Granulocyte/Macrophage Progenitor Cells From Peripheral Blood and Bone Marrow Differ in Their Response to Prostaglandin $E_1$

By Carol M. Richman and George D. Johnson

Prostaglandins of the E series ($PGE_1$) inhibit proliferation of normal bone marrow granulocyte/macrophage progenitors ($CFU-GM$). Circulating $CFU-GM$ are known to differ from marrow $CFU-GM$ in many characteristics, and in the present study, we compared the effect of $PGE_1$ on circulating and bone marrow progenitors in normals and in patients with chronic myelogenous leukemia (CML). $PGE_1$ caused a dose-dependent inhibition of normal marrow $CFU-GM$. Circulating $CFU-GM$ were inhibited only at concentrations of $10^{-9}$ and $10^{-8}$ mol/L. Bone marrow $CFU-GM$ from patients with CML were inhibited in a manner similar to that of normal bone marrow. Circulating cells from patients with CML were, however, less sensitive to $PGE_1$, inhibition than CML bone marrow cells and demonstrated a pattern intermediate between normal circulating and normal marrow progenitors. These studies suggest that peripheral blood and bone marrow contain different progenitor cell populations.

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EFFECTS OF PGE₁ ON CFU-GM

expressed as the percentage of control colonies: mean colonies with PGE₁ × 100/mean colonies without PGE₁. Statistical comparisons were performed by using Student’s t test or paired t test. Fifty percent inhibitory doses (ID₅₀) were determined by linear regression analysis using a Texas Instruments calculator.

Cytological evaluation of single colonies was performed by removing all the individual colonies from methylcellulose plates with a drawn-out Pasteur pipette, placing each colony on a marked portion of a glass slide (two to three colonies per slide), and staining with Wright’s stain. Each colony was classified as neutrophil, monocyte/macrophage, eosinophil, or mixed (neutrophil/macrophage) and the result expressed as the mean percentage of each colony type (± SEM).

RESULTS

PGE₁ effect on normal peripheral blood and bone marrow CFU-GM. A comparison of the effect of PGE₁ on normal bone marrow and peripheral blood CFU-GM is shown in Fig. 1. At prostaglandin concentrations between 10⁻¹⁰ and 10⁻⁴ mol/L, dose-dependent inhibition of marrow CFU-GM was observed. Depletion of adherent bone marrow cells before the assay had no significant effect on PGE inhibition of bone marrow CFU-GM (data not shown). Peripheral blood CFU-GM were, in contrast, stimulated by PGE₁ in this concentration range, and results differed significantly from controls at GM concentrations exceeding 10⁻⁴ mol/L. The differences observed at 10⁻⁵ and 10⁻⁶ mol/L are statistically significant at 10⁻⁶ and 10⁻⁵, respectively, paired t test). Maximal stimulation was achieved at 10⁻⁸ mol/L PGE₁; the response plateaued, and inhibition of circulating progenitors was observed only at PGE₁ concentrations exceeding 10⁻⁵ mol/L. The differences between the effects of PGE₁ on bone marrow and peripheral blood colony-forming cells were statistically significant for concentrations of 10⁻⁴, 10⁻³, and 5 × 10⁻³ mol/L (P < .001). Peripheral blood CFU-GM ID₅₀ occurred at a concentration of PGE₁ in excess of 10⁻⁵ mol/L, whereas the ID₅₀ for bone marrow cells is estimated to be 1.3 × 10⁻⁷ mol/L by linear regression analysis.

To determine whether the type of CSA used to stimulate CFU-GM had any effect on the action of PGE₁, we compared PHA-LCM to GCT-CM by using 5 × 10⁻⁴ mol/L PGE₁. For all five normal bone marrow samples, PGE₁ caused greater inhibition of CFU-GM when GCT was used as a source of CSA compared with PHA-LCM (P < .05). However, normal circulating progenitors were not inhibited by 5 × 10⁻⁴ mol/L PGE₁ with either source of CSA.

Effect of CSA and adherent cells on PGE₁ stimulation of circulating normal CFU-GM. To evaluate the mechanism of stimulation of normal circulating progenitors by PGE₁, studies were performed with and without exogenous CSA. In ten experiments, the mean CFU-GM/4 × 10⁵ cells was 26 ± 4 with CSA and 20 ± 6 without CSA, which suggested endogenous CSA production. In both CSA-stimulated and -unstimulated cultures, PGE₁ concentrations of 10⁻⁸ and/or 10⁻⁴ mol/L significantly increased colony formation (Fig 2). Maximum colony formation occurred at 10⁻⁴ mol/L PGE₁, and the mean CFU-GM/4 × 10⁵ cells were similar for cultures with and without exogenous CSA (40 ± 9 and 39 ± 8 respectively).

In ten experiments, nonadherent cells cultured with CSA (PHA-LCM) contained 34 ± 10 CFU-GM/4 × 10⁵ cells compared with 1 ± 0 CFU-GM for nonadherent cells cultured without CSA (indicating depletion of endogenous CSA-producing cells). When nonadherent cells were plated with CSA and PGE₁, the results were similar to those using...
whole mononuclear cells without adherence depletion, ie, PGE$_1$ at $10^{-6}$ mol/L caused significant enhancement of CFU-GM growth ($P < .05$).

In six of nine individuals studied, the maximum colony concentration in the nonadherent cells plated without an exogenous source of CSA was less than $5$ colonies/$4 \times 10^5$ cells with or without PGE$_1$. In one of the nine individuals, CFU-GM increased from 0 without PGE$_1$ to 6 with $10^{-6}$ mol/L PGE$_1$; in a second, CFU-GM increased from 0 to 12; and in a third, CFU-GM rose from 2 without to 51 with PGE$_1$.

**PGE$_1$ effect on circulating and bone marrow CFU-GM from patients with CML.** In patients with CML, CFU-GM from both the bone marrow and peripheral blood exceeded control values only at the lowest PGE$_1$ concentration ($10^{-10}$ mol/L, Fig 3). At concentrations higher than $10^{-10}$ mol/L, there was a dose-dependent inhibition for both circulating and bone marrow cells. Unlike normal cells, circulating CML cells were not stimulated by PGE$_1$. However, blood CFU-GM were more resistant to PGE$_1$ inhibition than were bone marrow progenitors ($P < .05$ at $10^{-6}$ mol/L and $P < .01$ at $5 \times 10^{-6}$ mol/L). For bone marrow, CFU-GM were 47% of control at $10^{-4}$ mol/L, whereas for circulating cells, values were still 58% of control at $5 \times 10^{-6}$ mol/L. PGE inhibition curves for normal (Fig 1) and CML bone marrow (Fig 3) were not significantly different except at $10^{-10}$ mol/L PGE$_1$, where CML CFU-GM was $114% \pm 6%$ of control whereas normal marrow CFU-GM was $97% \pm 5%$ ($P < .05$). For normal and CML marrow cells, 50% inhibition is projected by linear regression analysis to occur at 1.3 and $4.1 \times 10^{-6}$ mol/L respectively.

**Cytological evaluation of PGE$_1$ effects.** PGE$_1$ may inhibit macrophage colonies specifically,$^4$$^4$$^4$$^4$$^4$ although some reports suggest inhibition of both granulocyte and macrophage colonies.$^5$ To determine whether differences in the effects of PGE on circulating and bone marrow progenitors might be secondary to differences in the proportion of macrophage colonies, we characterized the type of colonies in control and PGE-exposed cultures. Individual Wright-stained colonies (see Methods) were analyzed from six peripheral blood and five bone marrow samples from normals (Table 1). There were significantly fewer macrophage colonies in the blood (19% ± 4%) than in bone marrow (38% ± 8%) ($P < .05$, paired $t$ test). PGE$_1$-containing cultures of marrow had significantly fewer macrophage colonies than did marrow cultures without PGE$_1$ ($P < .05$). The distribution of cytological types of circulating or bone marrow colonies was otherwise not significantly affected by the addition of PGE$_1$.

**DISCUSSION**

Normal peripheral blood and bone marrow granulocyte/macrophage progenitors differ significantly in their response to PGE$_1$. The inhibition of marrow CFU-GM by PGE$_1$ observed in the present study is similar to that reported by others.$^2$$^2$$^3$$^1$$^3$$^3$$^3$$^3$$^3$$^3$$^3$$^3$ Peripheral blood CFU-GM were not inhibited by PGE$_1$ and were, unexpectedly, stimulated by concentrations in the range of $10^{-8}$ to $10^{-6}$ mol/L, an effect not previously reported for normal peripheral blood. PGE$_1$ augmented CFU-GM in peripheral blood cultures containing an exogenous source of CSA, either PHA-LCM or GCT medium, as well as in cultures stimulated only by endogenous CSA production. The stimulatory effect of PGE$_1$ on peripheral blood progenitors was not significantly affected by depletion of adherent cells, thereby suggesting that these cells do not solely mediate the stimulatory effect. Nonadherent cells showed negligible spontaneous CFU-GM growth, which indicated adequate depletion of endogenous CSA-producing cells. In six of nine individuals studied, PGE$_1$ had no effect on nonadherent cells in the absence of exogenous CSA. In the other three, however, PGE$_1$ stimulated CFU-GM in the nonadherent cell fraction.

Stimulatory effects of PGE on CFU-GM have been noted variably in studies of circulating progenitors in patients with myelofibrosis and myeloid metaplasia (MMM).$^6$$^6$$^6$$^6$$^6$ The subpopulation of progenitors in MMM that are stimulated by PGE may be similar to normal circulating progenitors. One.

**Table 1. Effect of PGE$_1$ on Cytological Type of Circulating and Bone Marrow Colonies**

<table>
<thead>
<tr>
<th>Type of Colony</th>
<th>No PGE$_1$</th>
<th>With PGE$_1$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neutrophil</td>
<td>43 ± 8$^*$</td>
<td>40 ± 8</td>
</tr>
<tr>
<td>Monocyte/macrophage</td>
<td>19 ± 4</td>
<td>24 ± 5</td>
</tr>
<tr>
<td>Mixed</td>
<td>2 ± 2</td>
<td>4 ± 2</td>
</tr>
<tr>
<td>Eosinophil</td>
<td>36 ± 10</td>
<td>32 ± 10</td>
</tr>
<tr>
<td>Neutrophil</td>
<td>36 ± 11</td>
<td>42 ± 7</td>
</tr>
<tr>
<td>Monocyte/macrophage</td>
<td>38 ± 8</td>
<td>16 ± 4$^†$</td>
</tr>
<tr>
<td>Mixed</td>
<td>5 ± 1</td>
<td>10 ± 2</td>
</tr>
<tr>
<td>Eosinophil</td>
<td>21 ± 5</td>
<td>32 ± 7</td>
</tr>
</tbody>
</table>

$^*$Percentage of colonies of a given type (mean ± SEM). Six blood samples and five bone marrow samples from normal individuals were studied.

$^†$The difference between monocyte/macrophage colonies with and without PGE is significant at $P < .05$ when using the paired $t$-test.
might hypothesize that normal bone marrow contains primarily progenitors inhibited by PGE whereas normal blood contains CFU-GM stimulated by PGE and that the response of circulating CFU-GM to PGE would depend on the relative proportion of circulating marrowlike progenitors. Our results in CML showing effects of PGE on peripheral blood CFU-GM that are intermediate between normal bone and normal bone marrow CFU-GM support this notion because CML blood contains some cells that normally reside in the bone marrow. An alternative explanation is that circulating progenitors in these myeloproliferative diseases differ fundamentally from normal progenitors.

The cytological subtype of progenitors in peripheral blood and bone marrow differ, and our study is in agreement with other reports,22 ie, fewer macrophage and greater numbers of granulocytes. The observation that circulating progenitors of patients with CML have PGE responses that are intermediate between normal blood and bone marrow suggests that the blood CFU-GM are more primitive than bone marrow CFU-GM.19 It has been proposed23 that the situation is analogous to the more primitive erythroid precursor, BFU-E, found predominantly in the blood, and less primitive CFU-E, found predominantly in the marrow.30 Subpopulations of CFU-GM have been observed in murine bone marrow with varying responses to PGE. Progenitors forming large colonies (assumed to be more primitive) are relatively resistant to PGE inhibition, whereas the more prevalent marrow CFU-GM form smaller colonies and are several orders of magnitude more sensitive to inhibition by PGE.31 PGE in some reports appears to inhibit a subpopulation of CFU-GM that are in S phase and that express the Ia antigen.32 Because normal human marrow and peripheral blood are known to differ in cell cycle status,23 it is possible that such differences could account, in part, for the differences in PGE inhibition that we have observed.

CML bone marrow cells were, in the present study, as sensitive to PGE inhibition as were normal bone marrow cells. This result is in contrast to some reports indicating that CML progenitors from blood and/or bone marrow are extraordinarily resistant to PGE, with CFU-GM approximately 90% of control at PGE doses exceeding 10^{-6} mol/L.8,13-16,29 Although we noted that CML peripheral blood progenitors were more resistant to PGE than bone marrow progenitors, even the blood CFU-GM were more easily inhibited in our studies than in the studies mentioned earlier. Another report found, as we did, no difference between normal and CML marrow in the degree of prostaglandin-induced inhibition.33 Differences in culture technique may provide a partial explanation for these disparate results.

In the present study, normal peripheral blood and bone marrow CFU-GM differ significantly in their response to PGE. Circulating progenitors of patients with CML have PGE responses that are intermediate between normal blood and bone marrow. If circulating progenitors represent a more primitive cell than their bone marrow counterparts, as has been suggested,19,20,23,30 further studies of normal and pathological hematopoietic regulation by using circulating CFU-GM may supplement studies of bone marrow–derived progenitors.

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