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Regulation of Early Human Hematopoietic (BFU-E and CFU-GEMM) Progenitor Cells In Vitro by Interleukin 1–Induced Fibroblast-Conditioned Medium

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Stimulators of human erythroid burst-forming units (BFU-E) and multipotential colony-forming cells (CFU-GEMM) can be produced by a number of different cell types. A product of human peripheral blood monocytes, interleukin 1 (IL-1), was evaluated for its ability to stimulate fibroblast cultures to produce stimulators of human bone marrow BFU-E and CFU-GEMM colony formation. BFU-E and CFU-GEMM colony formation was evaluated using low-density, nonadherent low-density, and T lymphocyte–depleted non-adherent low-density human bone marrow cells cultured in the presence of a source of pure human erythropoietin. Both human monocyte conditioned medium (MCM) and human recombinant IL-1 (hrIL-1) induced fibroblasts to produce stimulators of BFU-E and CFU-GEMM in a dose-dependent fashion with maximal colony formation occurring when fibroblasts were stimulated by 25% MCM or 140 ng/mL ROO6B hrIL-1, or 1.25 to 5 ng/mL ROO6T hrIL-1. Preincubation of MCM and hrIL-1 with an antibody to IL-1 inactivated the ability of MCM and hrIL-1 to induce the release of erythroid and multipotential colony stimulating activity from fibroblasts. These results suggest that monocyte-derived IL-1 is involved in regulating the production of humoral stimulators of early human hematopoietic progenitors.

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GROWTH OF HUMAN hematopoietic progenitor cells in clonal cultures requires the presence of specific soluble stimulating factors. Besides erythropoietin, erythroid burst-forming units (BFU-E) and multipotential (CFU-GEMM) progenitor cells require factors present in medium conditioned by blood leukocytes or other sources of burst promoting activity (BPA).[1,2] A variety of cell types have been implicated in BPA production. These include monocytes and/or macrophages,[3,4] T lymphocytes,[5,6] and endothelial cells.[7] Similar cell types have also been reported to be involved in GM-CSA production.[8–10] Recent studies have focused on the monocyte as a central cell involved in the regulation of GM-CSA and BPA production.[11,12] These studies report that monocytes produce a monokine or monokines which function by recruiting other accessory cells to produce GM-CSA and/or BPA.

We have recently identified interleukin 1 (IL-1) as one monocyte product which induces confluent fibroblast cultures to produce and release GM-CSA.[13] Both purified human monocyte–derived IL-1 and human recombinant IL-1 (hrIL-1) reproduce the biologic activity found in this monocyte conditioned medium (MCM). In addition, a polyclonal antibody to IL-1 abolished this biologic activity. In the present study, we demonstrate that confluent fibroblast cultures, when stimulated by a factor present in MCM or by hrIL-1 also produce stimulators of colony formation by BFU-E and CFU-GEMM. Furthermore, the stimulation of fibroblast cultures by MCM or hrIL-1 can be blocked by the addition of antibody to IL-1.

MATERIALS AND METHODS

Isolation of peripheral blood monocytes. Peripheral blood cells were obtained from normal donors who had given informed consent. Peripheral blood leukocytes were isolated following semicontinuous flow pheresis[14] and the buffy coat was diluted with two volumes of Hanks' balanced salt solution without calcium and magnesium prior to removing platelets by centrifugation at 150 × g for ten minutes. This cell suspension was centrifuged over Ficoll-Hypaque (specific gravity 1.078) to remove contaminating granulocytes and erythrocytes. The mononuclear cells at the interface were collected and separated into two populations, monocytes and lymphocytes, by elutriation centrifugation as previously reported.[13,15] Using this technique, monocytes were collected as a homogeneous cell population and further subjected to 2-aminoethylisothiouronium bromide hydrobromide (AET)–treated sheep erythrocyte depletion to remove any contaminating T lymphocytes. Nonrosetting cells were separated by centrifugation through Ficoll-Hypaque. The purity of nonrosetting cells was determined using OKT3 (Ortho Diagnostics, Raritan, NJ) and LeuM3 (Becton Dickinson, Mountain View, CA) monoclonal antibodies and indirect immunofluorescence with affinity-purified goat antimouse IgG $F(ab')_2$-FITC (Cappel, West Chester, PA). The cells were analyzed using both fluorescence microscopy and a Coulter Model C flow cytometer. Background staining was determined on cells treated with nonimmune mouse immunoglobulins and second antibody. In addition, monocytes were identified on cytocentrifuge-prepared slides stained with Wright's-Giemsa and nonspecific esterase using alpha naphthyl acetate as substrate.[16] The monocyte populations used in all experiments described contained <2% OKT3-positive, >98% LeuM3-positive, and >98% nonspecific esterase–positive cells.

Monocyte-conditioned medium preparation. Fresh or cryopreserved separated monocyte populations were cultured at 5×10^4 cells/mL for two days in alpha minimum essential medium (GIBCO, Grand Island, NY) containing 6% fetal bovine serum (Hyclone, Logan, UT) and 1 ng/mL endotoxin (LPS) (lipopolysaccharide B, *S. typhosa.* (DIFCO). The conditioned media were harvested and stored frozen at −20 °C for assay.

Recombinant human IL-1. Human recombinant IL-1 (hrIL-1)

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Submitted March 19, 1986; accepted July 15, 1986.

Supported by Public Health Service Grants CA 36740 and CA 36464 to H.E.B. and National Institutes of Health Grant No. AI 15614 to C.A.D.

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0006–4971/87/6901–0011$3.00/0

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containing amino acids 71 through 269 and having a molecular weight of 24.5 kD (ROO6B) or another recombinant preparation (ROO6T) containing amino acids 112 through 269 and having a molecular weight of 17.0 kD were obtained as previously described.[13,17] Briefly, hrIL-1–producing bacteria were grown at 37 °C and lysed, and the hrIL-1 was purified by sequential ion exchange and either gel-filtration chromatography or high-performance liquid chromatography (HPLC). The hrIL-1 was stored at −70 °C in a 0.15 mol/L phosphate-buffered saline, pH 5.8. The identity of the purified hrIL-1 was confirmed by amino acid composition and sequence of the amino terminal heptapeptide. Protein concentration was determined by the Bradford method using bovine serum albumin (BSA) as standard. Purity was assessed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) with Coomassie blue or silver staining. The hrIL-1 was estimated to be greater than 99% pure.

Fibroblast conditioned medium preparation. Human lung fibroblasts (CCL 202) were obtained from the American Type Tissue Culture Collection (Rockville, MD) and cultured in Dulbecco's Modified Eagle's Medium (DMEM) (GIBCO) supplemented with 10% fetal bovine serum (Hyclone). Fibroblasts from the first to the tenth passage were used, and the cell density ranged from 1 to 5 $\times 10^4$ cells per square centimeter. Cells were subcultured by brief (one-minute, 37 °C) treatment with 0.05% trypsin/0.02% disodium EDTA (wt/vol in PBS) and resuspended in fresh DMEM plus 10% fetal bovine serum and reseeded at a 1:5 dilution in 35-mm culture plates. At least 24 hours were allowed to elapse after initial seeding or subculture to obtain confluence before an experiment was begun. For assay, the fibroblast cell culture was replaced with 10% to 50% monocyte-conditioned medium, with or without a 1:100 dilution of the IgG fraction of rabbit antihuman Il-1,[18] various concentrations of hrIL-1, or control medium diluted with fibroblast culture medium (FCM), and the cultures were returned to the incubator for an additional 24 hours. As controls, hrIL-1 alone, MCM alone, and FCM in the absence of hrIL-1 or MCM were incubated for 24 hours prior to assay. After 24 hours, the medium was aspirated and centrifuged (200 × g, five minutes), and the supernatants stored at −20 °C until tested.

Bone marrow cell preparation. Bone marrow cells were obtained by aspiration from the posterior iliac crest of healthy volunteers who had given informed consent. Unseparated nucleated buffy coat cells were obtained by centrifugation of whole bone marrow aspirates at 1,500 rpm for ten minutes, and isolation of the white cells that overlay the red cell layer. Low-density (LD) cells (1.077 g/mL) were obtained after separation on Ficoll-Hypaque (Pharmacia, Uppsala) at 1,500 rpm for 30 minutes, washed three times, and resuspended in Iscove's modified Dulbecco medium (IMDM) (GIBCO) containing 10% fetal bovine serum. Cells were further separated into nonadherent low-density (NALD) cells after incubation on plastic tissue culture dishes (Falcon, Oxnard, CA) for 90 minutes at 37 °C and 5% CO_2. NALD cells were collected by gently swirling the dishes and slowly removing the suspension cells. NALD cells prepared in this manner routinely contained <2% nonspecific esterase–positive cells. To deplete T lymphocytes, NALD cells were further separated into E-rosette–positive (E+) populations according to a method modified from Weiner et al[19] as described previously.[20] Nonrosetting cells were separated by centrifugation through Ficoll-Hypaque. The purity of the NALD T lymphocyte–depleted fraction ($NALDT^-$) was analyzed by indirect immunofluorescence with OKT3 or OKT11a as described above. The $NALDT^-$ marrow cell fraction contained <2% OKT11a-positive cells.

BFU-E and CFU-GEMM assay. Bone marrow cells were plated at 1×10^5 for LD and NALD cells and 4×10^4 for $NALDT^-$ cells in 35-mm Lux standard tissue culture dishes containing a 1-mL mixture of IMDM, 1% methylcellulose, 30% FBS, and 5×10^{-5} mol/L 2-mercaptoethanol. Cultures contained 0.1 mmol/L bovine hemin (Eastman Kodak Co, Rochester, NY) for CFU-GEMM colonies, while BFU-E were cultured without hemin.[20,21] Human urinary erythropoietin (Toyoba, New York) or recombinant erythropoietin (Amgen, Thousand Oaks, CA) 1 unit/mL and the conditioned mediums to be tested were added to the dishes on day 0. Results were comparable using the two preparations of erythropoietin. Dishes were incubated at 37 °C in a humidified atmosphere flushed with 5% CO_2 and lowered (5%) O_2 for 14 days, at which time colonies were scored for BFU-E and CFU-GEMM. Lowered oxygen

Table 1. Influence of FCM (MCM) and FCM (IL-1) on Colony Formation From BFU-E and CFU-GEMM by 10^5 Low-Density Normal Human Bone Marrow Cells (LD)

	BFU-E* Experiments		CFU-GEMM† Experiments	
Additions	1	2	1	2
Control medium	56 ± 2‡	32 ± 2	0	0
MCM§ (25%)	69 ± 2		3 ± 1	
MCM (50%)		32 ± 4		0
hrIL-1 (350 ng/mL)	58 ± 3	27 ± 2	0	0
FCM (control)	53 ± 2	30 ± 3	0	0
FCM (MCM 10%)‖	77 ± 3		7 ± 1	
FCM (MCM 25%)	98 ± 1		11 ± 1	
FCM (MCM 50%)		51 ± 3		6 ± 1
FCM (hrIL-1 350 ng/mL)¶	95 ± 7	59 ± 2	9 ± 1	7 ± 1
FCM (hrIL-1 140 ng/mL)	77 ± 2		8 ± 1	
FCM (hrIL-1 70 ng/mL)	73 ± 5		5 ± 1	
FCM (hrIL-1 35 ng/mL)	56 ± 4		1 ± 1	
FCM (control) + MCM (50%)	29 ± 1		0	
FCM (MCM 50%) + FCM (Control)		53 ± 4		7 ± 1
FCM (MCM 50%) ± MCM (50%)	55 ± 1		6 ± 0	

*BFU-E were cultured in the presence of 1 unit/mL erythropoietin (Epo) without hemin.

†CFU-GEMM were cultured in the presence of 1 unit/mL erythropoietin (Epo) and 0.1 mmol/L hemin.

‡Results were expressed as mean ± 1 SEM of four or five replicate cultures.

§Monocyte-conditioned medium.

‖Resulting fibroblast-conditioned medium after stimulation by monocyte-conditioned medium.

¶Resulting fibroblast-conditioned medium after stimulation by human recombinant IL-1.

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Table 2. Influence of FCM (MCM) and FCM (hrIL-1) on Colony Formation From BFU-E and CFU-GEMM by 10^5 Nonadherent Low-Density Normal Human Bone Marrow Cells (NALD)

Additions	BFU-E* Experiments		CFU-GEMM† Experiments	
	1	2	1	2
Control medium	48 ± 3‡	39 ± 2	0	0
MCM§ 50%	62 ± 3	45 ± 5	0	0
hrIL-1 350 ng/mL	50 ± 2	40 ± 1	0	0
FCM (Control)	49 ± 1	40 ± 2	0	0
FCM (MCM‖ 10%)	91 ± 8	89 ± 9	14 ± 1	5 ± 0
FCM (MCM 25%)	134 ± 2	100 ± 1	22 ± 2	13 ± 3
FCM (MCM 50%)	110 ± 9	82 ± 3	27 ± 4	15 ± 2
FCM (MCM 25% + ab IL-1)¶	63 ± 1	38 ± 1	0	0
FCM (hrIL-1# 350 ng/mL)	101 ± 5	67 ± 2	36 ± 1	15 ± 2
FCM (hrIL-1 140 ng/mL)	101 ± 5	92 ± 5	27 ± 2	21 ± 2
FCM (hrIL-1 70 ng/mL)	87 ± 5	77 ± 3	15 ± 1	1 ± 0
FCM (hrIL-1 35 ng/mL)	77 ± 5	55 ± 2	6 ± 2	0
FCM (hrIL-1 70 ng/mL + ab IL-1)**	54 ± 4	42 ± 1	0	1 ± 0

*,†,‡,§,‖See footnotes, Table 1.

¶Resulting fibroblast conditioned medium after stimulation by monocyte-conditioned medium previously incubated with antibody to IL-1.

#See Table 1, footnote ¶.

**Resulting fibroblast-conditioned medium after stimulation by hrIL-1 previously incubated with antibody to IL-1.

tension was obtained using an oxyreducer (Reming Bioinstruments, NY).[21] The criteria for evaluating BFU-E colonies have been given elsewhere.[20,21] Mixed (CFU-GEMM) colonies contained erythroid cells with granulocytes and macrophages and sometimes megakaryocytes.

Statistical analysis. Levels of significance for comparisons between samples were determined using the Student's *t* distribution. The results are expressed as mean ±1 standard error of the mean (SEM) of four or five plates per point for each experiment.

RESULTS

Both human monocyte–conditioned medium (MCM) and human recombinant interleukin 1 (hrIL-1) were shown to induce confluent fibroblast cultures to produce and release stimulators of BFU-E and CFU-GEMM in nine different experiments using seven different target bone marrow preparations. Stimulation occurred whether the target bone marrow was depleted of high-density cells (Table 1, two separate experiments using two different target bone marrow populations), high-density cells plus adherent cells (Table 2, two separate experiments using two different target bone marrow cells), or high-density cells plus adherent cells plus T lymphocytes (Table 3, five different experiments using three different target bone marrow cells). Colony growth was significantly greater ($P < 0.005$) when bone marrow cultures were stimulated by supernatants of fibroblasts cultured in the presence of MCM or hrIL-1 when compared to supernatants of fibroblasts or monocytes cultured in medium alone.

Table 3. Influence of FCM (MCM) and FCM (hrIL-1) on Colony Formation From BFU-E and CFU-GEMM by 4 × 10^4 Nonadherent Low-Density T Cell–Depleted Human Bone Marrow Cells (NALDT)$^-$

Additions	BFU-E* Experiments			CFU-GEMM† Experiments		
	1	2	3	1	2	3
Control medium	27 ± 1‡	46 ± 1	45 ± 4	0	2 ± 1	1 ± 0
MCM§ 50%	26 ± 1	49 ± 2	46 ± 2	0	2 ± 1	2 ± 0
hrIL-1 1X	27 ± 1	48 ± 2	47 ± 2	0	2 ± 0	1 ± 1
FCM (Control)	29 ± 1	51 ± 2	47 ± 2	0	2 ± 1	2 ± 0
FCM (MCM‖ 10%)	103 ± 1			17 ± 2		
FCM (MCM 25%)	124 ± 5	96 ± 7	99 ± 5	31 ± 3	24 ± 2	27 ± 1
FCM (MCM 50%)	90 ± 6			33 ± 2		
FCM (MCM 25% + ab IL-1)¶	37 ± 2	48 ± 1	47 ± 2	0	3 ± 1	2 ± 1
FCM (hrIL-1)# 1X	93 ± 12	86 ± 4	113 ± 6	25 ± 5	32 ± 2	29 ± 2
FCM (hrIL-1) 1:2	118 ± 8	95 ± 2	103 ± 6	31 ± 3	29 ± 1	28 ± 2
FCM (hrIL-1) 1:4	98 ± 6	92 ± 7	63 ± 3	18 ± 5	19 ± 2	14 ± 1
FCM (hrIL-1) 1:8	47 ± 4	67 ± 2	47 ± 2	3 ± 1	12 ± 1	9 ± 1
FCM (hrIL-1) 1:2 (+ IL-1)**	33 ± 1	46 ± 2	45 ± 2	0	2 ± 0	2 ± 1

*,†,‡,§,‖See footnotes, Table 1.

¶Resulting fibroblast-conditioned medium after stimulation by monocyte-conditioned medium previously incubated with antibody to IL-1.

#Experiment 1, human recombinant IL-1 (a 24.5-kD molecule containing amino acids 71 through 269) 1x = 350 ng/mL. Experiments 2 and 3, human recombinant IL-1 (a 17.0-kD molecule containing amino acids 112 through 269) 1x = 5 ng/mL.

**Resulting fibroblast-conditioned medium after stimulation by human recombinant IL-1 previously incubated with antibody to IL-1.

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Erythroid BFU-E colonies stimulated with FCM(MCM) or with FCM(hrIL-1) were much larger in size than the erythroid colonies in the absence of FCM(MCM) or FCM(hrIL-1). The effects of MCM or hrIL-1 required intact fibroblasts, since mixtures of FCM and MCM contained no more stimulatory activity than FCM or MCM alone (Group 13, Table 1). In the absence of Epo or Epo plus hemin, FCM(MCM) or FCM(hrIL-1) did not stimulate any BFU-E or CFU-GEMM colonies, but they did stimulate numerous CFU-GM colonies. Thus, in experiment 1 reported in Table 2, FCM(MCM 25%) stimulated 112 ± 3 day 7 and 64 ± 1 day 14 CFU-GM colonies while FCM(hrIL-1, 140 ng/mL) stimulated 108 ± 3 day 7 and 59 ± 4 day 14 CFU-GM colonies, respectively. Similar results were previously reported for the stimulation of CFU-GM by IL-1–induced fibroblast-conditioned medium.[13]

To rule out the possibility that monocyte-conditioned medium or fibroblast-conditioned medium contained inhibitors for BFU-E and CFU-GEMM growth which were inactivated, allowing for the expression of enhanced colony growth, the following study was performed (Table 4). We assessed the effects of MCM, FCM and FCM(MCM) on erythroid and mixed colony formation stimulated by Epo or Epo plus hemin in the presence of 5637-conditioned medium. Medium conditioned with 5637 has been shown to be appropriate for the detection of suppressor factors.[22] As seen in Table 4, none of the conditioned medium–suppressed colony formation by 5637-conditioned medium and the FCM(MCM) actually enhanced colony formation to a level equal to or slightly higher than that of FCM(MCM) in the absence of 5637-conditioned medium. (Compare group 6, experiments 2 and 3, Table 3 with group 4, Table 4).

Maximal colony formation by BFU-E and CFU-GEMM occurred when fibroblast cultures were stimulated by 70 to 140 ng/mL hrIL-1 (24.5 kD), 1.25 to 5.0 ng/mL hrIL-1 (17.0 kD) or 25% to 50% MCM. Conditioned medium obtained from fibroblasts cultured in the presence of increasing concentrations of hrIL-1 (35 to 140 ng/mL or 0.625 to 5.0 ng/mL) and MCM (10% to 25%) resulted in dose-

Table 4. Assessing MCM, FCM, and the Resulting Fibroblast-Conditioned Medium After Stimulation by Monocyte-Conditioned Medium [FCM(MCM)] for Inhibitory Activity Against Colony Formation by BFU-E and CFU-GEMM.*

Additions to 5637 CM†	BFU-E‡ Experiments		CFU-GEMM§ Experiments	
	1	2	1	2
Control medium	107 ± 4‖	108 ± 7	8 ± 1	8 ± 0
MCM	105 ± 4	109 ± 4	9 ± 1	9 ± 1
FCM	106 ± 3	113 ± 8	9 ± 1	9 ± 1
FCM (MCM)	112 ± 4	116 ± 6	28 ± 2	27 ± 2

*Similar results were seen with a second target bone marrow in two separate experiments.

†5637 human bladder carcinoma-conditioned medium.

‡BFU-E were cultured in the presence of 1 unit/mL Toyoba Epo and 0.1 mmol/L hemin.

§CFU-GEMM were cultured in the presence of 1 unit/mL Toyoba Epo and 0.1 mmol/L hemin.

‖Results are expressed as mean ± 1 SEM of four plates per point.

response increases in colony formation by BFU-E and CFU-GEMM.

As further proof that the activity found in monocyte-conditioned medium was due to IL-1 production by monocytes, studies were conducted using an antibody to IL-1. A 1:100 dilution of anti–IL-1 completely abolished the stimulation of CFU-GEMM by FCM(MCM 25%), while significantly reducing the enhancement of BFU-E colony formation. This same antibody completely abolished the stimulation of CFU-GEMM and BFU-E seen with fibroblast-conditioned medium collected in the presence of hrIL-1 (Tables 2 and 3). In addition, using this same IL-1 antibody, we tested to see if background erythroid colony formation stimulated in the presence of Epo or Epo plus hemin could be reduced by preincubation of the cells with IL-1 antibody. Cells were incubated for one hour with antibody to IL-1 (1:100 final dilution) in the presence of Epo or Epo plus hemin, or were incubated for one hour with McCoy's medium in the presence of Epo or Epo plus hemin. The cells were then plated without washing so that the antibody to IL-1 or control medium were plated also. Erythroid growth and CFU-GEMM growth were unchanged (BFU-E 63 ± 3 *v* 64 ± 3 and CFU-GEMM 5 ± 1 *v* 5 ± 1) when cells were incubated in the absence or presence of antibody to IL-1, respectively.

DISCUSSION

Assays are available to detect early human clonogenic progenitors that give rise to large erythroid colonies (BFU-E),[1] and colonies containing erythroid cells, granulocytes, megakaryocytes, and macrophages (CFU-GEMM).[2] Growth of these early progenitor cells depends on the presence of a source of burst-promoting activity (BPA) or conditioned medium obtained from leukocytes of patients with hemochromatosis cultured in the presence of 1% phytohemagglutinin. A number of different cell types including monocytes or macrophages,[3,4] T lymphocytes,[5,6] and endothelial cells[7] have been reported to produce BPA. Recent studies on regulatory cell interactions have reported that monocytes produce a factor (monokine) which stimulates production of BPA by endothelial cells.[12] A similar cell-to-cell relationship has been proposed for GM-CSA production[11] and we have recently identified interleukin 1 (IL-1) as one monocyte product which stimulates fibroblasts to produce GM-CSA.[13] The results of the present study show that fibroblasts are also capable of producing stimulating activities for BFU-E and CFU-GEMM colony formation. The production of this stimulating activity by fibroblasts requires prior induction by a monocyte product which we have identified as IL-1. Fibroblasts cultured in the presence of monocyte-conditioned medium or human recombinant IL-1 stimulated significantly greater BFU-E and CFU-GEMM colony growth when compared to supernatants of fibroblasts, monocytes, or IL-1 cultured alone. Both FCM(MCM) and FCM(hrIL-1) stimulated BFU-E and CFU-GEMM colony formation in a dose-dependent fashion with maximal colony formation occurring when fibroblasts were stimulated by 25% to 50% MCM, 70 to 140 ng/mL hrIL-1 (ROO6B), or 1.25 to 5

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ng/mL hrIL-1 (ROO6T). In addition, a polyclonal rabbit antibody specific for human monocyte IL-1 completely abolished CFU-GEMM colony-stimulating activity while completely reducing the enhancing activity for BFU-E by FCM(hrIL-1) and almost completely reducing the enhancing activity for BFU-E by FCM(MCM).

The presence of inhibitors in monocyte-conditioned medium or fibroblast conditioned medium to erythroid and mixed colony growth appear unlikely, since neither MCM nor FCM suppressed colony growth stimulated by 5637 human bladder carcinoma–conditioned medium. The action of FCM(MCM) and FCM(hrIL-1) on BFU-E and CFU-GEMM colony growth do not appear to require monocytes or T lymphocytes in the marrow population containing BFU-E and CFU-GEMM, since results were similar when the target bone marrow population was depleted of both adherent cells and T lymphocytes.

Because FCM(hrIL-1) contains stimulators for CFU-GM, BFU-E, and CFU-GEMM, it is possible that all of these actions are brought about by the presence of one or more factors. In this regard, human recombinant GM-CSF has been shown to stimulate BFU-E as well as CFU-GM.[23] Similarly, a pluripotent hematopoietic colony-stimulating factor (pluripotent CSA) has been purified from conditioned medium of the human bladder carcinoma cell line 5637.[24] This pluripotent CSF supports the growth of human mixed colonies, granulocyte-macrophage colonies, and early erythroid colonies and induces differentiation of the human promyelocytic leukemic cell line HL-60 and the murine myelomonocytic leukemic cell line WEHI-3B (D+). Whether the resulting fibroblast-conditioned medium stimulated by IL-1 and reported here contains one or more of these CSFs must await purification studies.

ACKNOWLEDGMENT

We wish to thank Scott Cooper for excellent technical assistance in setting up the bone marrow colony assays and Jeanie Wood for preparation of the manuscript.

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