Role for Monocyte-Macrophage-derived Colony-stimulating Factor and Prostaglandin E in the Positive and Negative Feedback Control of Myeloid Stem Cell Proliferation

By J. I. Kurland, H. E. Broxmeyer, L. M. Pelus, R. S. Bockman, and M. A. S. Moore

Murine peritoneal macrophages and human peripheral blood monocytes stimulated the proliferation in vitro of committed myeloid stem cells (CFU-C) by the elaboration of colony-stimulating factors (CSF). High numbers of monocyte-macrophages possessed little stimulating activity and were markedly suppressive due to the accumulation of a dialyzable, non-species-specific inhibitor of CFU-C proliferation. This inhibitory principle was identified as prostaglandin E (PGE) by means of a sensitive radioimmunoassay, and the levels of PGE correlated inversely with myeloid colony incidence. Suppression of PGE synthesis by indomethacin markedly increased the number and size of myeloid colonies stimulated by monocytes and macrophages and permitted a more accurate estimate of CFU-C incidence. Indomethacin had no direct effect on CFU-C in the absence of a biosynthetic source of PGE and did not influence CSF production occurring in the absence of prostaglandin synthesis. Dose-dependent stimulation of PGE synthesis by macrophages in response to increasing concentrations of CSF was concomitantly associated with progressive CFU-C inhibitory activity. Conversely, monocyte-macrophage PGE synthesis was significantly reduced following exposure to an extract of human granulocytes that decreased CSF production. These studies indicate a unique regulatory function of the monocyte and macrophage in the positive and negative feedback control of committed myeloid stem cell proliferation. The balance between CSF and PGE, factors with mutually opposing actions, is ultimately determined by an afferent feedback mechanism operating via the intrinsic modulation of macrophage PGE synthesis in response to local elevations in CSF concentrations.

The culture assay in vitro of Bradley and Metcalf led to the recognition of a class of hematopoietic progenitors restricted to granulocyte and macrophage differentiation. Essential to the clonal proliferation of this population of bipotentially committed progenitor cells (colony-forming units culture, CFU-C) is the presence of regulatory macromolecules operationally termed colony-stimulating factors (CSF). CSF is demonstrable in normal sera and is generally elevated in conditions associated with increased granulopoiesis and monocyte-macrophage production as well as following the injection of antigens and bacterial endotoxin, during acute viral and bacterial infections and both preceding and during active myelopoietic regeneration. In contrast, serum CSF is depressed in germ-free mice. Recognition that


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CSF is produced by blood monocytes and tissue macrophages, the elaboration of which is markedly augmented by exposure to endotoxin and that serum CSF levels coincide with the peak in peripheral blood monocyte numbers introduces the problem of mechanisms designed to counterbalance the positive feedback drive of monocyte-macrophage-derived CSF.

Limitation of CSF-dependent myelopoiesis may theoretically be mediated by activities inhibiting CSF production by mononuclear phagocytes, by direct inactivation of the CSF molecule, or by alteration in the responsiveness of the granulocyte-macrophage progenitor cells to CSF. We recently reported that the E-series prostaglandins (PGE) PGE, and PGE, (but not PGF, or PGF,) profoundly inhibit CFU-C proliferation in vitro. This inhibition can be effectively prevented by preincubation or coincubation of bone marrow cells with the dibenzoxapine hydrazide prostaglandin (PG) antagonist SC-19220, thereby providing evidence for the existence of PG receptors on the granulocyte-macrophage progenitor cell. In the presence of PGE, CFU-C, as well as a population of peritoneal macrophage precursor cells (CFU-PM), require an eight- or ninefold greater concentration of CSF in order to proliferate to the same extent as in the absence of PGE. Conversely, increasing the concentration of CSF counteracts the effectiveness of PGE-mediated inhibition, indicating that the control of production of both CSF and PGE may provide a dualistic modulation of committed stem cell proliferation.

The physiologic relevance of PGE in myelopoiesis is suggested by the observation that the phagocytic mononuclear cells of human or murine origin actively synthesize and release PGE in vitro. In contrast, pure populations of lymphocytes and polymorphonuclear leukocytes do not possess detectable PG synthetase activity, and the level of PGE production by both normal and neoplastic monocyte-macrophages is dependent upon their state of stimulation.

The contention that the mononuclear phagocyte possesses the capability to regulate the proliferation of myeloid progenitor cells by the elaboration of two factors with mutually opposing activities is confirmed by the present paper. In addition, the present study provides further evidence for the central regulatory functions of mononuclear phagocytes, indicating that an important mechanism limiting inappropriate myelopoiesis is CSF-dependent macrophage PGE synthesis.

**MATERIALS AND METHODS**

**Mice.** Female B6D2F, mice (Jackson Laboratories, Bar Harbor, Me.), 2-3 mo of age, were used as a source of bone marrow cells and peritoneal macrophages.

**Preparation of bone marrow and fetal liver cell suspensions.** Mouse bone marrow was steriley collected by perfusing the femurs from at least three mice with ice-cold serum-free McCoy's 5A medium (Grand Island Biological, Grand Island, N.Y.) Modified medium (Grand Island Biological, Grand Island, N.Y.). The cells of each mouse were pooled, prepared into single cell suspensions, and washed three times.

Human bone marrow was obtained from consenting normal healthy adult volunteers by iliac crest aspiration and separated on the basis of differential buoyant density by centrifugation in bovine serum albumin (BSA) (density 1.070 g/cm³) and active adherence to plastic culture dishes in the presence of 15% fetal calf serum (FCS; Microbiological Associates, Bethesda, Md.) at 37°C for 1.5 hr. The nonadherent, light-density (<1.070 g/cm³) human bone marrow cells were used for the target CFU-C population in all human bone marrow cultures and were devoid of...
endogenous CSF-producing cells as determined by their inability to clonally proliferate in the absence of a source of human CSF. 

An enriched population of human CFU-C devoid of CSF-producing cells was provided by 14-wk-gestation human fetal liver obtained at the time of therapeutic transplantation in a child with severe combined immunodeficiency. A small portion of the fetal liver was prepared into a single-cell suspension and separated by buoyant density centrifugation in human-tonicity BSA (density 1.062 g/cm³). The light-density (<1.062 g/cm³) fetal liver cells were washed three times, suspended in McCoy’s 5A modified medium containing 20% FCS, and added to equal volumes of 20% dimethylsulfoxide (DMSO) to give a final cell concentration of 10 × 10⁶/mL. Final 0.5-mL aliquots were dispensed in polypropylene serum vials (Nunc, Copenhagen, Denmark) and frozen in liquid nitrogen. When needed, the vials were thawed in a water bath, the contents were rapidly removed and diluted 20:1 in McCoy’s 5A modified medium containing 15% FCS and washed three times by centrifugation, and viable nucleated cell counts were performed.

Bone marrow CFU-C assay. Semisolid agar cultures of murine and human bone marrow cells were prepared as previously described. Briefly, 7.5 × 10⁶ mouse bone marrow cells or 1.5 × 10⁷ nonadherent light-density human bone marrow cells were suspended in 0.3% Bacto agar (Difco Laboratories, Detroit, Mich.) prepared in McCoy’s 5A modified medium containing 15% FCS. Then 1-mL aliquots of the bone marrow–agar suspensions were dispensed into 35-mm tissue culture dishes (Lux Scientific, Newbury Park, Calif.), allowed to gel, and, following 7 days incubation at 37°C in an atmosphere of 10% CO₂ in humidified air, scored for the presence of colonies (greater than 40 cells) and clusters (3–40 cells) under 25× magnification. Murine CSF was provided by concentrated conditioned medium of a murine myelomonocytic leukemia cell line (WEHI-3). Serum-free medium conditioned by WEHI-3 cells was chromatographed on ion-exchange resin. Fractions containing granulocytic or macrophage-specific colony-stimulating activity (CSA) were obtained. The procedure for isolation of the WEHI-3 cell line–derived CSA and their partial characterization are to be published elsewhere. Human bone marrow cultures were stimulated by CSF provided in the form of concentrated human monocyte-conditioned medium or by 0.5% agar feeder layers containing 1 × 10⁶ washed human peripheral blood leukocytes prepared according to the method of Pike and Robinson. In order to investigate the effects of mononuclear phagocytes on colony formation, the bone marrow cells in 0.3% agar medium were placed over 0.5% agar underlayers containing either adherent murine peritoneal macrophages or human peripheral blood monocytes (see below). Alternatively, the 0.3% agar bone marrow suspension was used as a single-layer system when assaying soluble supernatants from short-term liquid cultures of monocytes or macrophages or a soluble source of CSF.

Preparation of adherent murine macrophage and human monocyte underlayers. Murine peritoneal macrophages were obtained by washing out the peritoneal cavities of B6D2F₁ mice with 8 mL ice-cold serum-free McCoy’s medium containing 1 unit heparin/mL as described previously. The peritoneal exudate cells (PEC) were washed three times by centrifugation at 4°C, nucleated cell counts were performed, and the PEC were diluted to the appropriate cell concentrations in McCoy’s 5A modified medium containing 15% FCS. Various numbers of PEC were allowed to adhere to 35-mm tissue culture dishes (Lux) for 1.5 hr at 37°C, at which time the nonadherent cells were removed and the cells remaining adherent were washed three times with serum-free phosphate-buffered saline (PBS). The adherent peritoneal cell population comprised approximately 20–30% of the initial PEC population and consisted exclusively of macrophages on the basis of morphology and phagocytosis of latex beads, as well as specific staining with neutral red and for cytoplasmic nonspecific α-naphthyl acetate esterase. Human monocytes were obtained by differential centrifugation of normal human peripheral blood in BSA (density 1.070 g/cm³). The light-density (<1.070 g/cm³) mononuclear cell population containing lymphocytes and monocytes was washed three times, diluted to the appropriate cell concentration in McCoy’s 5A modified medium containing 15% FSC, and allowed to adhere in 35-mm plastic culture dishes, as described previously for mouse peritoneal macrophages. After 1.5 hr the nonadherent cells were removed and the adherent light-density mononuclear cells were washed three times with PBS. The human mononuclear cells remaining adherent were 99% monocytes as judged by α-naphthyl acetate esterase staining. In the case of murine macrophages, the numbers of adherent cells are described as the initial number of PEC permitted to adhere, whereas the true numbers of human monocytes were determined by counting the actual number of adherent esterase-positive cells in replicate dishes, as described previously.
Adherent murine and human mononuclear cells were overlayered with 1 ml cell-free 0.5\% agar prepared in McCoy’s 5A modified medium containing 15\% FCS. After the agar underlayers were allowed to gel, either murine or human bone marrow cell suspensions prepared in 0.3\% soft agar medium were overlayered. In this manner, the macrophages or monocytes adherent to the bottom of the culture dish were separated from the overlying 0.3\% agar-bone marrow cell suspension by an intervening 1-ml cell-free 0.5\% agar interface, thereby preventing physical contact but allowing diffusible interactions between the adherent mononuclear cells and bone marrow cells. In some cases, the PG synthetase inhibitor indomethacin or the appropriate ethanol diluent were incorporated within the mononuclear cell underlayers prior to the addition of the bone marrow overlayers.

Preparation of human granulocyte extract. Normal human polymorphonuclear leukocytes (PMN) were separated as previously described and the cells were lysed by rapid freeze-thawing. The cell extracts released by this method, corresponding to 16 × 10^6 PMN/ml, were centrifuged at 160,000 g, sterilized by Millipore filtration (0.45 μm pore size; Millipore, Bedford, Mass.), and frozen until use. The non-species-specific inhibitory actions of the granulocyte extract on monocyte-macrophage production of CSF have been published elsewhere.

Preparation of murine macrophage and human monocyte and lymphocyte supernatants for assay of CSF and PGE. Various numbers of adherent murine peritoneal macrophages and human monocytes, as well as nonadherent light-density human peripheral blood lymphocytes, were incubated for 48 hr or 5 days in McCoy’s 5A modified medium containing 15\% FCS. Agents tested for their effects on CSF and PGE production were indomethacin, synthetic PGE, (a generous gift of Dr. John Pike, UpJohn, Kalamazoo, Mich.), human granulocyte extract, and various preparations of murine CSF derived from WEHI-3-conditioned media. The culture fluids were harvested, cells removed by centrifugation at 1600 g for 15 min, and Millipore filtered (0.45 μm pore size). The supernatant media were assayed for CSA against murine or human bone marrow cells and for PGE by radioimmunoassay. In some cases, macrophages were incubated in the presence of various concentrations of murine CSF provided by WEHI-3-conditioned media, half of the samples were dialyzed against six changes of PBS for 2 days at 4°C, and the samples were tested for their effects on murine bone marrow cultures and human bone marrow cells stimulated by a human leukocyte feeder layer.

PGE radioimmunoassay. Murine peritoneal macrophage or human blood monocyte or lymphocyte supernatants were assayed for PGE by radioimmunoassay according to the method of Levine et al. Briefly, to freshly obtained culture supernatants were added 10^-12 g 3H-PGE in order to permit calculation of the percentage recovery. The samples were initially extracted with petroleum ether to remove neutral fats, then reextracted with acidified ethyl acetate-isopropanol. The organic phase was dried, resolubilized in benzene-ethyl acetate-methanol, and applied to silicic acid columns. The columns were sequentially eluted with benzene-ethyl acetate and increasing concentrations of methanol. The column effluent containing PGE was dried, resolubilized in gelatin-Tris buffer, and then alkaline hydrolized to quantitatively convert PGE\(_1\) and PGE\(_2\) to PGH. Competitive binding between 3H-PGB and standard PGE, or the test supernatant, and specific anti-PGB antibody was measured using a double-antibody radioimmunoassay made commercially available by Clinical Assays (Cambridge, Mass.). Since the silicic acid columns clearly separate PGE from PGB, the assay specifically detects PGB derived from alkaline-hydrolyzed PGE.

RESULTS

Effect of peritoneal macrophages on colony formation by mouse bone marrow CFU-C. The effects of adherent murine peritoneal macrophages on the proliferation of mouse bone marrow CFU-C was investigated using the two-layer soft agar culture system, as described in Materials and Methods. In the absence of an exogenous source of CSF, colony formation initially increased as a function of the numbers of adherent macrophages in the culture underlayer (Fig. 1A). Peak stimulation of colony numbers was provided by macrophages from 0.25 × 10^6 PEC. Greater numbers of macrophages produced a decrease in CFU-C numbers, and no colony formation was observed at the highest macro-
Fig. 1. (A) Effect of indomethacin on influence of murine macrophage-derived diffusible factors on proliferation of syngeneic bone marrow CFU-C. $7.5 \times 10^6$ B6D2F1 bone marrow cells suspended in 0.3% soft agar medium in the absence of an additional source of CSF were cultured over 0.5% agar underlayers containing adherent macrophages prepared from various concentrations of B6D2F1 peritoneal exudate cells (PEC) in the presence of indomethacin or control diluent. Triplicate cultures scored for each point at 7 days of incubation; results expressed in terms of capacity of underlaying macrophages to stimulate proliferation of CFU-C to form colonies. (B) Effect of indomethacin on murine macrophage-mediated suppression of exogenously CSF-stimulated bone marrow CFU-C. $7.5 \times 10^6$ B6D2F1 bone marrow cells were suspended in soft agar medium containing 1270 units CSF provided by WEHI-3-conditioned medium in presence of adherent peritoneal macrophages prepared as in A. Indomethacin or control diluent added to macrophage underlayers prior to addition of soft agar–bone marrow suspension; cultures scored for presence of colonies 7 days later. Results expressed as percentage of maximum number of CFU-C stimulated to form colonies by CSF in the absence of macrophages. Triplicate cultures scored for each point.

phage concentration tested. However, when indomethacin, an inhibitor of PG synthesis, was added to the adherent macrophage underlayers, an increase in colony stimulation was observed even at concentrations of macrophages that generally suppressed all colony formation. Indomethacin was unable to directly stimulate CFU-C and augmented their proliferation only in the presence of macrophages.

In order to determine if the reduction in colony formation in the presence of high numbers of macrophages was due to decreased colony stimulation or direct inhibition, competition experiments between increasing concentrations of macrophages and exogenous CSF were performed. Mouse bone marrow cultures were prepared as in Fig. 1A, except for the addition of WEHI-3-conditioned medium at concentrations that stimulated a maximum number of CFU-C. As shown in Fig. 1B, those cultures containing adherent macrophages from greater than $0.1 \times 10^6$ PEC showed a progressive decline in the numbers of exogenously CSF-stimulated CFU-C. Incorporation of indomethacin within the macrophage underlayers largely prevented the suppression of colony formation but had no effect on the stimulation of CFU-C by WEHI-3-conditioned medium in the absence of macrophages.

Ability of mouse macrophages to inhibit colony formation by normal human bone marrow CFU-C. In order to examine the effect of the macrophage-derived inhibitor(s) on CFU-C independently of the contribution of macrophage derived CSF, we took advantage of the species-specific action of CSF. Normal human bone marrow cells stimulated by a source of CSF from human mono-
Fig. 2. Effects of indomethacin and adherent murine peritoneal macrophages on normal human bone marrow CFU-C proliferation. Soft agar cultures containing $2 \times 10^6$ normal human bone marrow cells and 10% v/v human monocyte-conditioned medium as a source of CSF were prepared above 0.5% agar underlayers containing adherent murine macrophages from various numbers of B6D2F1 PEC. Indomethacin or control diluent added to macrophage underlayers as with mouse CFU-C cultures. Results expressed as number of human CFU-C that proliferated to form colonies after 7 days of culture. Triplicate cultures scored for each point.

cyte-conditioned medium were cultured in soft agar medium in the presence of varying numbers of adherent murine peritoneal macrophages. As shown in Fig. 2, increasing numbers of murine macrophages progressively inhibited the clonal proliferation of human CFU-C; indomethacin largely prevented this inhibition. As with mouse bone marrow, indomethacin had no effect on the stimulation of human CFU-C by CSF in the absence of macrophages.

**Indomethacin augments the CSA of human leukocyte feeder layers.** The effects of indomethacin on the capacity of human blood leukocyte feeder layers to support human adult bone marrow and fetal liver CFU-C were examined. Variable colony formation by both adult and fetal CFU-C was stimulated by the leukocyte feeder layers, and the presence of indomethacin markedly increased both the numbers (Fig. 3A) and size (not shown) of colonies. This effect of the PG synthesis inhibitor was more apparent in the presence of an additional biosynthetic source of PG such as provided by adherent mouse peritoneal macrophages. Both human adult and fetal CFU-C proliferation were suppressed by the presence of murine macrophages placed beneath the human leukocyte feeder layers; again the inclusion of indomethacin largely prevented this inhibition.

The kinetics of production of the indomethacin-sensitive factor that limited the stimulatory activity of human leukocyte feeder layers was investigated. As shown in Fig. 3B, the inclusion of indomethacin during the first 2 days following the preparation of the leukocyte feeder layers significantly increased the numbers of adult bone marrow and fetal liver colonies that developed. Delaying the addition of indomethacin, however, reduced the potentiating effects on colony formation.

**Cellular specificity of indomethacin: Relationship between lymphocyte and monocyte production of CSF and PGE.** We employed cell separation procedures to examine the cell(s) within the leukocyte feeder layer whose colony stimulatory or inhibitory activity was sensitive to indomethacin. In order to show a correlation between the potentiating effect of indomethacin and the constitutive levels of PGE, radioimmunologic determination for PGE was performed on the culture supernatants of these separated cells. Fractions of nor-
Fig. 3. (A) Effect of indomethacin on colony-stimulating ability of human leukocyte feeder layers. Human peripheral blood leukocyte feeder layers containing $1.4 \times 10^{-7}$ M indomethacin or control diluent were prepared in 35-mm culture dishes in the absence (A) and presence (B) of adherent murine macrophages from $1 \times 10^6$ B6D2F1 PEC, $1 \times 10^5$ human adult nonadherent bone marrow cells or human fetal liver cells (density <1.062 g/cm$^3$) were incorporated in the top 0.3% agar layer; number of CFU-C stimulated to proliferate and form colonies ($\pm$SEM) per triplicate cultures determined after 7 days of incubation. (B) Effect of adding indomethacin at varying times on colony-stimulating ability of human leukocyte feeder layers. Indomethacin ($1.4 \times 10^{-7}$ M) or control diluent were added to human peripheral blood leukocyte feeder layers on either the same day of their preparation (day 1) or on various times thereafter. Following the day-7 addition of indomethacin $1 \times 10^4$ either of normal human bone marrow or fetal liver cells suspended in 0.3% soft agar medium were added and the number of colonies scored 7 days later. Results expressed as mean ($\pm$ SEM) CFU-C stimulated to proliferate per triplicate cultures.

Normal human peripheral blood enriched for lymphocytes and monocytes were cultured at various cell concentrations in the absence and presence of indomethacin and the conditioned media tested for colony- and cluster-stimulating activity in normal human bone marrow cultures and for PGE by radioimmunoassay.

Media conditioned by the nonadherent mononuclear cells (predominantly lymphocytes) stimulated only cluster formation and were unable to support the formation of colonies (Fig. 4). Despite the progressive stimulation of cluster formation by the media conditioned by increasing numbers of lymphocytes, no PGE was detectable in any of the lymphocyte supernatants tested. Indomethacin had no effect on the production of the cluster-stimulating activity by lymphocytes, presumably owing to the inability of these cells to synthesize PGE.

The adherent monocyte-conditioned media supported the formation of both human bone marrow colonies and clusters. Peak colony and cluster numbers were stimulated by media conditioned by $1 \times 10^6$ adherent mononuclear cells/ml. Permitting greater numbers of monocytes to condition media resulted in a progressive decline in colony and cluster formation. PGE was first detectable in the medium conditioned by the same concentration of monocytes that stimulated peak colony and cluster formation. At greater monocyte concentrations PGE levels continued to increase; this was associated with a decrease in both colony and cluster numbers. Indomethacin completely suppressed the production of monocyte PGE and augmented both colony and cluster formation approximately three- to fourfold. The efficacy of indomethacin in poten-
MACROPHAGE REGULATION OF MYELOPOIESIS

Fig. 4. Effect of indomethacin on production of CSF and PGE by human peripheral blood monocytes and lymphocytes. Various numbers of human peripheral blood mononuclear cells obtained by buoyant density centrifugation in BSA (density < 1.070 g/cm³) were allowed to adhere to 35-mm culture dishes to obtain varying concentrations of adherent monocytes. Nonadherent lymphocytes were removed, washed, and dispensed into replicate dishes at concentrations corresponding to mononuclear cell numbers initially permitted to adhere. Following 5 days incubation in McCoy’s 5A modified medium containing 15% FCS and 1.4 x 10⁻⁷ M indomethacin or control diluent monocyte and lymphocyte culture supernatants were harvested and tested for both CSF and PGE. CSF was determined by testing 0.1 ml culture supernatants in soft agar cultures of L5 x 10⁵ normal human bone marrow cells; number of CFU-C stimulated to form colonies in four individual cultures were scored after 7 days incubation. Duplicate measurements of PGE in test supernatants were determined by radioimmunoassay; results expressed as pg PGE/0.1 ml supernatant added to human bone marrow cultures.

Indomethacin increased the production of CSF by human monocytes and lymphocytes. Various concentrations of indomethacin were added to monocyte and lymphocyte cultures, and the production of CSF and PGE was determined. The effect of indomethacin on CSF production was concentration-dependent, with a maximum increase observed at 1.4 x 10⁻⁷ M.

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Effects of synthetic PGE₁ on the production of CSF by murine macrophages. We examined whether or not the potentiating effect of indomethacin on colony formation might in part derive from a direct inhibitory action of PGE on macrophage production of CSF. B6D2F₁ peritoneal macrophages were incubated for 72 hr in the absence and presence of varying concentrations of synthetic PGE₁; following dialysis against six changes of PBS containing 5% FCS at 4°C in order to remove residual PGE₁, the culture supernatants were tested for CSF at 10% v/v in B6D2F₁ soft agar bone marrow cell cultures. As shown in Table 1, CSF production, albeit slightly inhibited by 10⁻⁶ M PGE₁, was significantly increased in the presence of 10⁻⁷–10⁻¹⁰ M PGE₁.

Action of a granulocyte-derived inhibitory extract on monocyte-macrophage production of CSF and PGE. It was recently reported that an activity in extracts of human granulocytes inhibits the proliferation of CFU-C by interfering with the production of CSF by mononuclear phagocytes.²⁹,³⁰ We carried out a series of experiments designed to compare the effects of the granulocyte extract (GE) and indomethacin on both human bone marrow colony formation and monocyte production of CSF and PGE.
Table 1. Effects of PGE₁ on CSF Production by Murine Peritoneal Macrophages

<table>
<thead>
<tr>
<th>Molar Concentration of PGE₁</th>
<th>CFU-C per 7.5 x 10⁴ B6D2F₁ Bone Marrow Cells</th>
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<tbody>
<tr>
<td>None</td>
<td>98 ± 4</td>
</tr>
<tr>
<td>10⁻⁷</td>
<td>71 ± 5</td>
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<tr>
<td>10⁻⁸</td>
<td>106 ± 5</td>
</tr>
<tr>
<td>10⁻⁹</td>
<td>133 ± 8</td>
</tr>
<tr>
<td>10⁻¹⁰</td>
<td>152 ± 4</td>
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<tr>
<td>10⁻¹²</td>
<td>113 ± 3</td>
</tr>
<tr>
<td>10⁻¹⁵</td>
<td>96 ± 3</td>
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B6D2F₁ peritoneal macrophages (5 x 10⁵) were incubated for 48 hr in the absence and presence of varying concentrations of PGE₁. The culture fluids were harvested, dialyzed against six changes of PBS containing 5% FCS at 4°C, and assayed for colony-stimulating activity at 10% v/v in soft agar cultures of 7.5 x 10⁴ B6D2F₁ bone marrow cells.

Feeder layers of various numbers of adherent human peripheral blood mononuclear cells (density <1.070 g/cm³) prepared in the absence and presence of 5% v/v GE, 1.4 x 10⁻⁷ M indomethacin, or both agents were used to stimulate colony formation by nonadherent human bone marrow cells. Conditioned media prepared from the same numbers of adherent mononuclear cells cultured in the absence and presence of the different agents were tested for PGE by radioimmunoassay. As shown in Fig. 5A, colony formation by human CFU-C initially increased as a function of the number of adherent monocytes present in the underlying feeder layers but then declined in the presence of high concentrations of monocytes (control). Feeder layers prepared with the GE stimulated fewer human CFU-C than control feeder layers alone, but at high num-

Fig. 5. Modulation of human monocyte production of CSF and PGE by indomethacin and human granulocyte extract. Various numbers of adherent human monocytes were overlaid with 1 ml 0.5% agar medium in absence and presence of 1.4 x 10⁻⁷ M indomethacin and 0.05 ml human granulocyte extract (GE). Target cell suspensions (1 ml) of 1.5 x 10⁵ nonadherent normal human bone marrow cells (density <1.070 g/cm³) in 0.3% soft agar medium were added above adherent monocyte underlayers; number of CFU-C that proliferated to form colonies in bone marrow overlays determined after 10 days culture. Quadruplicate cultures scored for each point. Replicate liquid cultures containing same number of adherent monocytes as in the agar underlayers were used to obtain supernatant media for determination of PGE production by radioimmunoassay; results expressed in pg PGE as shown in bar graphs.
bers of adherent cells the effectiveness of the GE decreased. In contrast, feeder layers containing indomethacin provided a complete linear stimulation of CFU-C over the range of monocyte numbers tested. Colony formation stimulated by the monocytes in the combined presence of both indomethacin and GE nearly approximated the average colony formation observed in cultures containing the individual agents alone.

PG radioimmunoassays were performed on the media conditioned by the same numbers of adherent monocytes as were used in the feeder layers. As shown in Fig. 5B, media from control adherent monocyte cultures contained levels of PGE that increased in a linear fashion with respect to the number of monocytes. At the monocyte concentration at which PGE first became detectable, no further increase in colony formation was observed (Fig. 5B) and colony size was markedly decreased (not shown). Monocyte-conditioned media prepared with indomethacin or with indomethacin and GE contained no detectable PGE. Of particular interest was the observation that in the presence of the GE alone PG production by the monocytes was significantly reduced.

Similar findings to those shown in Fig. 5A were obtained by analyzing 48-hr supernatants from various numbers of adherent human monocytes cultured in the absence and presence of indomethacin, GE, or both agents (Fig. 6). The supernatants were tested for the abilities to stimulate the number as well as the size of developing human bone marrow CFU-C colonies. As before, GE significantly reduced and indomethacin markedly increased the levels of detectable CSF in human monocyte-conditioned media. The GE decreased the cluster-stimulating activity more effectively than colony-stimulating activity of the monocyte supernatants. Indomethacin, however, augmented equally the formation of both colonies and clusters that were stimulated by the media conditioned by high numbers of monocytes. Again, the combination of both GE and indomethacin present during the preparation of the monocyte-conditioned media resulted in the formation of colonies and clusters equal to the average of the individual effects of the GE and indomethacin alone.

Fig. 6. Effect of indomethacin and human granulocyte extract on human monocyte production of CSF. Adherent monocytes of various numbers of normal human peripheral blood mononuclear cells (density < 1.070 g/cm³) incubated for 5 days in McCoy’s 5A modified medium containing 15% PCS and 1.4 x 10⁻⁷ M indomethacin, control diluent, or 5% v/v human GE and the supernatant media tested for CSF in soft agar cultures containing 1.5 x 10⁵ nonadherent human bone marrow cells. Both the number of colonies (>40 cells) and clusters (3-40 cells) that developed after 10 days incubation were determined in quadruplicate cultures. Results expressed as number of colonies or total colonies and clusters per 1.5 x 10⁵ nucleated human bone marrow cells (+ SEM) stimulated by 0.1 ml of various monocyte-conditioned media.
Production of a dialyzable and non-species-specific inhibitor of granulopoiesis by mouse peritoneal macrophages in response to increasing concentrations of CSF. To determine if the macrophage was capable of modulating its production of the indomethacin-sensitive CFU-C inhibitory factor in response to the local concentration of CSF, we again took advantage of the species-specific action of CSF on CFU-C. In addition, because of the extremely low molecular size of PG, we attempted to separate CSF from PGE by a simple preparative dialysis. Adherent murine peritoneal macrophages were incubated for 48 hr in the absence and presence of increasing concentrations of murine-active WEHI-3 CSF, and half of the pooled material from quadruplicate cultures was dialyzed against six changes of PBS and 5% FCS for 48 hr at 4°C. Both dialyzed and nondialyzed culture fluids were then assayed for CSA against B6D2F1 mouse bone marrow (Fig. 7A) as well as for non-species-specific inhibitory activity against normal human bone marrow cells stimulated by a leukocyte feeder layer (Fig. 7B). Similar procedures were performed on media containing the respective concentrations of WEHI-3 CSF but not exposed to peritoneal macrophages.
As shown in Fig. 7A, the supernatants from macrophage-free cultures stimulated mouse CFU-C colony formation as a function of the concentration of WEHI-3 CSF initially added. However, when these same concentrations of CSF were exposed for 48 hr to the murine macrophages prior to being assayed for CSF activity, a dramatic reduction in the stimulatory activity was observed. This diminution was, however, not due to the degradation of WEHI-3 CSF by the macrophages, since following dialysis stimulatory levels approached those found in the CSF concentrations not exposed to the macrophages. Similarly, the endogenous CSA of the mouse macrophages was suppressed in the presence of the WEHI-3 CSF added, but following dialysis net stimulation was unmasked. Note that no further increase in the stimulatory activity of medium containing the WEHI-3 CSF alone was observed following dialysis (not shown).

Normal human bone marrow colony formation stimulated by human leukocyte feeder layers was unaffected by either murine peritoneal macrophage-conditioned media or the various concentrations of murine WEHI-3 CSF (Fig. 7B). However, media conditioned by macrophages in the presence of WEHI-3 CSF progressively inhibited human colony formation as a function of the concentration of WEHI-3 CSF to which the macrophages were exposed. Following dialysis, these same supernatants were no longer inhibitory, suggesting that murine peritoneal macrophages elaborated a dialyzable, non-species-specific inhibitor of CFU-C proliferation, in response to varying concentrations of CSF.

Ability of macrophages to increase the production of PGE in response to CSF. In order to implicate macrophage-derived PGE in the CSF-induced suppression of CFU-C, we examined the effects of WEHI-3 CSF on macrophage production of PGE. Various concentrations of WEHI-3 CSF were added to liquid cultures containing adherent peritoneal macrophages from $1 \times 10^5$ peritoneal exudate cells, and the supernatant media harvested after 48 hr was assayed for PGE. This concentration of macrophages was selected in order to reduce the background level of detectable PGE. As shown in Fig. 8, WEHI-3 CSF stimulated the linear production of PGE by the macrophages. For purposes of comparison, stimulation of mouse bone marrow colony formation by an equivalent concentration of WEHI-3-conditioned medium is shown. Whereas colony formation approached plateau levels at high concentrations of WEHI-3 CSF, the synthesis of PGE was progressively stimulated over all of the concentrations of CSF tested. Note that the concentration of CSF capable of stimulating macrophage PGE synthesis was greater than that required to stimulate CFU-C proliferation.

Similar findings were obtained using L-cell-conditioned medium (not shown) and more purified preparations of serum-free WEHI-3 CSF (Table 2): $1 \times 10^4$ murine peritoneal macrophages were incubated in the absence and presence of fractions of serum-free WEHI-3-conditioned medium differing in their capacity to stimulate the formation of granulocytic or macrophage colonies. The culture fluids were harvested after 48 hr, and PGE levels were determined by radioimmunoassay. Macrophage PGE synthesis was significantly stimulated only in the presence of those fractions of serum-free WEHI-3-conditioned medium
that also supported the proliferation of murine bone marrow CFU-C. Of particular interest, however, was the observation that the stimulation of macrophage PGE synthesis was independent of whether the CSF induced the formation of colonies comprised of primarily granulocytes or macrophages. Conversely, pooled fractions of the WEHI-3-conditioned medium without CSA had only an insignificant effect on macrophages. Note that the various WEHI-3 preparations themselves contained no detectable PGE.

The quantitative radioimmunoassay measurements of PGE were supplemented by methods that permitted the qualitative examination of the effects of the various WEHI-3 CSF preparations on the biosynthetic conversion of \(^{3}H\)-arachidonic acid into \(^{3}H\)-PGE\(_2\) and \(^{3}H\)-PGF by murine macrophages.\(^{42}\) As

| Table 2. Effects of Granulocytic and Macrophage CSF on Macrophage PGE Synthesis |
|-------------------------------|-----------------|-------------------|
|                               | PGE (pg)        | CFU-C/7.5 x 10\(^6\) B6D2F\(_1\), Bone Marrow Cells |
| Macrophages alone             | 167 ± 11        | —                 |
| Macrophages + WEHI-3 CSF\(_{GM}\) | 6407 ± 241    | —                 |
| Macrophages + WEHI-3 CSF\(_{M}\) | 2058 ± 380    | —                 |
| Macrophages + WEHI-3 CSF\(_{D}\) | 1749 ± 175    | —                 |
| Macrophages + CSF-free        | 280 ± 63        | —                 |
| WEHI-3-conditioned medium     | 263 ± 16        | —                 |
| WEHI-3 CSF\(_{GM}\) alone     | 0               | 162 ± 8           |
| WEHI-3 CSF\(_{M}\) alone      | 0               | 121 ± 16          |
| WEHI-3 CSF\(_{D}\) alone      | 0               | 0                 |
| CSF-free WEHI-3-conditioned medium alone | 0 | 0 |

PGE was determined by radioimmunoassay in 48-h-conditioned media from 1 x 10\(^6\) C3H/HeJ peritoneal macrophages incubated in the absence and presence of 10% v/v of various preparations of serum-free WEHI-3-conditioned medium previously separated into fractions that stimulated the formation of either predominantly granulocytic or macrophage colonies. Subscripts of the CSF nomenclature refer to the morphologic type of colony stimulated by the particular fraction of WEHI-3-conditioned medium.\(^{32}\) CSF\(_{GM}\), 10%-15% granulocytic (G), 20%-25% mixed, and 55%-65% macrophage (M); CSF\(_{M}\), 80%-90% granulocytic, 10%-20% mixed, and 0% macrophages; CSF\(_{D}\), 0% granulocytic, 15% mixed, and 85% macrophage; CSF-free, pooled fractions of WEHI-3-conditioned medium without detectable colony-stimulating activity.
Table 3. Effects of Various CSF Preparations on Biosynthetic Conversion of Arachidonic Acid to PGE\(_2\) and 6-keto-PGF\(_{1\alpha}\) by Murine Macrophages

<table>
<thead>
<tr>
<th>Condition</th>
<th>(^{3}\text{H-PGE}_2) (cpm)</th>
<th>(^{3}\text{H-PGF}) (cpm)</th>
<th>(^{3}\text{H-Arachidonic Acid}) (cpm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Macrophages alone</td>
<td>176 ± 4</td>
<td>99 ± 23</td>
<td>2995 ± 5</td>
</tr>
<tr>
<td>Macrophages + WEHI-3 CSFGM</td>
<td>3495 ± 152</td>
<td>672 ± 93</td>
<td>12,140 ± 445</td>
</tr>
<tr>
<td>Macrophages + WEHI-3 CSFM</td>
<td>2076 ± 7</td>
<td>422 ± 79</td>
<td>3433 ± 103</td>
</tr>
<tr>
<td>Macrophages + WEHI-3 CSFG</td>
<td>1903 ± 46</td>
<td>527 ± 86</td>
<td>3788 ± 83</td>
</tr>
<tr>
<td>Macrophages + CSF-free WEHI-3-conditioned medium</td>
<td>369 ± 46</td>
<td>184 ± 35</td>
<td>2565 ± 69</td>
</tr>
<tr>
<td>Macrophages + serum equivalent of WEHI-3-conditioned medium</td>
<td>80 ± 42</td>
<td>136 ± 79</td>
<td>7024 ± 677</td>
</tr>
</tbody>
</table>

Murine peritoneal macrophages (4 x 10⁶) were preincubated in medium 199 containing 1 μCi \(^{3}\text{H-arachidonic acid}\) for 4 hr, after which time the adherent cells were exhaustively washed and fresh medium added. The prelabeled macrophage cultures were exposed for 18 hr to the same CSF preparations of WEHI-3-conditioned medium as in Table 2 as well as to a concentration of FCS equivalent to that in the unseparated but concentrated FCS-containing WEHI-3 (CSFGM)-conditioned medium. The macrophage supernatant media were harvested and the radioactive oxygenation products separated by silicic acid (50-81) chromatography and identified on the basis of their mobilities as compared to the standard reference products PGE\(_2\), PGF\(_{2\alpha}\), and arachidonic acid according to the method of Humes et al.\(^{42}\) Counts per minute (cpm) were corrected for the nonspecific oxidation products of arachidonic acid that co-chromatographed with PGE\(_2\) and PGF\(_{2\alpha}\), as determined by subtracting the cpm difference in material obtained from indomethacin (1.4 x 10⁻⁶ M)-treated and untreated control cultures. The chromatographic methods used in these experiments clearly separated PGE\(_2\) from PGF. However, the PGF initially thought to be PGF\(_{2\alpha}\) has tentatively been identified by Humes et al.\(^{42}\) to represent 6-keto-PGF\(_{1\alpha}\), the stable metabolite of PGI\(_2\) (prostacyclin).

As shown in Table 3, murine peritoneal macrophages prelabeled with \(^{3}\text{H-arachidonic acid}\) were markedly stimulated by the various CSF-containing WEHI-3-conditioned medium fractions to release radioactive substrate and convert it to \(^{3}\text{H-PGE}_2\) and \(^{3}\text{H-6-keto-PGF}_{1\alpha}\). In contrast, the WEHI-3-conditioned medium fraction devoid of any CSA had only a slight effect on the conversion of arachidonic acid to PG. However, the unseparated WEHI-3 (CSFGM)-conditioned medium prepared in the presence of FCS stimulated the release of arachidonic acid markedly in excess of the levels of PGE\(_2\) and PGF synthesized. This was not the case with the serum-free chromatographed WEHI-3 (CSFG and CSFM)-conditioned medium fractions, which stimulated the biosynthetic conversion of arachidonic acid into PGE\(_2\) and 6-keto-PGF\(_{1\alpha}\) despite a small but significant effect on arachidonic acid release. We examined whether or not this might reflect the concentration of serum used in the preparation of the conditioned media. It was found that in the absence of CSF the addition of FCS concentration equivalent to that present in the unseparated WEHI-3 (CSFGM)-conditioned medium stimulated arachidonic acid release by macrophages but had no effect on the synthesis of PGE\(_2\) and 6-keto-PGF\(_{1\alpha}\).

DISCUSSION

Human and murine adherent mononuclear cells of light buoyant density elaborated CSF that stimulated the proliferation of a class of hematopoietic progenitor cells restricted to granulocyte and monocyte-macrophage differentiation. Species-specific stimulation of CFU-C was provided by culture feeder layers containing highly enriched populations of human peripheral blood...
monocytes and murine peritoneal macrophages or media conditioned by such cells. The magnitude of stimulation (or the concentration of CSF) increased with the number of these mononuclear phagocytes. However, the relationship between colony numbers and the concentration of mononuclear phagocytes was not linear, since fewer CFU-C commenced colony formation in the presence of increasing numbers of monocytes or macrophages. The decline of colony formation in cultures containing high numbers of phagocytic mononuclear cells could not be simply attributed to reduced CSF production, since the inhibition of CFU-C was also noted in the presence of an exogenous source of CSF. The species specificity of CSF prevents murine peritoneal macrophages from supporting colony formation by human CFU-C. However, the ability of murine macrophages to profoundly inhibit both human adult bone marrow and fetal liver CFU-C substantiated the existence of an active inhibitory process that was independent of the species and tissue of origin of the progenitor cell.

These results indicate the ability of the mononuclear phagocyte to stimulate as well as inhibit the proliferation of the committed myeloid progenitor cell. However, this dualistic influence by monocytes and macrophages, being so highly dependent upon their cell concentrations, suggested the differential accumulation of two factors with mutually opposing actions elaborated by a given number of mononuclear phagocytes. The ability of indomethacin to both increase the colony-stimulating ability of high numbers of mononuclear phagocytes and largely prevent their inhibition of colony formation suggested a role for prostaglandin in antagonizing the stimulatory actions of monocyte- and macrophage-derived CSF.

By means of a sensitive radioimmunoassay we showed a positive correlation between the concentration of PGE produced by a given number of blood monocytes and the inhibition of colony formation. Within a critical concentration of monocytes, CSF production occurred in the absence of PGE synthesis, and slight increases in the number of monocytes permitted a progressive stimulation of CFU-C proliferation. Increasing the numbers of monocytes above this critical concentration was associated with the accumulation of radioimmunologically detectable PGE, which rose in proportion to the number of monocytes and prevented any further increase in colony formation. Thus cessation of further CFU-C proliferation corresponded to the monocyte concentration at which PGE synthesis was first detectable. Only above this critical concentration did indomethacin suppress the synthesis of monocyte PGE and augment colony formation. The accumulation of monocyte-derived PGE therefore limited the numbers of CFU-C that commenced colony formation in response to a given concentration of monocyte-derived CSF. Thus the proliferation of the myeloid progenitor cell varied proportionally with the level of CSF but inversely with the concentration of PGE in the culture system. By preventing the constitutive synthesis of PGE, indomethacin served to permit an unopposed level of CSF to initiate the proliferation of a greater number of CFU-C and allowed a more accurate estimate of the incidence of potentially clonable progenitor cells. The identification of an inhibitor derived from phagocytic mononuclear cells as prostaglandin is consistent with our previous observations that the synthetic E-series prostaglandins (PGE₁ and PGE₂) effectively inhibit the cloning of
myelopoietic progenitor cells in vitro and that this inhibition is effectively prevented by the dibenzo napine hydrazide prostaglandin antagonist SC-19220.

Inhibition of PGE synthesis by indomethacin resulted in marked augmentation of the net colony-stimulating ability of human leukocyte feeder layers and counteracted the inhibition of human CFU-C when an additional biosynthetic source of PGE such as murine peritoneal macrophages was present. Indomethacin was most effective when added at the time of preparation of the leukocyte feeder layers, suggesting that PGE synthesis and CFU-C inhibition are early events in vitro. This contention is substantiated by our previous observations that the early addition of synthetic PGE to CSF-stimulated murine bone marrow cultures was significantly more inhibitory to CFU-C than when added later during culture. Indomethacin had no direct effect on CFU-C and did not influence the colony-stimulating capacity of human lymphocytes, consistent with the absence of detectable PGE synthetic activity by this cell population.

Regulation of colony formation and production of CSF appears to occur by several mechanisms. We have shown that the human monocyte can be selectively modulated by an extract of human polymorphonuclear granulocytes (GE) in a manner distinct from that of indomethacin. GE inhibited colony formation by reducing the synthesis and/or release of CSF by human monocytes, whereas indomethacin potentiated the proliferation of myeloid progenitor cells by interfering with the synthesis of PGE. The combined effects of the GE and indomethacin on colony formation was not significantly different than the average of the individual effects of simultaneously reducing CSF production and inhibiting PGE synthesis. These observations therefore serve to indicate that selective manipulating of the elaboration of opposing influences by the mononuclear phagocyte can result in either stimulation or inhibition. Indeed, provided both stimulatory and inhibitory factors were concomitantly reduced, there was no net change in the numbers of CFU-C that commenced proliferation.

These findings indicate that the proliferation in vitro of the committed granulocyte-macrophage progenitor cell is regulated by both positive and negative feedback controls involving monocyte-macrophage-derived CSF and PGE, respectively. Such evidence is certainly sufficient to suggest that the constitutive elaboration of CSF and PGE may account for the steady-state regulation of myelopoiesis. However, evidence is presented that indicates that the mononuclear phagocyte possesses an inherent surveillance capability that allows it to modulate the synthesis of the indomethacin-sensitive inhibitory factor in response to varying concentrations of CSF. Murine peritoneal macrophages exposed to increasing concentrations of a conditioned medium containing murine active CSF became progressively less stimulatory for murine CFU-C by elaborating an inhibitory factor that counteracted the action of CSF on CFU-C. Similarly, murine macrophages at concentrations that otherwise had no effect on human CFU-C became profoundly suppressive as a function of the concentration of CSF to which they were exposed. In both instances, the inhibitory activity was lost following dialysis.

Radioimmunoassay measurements of murine macrophages incubated with
increasing concentrations of CSF showed a linear stimulation of PGE synthesis. In the absence of the additional CSF, the low concentrations of macrophages employed in these experiments failed to elaborate detectable levels of PGE and did not inhibit colony formation. However, only in the presence of CSF concentrations that induced the synthesis of PGE did the low numbers of macrophages show profound suppressive activity. Since CSF is an apparently heterogeneous group of factors with colony-stimulating capacity, it was of particular interest to observe that more purified murine CSF, separated into fractions that stimulated the formation of either predominantly granulocytic or macrophage colonies, were equally capable of stimulating the synthesis of PGE by murine peritoneal macrophages (Table 2). In contrast, pooled fractions of WEHI-3-conditioned medium that failed to show any colony-stimulating activity did not significantly affect macrophage PGE synthesis.

Prostaglandin biosynthesis is dependent upon the availability of arachidonic acid and its conversion by cyclooxygenase to the endoperoxide intermediates and subsequently to the stable prostaglandins PGE$_2$ and PGF$_{2\alpha}$, the thromboxanes, or to the stable metabolite of prostacyclin (PGI$_2$), 6-keto-PGF$_{1\alpha}$. However, we have shown (Table 3) that the stimulation by CSF of both PGE$_2$ and 6-keto-PGF$_{1\alpha}$ synthesis by macrophages is not solely the result of a nonspecific stimulation of arachidonic acid release from cellular phospholipid. This dissociation between the magnitude of arachidonic acid release and the subsequent synthesis of both PGE$_2$ and 6-keto-PGF$_{1\alpha}$ was shown by the observations that the addition of a high concentration of serum to macrophages stimulated the release of arachidonic acid but had no effect on prostaglandin synthesis. The ability of serum to release arachidonic acid from the phospholipid of tritium-labeled methylcholanthrene-transformed mouse 3T3 cells has been reported. In contrast, the dramatic effect of the serum-free granulocytic and macrophage CSF preparations on macrophage PGE$_2$ and 6-keto-PGF$_{1\alpha}$ synthesis was observed in the absence of nonspecific arachidonic acid release. Thus stimulation by CSF of macrophage prostaglandin synthesis may derive from an action on phospholipase as well as more proximally, such as on cyclo-oxygenase. This phenomenon of CSF-dependent macrophage PGE synthesis is presently under investigation.

Our interpretation that PGE is the indomethacin-sensitive inhibitory principle elaborated by mononuclear phagocytes is based upon direct evidence (1) that synthetically pure PGE$_1$ and PGE$_2$ inhibit CFU-C by reducing their responsiveness to a soluble source of CSF, (2) that this effect is antagonized by a dibenzoxapine hydrazide derivative reported to inhibit the action of prostaglandin, (3) that human monocytes and murine macrophages actively synthesize and release PGE, and (4) that they elaborate a diffusible and dialyzable inhibitor of CFU-C in vitro. Despite the contention of the present-day literature that indomethacin is specific in its inhibitory actions for cyclooxygenase, it may be argued that the ability of indomethacin to inhibit macrophage PGE synthesis and largely prevent their suppression of CFU-C are probably not related. It is possible that what we are determining by both radioimmunoassay and $^3$H-arachidonic acid conversion methods as PGE may also represent a separate, but synthetically related, group of cyclooxygenase depen-
dent arachidonic acid oxygenation products, such as the extremely short-lived but biologically active thromboxanes. Since we and others cannot show an effect of indomethacin on the release of β-glucuronidase, N-acetylglucosaminidase (Pelus L, et al: unpublished observation), and lysozyme (Ralph P: unpublished observation) from macrophages, it is an unlikely alternative that indomethacin may effect the elaboration of cyclooxygenase-independent factors by mononuclear phagocytes, particularly those that may influence CFU-C proliferation.

These findings document the existence of an afferent feedback control in which the macrophage can regulate the synthesis of PGE by sensing the CSF concentration in its external milieu, which in turn antagonizes the action of CSF on CFU-C. In addition, a CSF requirement for basal PGE synthesis by mononuclear phagocytes is suggested by our present finding that the reduction of endogenous human monocyte CSF production by treatment with extracts of mature granulocytes coincidently decreased the elaboration of PGE. Although CSF production is not dependent upon PGE synthesis, since it occurs in the presence of indomethacin, our preliminary evidence suggests that low concentrations of synthetic PGE increase the synthesis and/or release of CSF by murine macrophages. Thus the ability of CSF to stimulate macrophage PGE synthesis may be balanced by a second feedback loop associated with the stimulation of CSF production by a critical concentration of PGE.

The synthesis and release of CSF and PGE by the mononuclear phagocyte suggests its central role in the regulation of steady-state production of granulocytes, monocytes, and macrophages as well as the potential to reestablish the appropriate balance between CSF and PGE following a variety of imposed stimuli that elevate systemic levels of CSF (Fig. 9). The lability of the PGE molecule, coupled with its efficient clearance by the lung and presumably other tissues, dictates that the effects of PGE on the responsiveness of the myeloid progenitor cell to CSF occur via short-range cellular interactions and are transitory in nature. The continuation of the myelopoietic modulating effects of PGE would therefore be subject to a persistent elevation in local CSF levels, which in turn maintains the mononuclear phagocyte in an accelerated state of

![Fig. 9. Macrophage control of myelopoiesis.](image-url)
PGE synthesis. The intimate association between the synthesis of CSF and PGE by the monocyte and macrophage suggests that the normal cyclic variation in myelopoiesis may have its origins in a biologic regulatory system operating via macrophage surveillance of CSF and the modulation of PGE synthesis.

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Role for monocyte-macrophage-derived colony-stimulating factor and prostaglandin E in the positive and negative feedback control of myeloid stem cell proliferation

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