Prostaglandin E2 Production by Human Bone Marrow Cells: a Comparison with Peripheral Blood Mononuclear Cells

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Nakayama, Y., Shindo, T., Akihama, T. and Miura, A.B. Prostaglandin E2 Production by Human Bone Marrow Cells: a Comparison with Peripheral Blood Mononuclear Cells. Tohoku J. exp. Med., 1984, 144 (4), 417-423 — The amount of prostaglandin E2 (PGE2) in the supernatant of human bone marrow cell cultures was measured by radioimmunoassay. Bone marrow cells were obtained from adult patients without hematological malignancies. Marrow mononuclear cells (BMMNC) (0.5 x 10^6) and marrow nonadherent cells (BMNAC) (0.5 x 10^6) were cultured at 37°C in 5% CO2 in 0.5 ml RPMI 1640 supplemented with L-glutamine, 10% fetal calf serum and 10 mM Hepes buffer. The levels of PGE2 in the supernatant of BMMNC after 24 hr and 48 hr incubation were 3,000±2,320 pg/ml (n =10, mean ± S.D.) and 4,470± 3,510 pg/ml (n =12), respectively. The kinetics of PGE2 production by BMMNC was different from that of peripheral blood mononuclear cells. The level of PGE2 in the supernatant of BMNAC after 48 hr incubation was only 1/18 when compared to that of BMMNC. These data suggest that the major source of PGE2 in the supernatant of marrow cell cultures may be bone marrow mononuclear phagocytes and that admixed blood mononuclear cells in BMMNC may produce only a small amount of PGE2 — — — prostaglandin E2; bone marrow cell; macrophage

The prostaglandin E (PGE) inhibits CFU-C proliferation (Kurland et al. 1978) and enhances BFU-E and CFU-E growth in vitro (Chan et al. 1980). Bone cultures exposed to prostaglandin E2 (PGE2) reveal an increase in calcium release from bone to medium and an increase in osteoclast number compared to control bones (Rifkin et al. 1980). Since over 90% of PGE is inactivated in a single transit through the pulmonary circulations, it is unlikely that PGE has a function as circulating hormones (Piper et al. 1970). The PGE produced locally within the bone marrow environment may serve to regulate the proliferation of hematopoietic stem cells (Kurland and Moore 1977), and the bone resorption by osteoclasts. The level of PGE2 in the marrow is unknown, but may be determined by the PGE2 production of marrow cells and rate of its flow into the sinus
vein. The aim of this report is to evaluate the source of PGE₂ in the marrow using marrow liquid culture technique.

**Materials and Methods**

**Subjects**

Marrow was obtained from 20 patients without hematological malignancies, and blood was obtained from 5 healthy volunteers.

**Preparation of marrow cells**

Marrow samples (0.5–2 ml) were aspirated from sternum or iliac crest into a plastic tube containing preservative-free heparin. Marrow samples were diluted with 5–10 volumes of Hanks’ balanced salt solution without Ca²⁺ and Mg²⁺ (HBSS), and marrow mononuclear cells (BMMNC) were recovered after density gradient centrifugation over Ficoll-Conray. Cells at the interface were collected, washed 3 times with HBSS, and suspended in 2.5 ml of RPMI 1640 containing 10% (v/v) heat inactivated fetal calf serum (FCS) to a concentration of 1 × 10⁶ cells/ml.

The remaining marrow cells were used to obtain nonadherent and adherent marrow cells. Marrow mononuclear cells were suspended in 5 ml of RPMI 1640 containing 20% fetal calf serum, and plated in 60 × 15 mm plastic culture dish (Falcon 3002), and allowed to incubate for 90 min at 37°C in a humid 5% CO₂-air atmosphere. The nonadherent cells (BMNAC) were collected from the plastic dish, and washed three times with HBSS. Then, the dish was washed five times with warmed HBSS with 2% FCS. Residual adherent cells (BMAC) were detached from the dish by vigorous pipetting after exposure for several minutes to 30 mM buffered lidocaine solution. Recovered cells were washed 3 times with HBSS.

**Preparation of peripheral blood mononuclear cells**

Normal mononuclear cells were isolated from blood of healthy volunteers by Ficoll-Conray centrifugation. Mononuclear cells (PBMNC) were washed three times with HBSS.

**Cell cultures**

Each cell suspension mentioned above was resuspended in RPMI 1640 containing 10% FCS to a concentration of 1 × 10⁶ cells/ml. 0.5 ml of the cell suspension was transferred into a 12 × 75 mm round-bottomed plastic culture tube (Falcon 2054), and incubated in an atmosphere of 5% CO₂ in air at 37°C for various times. Each cell suspension was always cultured in duplicate.

After incubation, supernatants were taken, and frozen at −20°C until the measurement of PGE₂.

**Radioimmunoassay of PGE₂**

The concentration of PGE₂ was directly assayed by radioimmunoassay without extraction procedures. The immunoassay reaction mixture consisted of 100 μl of the standards or the samples, 100 μl of anti-PGE₂ antiserum, 700 μl of phosphate buffer saline (pH 7.4) with 0.1% gelatin, and 100 μl of [3H]-PGE₂. This mixture was incubated for 18–22 hr (overnight) at 4°C. At the end of this incubation, 10 mg of dextran coated charcoal was added to each tube. Tubes were then incubated for 15 min at 0°C, and centrifuged at 3500 rpm for 5 min to separate antibody bound (supernatant) from free [3H]-PGE₂ (charcoal absorbed). A 0.5 ml aliquot of the supernatant was solubilized in xylene-based scintillation solution (PCS, Amersham) and counted in an Aloka LSC 703 liquid scintillation system (Aloka, L). Bound [3H]-PGE₂ in sample was expressed as a percentage of the total [3H]-PGE₂. Assay tubes without antiserum to PGE₂ were employed to correct for the nonspecific appearance
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of $^3$H-PGE$_2$ in the supernatant after charcoal adsorption. With this assay system, 10 pg/ml of PGE$_2$ could be detected.

The antibody crossreacted approximately 6% with PGE$_1$, but did not crossreact with PGA$_2$, PGB$_2$, or PGF$_{2\alpha}$ (Nakayama et al. 1984). All results were expressed as PGE$_2$, because the level of PGE$_1$ in human monocyte culture fluid was only 1/16 that of PGE$_2$ synthesized by the same cells (Goldyne and Stobo 1979).

Statistics

Values are expressed in terms of mean±s.d. The significance of differences between means was determined by Student’s t test after logarithmic conversion of raw data.

RESULTS

The level of PGE$_2$ in BMMNC after 24 hr incubation was $3,000\pm 2,320$ pg/ml ($n=10$), and that after 48 hr incubation was $4,470\pm 3,510$ pg/ml ($n=12$)(Fig. 1). The difference was not statistically significant.

Fig. 2 shows the kinetics of PGE$_2$ concentration in the supernatants of blood mononuclear cell cultures. The PGE$_2$ concentration in the supernatants of PBMNC cultures increased over the first 24 hr of culture, after which it diminished gradually. The PGE$_2$ production by PBMNC after 24 hr and 48 hr incubation was $13,100\pm 7,000$ pg/ml ($n=5$) and $10,400\pm 5,800$ pg/ml ($n=5$), respectively. The PGE$_2$ production by PBMNC was 2- to 4-fold higher than that of BMMNC.

The kinetics of PGE$_2$ production by BMMNC is illustrated in Fig. 3. The PGE$_2$ level in the supernatants increased over the first 48 hr of culture, followed by a plateau until 72 hr. The level of PGE$_2$ after 48 hr and 72 hr incubation in

![Figure 1](image.png)

Fig. 1. Production of PGE$_2$ by bone marrow mononuclear cells. The levels of PGE$_2$ in the supernatant of $0.5\times10^6$ cells in 0.5 ml medium after 24 hr and 48 hr incubation.
Fig. 2. Kinetics of PGE₂ level in supernatants of peripheral blood mononuclear cell cultures. 0.5 × 10⁶ cells in 0.5 ml media were cultured for 0 to 72 hr. After the incubation, the concentration of PGE₂ in supernatants was measured by RIA.

Fig. 3. Kinetics of PGE₂ level in supernatants of bone marrow mononuclear cell cultures. 0.5 × 10⁶ cells in 0.5 ml media were cultured for 24 to 72 hr. After the incubation, the concentration of PGE₂ in supernatants was measured by RIA.
BMMNC cultures was significantly higher than that after 24 hr incubation \((p = 0.007, p = 0.01\), respectively\). Significant difference was found in the kinetics of PGE\(_2\) production between PBMNC and BMMNC.

In order to determine the PGE\(_2\) producing cells, we studied the PGE\(_2\) release from BMMNC and BMNAC (Fig. 4). The PGE\(_2\) production of BMMNC after 48 hr incubation was \(4,220 \pm 5,100 \text{ pg/ml} (n = 7, \text{ mean } \pm \text{ s.D.})\), while in the supernatants of the BMNAC cultures the level of PGE\(_2\) was \(235 \pm 290 \text{ pg/ml} (p = 0.002)\). PGE\(_2\) production by marrow nonadherent cells (adherent cell depleted BMMNC) was reduced to 5.6% of control PGE\(_2\) release from unfractionated bone marrow mononuclear cells. The level of PGE\(_2\) of BMAC was measured only in a patient with hemolytic anemia because sufficient number of BMAC was not obtained from other patients. PGE\(_2\) production by BMAC of the patient after 24 hr incubation was 15,000 pg/ml. In contrast, that by BMMNC of the same patient at 24 hr was 2,500 pg/ml. In comparison to BMMNC, a 6-fold increase was observed in BMAC.

**DISCUSSION**

It has been demonstrated that PGE\(_2\) may influence the hemopoietic stem cell cultures (Kurland et al. 1978 ; Chan et al. 1980), and increase the calcium release from bones by enhancing the proliferation and activation of osteoclasts in in vitro bone cultures (Rifkin et al. 1980). Kurland and Moore (1977) have proposed that
PGE produced locally within the bone marrow may serve to limit the proliferation of committed granulocyte-macrophage stem cells. However, there are only a few reports on the local PGE₂ production by bone marrow cells from animals. Jones and Lange (1983) have demonstrated that in the cyclic hematopoiesis dog, the supernatant fluids from cultures of marrow collected in each of 13 cycles days have a cyclic fluctuation of PGE. DeGowin and Gibson (1981) have measured the PGE₂ concentration in media conditioned by marrow stromal cells from mice by use of RIA. Little or no information is available about the impact of the bone marrow PGE₂ production in human upon the hemopoiesis and hypercalcemia.

To determine whether the PGE₂ level in marrow might influence the hematopoietic stem cell growth and osteolysis, we examined the PGE₂ production by human marrow mononuclear cells. When marrow aspirates are used for the PGE₂ production by marrow cells, peripheral blood contamination may have a considerable influence on the final results. In marrow aspirates, 97% of the hemoglobin count appears to be derived from peripheral blood (Holdrinet et al. 1980). Therefore, the PGE₂ level of marrow aspirates does not mean the PGE₂ concentration in marrow intercellular fluid. The fraction of peripheral nucleated cells in marrow aspirates is 14±8% (Holdrinet et al. 1980). In this report, we have examined the PGE₂ production by human marrow mononuclear cells, and compared them with PGE₂ release from human blood mononuclear cells.

The PGE₂ production by PBMNC after 24 hr incubation was 13,100±7,000 pg/ml and that of BMMNC was 3,000±2,300 pg/ml. The PGE₂ production by PBMC and BMMNC after 48 hr incubation was 10,400±5,800 pg/ml and 4,470±3,510 pg/ml, respectively. In general, PGE₂ production by PBMC was 2 to 5 times greater than that of BMMNC. However, the observed elevation of PGE₂ production in PBMC does not imply that the PGE₂ level is higher in the blood than in the marrow. Because nucleated cell count is about 30-fold higher in the bone marrow than in the peripheral blood, the PGE₂ production by BMMNC per cubic mm is higher than that of PBMC.

The PGE₂ concentration in the supernatants of PBMC cultures increased over the first 24 hr of culture, then diminished gradually, while that of BMMNC cultures increased over the first 48 hr, followed by a plateau until 72 hr. The difference of the kinetics of PGE₂ concentration between PBMC culture fluids and BMMNC culture fluids suggested that PGE₂ in BMMNC culture fluid was mainly produced by marrow cells, even if a small amount of PGE₂ was produced by contaminated blood mononuclear cells.

The PGE₂ production of BMAC was only 6% of the control PGE₂ release from BMMNC. PGE₂ production by BMAC was 6 times greater than that of BMMNC. In conclusion, PGE₂ producing cells in the bone marrow are macrophages that can adhere to plastic dishes.
References


