Regulation of Human Monocyte DNA Synthesis by Colony-Stimulating Factors, Cytokines, and Cyclic Adenosine Monophosphate

By Daisy L. Cheung and John A. Hamilton

It is reported in this study that a subpopulation of highly purified human peripheral blood monocytes can proliferate in response to colony-stimulating factor-1 (CSF-1), granulocyte-macrophage colony-stimulating factor (GM-CSF), and interleukin-3 (IL-3). Both GM-CSF and IL-3 synergized with CSF-1 for the induction of DNA synthesis. Given the DNA synthesis levels attained, we were able to test the effects of certain cytokines and cyclic adenosine monophosphate (cAMP)-elevating agents, which have been shown to modulate in vitro human myelopoiesis and murine macrophage proliferation. The cytokines, interferon-γ (IFN-γ), interleukin-4 (IL-4), and tumor necrosis factor-alpha (TNF-α), as well as cAMP-elevating agents, 8-bromoadenosine 3':5'-cyclic monophosphate (8BrCAMP), cholera toxin (CT), and prostaglandin E2 (PGE2), suppressed the monocyte DNA synthesis due to CSF-1. These results parallel those reported with human bone marrow progenitors, as well as murine macrophage populations. The cycling human monocyte population could provide a model cell type to understand the molecular events controlling human myelopoiesis. © 1992 by The American Society of Hematology.

Most of the current knowledge of the signals involved in the control of the proliferation of macrophages and their progenitors by CSFs has been obtained from the findings of experiments in which CSF-1–dependent immortalized murine cell lines and murine BMM have been used as cell models. Among the important biochemical signals implicated in the negative control of CSF-mediated proliferation are those that result from elevations in cyclic adenosine monophosphate (cAMP) levels, for example, in the inhibition of BMM DNA synthesis stimulated by CSF-1, granulocyte-macrophage CSF (GM-CSF), or interleukin-3 (IL-3) (also known as multi-CSF), and that of BAC1.2F5 cells, stimulated by CSF-1. Indirect evidence of the negative regulatory role of cAMP in human myelopoiesis comes from the finding that prostaglandin E2 (PGE2), which elevates intracellular cAMP concentrations in human monocytes and murine BMM, can inhibit human myeloid cell colony formation mediated by human monocyte–derived CSF(s). Recently, several cytokines have been recognized to be involved in the regulation of hematopoiesis, either as cofactors with CSF or as colony-stimulating activities themselves. Interleukin-1 (IL-1), previously known as hemopoietin-1, promotes the survival of human myeloid progenitors and of murine myeloid progenitors. Interleukin-4 (IL-4) inhibits macrophage colony formation in human bone marrow cultures. Other cytokines that have been involved in the regulation of hematopoiesis include interferon-γ (IFN-γ), tumor necrosis factor (TNF), and interleukin-6.

The present studies demonstrate that a subpopulation(s) of human peripheral blood monocytes can undergo DNA synthesis in response to CSF-1. With the level of DNA synthesis observed, we were able to explore the actions of a series of agents that are known to modulate human myelopoiesis in vitro.

MATERIALS AND METHODS

Monocyte isolation and culture. Buffy coats from normal donors for isolating peripheral blood monocytes were kindly provided by the Melbourne Red Cross Blood Bank. Monocytes were isolated by density gradient centrifugation followed by countercurrent centrifugal elutriation (Beckman JE-6B Elutriation System, Palo Alto, CA) as described by Hart et al. Monocyte-enriched fractions (≥95% purity) were resuspended in Alpha-Modified Eagle's Medium (αMEM; Flow Laboratories, McLean, VA), supplemented with 20 mmol/L L-3-(N-morpholino)propane-sulfonic acid.
To cyt centrifuged preparations and by nonspecific esterase stain-

phosphate-buffered saline, pH 7.4 (PD). The adherent cells were

were seeded in 96-well microwell plates (5-mm well diameter;

Brookvale, Australia) in PD.ZS Released nuclei were harvested and

lysed with zapoglobin (10% vol/vol) (Coulter Electronics,

water and vacuum suction. Radioactivity associated with the filters

chamber; Miles Scientific, Naperville, IL) at a density of 10s

in the washing buffer was determined by trypan blue dye exclusion.

For cells cultured in microtiter plates, cellular macromolecules

fraction, an equal volume of 20% trichloroacetic acid (TCA) was

for each experiment. It was because of this reason that the latter

indicated.

Measurement of DNA synthesis. As described previously, the
development of DNA synthesis was monitored by measuring the incorpora-
tion of 3H-thymidine (3H-Tdr) into stimulated monocytes that

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(5 min/room temperature). The process was concluded by counter-

was taken as the percentage of cells with 60 or more grains per nucleus. A

minimum of 500 cells per culture well was evaluated under 400× magnification. Results are expressed as the mean ± SEM of triplicate cultures per stimulus.

Preparation of platelet-poor plasma-derived HS. As described by Musson,27 heparinized whole AB blood from normal donors (Melbourne Red Cross Blood Bank) was centrifuged at 2,000 × g for 30 minutes at 10°C to remove cell fractions and platelets. The platelet-poor human plasma was collected and incubated with 20 mmol/L CaCl₂ at 37°C until coagulation occurred, thereby depleting any coagulation factors present. The clot was spun down (2,000 × g, 30 minutes, 10°C) and removed. The platelet-poor plasma-derived HS fraction was stored at −20°C until use.

Reagents. Highly purified recombinant human CSFs were obtained as gifts from the following: CSF-1, 4.2 × 10⁹ U/mg (Department of Process and Product Development, Cetus, Em-

eryville, CA)—CSF-1 bioactivity was measured using CSF71/1 bone marrow cells in semisolid agar medium assigning 50 U/mL to the

cellular growth.

The viability of cells present in the aspirated media and

if any, difference in the pattern of response (data not shown). The

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minimum of 500 cells per culture well was evaluated under 400× magnification. Results are expressed as the mean ± SEM of triplicate cultures per stimulus.
Fig 1. Effects of CSF-1 on DNA synthesis and cell number. Purified monocytes from a typical donor were seeded in 24-well plates and allowed to adhere for 90 minutes. Nonadherent cells were removed by multiple washes and the remaining adherent cell monolayers were treated with 3% HS together with CSF-1 (3,000 U/mL) (■) or 3% HS alone (○) for up to 11 days. (A) Cells were pulsed with [3H]-Tdr (2.5 pCi/mL) for the last 24 hours of incubation. At the time points indicated, cultures were terminated and DNA synthesis was monitored by measuring the incorporation of [3H]-Tdr as described in the Methods. Error bars have been omitted when errors were within the size of the symbol.

RESULTS

Stimulation of DNA synthesis and increase in cell number by CSF-1. When cultured in 3% HS for 3 days, human monocytes had a rounded appearance. In contrast, when CSF-1 was added, the cells enlarged, spread, and formed elongated cytoplasmic processes. To determine to what extent monocytes respond to CSF-1 for DNA synthesis when cultured under these conditions, [3H]-Tdr incorporation was measured over a 12-day period. The data in Fig 1A are representative of four experiments. CSF-1 (3,000 U/mL; 100 pmol/L) increased the level of [3H]-Tdr incorporation beginning between days 2 and 4, with the degree of incorporation peaking between days 6 and 8. It is unlikely that the decline at later time points was due to the depletion of CSF-1, as the addition of fresh CSF-1 (3,000 U/mL) every second day did not maintain the level (data not shown). When the increasing levels of [3H]-Tdr incorporation upon CSF-1 treatment were expressed on a per cell basis, they were also significantly greater than that of the control cultures, indicating that the increase of cellular DNA synthetic activity was not due to the presence of more cells in the CSF-1-containing cultures. The incorporation of [3H]-Tdr into contaminating lymphocytes could not account for the increase detected, since the lymphocyte-enriched fractions, obtained by elutriation, did not respond to CSF-1 for DNA synthesis (data not shown). In agreement with previous finding in murine BMM, no DNA synthesis was observed in serum-free cultures, even in the presence of CSF-1 (data not shown).

In the same experiment, the effect of CSF-1 on cell number was also examined. For control cultures incubated with 3% HS alone, less than 50% of the initial count remained adherent after 2 days of incubation (Fig 1B). For the detached cells, approximately 30% of them were viable and the percentage of viability decreased gradually with time (data not shown). On the contrary, 80% of the initial cell population in cultures treated with CSF-1 remained adherent after 2 days. The number of adherent cells increased between days 6 and 8; the original cell number doubled by day 10. Similar kinetics were observed in two other experiments. The addition of fresh CSF-1 every second day did not enhance the increase (data not shown). Increased [3H]-Tdr incorporation induced by CSF-1 was dose-dependent (Fig 2). Monocytes attained maximal level
of DNA synthesis in response to CSF-1 at doses greater than or equal to 3,000 U/mL (≥ 100 pmol/L).

**GM-CSF, IL-3, and human monocyte proliferation.** Other CSFs, such as GM-CSF and IL-3, were assessed for their ability to cause DNA synthesis in human monocytes cultured under the same conditions. The induction of DNA synthesis by GM-CSF or IL-3 was dose-dependent (Fig 3). GM-CSF exerted its maximal effect at doses of 100 U/mL or greater (0.11 nmol/L) (Fig 3A), whereas for IL-3 it was at 1 U/mL (0.05 nmol/L) as determined in a separate donor (Fig 3B). It should be noted that 10 U/mL (0.5 nmol/L) of IL-3 was required in some donors to achieve maximal effect. The relative effects of the CSFs on DNA synthesis were compared. For cells from three of four donors, CSF-1 enhanced 'H-TdR incorporation at a significantly greater degree than GM-CSF or IL-3 (P < .001) (Fig 4A). Any difference in the degree of response induced by the growth factors was not detected in the other donor.

![Graph](image1)

**Fig 3. Effects of GM-CSF and IL-3 on DNA synthesis.** Purified monocytes from two separate donors were seeded in 24-well plates and allowed to adhere for 90 minutes. Nonadherent cells were removed by multiple washes and the remaining adherent cell populations were exposed to 3% HS alone or together with either GM-CSF (10 to 1,000 U/mL) or IL-3 (0.01 to 10 U/mL) for up to 5 days and pulsed with 'H-TdR (2.5 μCi/mL) for 24 hours. At the end of the labeling period, the levels of 'H-TdR incorporation were determined as described in the Methods. Each point represents the mean of triplicate cultures (±SEM). Error bars have been omitted when errors were within the size of the symbol.

**Fig 4. Effects of CSF-1, GM-CSF, and IL-3 human monocyte DNA synthesis and cell number.** (A) Purified monocytes were seeded in 24-well plates and allowed to adhere for 90 minutes. Nonadherent cells were removed by multiple washes and the remaining adherent cell monolayers were exposed to 3% HS alone or together with either CSF-1 (1,000 U/mL), GM-CSF (1,000 U/mL), or IL-3 (10 U/mL) for 5 days followed by a 24-hour labeling period. The levels of 'H-TdR incorporation were determined as described in the Methods. (B) Monocytes purified from a different individual were prepared as described in (A); the adherent cell populations were treated with serum alone or together with either CSF-1 (3,000 U/mL), GM-CSF (1,000 U/mL), or IL-3 (10 U/mL) for 11 days. The dashed line represents the cell number at the time of addition of the various growth factors (day 0). The change in cell number of the adherent population was determined as described in the Methods. Relative to CSF-1-treated cultures: *P < .02, **P < .001. For both (A) and (B), each point represents the mean of triplicate cultures (±SEM). Error bars have been omitted when errors were within the size of the symbol.

When changes in cell number were followed, all three growth factors caused a significant increase in cell number after 11 days in culture (P < .01). However, in two of three donors, CSF-1 treatment resulted in a greater degree of increase, as shown in Fig 4B. Perhaps not surprisingly, the proliferative response of human monocytes to any particular CSF is subject to donor-to-donor variation.

**Number of 'H-TdR-labeled monocytes.** In view of the finding that CSF treatment was associated with increased
with 'H-TdR (4 μCi/mL) for 24 hours. Table 1 depicts the value may have been overestimated due to a decrease in the donor, the dose of GM-CSF administered induced a higher monocytes treated with suboptimal doses of CSF-1 (100 to the 24-hour labeling period, compared with the corresponding control value of 1% to 2.5%. In the GM-CSF–treated and IL-3–treated groups, the percentage of labeled cells varied from 5% to 10% and 7% to 10%, respectively. The labeling index was not improved by lengthening the labeling period (data not shown). It should be noted that the control value may have been overestimated due to a decrease in the absolute number of cells remaining in cultures (see above).

Synergistic effects of the CSFs. To determine whether the CSFs could act synergistically, as has been reported for murine BMM,24 monocyte cultures were treated with a suboptimal dose of either GM-CSF (5 U/mL; 5 pmol/L) or IL-3 (0.1 U/mL; 5 pmol/L), together with different concentrations of CSF-1 (10 to 5,000 U/mL); 'H-TdR incorporation was measured at day 3. Both GM-CSF and IL-3 enhanced synergistically the levels of DNA synthesis by monocytes treated with suboptimal doses of CSF-1 (100 to 1,000 U/mL) in three donors tested. Figure 5 shows the response in one cell type; for cells from this particular donor, the dose of GM-CSF administered induced a higher level of 'H-TdR than IL-3 when these growth factors were added alone to cultures. The more potent effect of GM-CSF with cells from this particular donor may account for the observation that GM-CSF potentiated the effect of CSF-1 to a greater extent. It was also observed in this particular donor that GM-CSF and IL-3 could potentiate an optimal concentration of CSF-1 for the induction of DNA synthesis. It is unlikely that the increase was due to an earlier maximum level of DNA synthesis, since the presence of either GM-CSF or IL-3 did not alter the kinetics of CSF-1–induced DNA synthesis (data not shown).

Effect of cytokines on CSF-1–induced DNA synthesis. The in vitro development of human myeloid colonies from bone marrow progenitor cells in the presence of CSFs can be inhibited by IFN-γ,33 IL-4,17 and TNF-α,19 while CSF-1–stimulated murine BMM DNA synthesis can be suppressed by IFN-γ and TNF-α.21 We therefore determined whether these inhibitory cytokines could also reduce CSF-1–stimulated human monocyte DNA synthesis.

In Fig 6A, CSF-1–treated cultures were exposed to IFN-γ (0.02 to 20 U/mL) for the entire 4-day culture period. IFN-γ at doses between 2 and 20 U/mL completely abrogated the mitogenic effect of CSF-1, with the resulting level of DNA synthesis lower than the basal. The inhibitory effect of IFN-γ could not be overcome by using higher doses of CSF-1 (Fig 6B). This inhibitory effect of IFN-γ was seen in all three donors tested.

Cells from five donors were examined for the action of IL-4 and representative results are presented in Fig 7. Maximal inhibition of CSF-1–initiated DNA synthesis by IL-4 was achieved at the doses of 10⁻¹¹ to 10⁻¹⁰ mol/L (Fig 7A); a supraoptimal dose of CSF-1 did not overcome the suppressive effect of IL-4 (Fig 7B). This present finding unveils another biological effect that IL-4 and IFN-γ have in common on human monocytes, although they can also have opposite effects.34,35 Unlike IFN-γ and IL-4, whose potent inhibitory effects on DNA synthesis were seen in all donors, the suppressive effect of TNF-α on CSF-1–induced DNA synthesis was partial (Fig 8A). TNF-α at the dose of 10⁻⁹ mol/L displayed a maximal inhibitory effect (Fig 8A); its inhibitory action could be partially overcome by a supraoptimal dose of CSF-1 in two of four donors tested, an example of which is provided (Fig 8B).

It has been reported that IFN-γ and TNF-α can be added to CSF-1–stimulated murine BMM quite late in the G₁ phase of the cell cycle and still achieve their maximal inhibitory effects on DNA synthesis.36,37 Likewise, we demonstrate in Fig 9 that the administration of these cytokines, as well as IL-4, can be delayed for up to 48 hours after adding the CSF-1 to human monocytes and still can lower the level of DNA synthesis to basal, 'H-TdR incorporation again being measured by a 24-hour pulse at day 3.

Table 1. Number of 'H-TdR–Labeled Monocytes Treated with CSFs

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Labeled Cells (% ± SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Donor 1</td>
</tr>
<tr>
<td>Control</td>
<td>0.9 ± 0.07</td>
</tr>
<tr>
<td>CSF-1</td>
<td>16.3 ± 0.5</td>
</tr>
<tr>
<td>GM-CSF</td>
<td>9.5 ± 2.2</td>
</tr>
<tr>
<td>IL-3</td>
<td>9.2 ± 1.8</td>
</tr>
</tbody>
</table>

*Purified monocytes were cultured in Lab-Tek eight-chamber slides, and were treated with 3% HS or together with either CSF-1 (3,000 U/mL), GM-CSF (1,000 U/mL), or IL-3 (10 U/mL) after a 90-minute adherence period. The percentage of labeled nuclei was measured as in the Methods. The results are expressed as the mean of triplicate cultures ± SEM for four separate experiments.
judged by the number of viable cells scored at the termination of cultures, there was no evidence that the antiproliferative actions of IFN-γ, IL-4, or TNF-α could be explained by cytotoxic effects.

**cAMP and DNA synthesis.** Agents that elevate intracellular cAMP have been shown to inhibit murine macrophage colony formation from progenitor cells, as well as to block the mitogenic action of CSFs on murine BMM. Table 2 shows the effect of cAMP-elevating agents, 8BrcAMP, CT, and PGE₂, on CSF-1-induced DNA synthesis in human monocytes. All three agents (8BrcAMP [10⁻⁵ to 10⁻³ mol/L], CT [10⁻¹² mol/L], and PGE₂ [10⁻⁸ to 10⁻⁷ mol/L]) caused a partial suppression of the mitogenic action of CSF-1 (Table 2). When the doses of agents used exceeded those indicated below, they were found to have a cytotoxic effect over the overall 4-day culture period, as judged by cell morphology and the number of viable cells (data not shown).

**DISCUSSION**

Using highly purified human peripheral blood monocytes, we demonstrated that these cells responded to CSF-1 with a gradual increase in DNA synthesis and this enhanced activity started to decline between days 6 and 8 in culture (Fig 1). ³H-TdR incorporation increased up to 50-fold upon treatment with an optimal dose of CSF-1 (3,000 U/mL)
Fig 8. Effects of TNF-α on CSF-1-induced DNA synthesis. (A) Purified monocytes were seeded in 96-well plates. After an overnight adherence period, cultures were subjected to gentle washes to remove nonadherent cells. The remaining adherent cell populations were treated with CSF-1 (3,000 U/mL) alone or together with different doses of TNF-α (10^{-11} to 10^{-8} mol/L) for the entire culture period (4 days). (B) Purified monocytes from a different donor were prepared as described in (A). The adherent cell populations were incubated with serial concentrations of CSF-1 alone (10 to 10,000 U/mL) (○) or together with TNF-α (10^{-7} mol/L) (●). For both (A) and (B), 3H-TdR (0.5 μCi/0.2 mL) was added to cultures at day 3 and cells were pulsed for 24 hours. The levels of 3H-TdR incorporation were measured as described in the Methods. Each point represents the mean of triplicate cultures (±SEM). Error bars have been omitted when errors were within the size of the symbol.

Fig 9. Effects of delayed addition of IFN-γ, IL-4, and TNF-α to CSF-1-treated cultures. Purified monocytes were seeded in 96-well plates and allowed to adhere overnight. Nonadherent cells were removed by gentle washes. Control cultures were incubated in 3% HS alone. The rest of the cultures contained CSF-1 (3,000 U/mL) together with serum. To some cultures, IFN-γ (100 U/mL), IL-4 (5 x 10^{-8} mol/L) or TNF-α (10^{-7} mol/L) was added following the addition of CSF-1 at the time points indicated (t). 3H-TdR (0.5 μCi/0.2 mL) was added to all cultures at day 3 and cells were pulsed for 24 hours. The levels of 3H-TdR incorporation were measured as described in the Methods. The results are expressed as the mean of triplicate cultures ± SEM. Error bars have been omitted when errors were within the size of the symbol. Relative to cultures treated with CSF-1 (3,000 U/mL) alone: *P < .001; **P < .02; ***P < .05.
Table 2. Effects of cAMP-Elevating Agents on CSF-1–Mediated DNA Synthesis

<table>
<thead>
<tr>
<th>Treatment*</th>
<th>^H-TdR Incorporation (cpm/well ± SEM)</th>
<th>P Value‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1,000 ± 70</td>
<td></td>
</tr>
<tr>
<td>CSF-1</td>
<td>8,600 ± 420</td>
<td></td>
</tr>
<tr>
<td>+ 8BrcAMP (10^-1 mol/L)</td>
<td>7,200 ± 70</td>
<td>&lt;.05</td>
</tr>
<tr>
<td>+ 8BrcAMP (10^-4 mol/L)</td>
<td>4,800 ± 140</td>
<td>&lt;.01</td>
</tr>
<tr>
<td>Control</td>
<td>2,000 ± 170</td>
<td></td>
</tr>
<tr>
<td>CSF-1</td>
<td>30,000 ± 1,800</td>
<td></td>
</tr>
<tr>
<td>+ CT (10^-15 mol/L)</td>
<td>32,900 ± 330</td>
<td>NS</td>
</tr>
<tr>
<td>+ CT (10^-15 mol/L)</td>
<td>16,400 ± 700</td>
<td>&lt;.01</td>
</tr>
<tr>
<td>Control</td>
<td>430 ± 20</td>
<td></td>
</tr>
<tr>
<td>CSF-1</td>
<td>10,500 ± 54</td>
<td></td>
</tr>
<tr>
<td>+ PGE₂ (10^-4 mol/L)</td>
<td>4,200 ± 500</td>
<td>&lt;.02</td>
</tr>
<tr>
<td>+ PGE₂ (10^-7 mol/L)</td>
<td>2,200 ± 370</td>
<td>&lt;.02</td>
</tr>
</tbody>
</table>

The effects of 8BrcAMP, CT, and PGE₂ on CSF-1–mediated monocyte DNA synthesis were examined separately in three different experiments. Data obtained from each experiment were compiled into this Table.

* Purified monocytes from three individuals were seeded in 96-well plates and allowed to adhere overnight. Nonadherent cells were removed by gentle washes. Control cultures were incubated in 3% HS alone. The rest of the cultures contained CSF-1 (3,000 U/mL) together with serum. To some cultures, a cAMP-elevating agent, 8BrcAMP, CT, or PGE₂, was added as indicated.

† Cells were pulsed with ^H-TdR (2.5 μCi/mL) over the last 24 hours of a 4-day culture. ^H-TdR incorporation was measured as described in the Methods. The results are expressed as the mean of triplicate cultures ± SEM.

‡ P values were determined by comparison with corresponding cultures treated with CSF-1 alone. NS, not significant, P > .05.

DNA synthesis were observed (Fig 5). In some but not all donors, the stimulatory effect of an optimal concentration of CSF-1 could be further enhanced by GM-CSF or IL-3 (Fig 5). Whether there are different subpopulations of cells which are selectively responsive to individual growth factors remains to be elucidated. Synergistic interaction between hematopoietic growth factors, when used at suboptimal concentrations, have also been reported in murine cells—BMM, PEM, and monocytes. It is unknown whether these synergistic interactions are a reflection of changes in receptor properties, although it has been shown in murine progenitor cells that CSF-1 receptor expression is upregulated by IL-3 and there is cross-talk between CSFs when acting on murine progenitor cells.

It was shown above that IFN-γ, IL-4, and TNF-α inhibited the mitogenic action of CSF-1 on the monocytes (Figs 6 to 8). These findings parallel those with human marrow progenitor cells which give rise to mature myeloid cells in the presence of CSFs. They are also similar to the action of IFN-γ and TNF-α on murine BMM proliferation treated with CSFs, although there is controversy in this system about the action of TNF-α. It is known that IFN-γ and IL-4 induce cultured monocytes/macrophages to undergo antigenic changes and formation of multinucleated giant cells (MGCs), which are indicative of cellular maturation. Whether the antimitogenic actions of IFN-γ and IL-4 as described above are related to the promotion of early maturation is unknown. In our experiments, formation of MGCs in IFN-γ- and IL-4–treated CSF-1–containing cultures was evident (data not shown). Despite the report that treatment of murine PEM with IFN-γ resulted in a decreased number of CSF-1 receptors, and that TNF-α caused a 90% downmodulation of CSF-1 receptors in various murine macrophage populations, it is unlikely to be the explanation for the inhibitory actions of IFN-γ, IL-4, and TNF-α we have described. This is because of the observations that CSF-1 was required only for the first 48 hours of a 4-day culture period to elicit a full-scale ^H-TdR incorporation (data not shown), and yet the addition of the antiproliferative cytokines at the end of this period was shown to inhibit the activity of DNA synthesis to a substantial degree (Fig 9). It is more likely that the cytokines are acting at the postreceptor level in the G1 phase of the cell cycle, possibly by the regulation of some critical event(s) in the mitogenic signaling pathway(s) as has been proposed for murine BMM.

The present study demonstrated that 8BrcAMP, PGE₂, and CT all suppressed CSF-1–mediated DNA synthesis (Table 2). Since PGE₂ and CT are able to increase intracellular cAMP levels in human monocytes, as in many cell types, these findings imply that cAMP-dependent protein kinase A activation provides a negative signal for human monocyte proliferation in response to CSF-1. These observations are in keeping with others using human bone marrow progenitors, murine BMM, and a CSF-1–dependent murine cell line. Evidence was presented in the latter two studies that the agents which elevated intracellular cAMP did not interfere with the interaction of CSF-1 with its receptor or with receptor function; whether this is the case in human monocytes requires further investigation.

It is known that elevation of intracellular cAMP is associated with human monocyte differentiation. Thus, it could be that the observed negative regulatory effects of the cAMP-elevating agents and the cytokines, IFN-γ, IL-4, and TNF-α, are a result of cellular differentiation.

In support of an earlier observation made by Imamura et al., it was found that pretreatment of monocyte cultures overnight with PT abolished the action of CSF-1 for the induction of DNA synthesis (data not shown). The involvement of a PT-sensitive G-protein(s) in the induction of signals from the occupied CSF-1 receptor to the initiation of DNA synthesis in these cells is therefore likely.

The findings reported above indicate that a subpopulation of human monocytes can proliferate in response to CSFs and various agents can be used to inhibit this growth. It is possible that only a certain fraction of the monocytes had time to undergo DNA synthesis during the assay period, although, as indicated, lengthening the labeling period did not increase the number of cells incorporating ^H-TdR. The actions of the various growth stimulators and inhibitors are similar on human bone marrow progenitor cells in vitro during the development of monocytes/macrophages, although the difficulty in obtaining sufficient numbers of enriched bone marrow progenitors makes biochemical analysis awkward; on this basis, we would like...
to suggest that the active monocyte population, particularly if it could be enriched further, might be a model cell type to understand the molecular events controlling human myelopoiesis. The noncycling populations may represent a more mature subpopulation. If these two putative subpopulations could be separated, it might be possible to compare their biochemical properties, and perhaps lead to a definition of the differences between them. The net result might be a better understanding of the events controlling human myeloid cell proliferation and differentiation. The results above also parallel those reported with murine macrophage populations, suggesting that the murine models may be appropriate for their human counterparts.

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