High Erythroid Burst-promoting Activity in Sera from Umbilical Cord Blood

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Abstract Using methylcellulose clonal cell cultures, we examined human erythroid burst-promoting activity (BPA) in sera obtained from umbilical cord blood. BPA was measured on the basis of the number of erythroid bursts (BFU-E) and hemoglobin synthesis when peripheral blood mononuclear cells were cultured with cord blood serum in the presence of 1.0 U/ml erythropoietin (Epo) at low fetal calf serum concentration (10%). Controls were cultured with adult serum. The number of BFU-E and hemoglobin synthesis increased in culture to which cord blood serum had been added, and the enhancing effects of cord blood serum on erythroid burst formation were more pronounced than in control cultures with 2.0 U/ml Epo. No erythroid bursts, however, grew in the cultures with cord blood serum in the absence of Epo. Increased sensitivity of erythroid burst formation to low concentrations of Epo was observed in cultures containing cord blood serum. Dose-relationships between the concentrations of cord blood serum and the number of BFU-E and hemoglobin synthesis were clearly observed. We therefore concluded that the stimulatory effects of cord blood serum on erythroid burst formation may be due to BPA, one of the factors required for the proliferation of erythroid precursors at an early stage.

Key Words: BFU-E, BPA.

The clonal erythropoietic cell culture method has made it possible to detect two classes of erythroid progenitor cells: early-stage erythroid progenitors (less differentiated), designated as burst-forming unit erythroids (BFU-E) or erythroid bursts, and late-stage erythroid progenitors (highly differentiated), designated as colony-forming unit erythroids (CFU-E) (EAVES *et al.*, 1979). These progenitor

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cells are distinguishable on the basis of their proliferation capabilities (OGAWA *et al.*, 1977), erythropoietin requirements, incubation periods (AXELRAD *et al.*, 1974), and fetal hemoglobin augmentation capabilities (KIDOGUCHI *et al.*, 1978). Generally, CFU-E are found only in bone marrow and BFU-E in both bone marrow and peripheral blood in the normal state.

Both CFU-E and BFU-E need the glycoprotein hormone, erythropoietin (Epo), for their colony formation *in vitro*. Similarly, CFU-E *in vivo* requires Epo for growth and proliferation. However, the number of BFU-E *in vivo* does not correlate with endogenous Epo levels (LAJTHA *et al.*, 1971; HARA and OGAWA, 1978; ISCOVE and GUILBERT, 1978), so it appears that humoral substances other than Epo may regulate the early stages of erythropoiesis. It has been reported that the addition of certain types of cell and/or cellular products referred to as burst-promoting activity (BPA) enhances the growth of BFU-E *in vitro*. The conditioned medium of human peripheral blood leukocytes (AYE, 1977) or bone marrow cells (PORTER and OGAWA, 1982), T-lymphocytes (NATHAN *et al.*, 1978), and macrophages (KURLAND *et al.*, 1980) increased the number of bursts observed in cultures of bone marrow or peripheral blood. In addition, we reported that extracts from the irradiated spleen cells of rats contained BPA-like activity (KASAI *et al.*, 1980). Subsequently, the presence of high levels of BPA was found in sera of patients with aplastic anemia (NISSEN *et al.*, 1979; KIGASAWA and NISHIHARA, 1980). Since fetal erythropoiesis is extremely active in spite of low Epo levels (HALVORSEN, 1963), we examined the effect of cord blood serum on erythroid burst formation. It was observed that cord blood serum promotes the growth of BFU-E in culture.

MATERIALS AND METHODS

Serum samples. Cord blood serum samples (5 ml) were drawn from umbilical cords of normal pregnant women at 38–42-week gestation. Adult serum samples (5 ml) were obtained by venipuncture from healthy volunteers.

Erythropoietic cell culture. Blood samples for culture were obtained from healthy volunteers of "O" blood group and the mononuclear cells were separated by the Ficoll-Isopaque method (Pharmacia Fine Chemical Corp., Sweden). Erythropoietic cell culture was performed for 14 days using a modification (OGAWA *et al.*, 1977) of the methylcellulose clonal assay developed by ISCOVE *et al.* (1974). Briefly, 3×10^5 blood mononuclear cells were plated in culture dishes in a 1 ml mixture containing α -medium (Flow Labs. Inc., Rockville, Md.), 0.8% methylcellulose (Fisher Scientific Co., Norcross, Ga.), 1% deionized bovine serum albumin (Sigma Scientific Co., St. Louis, Mo.), 10% fetal calf serum (Sterile System Inc., Logan, Ut.), and 1.0 U/ml of sheep-plasma erythropoietin (Connaught Lab. Ltd., Willowdale, Ontario, Canada, specific activity 15 U/mg protein) unless otherwise specified. Adult or cord-blood serum was added at a concentration of 10% unless otherwise specified. Cultures were incubated in a humidified atmosphere flushed

with air containing 5% CO₂. Erythroid bursts were scored at day 14 under an inverted microscope.

Hemoglobin synthesis. On day 13 of incubation, cultures were labelled with 0.2 ml of α -medium containing 50% fetal calf serum and 0.5 μ Ci of ⁵⁹FeCl₃ (NZE-037, New England Nuclear Corp., Boston, Ma.). After 24 hr of labelling, heme was extracted using acid-methylethylketone and the activity was measured with a gas flow counter (TEALE, 1959).

RESULTS

It is well known that erythroid burst-forming efficiency is dependent not only on the Epo concentration but also on the fetal calf serum concentration of a culture. TERASAWA *et al.* (1980) reported that fetal calf serum contained some BPA. Therefore, a culture system using a low concentration of fetal calf serum (10%) was employed to detect the BPA in cord blood serum without the influence of the BPA existing in fetal calf serum. When examining the effect of cord blood serum on BFU-E growth, we observed that it increased the number of BFU-E and the size of the colonies determined by hemoglobin synthesis in cultures of human adult peripheral blood mononuclear cells to a greater extent than in control cultures with adult serum (Fig. 1). The magnitude of the response of BFU-E colony formation and hemoglobin synthesis to cord blood serum varied from donor to donor, as did the bursts grown under conditions of low fetal calf serum. The relation between hemoglobin synthesis and plating efficiency was not always parallel because bursts appeared to be larger in the presence of cord blood serum. Furthermore, it is to be noted that cultures with cord blood serum and 1.0 U/ml of Epo revealed a greater number of BFU-E than control cultures with 2.0 U/ml of Epo (Fig. 1, Exp. 3 and Exp. 4). However, no erythroid colonies were formed in cultures with only 20% cord blood serum in the absence of Epo (Fig. 1, Exp. 4).

Figure 2 shows the well-hemoglobinized and large-size erythroid bursts observed in a culture with cord blood serum under the condition of low fetal calf serum concentration.

The effect of varying concentrations of cord blood serum on BFU-E growth in peripheral blood mononuclear cells in the presence of Epo is shown in Fig. 3. The cord blood serum induced dose-related increases in the number of BFU-E and in hemoglobin synthesis at a constant concentration of 1.0 U/ml of Epo. A maximum stimulation, in which about 2.5 times the number of BFU-E grown in control cultures was observed, was obtained with 10% cord blood serum. In the case of adult serum, however, no dose-response was observed. No dose-related inhibitory effect was seen under these conditions.

The effect of cord blood serum on peripheral blood BFU-E grown was also investigated at various concentrations of Epo (Fig. 4). It was observed that ery-

Fig. 1. Effect of cord blood serum on erythroid burst formation (lower) and hemoglobin synthesis (upper). Peripheral blood mononuclear cells (3×10^5 cells/ml) were plated in a 1 ml culture containing Epo, 10% FCS and 10% cord blood serum or 10% adult serum as control cultures (cont.). The initials on the columns represent the names of the pregnant women from whom the cord blood serum samples were collected. In Y.M., Exp. 4, cells were cultured with 20% cord blood serum in the absence of Epo. Results are expressed as mean $\pm$ S.D. (3 plates).

throid burst formation and hemoglobin synthesis tended to respond to low levels of Epo in the cultures with cord blood serum, in contrast to the controls.

DISCUSSION

In the present study, we demonstrated that umbilical cord blood serum enhanced erythroid burst formation in peripheral blood mononuclear cell cultures. It has been reported that the concentrations of Epo in cord blood serum are less than 0.1 U/ml (HALVORSEN, 1963) and that such low concentrations are insufficient for growth of erythroid bursts in culture. Our present data also demonstrated that no growth of BFU-E colonies were observable when cultures were incubated with cord blood serum without Epo, although we did not measure the concentration of Epo in cord blood serum. It is possible, therefore, that the stimulatory effect of cord blood serum on BFU-E colony formation may be due to BPA rather than Epo.

Fig. 2. The well-hemoglobinized and large erythroid bursts (BFU-E) in a culture containing 10% cord blood serum under the condition of low fetal calf serum (10%) observed by inverted microscope, $\times 200$.

Fig. 3.

Fig. 3. Effect of the addition of cord blood serum (case N.K) on erythroid burst formation (lower) and hemoglobin synthesis (upper). Peripheral blood mononuclear cells (3×10^5 cells/ml) were cultured in the presence of increasing concentrations of cord blood serum at constant concentrations of Epo (1.0 U/ml) and 10% FCS. Results are expressed as mean $\pm$ S.D. (3 plates).

Fig. 4

Fig. 4. Growth of erythroid bursts (lower) and hemoglobin synthesis (upper) from 3×10^5 peripheral blood mononuclear cells at varying concentrations of Epo in the presence of 10% cord blood serum, case T.O (●) or 10% adult serum (○). Results are expressed as mean $\pm$ S.D. (3 plates).

Although the physiological behavior of BPA is still obscure, the observation that bursts are always larger in culture with cord blood serum suggests that BPA possesses two kinds of actions; 1) stimulation of recruitment of BFU-E from stem cells, 2) enhancement of cell division during the process of erythroid burst formation. Recently, TERASAWA *et al.* (1980) demonstrated that BPA obtained from both bone marrow conditioned medium and T-lymphoblastic cell line (Mo) conditioned medium enhanced fetal hemoglobin synthesis in BFU-E colonies and suggested that BPA might be an important modulator of fetal hemoglobin synthesis and/or a regulator of F-cell (BOYER *et al.*, 1975) production. Cord blood BPA

may therefore play a role in active erythropoiesis or in the high level of fetal hemoglobin synthesis found in fetuses. Whether or not these actions are mediated by a single factor cannot be determined from the present study alone. The characterization and purification of BPA may provide further insight into the regulation of the early stages of erythropoiesis.

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