Different Stimulative Effects of Human Bone Marrow and Fetal Liver Stromal Cells on Erythropoiesis in Long-Term Culture

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The factors determining the predominantly erythroid direction of human fetal liver hematopoiesis are unknown. We compared the capacities of human fetal liver and bone marrow stromas to sustain fetal and adult hematopoiesis in long-term cultures. In various marrow-fetal liver combinations of stroma and rechange, the maintenance of erythroid (BFU-e) and myeloid (CFU-GM) precursors in the nonadherent phase was determined. The morphology of the fetal liver nucleated cells during culture was also examined. This study shows that fetal liver stromas efficiently support fetal BFU-e for 6 to 7 weeks in vitro. Bone marrow stromas were not able to maintain fetal BFU-e beyond 4 weeks. Significant numbers of marrow BFU-e were not sustained in vitro on either source of stroma. On the other hand, the stroma layers of fetal liver and marrow origin were equally effective in maintaining fetal CFU-GM and adult CFU-GM in long-term culture. These findings show that the human embryonic liver stroma is a preferential site for stimulating fetal erythropoiesis. They do not demonstrate differences in stroma function to explain the relative paucity of myelopoiesis in the fetal liver.

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the underlayer, as described, to determine the number of myeloid precursors (CFU-GM). Erythroid burst-forming units (BFU-e) were cultured in 1.1% methylcellulose in Iscove’s modified Dulbecco’s medium (GIBCO, Scotland), supplemented with crude human urinary erythropoietin (prepared from urine of patients with aplastic anemia), 30% fetal calf serum, bovine serum albumin (0.75%), egg lecithin (3 x 10⁻¹⁰ mol/L), Na₂ SeC₃ (10⁻¹⁰ mol/L), iron-saturated human transferrin (7.7 x 10⁻⁴ mol/L), and betamercaptoethanol (5 x 10⁻⁴ mol/L). One milliliter of this culture medium, containing 1 x 10⁵ fetal or bone marrow cells, was plated into four wells of a cluster dish (Costar, Cambridge, MA; 16-mm well diameter) and incubated at 37 °C in a fully humidified atmosphere in 5% CO₂ in air. Colonies containing more than 50 cells were counted on day 14 by use of an inverted microscope (Zeiss).

Table 1 presents the recoveries of CFU-GM numbers; the lower part, those of BFU-e numbers during long-term culture. Figures indicate corrected numbers of progenitors per flask. ND, not determined.

Fetal liver cells in Dexter-type cultures. In six experiments (Table 1) we recharged fetal liver-derived adherent cell layers with cryopreserved autologous fetal liver cells, to determine the maintenance of CFU-GM and BFU-e in long-term culture. A typical decline of CFU-GM numbers was seen in all instances during the first week, which, in four experiments, was subsequently followed by a maintenance of CFU-GM during another 5 to 6 weeks. In four experiments, CFU-GM numbers were still detectable at week 6 to 7 of culture, while in the two other experiments (2 and 6) CFU-GM extinguished rapidly. Numbers of BFU-e were also assessed throughout culture and were recovered until approximately week 6 of culture. BFU-e showed similar kinetics of recovery in vitro, i.e., a precipitous fall during week 1 and a maintenance of BFU-e numbers during the subsequent 6 weeks of culture. The data are not indicative of a relationship between fetal age of the stromas and their capacity to sustain CFU-GM and BFU-e during culture. Stromas derived from livers of week 14 and week 15 fetuses (Table 1, experiments 1, 2, and 3) and those from week 18 (Table 1, experiments 4, 5, and 6) gave equivalent results.

In order to investigate the capacity of fetal liver stroma layers to sustain the fetal liver hematopoiesis from different donors, we also evaluated the effect of allogeneic stroma layers under similar conditions. Two directly comparative experiments were performed, and the influence of the allogeneic fetal liver stroma on the recoveries of nucleated cells, CFU-GM, and BFU-e in long-term cultures was determined. Equivalent values of these parameters were measured on allogeneic and syngeneic fetal liver stroma layers.

In addition, four control experiments were performed in which fetal liver cells were cultured in the absence of a stroma layer. In these cultures no CFU-GM and BFU-e could be recovered at week 4 of culture. In two experiments, the cell layers were scraped and assayed for the presence of the cell population.
CFU-GM and BFU-e. This was done to verify whether significant numbers of progenitor cells were trapped in the adherent cell layer. At week 3 of culture, the fetal liver stroma layer contained 75 CFU-GM per flask and at the time of recharge (week 4) no CFU-GM were detected. At the same time points, no BFU-e were measurable in the fetal liver adherent cell layers.

*Is the bone marrow stroma layer capable of maintaining allogeneic fetal liver cells in long-term culture?* In a subsequent series of experiments, a comparison between the effects of bone marrow and fetal liver layers on fetal liver CFU-GM (Fig 1) and BFU-e (Fig 2) was made. These experiments were performed using allogeneic cells.

The myeloid progenitor cells from fetal liver were equally well maintained on fetal liver and bone marrow stroma layers until approximately week 9. No differences were seen as compared with cultures in which the stroma layers had been preirradiated, indicating that the CFU-GM measured in the nonadherent layer were not derived from the adherent layer. On the other hand, it appeared that bone marrow adherent cell layers were generally inferior to fetal liver stroma layers in maintaining fetal liver BFU-e numbers. This was apparent from the directly comparative experiments presented in Fig 2. Additional experiments revealed that considerable numbers of BFU-e were still recoverable at week 6 in five allogeneic fetal liver cultures (data not shown). However, when allogeneic fetal liver cells were cultured on a bone marrow stroma layer, BFU-e were measurable until 3 to 5 weeks only.

Thus these data indicate a stromal specificity of fetal liver and adult marrow for promoting the recoveries of the erythroid precursor cells but not myeloid precursor cells. Notably, preirradiation of the stroma layers did not influence the recoveries of the numbers of BFU-e from the cultures.

*Is a fetal liver stroma layer also capable of maintaining adult precursor cells in long-term culture?* In the last series of experiments, the capacity of fetal liver stromas to maintain marrow precursors (Table 2) in long-term cultures was examined. As compared with marrow stromas, fetal liver stromas were equally capable of maintaining marrow CFU-GM and BFU-e.

In conclusion, the favorable effect of a fetal liver stroma layer on the maintenance of erythroid progenitor cells is restricted to fetal liver BFU-e; it is not exerted on marrow BFU-e.

**Morphology of the nonadherent fetal liver cells during long-term culture.** The lineage-specific developments of the recharged fetal liver cells during long-term culture were assessed by cytological examination. Whereas the fetal liver is mainly erythropoietic at the time of inoculation, at 1 week of cultivation on a stroma layer, a switch to myelopoiesis was observed. After 4 to 5 weeks in vitro the nonadherent cell population was mainly composed of monocytes and macrophages. No differences were seen between cells grown on an autologous or allogeneic fetal liver stroma or those on a bone marrow stroma.

**Morphology of the fetal liver stromal layers.** The composition of the fetal liver stroma differed from that of the bone marrow stroma. The fetal liver fibroblasts were organized as confluent layers on the bottom of the flask. In contrast to the bone marrow cultures no patches or cobblestones were observed in cross sections of the fetal liver stroma. No fat cells appeared in the fetal liver stromal layer. Cytochemistry of these cells did not reveal differences and confirmed the presence of macrophages, myeloblasts, and more mature myelocytic cells.

**DISCUSSION**

We have performed experiments to examine the specific effects of fetal liver and bone marrow stroma layers on the proliferation and differentiation of fetal and adult hemo-
fibroblasts appear to suppress the formation of new microenvironment on erythroid progenitors was specific for production within the first week, but in the case of fetal liver, a stromal cultures BFU-e numbers dropped considerably marrow stromas on BFU-e. Both in fetal liver and marrow being responsible for the discrepant effects of fetal liver and influence the proliferation of marrow BFU-e.'6 Marrow-endothelial cells in human marrow stromal cultures do not differences. Evidence exists to suggest that macrophages and which cell types in the stroma layers account for these cultures. Total BFU-e numbers recovered were also higher in fetal liver demonstrated a more powerful stimulative effect of the fetal liver BFU-e. These cells may therefore be regarded as candidates capable of maintaining BFU-e in long-term cultures. The data presented in this study show that human fetal liver hematopoiesis in long-term culture, similar to human marrow hematopoiesis requires the establishment of an adherent cell layer. In direct comparative experiments it was demonstrated that the fetal liver stroma maintains fetal liver BFU-e better than the bone marrow stroma does. In contrast, marrow BFU-e, marrow CFU-GM, and fetal liver CFU-GM were supported equally well by the stroma layers of both tissues. Allogeneic effects between stromas and cell inoculates were not apparent.

The hematopoietic inductive microenvironmental sites within different organs are thought to have a role in regulating hematopoiesis from the pluripotent stem cells into committed precursors. The microenvironments in different organs (eg, marrow, spleen, embryo liver) appear to have different specificities in determining cell lineage maturation. We investigated whether fetal liver stroma layers were capable of maintaining BFU-e in long-term cultures. The answer to this question is considered of interest with respect to the lack of understanding of the predominantly erythropoietic nature of the human fetal liver. Direct comparisons between the effects of fetal liver and marrow stroma layers on fetal BFU-e demonstrated a more powerful stimulative capacity of the fetal microenvironment.

BFU-e were recovered for 6 to 7 weeks when seeded on a fetal stroma, whereas, in all instances, they had disappeared before 5 weeks after their inoculation into marrow stromal cultures. Total BFU-e numbers recovered were also higher in fetal liver than in marrow stromal cultures. It is unclear which cell types in the stroma layers account for these differences. Evidence exists to suggest that macrophages and endothelial cells in human marrow stromal cultures do not influence the proliferation of marrow BFU-e. Marrow-derived fibroblasts appear to suppress the formation of (new) BFU-e. These cells may therefore be regarded as candidates being responsible for the discrepant effects of fetal liver and marrow stromas on BFU-e. Both in fetal liver and marrow stromal cultures BFU-e numbers dropped considerably within the first week, but in the case of fetal liver, a production of erythroid precursors was seen beyond week 2. We noted that the stimulative effect of the fetal liver microenvironment on erythroid progenitors was specific for fetal BFU-e. When bone marrow cells were cultured on a fetal liver stroma layer, the number of adult BFU-e declined precipitously. These different values of fetal liver and marrow BFU-e on the same type of stroma are suggestive of cell dissimilarities existing between fetal liver BFU-e and marrow BFU-e and indicate alterations of the responsiveness of the erythroid precursors to stroma stimuli during human ontogeny.

Human fetal liver contains large numbers of committed myeloid progenitor cells. However, a minimal representation of granulopoiesis is apparent in histologic sections. Whether the scarce granulopoietic activity in the fetal liver organ is the result of different microenvironmental influences or instead a different responsiveness of the fetal progenitors to stimulation has not been defined. Our experiments do not reveal divergent efficiencies of fetal liver and marrow stromas as far as the production of CFU-GM is concerned. Quantitatively equivalent numbers of marrow- and fetal liver–derived CFU-GM were recovered from the fetal liver stromas (cultured for 5 to 7 weeks). In addition, fetal liver and marrow stroma layers permitted identical recoveries of CFU-GM numbers. Thus the data from these studies suggest that (a) fetal CFU-GM are susceptible to the stromal factors from both marrow and fetal liver, and (b) the fetal liver stroma layer is capable of stimulating the production of CFU-GM from adult and embryonic hematopoiesis to the same extent as bone marrow stroma layers do.

Morphological analysis revealed the predominance of myeloid cells, while erythroid cells had disappeared within 1 week of culture. As a matter of fact, it is likely that in vivo in the embryo, certain events inhibit granulopoiesis from the numerous myeloid precursors contained in the human fetal liver. A previous study has shown that mature granulopoietic elements from fetal liver may be detected in cultures on a bone marrow stroma layer for more than a year. This particular analysis did not include measurements of CFU-GM. We discontinued our cultures as soon as the CFU-GM had disappeared, and in our experience, at approximately week 5 of culture, the nonadherent cell population consisted mainly of macrophages, regardless of the tissue origin of the stroma layer or recharge.

We conclude from these experiments that with regard to
the regulation of erythropoiesis, both the function of the fetal liver microenvironment and the responsiveness of fetal liver progenitors (BFU-e) to stroma stimulation differ from those of the marrow stroma and marrow BFU-e. No differences were found to account for the scarcity of myelopoiesis in the fetal liver. While in vitro no active suppression of granulopoiesis exists, the rare granulopoiesis in vivo may primarily be the consequence of the erythropoietic advantage in the embryo liver.

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