The Effect of Prostaglandins E, and E2 on Macrophage Progenitor Cells With High Proliferative Potential in Mouse Bone Marrow In Vitro

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High proliferative potential macrophage progenitor cells (HPP-CFC) in 5-fluorouracil (FU) treated and normal mouse bone marrow (BM) have been shown to have a profound effect on the inhibition of proliferation by prostaglandins of the E series (PGE) than low proliferative potential macrophage progenitor cells (LPP-CFC) in normal BM in agar cultures. The growth of large colonies (diameter >0.5 mm) derived from HPP-CFC in FU BM, which require a combination of macrophage colony-stimulating factor (CSF-1) plus a new growth factor called synergistic activity (SA), are inhibited by 50% in the presence of 5.5 x 10⁻⁶ M PGE₁. On the other hand, LPP-CFC in normal BM, which form smaller colonies (diameter ≤0.5 mm) in the presence of CSF-1 alone, require only 5 x 10⁻⁶ M PGE₁, for the same level of inhibition. Addition of appropriate concentrations of PGE to the agar culture assay should improve detection of HPP-CFC by inhibiting the proliferation of LPP-CFC. These observations suggest that the apparent negative feedback control of macrophage production by PGE operates largely on the LPP-CFC, which respond to CSF-1 alone, and is probably not involved in the regulation of the more primitive HPP-CFC.

A population of granulocyte-macrophage progenitor cells, with high proliferative potential (HPP-CFC), has recently been demonstrated in post-5-fluorouracil (FU)-treated and normal mouse bone marrow (BM) in vitro, when a newly discovered growth factor (synergistic activity, SA), is combined with a macrophage colony-stimulating factor (CSF-1) as a proliferative stimulus. SA has been found in several sources, including human spleen and human placental conditioned media, and has now been partially purified. HPP-CFC can be detected in 4-day post-FU-treated and in normal BM by their ability to form large macrophage colonies (diameter >0.5 mm) in agar cultures in response to the combination of factors. In contrast, the granulocyte-macrophage progenitors in FU-treated and normal BM, which proliferate in response to CSF-1 alone, form smaller macrophage colonies (diameter ≤0.5 mm) in agar cultures and are referred to as low proliferative potential colony-forming cells (LPP-CFC). The ratio of HPP-CFC to LPP-CFC has been shown to be 0.06 in normal BM and to increase to 34 4 days after treatment with FU, which severely depletes the LPP-CFC population, whereas the HPP-CFC preferentially survive. Because FU kills cells in cycle, Bradley and Hodgson concluded that HPP-CFC were an earlier progenitor cell type that cycled more slowly than LPP-CFC.

The prostaglandins E₁ and E₂ (PGE₁ and PGE₂) have been shown to have a profound effect on the proliferation of granulocyte-macrophage colony-forming cells (LPP-CFC) in culture. Kurland et al. have shown that the presence of PGE₁ or E₂ in culture inhibited the CSF-stimulated LPP-CFC proliferation, and because CSF can induce macrophage elaboration of PGE₁ and E₂, they proposed that this inhibition may provide a self-regulatory feedback mechanism for myelopoiesis in both humans and mice. More recently, Verma et al. and Pelus have demonstrated that preincubation of human BM cells with PGE prior to culturing in agar can trigger noncycling LPP-CFC into cycle. It has thus been suggested by these authors that, in humans, the effects of PGE are biphasic. At high as well as low concentrations, short exposures to PGE result in the triggering of the noncycling LPP-CFC into cycle, which are then stimulated to proliferate and differentiate to mature granulocytes and macrophages under the influence of CSF. On the other hand, the continuous presence of high levels of PGE result in inhibition of LPP-CFC proliferation.

In this article, the inhibitory effects of PGE₁ and PGE₂ on the HPP-CFC and LPP-CFC in mouse BM in culture are compared.

**Materials and Methods**

**Growth Factors**

Extracts of whole placenta, membranes, uterus, and embryo (PMUE), prepared from pregnant (BALB/c x C57BL/6)F₁ mice as described by Bradley et al., were used as a CSF-1 source throughout this work. Synergistic activity (SA) was partially purified from human placental conditioned medium by gel filtration chromatography, as previously described. Purified CSF-1 from L cell conditioned medium was a gift from Dr. E. R. Stanley, Department of Microbiology and Immunology, Albert Einstein College of Medicine, Yeshiva University, Bronx, NY.

**Prostaglandins**

Prostaglandins were purchased from Sigma Chemical Company, St. Louis, MO, and were made up in ethanol at a concentration of...
10^{-3} M. These stock solutions were stored at -20°C. Serial dilutions were made in normal saline containing 0.3% polyethylene glycol 300 (PEG) and 0.01% Triton X-100. PEG and Triton X-100 were added to prevent the loss of PEG from dilute aqueous solutions by adsorption to apparatus during handling. The responses of normal serum, and hence from dilute aqueous solutions by the use of saline containing 0.3% PEG and Triton X-100 does not influence these responses. The highest concentration (10^{-4} M) of prostaglandin used in this study also contained ethanol diluted 100-fold. Control cultures indicated that this level of ethanol had no effect on either the number or size of colonies produced.

**Agar Cultures of FU-Treated Bone Marrow**

Post-FU bone marrow was taken from groups of 4 BALB/c, 3-mo-old male mice 4 days after a single intravenous injection of FU at 150 mg/kg body weight. Agar cultures of these cells (5 x 10^7 cells/culture) were grown essentially as described by Bradley et al.15 Double-layer cultures (1 ml underlay, 0.5 ml overlay) in 35-mm petri dishes were used throughout. PMUE was added to the medium at the minimum concentration required for optimal colony development by normal mouse marrow cells. SA was added to the underlayers at the minimum concentration required for optimal growth of colonies derived from HPP-CFC in FU BM. Control cultures without SA were included in each experiment. Aliquots (150 μl) of prostaglandin solutions (concentrations 10^{-5}-10^{-4} M) to be tested were added to the underlayers. Normal saline, containing 0.3% PEG and 0.01% Triton X-100, and ethanol diluted 100-fold in this same saline solution were added to control cultures containing SA in each experiment. The culture medium used was alpha modification of Eagle’s MEM (Flow Laboratories, Detroit, MI) supplemented with 20% newborn calf serum. The same serum batch (Flow Laboratories) was used throughout this work and was found to give optimal growth at a concentration of 20%. Cultures were incubated for 14 days at 37°C, after gassing in a mixture of 5% O2 and 10% CO2. All clones containing more than an estimated 50 cells were scored separately.

**Agar Culture of Normal Bone Marrow**

These assays were carried out exactly the same way as described above, except that 10^6 normal BM cells were plated per culture. Assays for LPP-CFC only were carried out using PMUE as the only growth stimulus, whereas assays for both LPP-CFC and HPP-CFC require both PMUE and SA.

**Statistical Treatment**

Data from single representative experiments are shown in all cases. However, all experiments have been carried out at least twice and have been shown to be reproducible. Data are presented as the mean ± standard error of the mean (SEM).

**Cell Morphology**

Two sets of cultures of FU-treated BM were grown in the presence of PMUE and SA with PGE1 concentrations varying from 0 to 10^{-4} M as in all the other experiments. One set was incubated for 7 days and the other for 14 days and then fixed with a 2.5% glutaraldehyde solution. Cultures were immersed in water and the agar layer transferred to glass slides (50 x 70 mm). The agar layers were covered with cellulose acetate paper and allowed to dry. When dry, the paper was removed and the slide stained with Luxol fast blue, a specific eosinophil stain, and counterstained with Harris’ hematoxylin.

In a parallel experiment, normal BM was grown in the presence of PMUE and PGE1 (0-10^{-4} M), and the cultures were mounted and stained as described above.

**RESULTS**

**Inhibitory Effects of PGE on HPP-CFC From FU-Treated BM and LPP-CFC From Normal BM**

Normal mouse BM was cultured in the presence of an extract of pregnant mouse uterus and embryo (PMUE, a source of macrophage colony-stimulating factor) and varying concentrations of PGE1 or PGE2, whereas 4-day post-FU-treated mouse BM was grown in the presence of PMUE, SA, and the same concentrations of PGE1 or PGE2. As can be seen in Fig. 1 (A and B), colonies (diameters ≤0.5 mm) derived from LPP-CFC in normal BM, and large colonies (diameter >0.5 mm) derived from HPP-CFC in 4-day post-FU BM show an increased growth inhibition with increasing concentrations of PGE1 or PGE2 in culture, although PGE1 appears to be slightly more active than PGE2 on a molar basis. In Fig. 1A, it can be seen that colonies derived from LPP-CFC in normal BM are inhibited to the same extent by PGE1, regardless of whether they are stimulated by PMUE or purified CSF-1 from L cell conditioned medium.

Because mature macrophages are known to elaborate PGE1 and E2, the effect of PGE1 on the proliferation of LPP-CFC in normal BM was determined after preincubation of BM cells in liquid culture to remove mature macrophages and other adherent cells. As can
be seen in Fig. 1A, the effect of PGE₁ on the proliferation of LPP-CFC in agar cultures is practically the same as in the case of normal BM containing adherent cells.

Although the proliferation of both LPP-CFC in normal BM and HPP-CFC in post-FU BM are inhibited by PGE₁ and E₂, the HPP-CFC are clearly less sensitive to inhibition by both these prostaglandins in agar cultures. Thus, whereas the proliferation of HPP-CFC in FU-treated BM is inhibited by 50% in the presence of $5.5 \times 10^{-6} \text{ M PGE}_1$, the proliferation of LPP-CFC in normal BM requires only $5 \times 10^{-8} \text{ M PGE}_1$ for the same level of inhibition.

Inhibitory Effect of PGE₁ on HPP-CFC and LPP-CFC From Normal BM

In order to determine what effect these prostaglandins have on colonies derived from HPP-CFC and LPP-CFC in normal BM, BM cells were cultured in the presence of both PMUE and SA and varying amounts of PGE₁. Again, it can be seen (Fig. 2) that, although the proliferation of both precursor cell types are inhibited by PGE₁, the HPP-CFC are less sensitive to inhibition. In this case, the ratio of the number of colonies formed by HPP-CFC to those formed by LPP-CFC was 0.13 in the absence of any added PGE₁ and increased with increasing PGE₁ concentration in culture (Fig. 2), reaching a maximum of 0.5 at a concentration of $10^{-6} \text{ M}$.

Inhibitory Effect of PGE₁ on HPP-CFC and LPP-CFC From FU-Treated BM

The ratio of HPP-CFC to LPP-CFC in 4-day post-FU BM varies in practice from one marrow sample to the next. From the data in Fig. 3, this ratio can be calculated to be 0.27 and 6.01 in A and B, respectively, when FU BM was cultured in the presence of PMUE plus SA in the absence of PGE₁. In most cases, the ratio is high (>2), as for example, in Fig. 3B. The reason for the occasional cases in which this ratio is unusually low, as for example in Fig. 3A, is not clear. One possible explanation is that, on these occasions, some of the mice in the particular group received

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**Fig. 2.** Effect of PGE₁ concentration on colony formation in agar cultures by HPP-CFC (Δ) and LPP-CFC (○) in normal bone marrow. Both colonies derived from HPP-CFC (diameter >0.5 mm) and from LPP-CFC (diameter ≤0.5 mm) were grown in the same cultures in response to the combined stimulus of PMUE plus SA. Each point represents the mean ± SEM of triplicate dishes.

**Fig. 3.** Effect of PGE₁ concentration on colony formation by HPP-CFC (Δ) and LPP-CFC (○) in FU-treated BM, cultured in the presence of PMUE plus SA. (A) In this experiment, the FU-treated BM contained a large number of LPP-CFC compared to HPP-CFC. (B) In these two experiments (1 and 2), the same FU-treated BM was used that contained few LPP-CFC compared to HPP-CFC. Experiment 1 (solid line) was carried out using PMUE and the usual amount of SA, and experiment 2 (dotted line) was carried out using PMUE plus twice the usual amount of SA. Each point represents the mean ± SEM of triplicate dishes, except exp. 1, in which 6 dishes were used for each point.
incomplete intravenous doses of FU, thus allowing a greater number of LPP-CFC to survive. Although the colonies derived from HPP-CFC are inhibited, as expected, with increasing PGE, concentrations. (Fig. 3), the smaller colonies behave differently in the two experiments. When a BM sample contains a large number of LPP-CFC compared to HPP-CFC (Fig. 3A), the LPP-CFC-derived colonies show an increased inhibition with increasing PGE, concentration up to $10^{-6} M$. At $10^{-3} M$ PGE, however, an increase in the number of small colonies (diameter ≤0.5 mm) is observed. As LPP-CFC in normal BM are completely inhibited at this concentration, it can be concluded from this that all the colonies with diameter ≤0.5 mm, which are able to grow in the presence of $10^{-5} M$ PGE, are in fact derived from HPP-CFC that have been prevented from attaining their full size. When the BM sample contains few LPP-CFC compared to HPP-CFC (Fig. 3B, experiment 1), the increase in the number of small colonies in the presence of $10^{-5} M$ PGE, is even more pronounced, whereas they show almost none of the expected inhibition at $10^{-7} M$.

When FU-treated BM was cultured with PMUE plus twice the usual amount of SA, no significant difference in the inhibitory effect of PGE, was observed (Fig. 3B, experiment 2).

**Effect of Prostaglandins F,α and F,α**

Four-day post-FU BM was cultured in agar cultures containing PMUE and SA plus the prostaglandins F,α and F,α at concentrations ranging from $10^{-4}$ to $10^{-8} M$. These prostaglandins, however, appeared to have no effect on the growth of HPP-CFC-derived colonies.

**Effect of PGE, on Cell Morphology**

Luxol fast blue-hematoxylin stained colonies from FU BM, grown in the presence of PMUE, SA, and PGE, ($0-10^{-3} M$), and similarly stained colonies from normal BM grown with PMUE and PGE, ($0-10^{-5} M$) were examined for the presence of eosinophils and cells with the typical lobulated nuclei of mature granulocytes. The vast majority of the 7-day and 14-day colonies from FU-treated and normal BM were found to contain mononuclear cells only, with an occasional colony made up of largely mononuclear cells plus a few cells that appear to have lobulated nuclei. No eosinophils were observed in any colonies. These observations indicate that the presence of PGE, does not alter the morphology of the progeny cells, which have been shown to be macrophages in both marrow types when PMUE plus SA are used as growth stimuli.

**DISCUSSION**

The results indicate that, although both HPP-CFC and LPP-CFC in normal and FU-treated mouse BM are inhibited by prostaglandins of the E series, the HPP-CFC are less sensitive to inhibition than LPP-CFC. It has been shown that CFC, which respond to CSF alone (LPP-CFC), are more sensitive to inhibition by PGE than the more mature CFC population that responds to hemolysate plus CSF. Maximum sensitivity to inhibition is thus displayed by LPP-CFC, whereas their precursors and progeny, the more primitive HPP-CFC and more mature lysate-dependent CFC, respectively, are less sensitive. Since PGE preferentially inhibit the proliferation of LPP-CFC, it should be possible to use this property to improve assays for HPP-CFC or SA.

When normal BM is grown in agar cultures with PMUE as the only growth stimulant, only 4% of the colonies derived from HPP-CFC are able to proliferate to form macrophage colonies in the presence of $10^{-6} M$ PGE, whereas at $10^{-5} M$, they are totally inhibited (Fig. 1). On the other hand, when FU BM is cultured in the presence of both PMUE and SA, the small colonies are less inhibited than expected at $10^{-6} M$, whereas at $10^{-5} M$ PGE, they are in fact enhanced (Fig. 3). It has been concluded from this that all the small colonies that are able to grow in the presence of $10^{-5} M$ and the extra small colonies that are able to grow at $10^{-6} M$ PGE, may, in fact, be derived from HPP-CFC, but have been prevented from attaining their full size. This conclusion is supported by the data presented in Fig. 3B, experiment 1, in which it is found that the number of large colonies formed at $10^{-5} M$ PGE, is exactly equal to the number of large colonies formed in the absence of PGE. This explanation could also account for the greater than expected numbers of small colonies that may not be dependent on HA at a concentration of $10^{-2}$ and $10^{-6} M$ in cultures of normal BM in response to PMUE plus SA (Fig. 2). This would suggest that the actual ratio of HPP-CFC to LPP-CFC-derived colonies from normal BM at $10^{-6} M$ PGE, is in fact greater than that shown in Fig. 2. A portion of the HPP-CFC in FU BM are still able to form large colonies in the presence of $10^{-5} M$ PGE, (Fig. 3). If the smaller colonies that are able to form at this concentration are, in fact, also derived from HPP-CFC as proposed, it would suggest that the HPP-CFC compartment may contain subpopulations of cells with differing sensitivities to PGE.
derived from a hitherto undetected population of progenitor cells requiring this combination of factors for proliferation.

It has previously been suggested that regulation by PGE of myelopoiesis is specific for the monocyte-macrophage lineage. This study indicates that this regulation operates mainly on a particular macrophage-progenitor cell compartment, namely, the LPP-CFC, which responds to CSF-1 alone.

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REFERENCES

The effect of prostaglandins E1 and E2 on macrophage progenitor cells with high proliferative potential in mouse bone marrow in vitro

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