Studies of Circulating Hemopoietic Progenitor Cells in Human Fetal Blood


High levels of committed erythroid and granulocytic/monocytic progenitor cells have been demonstrated in fresh blood obtained at fetoscopy. The fetal progenitor cells were more sensitive to appropriate stimuli (erythropoietin and colony-stimulating factor) than adult progenitor cells grown under the same conditions, and this was shown to be due to intrinsic differences in the progenitor cells at the different developmental stages.

DURING HUMAN DEVELOPMENT, hemopoiesis has four distinct phases. In the embryo, nucleated erythrocytes arising in the extraembryonic membranes synthesize the embryonic hemoglobins, Hb Gower 1, Hb Gower 2, α2γ2, and the relatively minor haemoglobin Hb Portland, α3γ2. From 6 wk gestation, erythropoiesis transfers to the fetal liver and the embryonic hemoglobins are replaced by the fetal hemoglobin, α2G.

Bone marrow hemopoiesis is established between the 11th and 22nd wk of gestation and probably arises by stem cell migration into appropriate marrow stroma. Hepatic hemopoiesis declines in the third trimester and ceases soon after birth. Finally at birth, fetal hemoglobin is replaced by adult hemoglobin, and this event is related not to birth but to the time of gestation.

This study was performed to examine the levels of progenitor cells in fetal blood and to compare their sensitivities to appropriate stimuli with adult progenitor cells.

MATERIALS AND METHODS

Samples

Fetal blood was obtained by aspiration from an umbilical vessel under fetoscopic control, as previously described. Fetoscopy was performed to exclude β-thalassaemia (10 cases), α-antitrypsin deficiency (1 case), amegakaryocytosis with absent radii (1 case), and prior to intraamniotic prostaglandin abortion in 4 cases. Only one fetus studied (study no. 16) was found to be affected (β-thalassaemia major) and was later aborted. Samples for study were taken after adequate diagnostic specimens had been obtained. Two-hundred microliters of blood were taken into 2 ml of tissue culture medium containing 40 U of preservative-free heparin.

Cell Separation

The diluted fetal blood samples were mixed with an equal volume of hydroxyethyl starch, and the red cells were allowed to sediment at room temperature. After 30-60 min, a red cell pellet formed, and the red-cell-depleted supernatant could be removed. This contained approximately 90% of the original nucleated cells. Nucleated cells from adult blood and marrow were obtained by buoyant density separation using Ficoll-Hypaque.

Cell Culture Methods

Granulocytic and monocytic progenitor cells (CFU-GM) were grown in a double nutrient agar system by a modification of the method of Pike and Robinson, dialyzed placental conditioned medium being added to the underlay (final volume 1.2 ml) as a source of colony-stimulating activity. Adult marrow was plated in the upper layer (0.5 ml) at 104 nucleated cells/dish. Primitive erythroid progenitor cells (BFU-E) were grown in the presence of crude erythropoietin (human urinary erythropoietin obtained from the NIH, Bethesda, or Connaught Step III sheep plasma erythropoietin), in 0.9% methylcellulose, with supplemented α-medium, 30% fetal calf serum, 1% deionized bovine serum albumin, and 2 x 10^-6 M β-mercaptoethanol. No other source of burst-promoting activity was added. Adult peripheral blood nucleated cells were plated at 2-4 x 10^5/ml and fetal blood nucleated cells at 1-4 x 10^6/ml. Cultures were performed in 1-ml aliquots when cell numbers were sufficient. For titration experiments, fetal blood samples were cultured in 400-μ1 aliquots in multiwell trays.

All cultures were performed in duplicate or triplicate if adequate numbers of cells were available. Cultures were counted using an Olympus dissecting microscope. Fetal CFU-GM were stained in the dish for 12 days in cultures by cytoenzymatic methods to differentiate the various colony types.

Studies of Globin Chain Synthesis

Fetal BFU-E were incubated for 24 hr with tritiated leucine. Cell lysates were analyzed by globin chain separation on CM-Sepharose.

RESULTS

The culture of fetal blood between the 12th and 19th wk of gestation reveals extremely high levels of circulating progenitor cells (Table 1). The average CFU-GM levels of 12,493/ml and BFU-E levels of 20,451...
Fetal blood CFU-GM consist of monocyte colonies (42%-68%), neutrophil colonies (27%-41%), and eosinophil colonies (5%-30%). Occasional mixed neutrophil-monocyte colonies are seen. The fetal blood CFU-GM are more sensitive to placental conditioned medium (source of colony-stimulating activity) than are adult bone marrow CFU-GM (Fig. 1). Occasional fetal CFU-GM were seen in the absence of added colony-stimulating activity. Fetal blood is compared with adult marrow, rather than with adult peripheral blood, because CFU-GM levels are so low in adult blood (average 4.4/10^3 in 7 experiments) that when adequate nucleated cells are plated, there is considerable endogenous colony-stimulating factor production.

Hemoglobinized fetal-blood-derived BFU-E are visible by the seventh day in culture and reach maximum numbers by day 10. Hemoglobinized adult BFU-E are not seen until days 10–12 in culture and do not reach maximum numbers before day 14. Fetal blood BFU-E by definition contain more than 64 cells but usually contain several thousand cells. Some BFU-E are large single colonies, but others consist of cell aggregates with multiple subcolonies. Fetal blood BFU-E are more sensitive to erythropoietin than adult BFU-E (Fig. 2), with peak fetal BFU-E levels seen at 0.5 U/ml of erythropoietin. No fetal BFU-E are seen in the absence of added erythropoietin. Adult BFU-E grown under the same conditions require 3 U of erythropoietin/ml for maximum growth, and when fetal and adult blood are mixed, maximum growth is also seen at an erythropoietin concentration of 2–4 U/ml (Fig. 3). The fetal BFU-E make almost exclusively HbF, as shown by globin chain synthesis analyses, similar to previous reports.  

**Table 1.** Committed Hemopoietic Progenitors in Fetal Blood

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>CFU-GM/10^3</th>
<th>CFU-GM/ml†</th>
<th>BFU-E/10^3</th>
<th>BFU-E/ml†</th>
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</table>

Mean ± SD: 295 ± 230, 12,493 ± 9,626, 444 ± 439, 20,451 ± 18,833

*Gestational age is time since fertilization, i.e., 2 wk less than time from last menstrual period. In all cases, the estimated gestational age was in agreement with the biparietal diameter measured by ultrasound.

†Calculated by multiplying the number of progenitors per 10^6 nucleated cells by the number of nucleated cells in whole blood expressed as millions per millimeter.

Fig. 1. The mean dose–response curve for 5 fetal samples is shown with standard deviations for each point. CFU-GM were scored at day 12. This is compared with the dose–response curve of 7 normal adult bone marrow cultures scored at days 12–14.
Fig. 2. The mean dose-response curve for 6 fetal blood samples is compared with 4 adult peripheral blood samples.

DISCUSSION

The high levels of hemopoietic progenitor cells circulating in human fetal blood demonstrated in this study support the concept that developing bone marrow is colonized by circulating stem cells, in agreement with the results of Barnes et al.\(^\text{15}\) in mice.

High levels of erythroid progenitor cells in human fetal liver have also been described. Stamatoyannopoulos et al.\(^\text{14}\) found BFU-E levels of 160/10\(^3\) fetal liver cells, and Hassan et al.\(^\text{16}\) and Rowley et al.\(^\text{17}\) found erythroid colony levels of about 1000/10\(^3\) fetal liver cells. The latter authors scored all aggregates greater than 8 cells as colonies and scored all BFU-E subcolonies individually, accounting largely for the discrepancy between the results from the three groups. Fetal liver BFU-E grow rapidly,\(^\text{14,16}\) as do those derived from fetal blood described in this article, indicating that fetal BFU-E cycle more rapidly than do their adult counterparts.

It is interesting that although granulocytes are not formed in large numbers in fetuses until shortly before birth,\(^\text{9}\) fetal blood CFU-GM levels are very high. This suggests that conditions for granulopoiesis are not present in the human fetus in the second trimester, possibly due to a lack of colony-stimulating factor.

Stamatoyannopoulos et al.\(^\text{14}\) and Hassan et al.\(^\text{16}\) noted a similar higher erythropoietin sensitivity of fetal liver erythroid colony-forming cells as was demonstrated in the blood-derived BFU-E in this article. The fetal liver is thought to be the major site of fetal erythropoietin production\(^\text{18}\) and may also produce other “promoting factors.” In the present study, fetal blood nucleated cells have been plated at only 2 \(\times 10^4\) cells/ml to reduce promoting factor effect. To demonstrate that this high sensitivity of fetal cells was not due to promoting factors, the coculture experiments were performed. The biphasic response curves (Fig. 3) indicate the presence of progenitors with a high and low intrinsic erythropoietin sensitivity, presumably the fetal and adult blood-derived cells, respectively.

The fetal progenitor cells with high erythropoietin (epo) sensitivity produce predominantly HbF, while the adult progenitor cells with a lower epo sensitivity produce mainly HbA. The latter produce some HbF with high erythropoietin,\(^\text{19,20}\) and this is postulated to be due to the stimulation of a more undifferentiated progenitor cell to produce red cells. The formation of HbF by these adult cells is therefore not analogous to the formation of HbF by fetal progenitor cells at low erythropoietin concentrations as described here. The adult stem cell may arise either by maturation of the fetal stem cell or from a “primordial” stem cell. The former hypothesis requires the demonstration of a progenitor cell with intermediate erythropoietin sensitivity as well as intermediate HbF production at the time of the switch. The demonstration of intermediate HbF production alone in cord blood\(^\text{21}\) does not necessarily indicate that adult progenitor cells are the progeny of fetal progenitor cells, as a newly developing clone would contain more of the less differentiated...
erythroid stem cells, and the “adult BFU-E” at this stage of ontogeny would synthesize more HbF than at a later stage.

REFERENCES

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DC Linch, LJ Knott, CH Rodeck and ER Huehns