular and molecular level. Colony-stimulating factors (CSFs) are glycoproteins involved in the production and activation of neutrophils and monocytes in vitro and in vivo. We have found that quiescent dermal fibroblasts constitutively release granulocyte–macrophage CSF (GM-CSF), granulocyte CSF (G-CSF), and macrophage CSF in culture, and that picomolar concentrations of the inflammatory mediator IL-1 stimulate by at least fivefold the transcription and release of GM-CSF and G-CSF. These findings establish the role of IL-1 in the hematopoietic response to inflammation through the stimulation of the production and release of GM-CSF and G-CSF.

Introduction

Colony-stimulating factors (CSFs)[1] are acidic glycoproteins required for the survival, proliferation, and differentiation of hematopoietic progenitors in semi-solid medium. Four human CSFs have been characterized—granulocyte–macrophage CSF (GM-CSF), granulocyte CSF (G-CSF), macrophage CSF (M-CSF or CSF-1), and multi-CSF or IL-3 (for review, see reference 1)— which differ in progenitor cell specificity and physicochemical properties. Originally recognized on the basis of their effects in vitro, GM-CSF, G-CSF, and multi-CSF have been reported to stimulate in vivo hematopoiesis as well (2–4), making these molecules likely physiologic regulators of blood cell production. Culture medium conditioned by several different tissues will support the growth of hematopoietic progenitor cells. When stimulated, fibroblasts, endothelial cells, smooth muscle cells, and lymphocytes secrete CSAs, making these cells candidate sources of hematopoietic growth factors in the marrow microenvironment.

Our understanding of the cells of origin of the CSFs and the details of the regulation of production of the various CSFs is incomplete, but a recent model suggests that soluble products of monocytes play a central role. Bagby et al. have reported that monocyte-conditioned medium stimulates the production and release of CSAs from fibroblasts (5), endothelial cells (6), and lymphocytes (7). This CSA(s) has the capacity to stimulate the growth of GM, G, megakaryocytic, and mixed-cell colonies as well as erythroid bursts. IL-1 is a family of related polypeptides produced primarily by monocytes and which is a key mediator of the host response to infectious, inflammatory, and immunologic challenges. At least two polypeptides possessing IL-1 activity (IL-1α and IL-1β) have been identified and cloned (8). T lymphocytes, B lymphocytes, fibroblasts, endothelial cells, hepatocytes, neutrophils, and myocytes can be stimulated by IL-1 to generate many features of the inflammatory reaction (for review, see references 9 and 10). The effects of acute and chronic inflammation on the hematopoietic system include release of neutrophils from the vascular marginal pool, premature release of neutrophils from the marrow into the circulation, enhanced neutrophil chemotaxis to, and activation at, sites of inflammation, and a sustained increase in neutrophil and monocyte production. Many of these responses are evoked by the various CSFs in vitro.

We are interested in the molecular basis for the effects of IL-1 on hematopoiesis and cells of the marrow microenvironment. In this report, we present the results of studies of the effect of IL-1 on quiescent human dermal fibroblasts. When stimulated by picomolar concentrations of purified recombinant human IL-1, fibroblasts elaborate multiple CSAs into the surrounding medium. The release of these CSAs coincides within a fivefold increase of GM-CSF and G-CSF transcripts in the cytoplasm of the cells, and the appearance of GM-CSF and G-CSF in the culture medium.

Methods

Cell culture. Tenth-passage human dermal fibroblasts (kindly provided by Elaine Raines) were cultured in RPMI 1640 medium (Gibco, Grand Island, NY), supplemented with 10% FCS (HyClone, Inc., Logan, UT), penicillin (100 U/ml), streptomycin (100 μg/ml), and nystatin (25 ng/ml), and grown to confluence. Fresh medium containing 2% platelet-poor plasma was added, and the cells incubated for 48 h (quiescent cultures). Finally, medium containing 10–1,000 pg/ml (10 pg, 1 U) of purified recombinant human IL-1α (Genzyme Corp., Boston, MA) was added, and the cells were cultured for 6–24 h. To prepare mitogen-stimulated lymphocytes and their conditioned media, peripheral blood mononuclear cells were cultured at 1×10^6 cells/ml in α-medium containing 10% FCS, antibiotics, and 1% PHA. Spent cul-

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1. *Abbreviations used in this paper:* BFU-E, erythroid burst-forming unit; CSF, colony-stimulating factor; G, granulocyte; GM, granulocyte–macrophage; M, macrophage, PHA-LCM, PHA-stimulated lymphocyte-conditioned medium; CFU-Meg, megakaryocytic CFU; CFU-Mix, mixed CFU.

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ture medium was collected after 7 d (11). To prepare a positive CSA control for murine colony-forming cells, the murine splenic lymphoma cell line LBRM (kindly provided by S. Gillis) was grown in the presence of 1% PHA for 24 h and the medium collected.

Marrow colony-forming cell assays. Human marrow cells were obtained from normal volunteers with their informed consent, and fractionated on a Ficoll-Hypaque density gradient (1.077 g/ml; Litton Bionetics, Inc.). Low density cells were depleted of adherent cells by double-plastic adherence and of T cells by E rosetting (7). The nonadherent, T-depleted cells (5×10^4/ml) were plated in α-medium (Gibco) containing 10% FCS, antibiotics, 0.1–10% by volume of the material to be assayed and the cultures were made semi-solid with 1% methylcellulose (Dow). Cultures were grown for 13 d in a humidified atmosphere containing 5% CO_2, and colonies were enumerated with an inverted microscope. 50 U of GM-CSA is defined by the dilution that stimulates half-maximal colony formation, compared with an optimal concentration of PHA-stimulated lymphocyte-conditioned medium (PHA-LCM) as a control. For the growth of megakaryocytic colonies (from megakaryocytic CFU [CFU-Meg]), 25% human plasma was used instead of FCS (11) and, for the growth of erythroid bursts (from erythroid burst-forming units [BFU-E]) and mixed erythroid–nonerythroid colonies (from mixed CFU [CFU-Mix]), 1 U of purified recombinant human erythropoietin (AMGen, Inc., Thousand Oaks, CA) was added to cultures on day 4 (12).

For murine granulocytic colony growth, LA/F_1 mice were killed by cervical dislocation and femoral bone marrow obtained. 1×10^5 cells were plated in α-medium with 10% FCS, antibiotics, 1–10% by volume of the conditioned medium to be assayed, and 0.3% agar. Cultures were incubated for 5 d. Whole 1-ml cultures were fixed and stained for chloroacetate esterase and counterstained with toluidine blue. Pure granulocytic colonies were enumerated by direct microscopy. 50 U of G-CSA were defined as for GM-CSA, except comparison was to an optimal concentration of PHA-stimulated LBRM-conditioned medium.

Nucleic acid probes. Anti-sense oligonucleotides, 40 bases in length and corresponding to bases 755 to 794 of the human GM-CSF gene (13), bases 151 to 190 of a human G-CSF cDNA (14), bases 232 to 271 of a human M-CSF cDNA (15), and bases 73 to 112 of the human multi-CSF gene (16) were synthesized by the phosphotriester method (model 380A; Applied Biosystems Inc., Foster City, CA). The oligonucleotides were purified by polyacrylamide gel electrophoresis and end labeled with [γ^{32}P]ATP and polynucleotide kinase ($2–4 \times 10^8$ cpm/μg sp act). These probes were specific for their respective growth factors as they contain no significant sequence homology and bind exclusively to appropriately sized transcripts in cells or cell lines producing M-CSF (MiaPaCa [15]), G-CSF (5637 [17]), GM-CSF (5637 [18]), or multi-CSF (PHA-stimulated lymphocytes [16]). Full-length cDNAs for human GM-CSF (19) or for human G-CSF were nick-translated to high specific activity ($1–4 \times 10^8$ cpm/μg) (20) and boiled before use.

RNA preparation and analysis. Quiescent or IL-1–stimulated fibroblasts were rinsed in PBS and lysed with 5 M guanidium thiocyanate (21). RNA was recovered from a 5.7 M CsCl step gradient and resuspended in diethylpyrocarbonate-treated water. Samples were enriched for poly A–containing RNA by chromatography over oligo (dT) cellulose (22). Aliquots were denatured with formamide and formaldehyde and size-fractionated by electrophoresis through 1.4% agarose–formaldehyde gels (23). The RNA was transferred to nitrocellulose, prehybridized at 42°C for 4–6 h in 5× SSC (1× SSC = 0.15 mM NaCl/15 mM Na citrate, pH 7.0), 2× Denhardt's, 50 mM Na phosphate, pH 7.0, 20% formamide, 10% dextran sulfate, 1 mM Na pyrophosphate, 50 μg/ml ATP, and 50 μg/ml sonicated salmon sperm DNA (24). Twenty-million counts per minute of probe were added to 10 ml of prehybridization solution and the blot was hybridized at 42°C for 12–16 h. For oligonucleotide probes, the blots were washed sequentially in 2× SSC/0.1% $NaDodSO_4$ at 20°C, 40°C, and 65°C and prepared for autoradiography. For hybridization to the cDNA probes, the blots were hybridized as above except for the presence of 50% formamide, and were washed sequentially in 0.2× SSC/0.1% $NaDodSO_4$ at 20°C, 40°C, and 65°C. The blots were probed sequentially with the M-CSF, GM-CSF, G-CSF, and the multi-CSF probes. Bound probe was removed by washing in water at 95°C for 20 min between each hybridization.

Anti–GM-CSF antiserum and Western blot analysis. A 15 amino acid peptide that contains 13 residues near the carboxyl terminus of human GM-CSF (ESFKENLKDFLLV) was crosslinked to keyhole limpet hemocyanin, emulsified with complete Freund's adjuvant, and used to immunize NZY rabbits. After three booster immunizations using incomplete adjuvant, high anti-peptide antibody titers were detected. GM-CSF was found to react with this antiserum on Western blots when the protein was denatured and reduced in situ (19). Proteins found to be nonreacting included insulin, transferrin, recombinant human G-CSF (AMGen, Inc.), recombinant platelet-derived growth factor (Zymogenetics, Inc., Seattle, WA), and recombinant human M-CSF (Cetus, Inc., Emeryville, CA). However, several high molecular weight plasma proteins crossreacted with this antiserum when the Western blot was handled as described. Fibroblast-conditioned media containing 2% platelet-poor plasma were concentrated 30-fold by ultrafiltration (Amicon PM-10), reduced by boiling in 0.5 M β-mercaptoethanol and 0.5% $NaDodSO_4$, and 10-μl aliquots were size-fractionated by electrophoresis through 15% polyacrylamide gels. Proteins were transferred to nitrocellulose by electroblotting (25) and were reduced on the blot as described (19). The Western blot was probed with a 1:200 dilution of antiserum and with a 1:1,000 dilution of ^{125}I-goat anti-rabbit IgG antiserum.

Results

Fibroblast-derived CSA. As shown in Table I, the culture medium conditioned by quiescent, confluent fibroblasts supports low level growth of hematopoietic progenitor cells in semi-solid culture. By dose-response analysis, a mean of 150 U/ml of CSA was detected in six separate experiments. In contrast, the addition of IL-1α at concentrations from 10 to 1,000 pg/ml greatly enhanced the release of CSAs into the medium. As shown, IL-1α at 100 pg/ml resulted in the maximal amount of CSA detected at 24 h. IL-1α alone at 10–100 pg/ml had a negligible effect on GM colony growth and no effect on erythroid burst or megakaryocyte colony growth (data not shown).

Table I. Dose Response and Time Course of Fibroblast-derived CSA Release

IL-1	Time	GM-CSA
pg/ml	*h*	*U/ml × 10³*
0	24	0.15±0.05
10	24	0.90±0.16
100	24	2.20±0.39
1,000	24	1.60±0.18
100	6	0.30±0.03
100	12	2.10±0.24
100	18	3.00±0.29
100	24	3.70±0.56

Serial dilutions of fibroblast-conditioned medium were plated in standard colony-forming assays. GM colonies were enumerated and 50 U of activity was defined by the dilution which produced a half-maximal number of GM colonies compared with an optimal concentration of PHA-LCM, which produced 46±4 colonies/5×10^4 low density, nonadherent, T-depleted cells. The results represent the mean±SEM of four separate experiments.

Next, the time course of CSA induction was studied using 100 pg/ml IL-1α. Within 6 h of the addition of IL-1α to quiescent, confluent fibroblasts, elevated levels of CSA were detectable in the culture medium. CSA continued to accumulate in the medium up to 24 h of culture.

To assess the ability of the fibroblast-derived growth factor(s) to stimulate in vitro erythropoiesis and megakaryopoiesis, assays for BFU-E, CFU-Meg, and CFU-Mix were performed. In addition to their stimulation of GM colonies, the CSAs released by IL-1α–stimulated fibroblasts support the growth of erythroid bursts, mixed erythroid–nonerythroid colonies, and megakaryocytic colonies. As shown in Fig. 1, stimulation of erythroid bursts occurred at all dilutions of conditioned medium that stimulated GM colony growth. In addition, a near-maximal number of mixed-cell colonies, and approximately half-maximal numbers of megakaryocytic colonies developed, compared with an optimal concentration of PHA-LCM.

RNA analysis. To determine the molecular basis of the apparent multiple CSAs elaborated by IL-1α–stimulated fibroblasts, RNA was extracted from these cells and from quiescent fibroblasts, and probed for the presence of known hematopoietic growth factor mRNA. Oligonucleotides complementary to the 5′ end of human multi-CSF, GM-CSF, G-CSF, or M-CSF mRNA were prepared and used to probe Northern blots of fibroblast RNA.

The Northern blot pattern of poly A RNA derived from cells known to produce M-CSF is complex, but growth conditions known to stimulate the production of M-CSF are associated with the presence of three to six hybridizing bands (15). Three hybridizing bands were seen in quiescent fibroblast RNA when the blot was probed for M-CSF–specific mRNA (Fig. 2).

A clear, low intensity signal was seen when unstimulated fibroblast RNA was probed for GM-CSF and for G-CSF–specific message. However, no signal was seen when the blot was hybridized with a probe for human multi-CSF (data not shown). When stimulated with IL-1α (100 pg/ml) for 8 h, strong signals for GM-CSF and G-CSF were seen. The signals seen with the GM-CSF and G-CSF oligonucleotide probes were confirmed with full-length cDNA probes (data not shown). The signal for M-CSF–specific RNA was little changed from that seen in unstimulated fibroblast RNA. Again, no signal for human multi-CSF was detected.

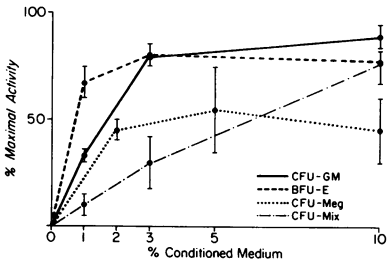

Figure 1. Dose-response analysis of fibroblast-conditioned medium. Increasing concentrations of IL-1α–stimulated fibroblast-conditioned medium were used to support the growth of BFU-E–CFU-GM–CFU-Meg–, and CFU-Mix–derived colonies. The data are expressed as the percent of colonies produced in response to an optimal concentration of PHA-LCM (range for GM colonies, 24 to 49, erythroid bursts, 42 to 47, megakaryocytic colonies, 5 to 8, and mixed-cell colonies, 3 to 8 per 5×10^4 low density, nonadherent, T-depleted marrow cells). The data are from a representative experiment. Similar results have been obtained in three separate experiments.

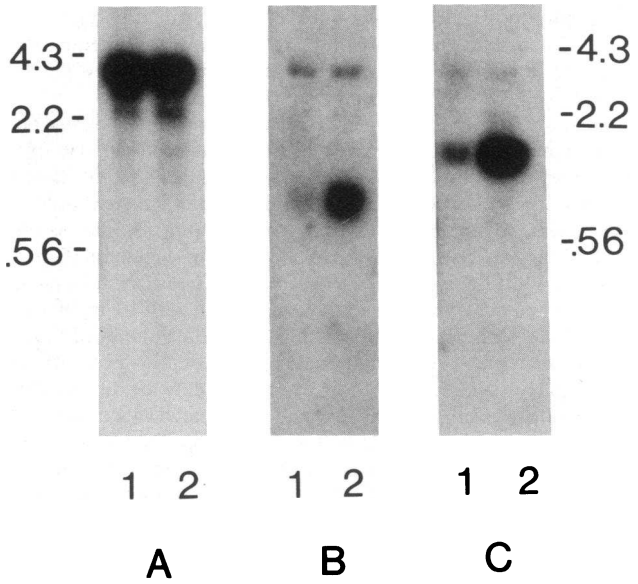

Figure 2. Northern blot analysis of fibroblast RNA. Five μg of poly A(+) RNA were denatured and size-fractionated by formaldehyde–agarose gel electrophoresis (20). Lane *1*, nonstimulated fibroblasts; lane *2*, IL-1-α (100 pg/ml)–stimulated fibroblasts. The blot was hybridized with oligonucleotide probes complementary to (*A*) M-CSF, (*B*) GM-CSF, or (*C*) G-CSF. The size markers represent fragments of λDNA digested with Hind III. The minor bands seen at ~ 4 kb in *B* and *C* represent radioactivity that could not be removed after hybridization with the M-CSF probe (*A*).

After autoradiography with the cDNA probes, and after rehybridization with the M-CSF probe, the hybridizing bands were cut from the blot and counted directly for β-emissions. Five times more GM-CSF or G-CSF probe hybridize with 5 μg of IL-1α–stimulated fibroblast poly A RNA than with the same amount of unstimulated fibroblast poly A RNA. The bands hybridizing to the M-CSF probe contained nearly the same amount of labeled probe.

Detection of specific CSFs in conditioned media. To detect human M-CSF, conditioned media were analyzed by radioimmunoassay (26). Medium conditioned by quiescent fibroblasts contained 119 U/ml of M-CSF. IL-1α–stimulated fibroblast-conditioned medium contained 155 U/ml M-CSF. These levels are significantly above the detectable level ($\geq$ 25 U/ml), but are not significantly different (error not greater than 20%).

To detect human G-CSF (17), cultures of murine bone marrow stimulated with fibroblast-conditioned medium were evaluated by cytochemical techniques for neutrophilic colonies. Human multi-CSF and human GM-CSF fail to stimulate the growth of murine CFU-GM–derived colonies. Although human M-CSF is a potent stimulus of murine CFU-M, few, if any, of the resultant colonies are purely neutrophilic. Thus, the growth of murine neutrophilic colonies in response to human fibroblast-conditioned medium suggests either the presence of an as yet undescribed hemopoietin or, more likely, the presence of human G-CSF. As shown in Table II, quiescent fibroblast-conditioned medium supports the growth of a small number of murine neutrophilic colonies. When compared with a standard source of recombinant human G-CSF, ~ 280 U/ml of G-CSF was detected. When stimulated with IL-1α, fibroblasts release increased quantities of G-CSF into their me-

Table II. Murine Neutrophilic Colony Assay

Addition	Neutrophilic colonies
Saline	2.0±1.6
PHA-LBRM	74.0±12.3
Human G-CSF	
100 U	22.3±2.1
1,000 U	39.7±1.2
Human GM-CSF	
800 U	0.3±0.5
FCM_0	
1%	3.3±1.7
10%	19.4±13.6
$FCM_{IL\text{-}1}$	
1%	22.5±1.5
10%	41.4±19.8

Dilutions of quiescent and IL-1α–stimulated fibroblast-conditioned medium (FCM) were plated in a murine colony assay. Only pure neutrophil colonies (chloroacetate esterase positive) were enumerated. Culture medium from the murine cell line LBRM stimulated with PHA served as a positive control, and purified recombinant human G-CSF (AMGen, Inc.) and recombinant human GM-CSF were used to demonstrate the specificity of the assay. The results represent the mean±SEM of four separate experiments. FCM_0 represents medium conditioned by unstimulated fibroblasts; $FCM_{IL\text{-}1}$ represents the medium conditioned by IL-1α–stimulated fibroblasts.

dium. When quantitated by dose-response analysis, 3,040 U/ml were detected.

Finally, to detect human GM-CSF protein, we used a rabbit antiserum raised against a human GM-CSF peptide to probe Western blots of fibroblast-conditioned medium. As shown in Fig. 3, GM-CSF was undetectable in 30-fold concentrated conditioned medium from quiescent fibroblasts. The lower limit of detection by this assay is ~ 400 U. Clear signals for glycosylated and nonglycosolated GM-CSF are present in 30-fold concentrated IL-1α–stimulated fibroblast-conditioned medium.

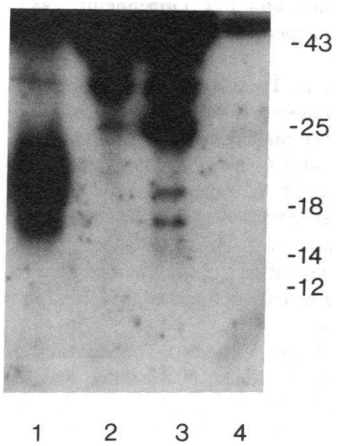

Figure 3. Western blot analysis of fibroblast-conditioned medium. Unstimulated (lane *2*) and IL-1-α–stimulated (lane *3*) fibroblast-conditioned media were concentrated 30-fold by ultrafiltration, reduced, and size-fractionated by electrophoresis. Proteins were transferred by electroblotting and the nitrocellulose probed for GM-CSF by an anti–GM-CSF peptide rabbit antiserum. Positive and negative controls included 8,000 U of recombinant human GM-CSF (lane *1*) and 100 ng of recombinant human G-CSF (lane *4*). Molecular weight markers are shown.

Discussion

Fibroblasts are cells that have diverse functions. They provide the scaffolding required for cellular organization, maintain the extracellular matrix required for tissue cohesion, and may function as antigen-presenting cells (27–29). Fibroblasts are present in the bone marrow, and have been postulated to function as part of an inductive microenvironment (30, 31). Fibroblasts are also effector cells of the host's response to injury. They are the primary cell of tissue repair, and respond to IL-1 by proliferation and collagen synthesis and assembly (9, 32). In support of their role in hematopoietic regulation as part of the marrow microenvironment, we report here that, when human fibroblasts are stimulated by concentrations of IL-1α as low as 10 pg/ml, large amounts of biologically active hematopoietic growth factor(s) are released.

Recently, Zucali et al. reported that IL-1β stimulates fibroblasts to release a GM-CSA (33). We confirm these results for IL-1α, and extend them. In addition to the stimulation of G and M colonies, medium conditioned by fibroblasts stimulated by picomolar concentrations of IL-1α supports the growth of megakaryocyte colonies and, in the presence of erythropoietin, erythroid bursts and mixed hematopoietic cell colonies.

Human GM-CSF is a 22-kD acidic glycoprotein that supports the growth of five lineages of hematopoietic cells—neutrophils, eosinophils, monocytes, erythrocytes, and megakaryocytes—in semi-solid medium. Human G-CSF is an 18-kD glycoprotein that primarily supports the growth of neutrophilic colonies but, at tenfold higher concentrations, can support the growth of erythroid and mixed hematopoietic cell colonies (17). Human M-CSF is a homodimer glycoprotein of 44 kD that exclusively supports the growth of monocyte–macrophage colonies (15). Human multi-CSF is a 15–25-kD glycoprotein that stimulates a wide range of hematopoietic cell types (16). We sought to investigate whether any of these well-characterized hematopoietic growth factors, or a novel growth factor, was responsible for the multilineage growth-promoting activity released by IL-1α–stimulated fibroblasts.

Using poly A–enriched cellular RNA from quiescent cultures of fibroblasts, and from cells stimulated with IL-1α, we probed for the presence of known hematopoietic growth factor–specific transcripts. No signal was detected for multi-CSF–specific RNA, a finding consistent with results obtained using murine tissues. To date, stimulated lymphocytes are the only physiologic source demonstrated to produce this multilineage hemopoietin (34). Using an oligonucleotide probe specific for human M-CSF, we found that quiescent fibroblast RNA contains M-CSF–specific transcripts and that IL-1α stimulation did not alter this level significantly. The hybridization pattern was complex, with three clear bands ranging from 1.8 to 4.0 kb detected. cDNA clones derived from both the 4.0- and 1.8-kb transcripts have been shown to direct the synthesis of biologically active M-CSF (15, 35). As the gene for human M-CSF is present in a single copy in the haploid genome, and since the previously published cDNA clones differ primarily by the deletion of 894 bp of coding sequence (35), these data taken together suggest that the multiple transcripts characteristic of cellular sources rich in M-CSF represent an example of alternate splicing of the primary transcription product.

Initially, we cultured quiescent fibroblasts in the presence of FCS. The conditioned medium was noted to contain low

levels of CSA, and the poly A–enriched RNA contained GM-CSF– and G-CSF–specific transcripts. To reduce or eliminate the possibility that mitogens or cytokines present in the FCS might be responsible for low level stimulation of these cultures, we reduced the protein content of the growth medium to 2%, and substituted platelet-poor plasma for serum. Under these conditions, quiescent endothelial cells (36) and unstimulated lymphocytes (Kaushansky, K., and W. Hammond, unpublished observations), cells that can be induced to produce GM-CSF in response to mitogens and cytokines including IL-1α, contain no detectable growth factor–specific mRNA. Thus, low level stimulation is not a likely explanation for our detection of CSA and GM-CSF– and G-CSF–specific transcripts in quiescent cultures of fibroblasts. When quiescent fibroblasts are stimulated with physiologic levels of IL-1α, the level of CSA rises substantially, and the signals for GM-CSF– and G-CSF–specific mRNA are enhanced fivefold.

Although the appearance of specific mRNA suggests the production of the corresponding polypeptide, RNA accumulation in the absence of protein secretion may occur, as has been demonstrated recently for tumor necrosis factor-α production by human monocytes (37). To confirm the conclusions based on the Northern blot analysis, we used a number of immunologic and functional assays to detect the presence of specific hematopoietic growth factors in quiescent or IL-1α–stimulated fibroblast-conditioned medium.

A sensitive radioimmunoassay detected low levels of M-CSF in both quiescent and IL-1α–stimulated fibroblast-conditioned medium. Using murine neutrophilic colony formation as an indication of the presence of human G-CSF, we found that the conditioned medium from quiescent fibroblasts contained low levels of G-CSF and, when fibroblasts were stimulated for 24 h with 100 pg/ml IL-1α, tenfold higher levels of CSF were detected. Finally, through the use of a specific antiserum, we detected GM-CSF in concentrated IL-1α–stimulated fibroblast-conditioned medium but not in medium conditioned by unstimulated fibroblasts. As the sensitivity of our Western blot system is 400 U, a low level of GM-CSF production by quiescent fibroblasts may have escaped detection.

From these findings, we conclude that fibroblasts increase the transcription, production, and release of GM-CSF and G-CSF in response to stimulation with IL-1α. In addition, small amounts of M-CSF, G-CSF, and GM-CSF are constitutively produced by these cells. No multi-CSF mRNA was detected in either quiescent or IL-1α–stimulated fibroblasts. Whether GM-CSF, G-CSF, and M-CSF account for all of the hematopoietic growth-promoting activity released by fibroblasts cannot be answered by these studies. Note that IL-1, at similar concentrations, stimulates the release of GM-CSF and G-CSF from endothelial cells, cells also found in the marrow microenvironment (36, 38), and from a marrow-derived murine fibroblast/endothelial–like cell line (39). Taken together, these data support the hypothesis that fibroblasts and endothelial cells are important components of the marrow microenvironment.

Monocytes have been shown to release numerous monokines at sites of infection, inflammation, and immunologic reaction. IL-1 can be detected in the plasma of patients with fever, inflammation, or even during physiologic stress, such as after vigorous exercise or ovulation (40, 41). These studies have used a moderately sensitive biological assay for IL-1. We have shown that picomolar concentrations of IL-1α augment the release in vitro of GM-CSF and G-CSF from candidate cells of the marrow microenvironment, and that IL-1 is at least one of the regulatory mechanisms that signals an increase in blood cell production in response to the inflammatory state.

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