CONCISE REPORT

Hemoglobin Biosynthesis in Individual Bursts in Culture: Studies of Human Umbilical Cord Blood

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We cultured human umbilical cord blood erythropoietic precursors in methyl cellulose clonal assay and analyzed the synthetic rates of Hb A and Hb F in individual erythropoietic bursts. Hemoglobin was labeled with 14C-amino acids in culture, separated by slab gel isoelectric focusing techniques, and quantitated by fluorographic methods. Almost all bursts exhibited both Hb A and Hb F in varying ratios. Frequencies of the individual bursts differing in percentage Hb F biosynthesis had normal distributions. Natal erythropoietic precursors appeared to be randomly committed to Hb F synthesis.

Recent developments in clonal cell culture techniques for erythropoietic precursors have made it possible to study hemoglobin (Hb) synthesis in culture in relation to the maturational stages of human erythropoietic precursors. Using such a clonal culture assay, Papayannopoulou et al.1,2 established fundamental concepts regarding Hb biosynthetic capabilities of adult human marrow erythropoietic precursors. They proposed that the Hb F biosynthetic capabilities of human erythropoietic precursors are inversely related to their maturational stages and that a single early erythropoietic precursor termed the burst-forming unit (BFU-E) is capable of expressing both fetal and adult globin genes in its differentiated progenies. We have shown that the Hb biosynthetic capabilities of adult human BFU-E in peripheral blood are identical to those of a BFU-E population in the marrow.3 We have also observed that approximately half of the Hb synthesized in culture by the umbilical cord blood BFU-E is Hb F.4 Using fluorographic methods, we have now succeeded in the characterization of Hb biosynthesis in individual erythropoietic bursts in culture. Studies of umbilical cord blood have revealed a heterogeneity in the Hb F biosynthetic capabilities of natal BFU-E.

MATERIALS AND METHODS

Erythropoietic Cell Cultures

After umbilical cords were severed from newborns, the cord blood was collected in 16-ml Falcon tissue culture tubes containing 400 units of heparin without preservatives. Mononuclear cells were
harvested from the blood by using slight modifications of the Ficoll-Isopaque techniques described by Boyum. Cell culture was carried out for 14 days using slight modifications of the methyl cellulose assay developed by Iscove et al. We used human urinary erythropoietin with specific activity of 40 units/mg that was kindly supplied by Dr. Anne P. Ball, Division of Blood Diseases and Resources, National Heart, Lung and Blood Institute, in a final concentration of 1.0 unit/ml.

Analysis of Hb Synthesis in Individual Bursts in Culture

Measurement of the rate of Hb synthesis was carried out during the last 2 days of culture. On day 12 of incubation 2 μCi of uniformly 14C-labeled amino acid mixture (NEC 445, New England Nuclear, Boston, Mass.) in 0.3 ml of phosphate-buffered saline (PBS) were layered over a dish containing 1 ml of tissue culture mixture. On day 14 of incubation individual bursts were identified by viewing through a magnifier; they were lifted from the methyl cellulose medium using an Eppendorf 10-μl pipette and placed in a microcentrifuge tube containing 200 μl of PBS and 0.5 μl of packed red blood cells prepared from the same samples. After washing, the samples were frozen at -70°C overnight. The frozen cell pellets were thawed at room temperature and lysed by the addition of 4 μl of 0.01-M KCN and 1 μl of 10% Nonidet P-40 solution. Hemolysates of individual bursts were subjected to isoelectric focusing that was carried out by using an LKB Multiphor apparatus as described previously. After completion of the run, the gels were fixed in 15% trichloroacetic acid, equilibrated with dimethylsulfoxide (DMSO), impregnated with 2,5-diphenyloxazole (PPO) in the presence of DMSO, and soaked in water. Gels were dried and then were placed in contact with Kodak RP X-Omat film that had been preexposed to a brief flash of light using an electronic photographic flash unit (Vivitar 283). The densities of fluorogram bands were determined from densitometric tracings that were carried out on a Joyce/Loebl 3CS microdensitometer. The preexposure to a flash of light had raised the level of background fog absorbance of the film by 0.15 O.D. and had established a linear relationship between radioactivity of a sample and density of the fluorogram band.

RESULTS

In total, 164 erythropoietic bursts obtained from four separate cord blood samples were analyzed for Hb content. Almost all bursts contained both adult and fetal Hb. Only six bursts exhibited either Hb A or Hb F. The ratio of fetal Hb to adult Hb varied among individual bursts, as is shown in a representative fluorogram (Fig. 1). The frequency distribution of bursts ranked according to their proportional Hb F synthesis (percentage Hb F synthesis per burst) approached a normal distribution. Analysis of goodness of fit to a normal distribution of a representative cord blood sample is shown in Fig. 2. Three additional cord blood

![Fig. 1. Portion of a fluorogram showing separation by isoelectric focusing of 14C-labeled Hb in seven individual bursts.](https://www.bloodjournal.org/content/bloodjournal/91/11/520/F1)

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samples also exhibited normal distributions, with standard deviations of 17.3%, 17.8%, and 12.5%.

In order to exclude the possibility of exchange of globin chains between labeled and carrier Hb, sickle cell erythrocytes were added to labeled cultures of normal peripheral blood mononuclear cells, and the samples were processed for fluorography. There was no radioactive peak in the area corresponding to Hb S, which suggests that under the conditions we employed there were no significant globin chain exchanges between labeled and unlabeled Hb.

**DISCUSSION**

Two models have been proposed as cellular mechanisms controlling physiologic Hb switching during perinatal periods. One model assigns particular Hb types to specific populations of erythroid precursors, with switching to changes in the cell populations during the perinatal period. The other model hypothesizes qualitative changes in a single stem cell line. Recently, Valet et al., through analysis of the volume distribution of erythrocytes from newborn sheep, presented an argument in support of the former model. Our data, on the other hand, clearly show that the natal BFU-E are randomly committed to Hb F synthesis, thus supporting the second model. Our studies also indicate that perinatal Hb switching in man is a gradual process, and they predict that Hb F distribution in cord blood erythrocytes is heterogeneous. While addressing somewhat different phenomena, our results are in basic agreement with the hypothesis of Papayannopoulou et al. that postnatal reactivation of Hb F synthesis in culture by human erythropoietic precursors involves intracellular mechanisms rather than population changes.

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REFERENCES


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