Deoxycytidine Stimulates the In Vitro Growth of Normal CFU-GM and Reverses the Negative Regulatory Effects of Acidic Isoferritin and Prostaglandin E₁

By Kapil Bhatia, John Cole, William MacLaughlin, Michael Baker, Zalmen Arlin, Gary Graham, and Steven Grant

We have examined the effect of supraphysiologic concentrations of the naturally occurring nucleoside deoxycytidine (dCyd) on the in vitro growth of normal (CFU-GM) and leukemic (L-CFU) myeloid progenitor cells. Bone marrow samples obtained from 34 consecutive patients undergoing routine diagnostic bone marrow aspirations for nonmalignant hematologic disorders exhibited nearly a twofold increment in CFU-GM when continuously cultured in the presence of 10⁻⁴ mol/L dCyd. Higher dCyd concentrations were associated with a smaller degree of enhancement of colony formation. In contrast, the growth of leukemic blast progenitors obtained from patients with acute nonlymphocytic leukemia were not enhanced by any of the dCyd concentrations tested. Coadministration of 10⁻⁴ mol/L tetrahydrouridine (THU), a cytidine deaminase inhibitor, failed to alter the relative inability of dCyd to enhance L-CFU colony growth. The stimulatory effect of dCyd on normal CFU-GM was not mediated by the adherent mononuclear cell population of the marrow, nor was it restricted to the subpopulation of CFU-GM in S phase at the time of initial exposure. Moreover, treatment of normal bone marrow cells with dCyd at concentrations ranging from 10⁻⁴ to 5 × 10⁻⁴ mol/L for 24 hours had only a minor effect on the fraction of CFU-GM in S phase. Coadministration of 10⁻⁴ mol/L dCyd was able to reverse the inhibitory effects of several putative regulators of normal myelopoiesis, including leukemia inhibitory activity (LIA), acidic isoferritins (AIF), and prostaglandin E₁ (PGE₁). Leukemic myeloblasts exposed to 10⁻⁴ mol/L dCyd exhibited substantial expansion of intracellular pools of dCyd triphosphate (dCTP), demonstrating that inability to metabolize dCyd could not be solely responsible for the absence of growth potentiation in these cells. These studies suggest that supraphysiologic concentrations of dCyd may confer a selective in vitro growth advantage upon normal v leukemic myeloid progenitor cells, and may free the former from the inhibitory effects of several potential negative regulators of myelopoiesis.

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Deoxycytidine (dCyd) is a naturally occurring pyrimidine nucleoside that is present in human plasma at concentrations ranging from 5 × 10⁻⁷ to 4 × 10⁻⁴ mol/L. It is metabolized intracellularly to its triphosphate derivative, dCTP, which is required by DNA polymerase for ongoing DNA synthesis and cell replication. The enzyme dCyd kinase catalyzes the initial phosphorylation of dCyd to its nucleoside monophosphate derivative, dCMP. This process represents the rate-limiting step in dCyd metabolism. Opposing the phosphorylation of dCyd is the degradative enzyme dCyd deaminase, which converts dCyd to deoxyuridine (dUrd). Alternatively, dCMP may be deaminated to deoxyuridylate (dUMP) by another degradative enzyme, dCMP deaminase. Resting cells maintain low dCTP levels, but exhibit increased activity of dCyd kinase and expand dCTP pools immediately prior to cell division. Since a balanced supply of deoxyribonucleotides is required for DNA synthesis, it is believed that a relative deficiency in intracellular dCTP concentrations may limit DNA replication and thereby modulate growth regulation.

Normal human bone marrow myeloid progenitors (CFU-GM) are known to exhibit a larger growth fraction than their leukemic counterparts and may preferentially utilize the pyrimidine salvage pathway for nucleic acid synthesis. Consequently, it is conceivable that quantitative differences in nucleotide metabolism may exist between normal and neoplastic elements. The aim of these studies was to determine whether such differences might be exploited in order to confer an in vitro growth advantage on normal cells. Specifically, we have examined the effect of supraphysiologic concentrations of dCyd on the soft agar growth of normal and leukemic myeloid progenitors. An additional goal was to determine whether high concentrations of dCyd might reverse the growth inhibitory effects toward normal cells of several putative regulators of granulopoiesis, including leukemia inhibitory activity (LIA), acidic isoferritins (AIF), and prostaglandin E₁ (PGE₁). Our results suggest that supraphysiologic concentrations of dCyd may preferentially stimulate the in vitro growth of normal CFU-GM, and may overcome the inhibitory effects of potentially important physiologic modulators of myelopoiesis.

MATERIALS AND METHODS

Drugs and chemicals. dCyd, thymidine (dThd), hydroxyurea (HU), indomethacin, and PGE₁ were purchased from Sigma Chemicals (St. Louis, Mo). Tetrahydrouridine (THU) was provided by Dr Kenneth Pauli, Drug Development Branch, NCI. AIF (pH 5.0 after isoelectric focusing) was prepared for the spleen of a patient with chronic myelogenous leukemia. This isoferritin (94% AIF) as determined by radioimmunoassay was kindly provided by Dr H. Brommeyer, University of Indiana School of Medicine. [³H]Thymidine (50 Ci/mmol/L) was purchased from Amersham Radiochemicals (Springfield, Ill). Drugs were stored as dry powders at −20°C and reconstituted in RPMI media immediately prior to use.

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solutions were filter sterilized utilizing 22 μm filter discs (Millipore Corporation, Cambridge, Mass).

Culture of normal human bone marrow granulocyte-macrophage progenitors. Human bone marrow myeloid progenitors were cultured by a previously described double-layer soft agar cloning method. Samples were obtained with informed consent from 34 consecutive patients undergoing routine diagnostic bone marrow aspirations for nonneoplastic hematologic disorders. These disorders consisted principally of iron deficiency anemia, immune thrombocytopenias, and the anemia of chronic disease. Specifically excluded were bone marrow aspirates from patients with myelodysplastic syndromes, drug-induced leucopenias, myelophthisic processes, acquired immune deficiency syndrome, or any other qualitative or quantitative disorder of myelopoiesis. After isolation of mononuclear cells, appropriate concentrations of dCyd were added prior to plating in soft agar and McCoy's 5a medium. GCT-conditioned media (10% v/v; GIBCO, Grand Island, NY) was used as a source of colony-stimulating activity. Additionally, in a separate set of experiments, cells were plated in the continuous presence of 10⁻³ mol/L THU (a concentration that maximally inhibits the deamination of dCyd) alone, and in conjunction with 10⁻⁴ mol/L dCyd. Alternatively, bone marrow mononuclear cells were treated for 24 hours with dCyd concentrations ranging from 10⁻⁴ to 5 x 10⁻³ mol/L, washed twice with McCoy's medium, and then plated in soft agar as above. In separate experiments, adherent bone marrow cells containing 90% or greater monocytes and macrophages were removed by incubation in tissue culture flasks in McCoy's medium with 10% fetal calf serum for one hour. Nonadherent mononuclear bone marrow cells were plated with various dCyd concentrations as described above. At the end of seven days incubation in a 37 °C, 5% CO₂ fully humidified incubator (NAPCO, Portland, Or), colonies, consisting of groups of 50 or more cells, were scored with an inverted microscope. The effect of various concentrations of dCyd on the fraction of clonogenic CFU-GM in S phase was examined utilizing a previously described "Crude" leukemia inhibitory activity, acidic isoferritin, and prostaglandin E₁, studies. Cells were cultured in the presence of 10⁻⁸ or 10⁻⁴ mol/L dCyd, along with each negative regulator of myelopoiesis. Cell-free "crude" LIA was prepared utilizing the method described by Broxmeyer et al. Maximal inhibitory concentrations of crude LIA (10% v/v), AIF (10⁻⁴ mol/L), or PGE₁ (10⁻⁴ mol/L) were added to each plate. In the case of PGE₁, indomethacin (10⁻⁴ mol/L) was added to the plates to prevent endogenous prostaglandin production by monocytes and macrophages, and appropriate controls assessed.

Deoxyribonucleotide determinations. Normal bone marrow mononuclear cells and leukemic myeloblasts were obtained as described previously, suspended in McCoy's 5a medium containing 10% fetal calf serum, and placed in 25 cm² tissue culture flasks along with varying concentrations of dCyd with and without 10⁻³ mol/L THU. After 18 hours incubation in a 37 °C, 5% CO₂ incubator, the cells were centrifuged, the cell pellet precipitated with 0.75 mL of cold 0.5N HCL0₄, and the supernatant neutralized with cold 4N KOH. The HClO₄ precipitate was removed and the neutralized acid soluble extracts assayed for dCTP levels utilizing the DNA polymerase assay of Grindey and Kinahan, described elsewhere in detail.

Statistical methods. Significant differences between experiment groups were assessed utilizing the Student's t test for paired or unpaired observations.

RESULTS

The effects of increasing concentrations of dCyd on CFU-GM and leukemic myeloid progenitor (L-CFU) colonies in soft agar are shown in Fig I. At physiologic concentrations of dCyd in the culture medium (10⁻⁴ mol/L), no stimulation in colony formation was observed. Exposure to 10⁻⁴ mol/L dCyd led to a significant increase in CFU-GM colony formation to 193 ± 11.5% of control (P < 0.001). At higher concentrations of dCyd, a decline in colony formation to control values was noted. For example, dCyd levels of 10⁻³ and 5 x 10⁻³ mol/L were associated with 139 ± 8.5 and 96.5 ± 4.6% of control colony growth, respectively. The degree of stimulation varied among different bone marrow samples. However, only 3 our of 34 bone marrow samples failed to exhibit significant enhancement of colony growth following exposure to 10⁻⁴ mol/L dCyd concentration, and no sample exhibited inhibition. A comparable degree of
stimulation was observed when 10d CFU-GM were scored instead of day 7 colonies (10 samples). A shorter dCyd exposure interval (24 hours) immediately prior to culture also resulted in a significant growth enhancement (201.7 ± 7.1% of control values; not shown). The degree of enhancement of colony formation by dCyd was not altered either by depleting the monocellular cell population of T lymphocytes or by utilizing PHA-LCM as a source of colony-stimulating activity. For example, when cells from 6 samples were plated under these conditions, the mean increment in colony formation in the presence of 10−4 mol/L dCyd was 198.6 ± 19.1% of control. This value was not significantly different from that obtained with dCyd-treated cells plated under standard conditions. A loss of expression of la-antigen on CFU-OM has been reported when the latter is incubated in suspension culture at 37 °C for six hours. Treatment of CFU-GM in this manner prior to plating in soft agar with 10−4 mol/L dCyd elicited a growth increment of 174.4 ± 12.3% of control (not shown). Removal of the adherent cell population also did not alter the increment in CFU-GM growth (212 ± 6.7% of control values; not shown).

In contrast to normal CFU-GM, leukemic myeloid progenitor colony growth was not enhanced by treatment with any of the dCyd concentrations tested (Fig 1). Moreover, at 5 × 10−3 mol/L dCyd, a decrease to 83.9 ± 3.4 of control leukemic colony formation was observed.

To assess what role cytidine deaminase might play in determining the response of normal and leukemic progenitor cells to supraphysiologic concentrations of dCyd, we examined the growth of CFU-GM and L-CFU in the presence of THU and dCyd (Table 1). Continuous exposure to 10−3 mol/L THU or 10−4 mol/L dCyd alone resulted in little change in the number of L-CFU. Furthermore, coadministration of THU in conjunction with dCyd did not result in enhanced leukemic cell colony formation. With respect to normal CFU-GM, THU did not increase the number of CFU-GM colonies relative to untreated control cells, nor did addition of THU to dCyd increase CFU-GM growth relative to dCyd (10−4 mol/L) administered alone.

In separate studies, dCTP pools were determined in leukemic blasts obtained from 14 patients with acute nonlymphocytic leukemia both in the unperturbed state and following exposure to several concentrations of dCyd for 18 hours (not shown). Untreated freshly procured myeloblasts exhibited a mean of 0.52 ± 0.09 pmol dCTP/106 cells, while exposure to 10−4 and 5 × 10−3 mol/L dCyd for 18 hours increased levels to 1.21 ± 0.27 and 3.38 ± 0.98 pmol dCTP/106 cells, respectively (P < 0.02). The effect of THU on intracellular dCTP pool expansion in leukemic myeloblasts exposed to high concentrations of dCyd was also evaluated (Table 2). Leukemic myeloblasts from 4 patients were exposed to 10−4 mol/L dCyd for 18 hours in the presence or absence of 10−3 mol/L THU and intracellular dCTP levels determined. In the presence of 10−4 mol/L dCyd, mean dCTP levels increased from 0.39 ± 0.1 to 0.68 ± 0.14 pmol/106 cells. Exposure to THU alone resulted in a smaller increment in dCTP levels (0.57 ± 0.9 pmol/106 cells). When cells were exposed to both THU and dCyd, dCTP levels (0.88 ± 0.29 pmol/106 cells) were not significantly different from those exposed to dCyd alone (P > 0.10). Identical experiments examining dCTP levels were performed on normal bone marrow mononuclear cells separated by Ficoll-Paque (Pharmacia, Piscataway, NJ) density gradient centrifugation. The results, in general, were quite similar to those involving leukemic myeloblasts, ie, THU did not enhance the ability of 10−4 mol/L dCyd to expand intracellular dCTP pools. It should be noted that for each condition, intracellular dCTP levels were greater for normal bone marrow mononuclear cells than for leukemic myeloblasts. However, these differences did not achieve the level of statistical significance (P > 0.10).

The effect of a 24-hour exposure to 10−4 and 10−3 mol/L dCyd on the percentage of clonogenic CFU-GM in S phase as determined by the thymidine suicide index is shown in Table 3. Neither dCyd concentration increased the percentage of CFU-GM synthesizing DNA. In addition, treatment of cells with 10−3 mol/L HU for one hour reduced CFU-GM colony formation to 69.5 ± 1.4% of control. However, addition of 10−4 mol/L dCyd significantly increased clonogenicity to 91.3 ± 4.2%, indicating that the effect of dCyd is not restricted to CFU-GM initially in S phase.

The effect of dCyd on the inhibitory activity of some of the known regulators of granulopoiesis is also shown in Table 3. In the absence of dCyd, a significant reduction in CFU-GM growth to 65.7, 64.5, and 55.0% of control values was observed following exposure to crude LIA (10%), AIF (10−9 mol/L), and PGE1 (10−9 mol/L), respectively. Coadministration of physiologic concentrations of dCyd (10−6 mol/L) did not alter the degree of inhibition by these agents. However, simultaneous treatment with 10−4 mol/L dCyd resulted in a significant increment in CFU-GM growth to values exceeding unperturbed control colony formation. The degree of reversal of the growth inhibition by 10−4 mol/L dCyd concentrations varied among different bone marrow samples. In general, the abolition of the inhibitory effect of crude LIA, AIF, and PGE1 was more pronounced in those CFU-GM samples that demonstrated a greater increment in colony growth following treatment with dCyd alone (data not shown).

| Table 1. Effect of Deoxycytidine (dCyd) and Tetrahydroxuridine (THU) on the Growth of Normal (CFU-GM) and Leukemic (L-CFU) Myeloid Progenitor Cells |
|-----------------------------------------------|------------------|
| Condition                                      | Percent Control Colony Formation ± SEM |
| dCyd (10−4 mol/L)                              | 193.0 ± 11.5     |
| THU (10−3 mol/L)                               | 99.6 ± 2.9       |
| dCyd (10−4 mol/L) + THU (10−3 mol/L)           | 168.8 ± 21.5     |

Bone marrow samples from 12 patients with nonmalignant hematologic disorders and 4 patients with acute nonlymphocytic leukemia were cloned in soft agar in the presence of dCyd (10−4 mol/L), THU (10−3 mol/L), or both agents simultaneously. At the end of seven days incubation in a 5% CO2, 37 °C incubator, normal (CFU-GM) and leukemic (L-CFU) myeloid colonies were scored with the aid of an inverted microscope as described in Materials and Methods. Values for each condition are expressed as the percentage of untreated control cell colony formation ± SEM.
Table 2. dCTP Pools in Human Leukemic Myeloblasts and Normal Bone Marrow Mononuclear Cells Exposed to Deoxycytidine (dCyd) and/or Tetrahydrouridine (THU)

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>Control</th>
<th>dCyd 10^-4 mol/L</th>
<th>THU 10^-3 mol/L</th>
<th>dCyd 10^-4 mol/L + THU 10^-3 mol/L</th>
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</thead>
<tbody>
<tr>
<td><strong>Leukemic Blasts</strong></td>
<td>1</td>
<td>0.18</td>
<td>0.23</td>
<td>0.51</td>
<td>0.62</td>
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<td></td>
<td>2</td>
<td>0.71</td>
<td>0.98</td>
<td>0.84</td>
<td>1.37</td>
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<tr>
<td></td>
<td>3</td>
<td>0.42</td>
<td>0.84</td>
<td>0.34</td>
<td>0.88</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>0.27</td>
<td>0.70</td>
<td>0.61</td>
<td>0.66</td>
</tr>
<tr>
<td><strong>Mean ± SEM</strong></td>
<td></td>
<td>0.39 ± 0.10</td>
<td>0.68 ± 0.14</td>
<td>0.57 ± 0.09</td>
<td>0.88 ± 0.29</td>
</tr>
<tr>
<td><strong>Normal Bone Marrow Mononuclear Cells</strong></td>
<td>1</td>
<td>0.29</td>
<td>1.79</td>
<td>0.68</td>
<td>2.76</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0.52</td>
<td>1.21</td>
<td>0.70</td>
<td>1.20</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>0.59</td>
<td>1.65</td>
<td>1.04</td>
<td>2.45</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>0.13</td>
<td>0.32</td>
<td>0.15</td>
<td>0.34</td>
</tr>
<tr>
<td><strong>Mean ± SEM</strong></td>
<td></td>
<td>0.38 ± 0.09</td>
<td>1.24 ± 0.28</td>
<td>0.64 ± 0.15</td>
<td>1.68 ± 0.48</td>
</tr>
</tbody>
</table>

Leukemic myeloblasts from 4 patients with acute nonlymphocytic leukemia and normal bone marrow mononuclear cells from 4 patients with nonmalignant hematologic disorders were incubated for 18 hours with 10^-4 mol/L dCyd alone, 10^-3 mol/L THU alone, and the two agents in combination. At the end of the incubation period, cold ethanol extracts were obtained and dCTP levels determined by the DNA polymerase assay as described in Materials and Methods. Values are expressed as pmol dCTP/10^6 cells.

DISCUSSION

In this report we describe the ability of supraphysiologic concentration (10^-4 mol/L) of exogenous dCyd to enhance the in vitro growth of normal CFU-GM, but not of leukemic blast progenitors. This stimulatory effect is apparently not mediated through increased elaboration of granulocyte-macrophage colony-stimulating factors (GM-CSF) from monocytes and macrophages in the adherent cell population of bone marrow. Since depletion of the T lymphocyte subpopulation did not reverse the enhancement of colony formation by dCyd, it is unlikely that these cells are implicated in this phenomenon. Previous studies have suggested that putative regulators of myelopoiesis affect only the Ia antigen bearing CFU-GM in S phase. In contrast, our results demonstrate that the dCyd-mediated stimulation of CFU-GM proliferation is not dependent on Ia antigen expression, nor is it limited to CFU-GM in S phase. However, since we have not extracted the Ia antigen-bearing cells from our marrow samples, we cannot rule out the possibility that such cells may play some role in determining the response of CFU-GM to supraphysiologic concentrations of dCyd. Finally, our results indicate that dCyd treatment does not, by itself, increase the percentage of CFU-GM in S phase. Therefore, the twofold increment in CFU-GM colony formation cannot be ascribed to a "recruitment" of myeloid progenitors by dCyd into the DNA synthesis phase of the cell cycle.

An explanation for the inability of dCyd to stimulate the in vitro growth of L-CFU is not readily apparent. One possibility is that leukemic myeloblasts may be highly efficient in their ability to deaminate dCyd by the enzyme cytidine deaminase. Previous studies by Chabner and colleagues have indicated that although cytidine deaminase activity tends to increase with granulocytic maturation, leukemic myeloid progenitors do not exhibit higher levels of cytidine deaminase activity than their normal counterparts at equivalent levels of differentiation. In addition, Coleman and coworkers have reported that dCyd kinase/cytidine deaminase activity ratios in the immature or myeloblast-enriched fractions from normal and leukemic bone marrow samples are quantitatively similar. Results of the present study demonstrate that coadministration of tetrahydrouridine in conjunction with dCyd fails to enhance significantly either the intracellular dCTP pools or the in vitro growth of L-CFU. This suggests

Table 3. Effect of 10^-4 mol/L and 10^-3 mol/L dCyd on the Inhibitory Activity of Various Agents on CFU-GM Colony Growth

<table>
<thead>
<tr>
<th>Conditions</th>
<th>Percent Control CFU-GM Growth</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Without dCyd</td>
</tr>
<tr>
<td>Hydroxyurea (10^-3 mol/L)*</td>
<td>69.5 ± 1.4†</td>
</tr>
<tr>
<td>[3H] Thymidine Suicide Index†</td>
<td>59.0 ± 2.2‡</td>
</tr>
<tr>
<td>&quot;Crude&quot; LIA (10%)</td>
<td>67.7 ± 1.6‡</td>
</tr>
<tr>
<td>Acidic Isoferritin (10^-4 mol/L)</td>
<td>64.5 ± 1.5‡</td>
</tr>
<tr>
<td>Prostaglandin E1 (10^-5 mol/L)</td>
<td>55.0 ± 1.6‡</td>
</tr>
</tbody>
</table>

ND, not done.

*Cells are incubated for one hour with hydroxyurea, washed, and then plated in the continuous presence of dCyd.
†Cells are incubated with [3H] thymidine (50 μCi/mL) for 25 minutes with and without a prior 24-hour incubation with dCyd. They are then washed and plated in the absence of drug.
‡Significantly different from control values (P < 0.05).
§Significantly different from colony growth in the absence of dCyd exposure (A) (P < 0.05).
that activity of cytidine deaminase in leukemic blasts is not solely responsible for their limited response to dCyd. Despite the results of these in vitro studies, it is possible that deamination may play an important role in determining the pharmacokinetics and biologic effects of dCyd in an intact animal and would have to be taken into consideration in any projected in vivo studies.

The apparent inability of supraphysiologic concentrations of dCyd to stimulate L-CFU growth might also result from a relative impairment in the capacity of leukemic cells to expand dCTP pools in response to exogenous dCyd. However, our studies demonstrate that leukemic blasts are capable of increasing intracellular dCTP pools significantly under these conditions and that this phenomenon is not associated with potentiation of colony formation. Other potential explanations include the possibilities that leukemic cells may actively degrade dCyd nucleotides utilizing enzymes such as S'-nucleotidase or higher dephosphorylases, or that intracellular dCTP levels might be less limiting for the in vitro growth of leukemic cells than for normal myeloid progenitor cells. Finally, it is conceivable that biochemical perturbations in normal and leukemic cell populations as a whole do not reflect events occurring in the small subset of clonogenic cells. For example, in our studies, intracellular levels of dCTP under various conditions tended to be greater for normal mononuclear cells than for leukemic myeloblasts, although the differences did not achieve statistical significance. However, it is known that the normal bone marrow mononuclear cell population separated by Ficoll-Paque density gradient centrifugation is heterogeneous and does not represent a pure population of clonogenic cells. Consequently, direct comparisons of biochemical differences between normal and leukemic bone marrow cells (eg, dCyd kinase or cytidine deaminase activity, perturbations in dCTP pools) might not predict the behavior of the small subset of myeloid progenitors. It is likely that advances in the purification of normal and leukemic stem cells will be necessary before definitive biochemical differences can be characterized.

AIF have been identified as the mediators of the inhibitory effects of LIA on CFU-GM growth. Both "crude" LIA and AIF are relatively incapable of inhibiting the in vitro proliferation of blast cells from patients with acute nonlymphocytic leukemia, leading to the hypothesis that AIF confers a proliferative advantage of leukemic blasts over normal myeloid progenitors. Our study demonstrates that supraphysiologic concentrations of dCyd (10^{-4} mol/L) in the culture medium reverses the inhibitory effect of AIF on CFU-GM. This effect was also observed with respect to PGE_{1}, which is produced by monocytes and macrophages, and suppresses colony formation by cycling, Ia antigen positive, macrophage progenitor cells. Whether dCyd directly antagonizes the activity of these factors or stimulates populations of cells immune to their actions remains to be established.

The mechanism(s) underlying in vivo suppression of normal hematopoiesis in leukemia are not clearly understood. In vitro studies have recognized several growth promoters for normal hematopoiesis such as interleukin 3, GM-CSF, and colony-stimulating activity derived from fibroblasts. On the other hand, the inhibitory influences of AIF, PGE_{1}, and lactoferrin are also well characterized. It is not currently known whether it is inadequate expression of growth factors or enhanced manifestation of the inhibitors that favors leukemic progenitor cell growth. Our study provides in vitro evidence that supraphysiologic concentrations of dCyd may protect normal myeloid progenitors from inhibitory regulators and may directly stimulate their proliferation. It also raises the possibility that modulation of exogenous dCyd concentrations may confer a selective growth advantage on normal myelopoiesis. In view of the potential therapeutic benefits of dCyd in leukemia, animal studies designed to assess the in vivo relevance of these findings appear warranted.

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dCyd STIMULATES AND PROTECTS CFU-GM

1141


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