Published in final edited form as:

Early Hum Dev. 2001 December ; 65(2): 159–163.

Cell cycle status of CD34+ cells in human fetal bone marrow

Joyce M. Koenig*, Benjamin Luttge, Neal A. Benson, and Robert D. Christensen

Division of Neonatology, Department of Pediatrics, University of Florida College of Medicine, P.O. Box 10096, Gainesville, FL 32610-0296, USA

Abstract

We used flow cytometric analysis to determine the cell cycle characteristics of human CD34+ cells from fetal bone marrow (BM), adult BM, and umbilical cord blood (UCB) samples. Fetal BM had three-fold more cells in the S-phase than did adult BM or UCB.

Keywords

Cell cycle status; CD34+ cells; Bone marrow

1. Introduction

Neonates have a particularly high risk of developing neutropenia during infection [1]. Infection in adult rats induces the recruitment of quiescent granulocyte progenitors into the cell cycle, and this is one mechanism for accelerating neutrophil production [2]. However, in neonatal rats, a high proportion of cycling hematopoietic progenitors in the basal, non-infected state limits their capacity to further accelerate granulocytopoiesis in response to increased demand for neutrophils [2]. Thus, in this animal model, the rapid cycling of hematopoietic progenitors in the non-infected pups contributes to the development of neutropenia during infection [3]. Whether a similar restriction is contributory to the frequent neutropenia in infected premature human neonates is unknown. To test the hypothesis that fetal bone marrow (BM) has a high proportion of cycling progenitors in the basal state, we used flow cytometry to determine the cell-cycle status of CD34+ progenitor cells in the BM of human fetuses and adults.

2. Methods

2.1. Subjects

Adult BM cells were aspirated from the anterior superior iliac crest of 10 healthy volunteers. Fetal BM cells (gestational ages, 14–18 weeks) were collected by flushing the femurs and humeri following five elective pregnancy terminations. Umbilical cord blood (UCB) samples, obtained from five term (37–41 weeks) and three preterm (32–34 weeks) gestations, were collected immediately after delivery. All studies were performed according

to protocols approved by the University of Florida Institutional Review Board, and informed consent was obtained from the adult volunteers.

2.2. Preparation of CD34+ enriched populations

Light-density mononuclear cells (specific gravity < 1.077) were isolated by density centrifugation (Ficoll-Hypaque; Upjohn Pharmacia, Piscataway, NJ). Cell suspensions were enriched for their content of CD34+ cells using an avidin anti-CD34+ column (Cell Pro, Bothell, WA), according to the manufacturer's instructions. The CD34+ content of the cell suspensions was similar in bone marrow and umbilical cord blood samples, and varied between 45% and 60%.

2.3. Cell-cycle status

Flow cytometric analysis of the cell-cycle distribution of CD34+ cells was performed using a modification of an established propidium iodide technique [4]. Briefly, trypsinized cells were stained with propidium iodide (CycleTEST™ PLUS DNA Reagent Kit; BD Biosciences, San Jose, CA), according to the manufacturer's instructions, and cell samples analyzed within 1 h of staining. Cell doublets and aggregates were excluded from analysis by their abnormal fluorescence width (observed as a single event) vs. total fluorescence area. At least 10,000–30,000 events were acquired per analysis using a FACScan flow cytometer and the Lysis II software (both from Becton Dickinson). Computer data analysis was performed using the CellFit Cell-Cycle Analysis software (BD Biosciences) or the Modfit Cell Cycle analysis software (Verity Software House, Topsham, ME).

2.4. Statistical analysis

Student's *t*-test was used for comparative analysis of data. A *P* value less than 0.05 was considered significant.

3. Results and discussion

Cells synthesizing DNA (S-phase), or those in the post-replicative (G_2) or mitotic (M) phases of the cell cycle, contain more DNA than do cells containing an unreplicated complement of DNA (G_1 phase) or that are quiescent (G_0). We observed the highest proportion of cells in the S-phase in fetal BM ($16.5 \pm 1.5\%$; $X \pm SD$) (Figs. 1 and 2), threefold higher than in adult BM samples ($5.3 \pm 2.5\%$) and nearly 11-fold higher than in UCB samples ($1.5 \pm 0.8\%$). Data for preterm and term UCB were combined, as no differences between groups were observed for any portion of the cell cycle analyses. The percentage of CD34+ cells in G_2/M was three-fold higher in fetal BM cells ($6.3 \pm 2.6\%$) than in adults ($1.9 \pm 1.2\%$) or in UCB ($1.8 \pm 1.2\%$) samples; no differences were noted between adult BM and UCB ($P = 0.13$). Fetal BM contained a lower proportion of cells in G_0/G_1 phase ($77.2 \pm 3.2\%$) than did adult BM ($92.8 \pm 3.3\%$, $P < 0.001$). Conversely, the highest proportion of cells in the G_0/G_1 phase was found in UCB ($96.7 \pm 1.2\%$, $P < 0.01$ vs. fetal BM or adult BM). The higher proportions of CD34+ cells in both S-phase and G_2/M phase in fetal BM most likely reflects rapid production of blood cells in the developing fetus [5]. Despite this high cycling rate, fetuses can increase erythrocyte production during erythroblastosis fetalis [6], although whether the response to fetal anemia occurs as

efficiently as in anemic adults is unknown. Conversely, a fetal capacity to increase myelopoiesis in response to neutropenia has not been specifically reported, although increased neutrophil production was observed in preterm neonates whose mothers received granulocyte colony-stimulating factor prior to delivery [7].

In agreement with previous reports [8,9], we observed a higher proportion of cord blood CD34+ cells in the G₀/G₁ phase compared with those from adult marrow. Stimulated cord blood hematopoietic progenitors rapidly exit G₀/G₁ than do adult BM progenitors [8], possibly due to a higher proportion of CD34+ cells in G₀-phase [10]. In contrast, fetal BM contains a lower proportion of CD34+ cells in G₀/G₁ than is found in adult BM or in UCB. Our findings suggest that in the fetus, a smaller proportion of quiescent progenitors are available to enter the cell cycle in response to an increased need for neutrophils, a possibility that is concordant with observations in neonatal vs. adult rats [2]. Thus, low availability of myeloid progenitors, by limiting rapid up-regulation of neutrophil production during overwhelming infection, might be a contributing factor to the neutropenia commonly observed in septic premature neonates [3]. However, accelerated neutrophil destruction or utilization, or inhibition of myelopoiesis, are probably more common mechanisms causing neonatal neutropenia [3].

The mechanism(s) responsible for the higher cycling rates that we observed in CD34+ cells from fetal marrow compared with those of adult marrow are unclear. These differences might reflect properties unique to the fetal hematopoietic microenvironment [11], such as a differing proportion of stimulatory factors [12] or hematopoietic inhibitors [13]. Alternatively, fetal progenitors might possess cell cycle characteristics that are intrinsically different from those of their adult counterparts [14]. The continued study of fetal stem cell biology will likely provide mechanistic insights into the frequent cytopenias of extremely premature neonates.

Acknowledgements

The authors thank the research nurses at the University of Florida Clinical Research Center, Pam Connolly, R.N. and Ann Cothran, R.N., for their assistance with adult bone marrow aspirations, and the Labor & Delivery staff at Shands Hospital for their help with cord blood samples. We also thank DeFang Luo and Joe Stegner for their technical supports, and the Interdisciplinary Center for Biotechnology Research at the University of Florida for providing access to their flow cytometer. This work was supported in part by funds from the Children's Miracle Network (J.M.K.), STOP! Children's Cancer, (J.M.K.), and the National Institutes of Health (HL-44951, HL-61798, and RR-00083, R.D.C.). J.M.K. is a recipient of an NIH Clinical Investigator Development Award (K08 HD-1062) and a University of Florida Howard Hughes Research Resources grant.

References

1. Christensen RD, Calhoun DA, Rimsza LM. A practical approach to evaluating and treating neutropenia in the neonatal intensive care unit. *Clin Perinatol*. 2000; 27:577–601. [PubMed: 10986630]
2. Christensen RD, Macfarlane JL, Taylor NL, et al. Blood and marrow neutrophils during experimental group B streptococcal infection: quantification of the stem cell, proliferative, storage and circulating pools. *Pediatr Res*. 1982; 16:549–553. [PubMed: 7050869]
3. Christensen RD. Neutrophil kinetics in the fetus and neonate. *Am J Pediatr Hematol/Oncol*. 1989; 11:215–223.

4. Vindeløv LL, Christensen IJ. A review of techniques and results obtained in one laboratory by an integrated system of methods designed for routine flow cytometric DNA analysis. *Cytometry*. 1990; 11:753–770. [PubMed: 2272241]
5. Christensen RD. Hematopoiesis in the fetus and neonate. *Pediatr Res*. 1989; 26:531–535. [PubMed: 2689984]
6. Gilmour JR. Erythroblastosis fetalis. *Arch Dis Child*. 1944; 19:1.
7. Calhoun DA, Christensen RD. A randomized pilot trial of administration of granulocyte colony-stimulating factor to women before preterm delivery. *Am J Obstet Gynecol*. 1998; 179:766–771. [PubMed: 9757987]
8. Traycoff CM, Abboud MR, Laver J, Clapp DW, Srouf EF. Rapid exit from G0/G1 phases of cell cycle in response to stem cell factor confers on umbilical blood CD34+ cells an enhanced ex vivo expansion potential. *Exp Hematol*. 1994; 22:1264–1272. [PubMed: 7525328]
9. Leitner A, Strobl H, Fischmeister G, Kurz M, Romanakis K, Haas OA, et al. Lack of DNA synthesis among CD34+ cells in cord blood and in cytokine-mobilized blood. *Br J Haematol*. 1996; 92:255–262. [PubMed: 8602983]
10. Lucotti C, Malabarba L, Rosti V, Bergamaschi G, Danova M, Invernizzi R, et al. Cell cycle distribution of cord blood-derived haematopoietic progenitor cells and their recruitment into the S-phase of the cell cycle. *Br J Haematol*. 2000; 108:621–628. [PubMed: 10759722]
11. Van Den Heuvel R, Schoeters G, Leppens H, Vanderborght O. Stromal cells in long-term cultures of liver, spleen, and bone marrow at different developmental ages have different capacities to maintain GM-CFC proliferation. *Exp Hematol*. 1991; 19:115–121. [PubMed: 1991493]
12. Kale VP, Limaye LS. Stimulation of adult human bone marrow by factors secreted by fetal liver hematopoietic cells: in vitro evaluation using semisolid clonal assay system. *Stem Cells*. 1999; 17:107–116. [PubMed: 10195571]
13. Roy V, Verfaillie CM. Soluble factors produced by adult bone marrow stroma inhibit in vitro proliferation and differentiation of fetal liver BFU-E by inducing apoptosis. *J Clin Invest*. 1997; 100:912–920. [PubMed: 9259591]
14. Moore MAS, Williams N. Analysis of proliferation and differentiation of foetal granulocyte-macrophage progenitor cells in haemopoietic tissue. *Cell Tissue Kinet*. 1973; 6:461–476. [PubMed: 4201113]

Fig. 1.

Cell cycle status of CD34+ cells from adult bone marrow (BM), fetal BM, and umbilical cord blood (CB). Cells were stained with propidium iodide and analyzed by flow cytometry for determination of cell cycle status. Data are shown as percentages of the analyzed cell population; $\bar{X} \pm \text{SD}$. * $P < 0.01$, fetal BM vs. adult BM, CB.

Fig. 2. DNA fluorescence of CD34+ cells. Shown are representative histograms of DNA fluorescence in CD34+ cells stained with propidium iodide. To enhance detail, the Y-axis is truncated at 100 cells. Samples are from adult BM (A), fetal BM (B), and umbilical CB (C).