Ontogeny-related Changes in Proliferative Potential of Human Hematopoietic Cells

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Summary

Blood cells originate from hematopoietic stem cells that are located at different sites during ontogeny. Production of human stem cells and their progeny in culture is expected to have important implications for experimental therapeutic strategies involving gene transfer and transplantation. Here we report striking differences between primitive hematopoietic cells purified from adult bone marrow, umbilical cord blood, and fetal liver in cytokine-supplemented, serum-free cultures. In such cultures both the fraction of responding cells and their ability to produce CD34+ progenitor cells decreased markedly with the age of the cell donor. These results document extensive, ontogeny-related functional differences between primitive hematopoietic cells.

Materials and Methods

Bone marrow cells were retrieved from vertebral bodies of cadaveric organ donors (20). Cord blood samples were obtained from clamped umbilical cords at the time of birth of normal full-term pregnancies. Fetal liver cells were obtained from elective, therapeutic abortions in the 10–16th wk of gestation. The use of human material in this study was approved by local Institutional Review Boards as well as the Ethical Screening Committee of the University of British Columbia. All cells were previously frozen, separated using Ficoll-Hypaque, and processed for flow cytometry cell sorting as described previously (20, 21).

Results and Discussion

CD34+ CD45RA+ CD71+ cells purified from various tissues were cultured in serum-free liquid cultures (20, 21) supplemented with IL-3 (20 ng/ml), IL-6 (10 ng/ml), Steel factor

1 Abbreviation used in this paper: SCC, stem cell candidates.
bone marrow remained brightly labeled with PKH26 (indistinguishable) after 7 d of culture. Importantly, loss of PKH26 indicating no or single-cell divisions), whereas most CD34 §

cells from cord blood and especially those from fetal liver progenitors increased 31-250-fold and 490-3,200-fold, respectively, over the same culture period (results from three separate experiments). CD34 § cells that were recovered from the cultures had a cloning efficiency in methylcellulose medium that ranged from 14 to 46%, and the production of colony-forming cells corresponded to the production of CD34 § cells (Table 1). Interestingly, a clear shift towards the production of myeloid colony-forming cells was observed with time, similar to that described by others (11, 22). These results indicate that the culture conditions used do not support true self-renewal of fetal-derived stem cell “candidates” or, alternatively, that the latter were increasingly diluted by CD34 § cells with a more limited proliferative potential.

The large and striking differences in culture between stem cell “candidates” purified from adult bone marrow, fetal liver, and cord blood are further illustrated in Fig. 2. In this representative experiment purified candidate stem cells were labeled with the fluorescent tracking dye PKH26 (23) and analyzed for CD34 expression and PKH26 fluorescence before and after 7 d of culture. Note that most CD34 § cells from bone marrow remained brightly labeled with PKH26 (indicating no or single-cell divisions), whereas most CD34 § cells from cord blood and especially those from fetal liver had decreased PKH26 fluorescence (indicative of multiple divisions) after 7 d of culture. Importantly, loss of PKH26 fluorescence of adult bone marrow cells coincided with loss of CD34 expression, whereas large numbers of CD34 §

PKH26 § cells were recovered from cultures initiated with fetal liver or umbilical cord blood cells. These results indicate that the differences between stem cell “candidates” purified from different sources were not restricted to the fraction of responding cells, but included qualitative differences in the ability to generate CD34 § daughter cells as well.

Taken together, these observations indicate large functional differences between phenotypically similar hematopoietic cell populations derived from blood-forming tissues at different stages in ontogeny. Differences in the developmental potential between purified fetal and adult hematopoietic cells were previously described for murine cells with respect to thymic maturation potential (24). An increased reinitiating potential of human CD34 § cells from umbilical cord blood compared with CD34 § cells from bone marrow was reported in a recent study (22). Our findings are in agreement with those previous studies and suggest that the proliferation and differentiation potential of primitive hematopoietic cells may change extensively during ontogeny. In this respect, it will be of interest to determine if the common precursor for the hematopoietic microenvironment and hematopoietic stem cells that was recently isolated from fetal bone marrow (25) can also be found in adult tissue. In view of the ontogeny-related differences in the functional properties of purified primitive hematopoietic cells reported here, a search for the genes that are differentially expressed in such cells is indicated (26, 27). A possible link between the limited proliferative potential of adult hematopoietic cells and the length of their telomeres (28) also warrants further study.

From a practical point of view it appears of interest to pursue numerical expansion of both cord blood and fetal liver CD34 § progenitor cells for potential clinical use, as has been suggested for cord blood cells by others (29, 30). The number of nucleated (Ficollled) cells that can be obtained from a fetal

![Figure 1](image-url)
Table 1. Ontogeny-related Differences in Production of Clonogenic Cells

| Cell type | Day | Cloning efficiency % | CFU-c | BFU-e | CFU-mix |
|-----------|-----|----------------------|-------|-------|---------|
| BM SCC    | 0   | 13                   | 494   | 494   | 312     |
|           | 7   | 26                   | 2,602 | 575   | 203     |
|           | 16  | 15                   | 2,967 | 103   | 379     |
|           | 25  | 14                   | 2,604 | 0     | 196     |
| CB SCC    | 0   | 25                   | 1,050 | 550   | 900     |
|           | 7   | 30                   | 20,250| 1,580 | 675     |
|           | 16  | 22                   | 69,960| 0     | 0       |
|           | 25  | 17                   | 187,000| 0    | 0       |
| FL SCC    | 0   | 46                   | 1,472 | 1,288 | 1,840   |
|           | 7   | 33                   | 187,968| 6,336| 16,896  |
|           | 16  | 25                   | 878,600| 38,200| 38,200  |
|           | 25  | 17                   | 3,128,000| 0   | 0       |

SCC from bone marrow (BM), umbilical cord blood (CB), and fetal liver (FL) were purified and CD34+ cells recovered from the liquid cultures as described in the legend to Fig. 1. Sorted CD34+ cells were plated in methylcellulose medium for measurements of clonogenic cells (20). Colonies were scored 14-21 d later and the results of a representative experiment are shown. The absolute number of myeloid- (CFU-c; including CFU-G, CFU-M, and CFU-GM), erythroid- (burst forming unit-erythroid, BFU-e), and mixed myeloid-erythroid- (CFU-mix) colony-forming cells present at each time point was calculated from the fraction of CD34+ cells plated and the calculated number of CD34+ cells present in the cultures (see also legend to Fig. 1). Note the differences in clonogenic cell production between SCC from BM, CB, and FL and the shift towards production of myeloid colony-forming cells upon prolonged culture.

Figure 2. Ontogeny-related differences in response to a mixture of growth factors by highly purified hematopoietic cells. Fetal liver, cord blood, and adult bone marrow SCC with a CD34+ CD45RA- CD71lo phenotype were purified by FACS® labeled with the membrane label PKH26 (21), and cultured in serum-free medium supplemented with IL-6, IL-3, MGF, and Epo. PKH26 is a bright fluorescent dye that is stably incorporated into the lipid bilayer of cell membranes and diluted among daughter cells upon division of the parental cells (21). In this representative experiment (n = 4), PKH26 was incorporated to different degrees in the three target cell populations resulting in differences in PKH26 fluorescence at day 0 (top). PKH26 fluorescence was measured again at day 7 (bottom) and plotted against CD34 fluorescence. Note that the majority of adult bone marrow CD34+ cells maintained their PKH26 fluorescence, indicating little turnover during the 7 d of culture and a weak response to the growth factor mixture. In contrast, practically all CD34+ cells from umbilical cord and fetal liver showed decreased levels of PKH26 fluorescence at day 7, indicating an active proliferative response that included production of CD34+ cells. Note that fetal liver CD34+ cells had a decreased PKH26 fluorescence compared with cord blood CD34+ cells, indicating a higher proliferative rate of the fetal cells. The numbers in the boxed areas represent the number of cells used to initiate the experiment (top) or the number of cells with the indicated phenotype that were recovered at day 7. This number was calculated from total cell counts and phenotypic analysis.
liver (8–16 wk of gestation) ranges from $10^7$ to $10^8$ cells, of which 1–4% are CD34+ CD45RA+ CD71+ (10^6 SSC/liver). For cord blood, minimal estimates (29) of these figures are $50 \times 10^6$ cells total, of which 0.5–1% are CD34+ CD45RA+ CD71+ (also $\sim 10^6$ SCC/cord blood sample). Upon culture for 25 d with the combination of growth factors used in this study, purified SCC from fetal liver and cord blood would be expected to yield, respectively, $10^9$ and $10^8$ CD34+ cells, assuming that all CD34+ cells could be recovered from the cultures and logistics of large scale cultures were established. These numbers are comparable to the number of CD34+ cells in a typical allogeneic bone marrow graft (1–2 $\times 10^8$ total nucleated cells, of which 1–2% are CD34+, for a total of $\sim 2 \times 10^8$ CD34+ cells/allograft) and are also comparable to the calculated number of CD34+ cells that could possibly be obtained by culture of peripheral blood with interleukin-3, granulocyte colony-stimulating factor, or granulocyte-macrophage colony-stimulating factor. Blood. 77: 2316.

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