Surface fucosylation of human cord blood cells augments binding to P-selectin and E-selectin and enhances engraftment in bone marrow

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Murine hematopoietic stem and progenitor cells (HSPCs) home to bone marrow in part by rolling on P-selectin and E-selectin expressed on endothelial cells. Human adult CD34+ cells, which are enriched in HSPCs, roll on endothelial selectins in bone marrow vessels of nonobese diabetic/severe combined immune deficiency (NOD/SCID) mice. Many human umbilical cord blood (CB) CD34+ cells do not roll in these vessels, in part because of an uncharacterized defect in binding to P-selectin. Selectin ligands must be α1-3 fucosylated to form glycan determinants such as sialyl Lewis x (sLex). We found that inadequate α1-3 fucosylation of CB CD34+ cells, particularly CD34+CD38low cells that are highly enriched in HSPCs, caused them to bind poorly to E-selectin as well as to P-selectin. Treatment of CB CD34+ cells with guanosine diphosphate (GDP) fucose and exogenous α1-3 fucosyltransferase VI increased cell-surface sLe^a determinants, augmented binding to fluid-phase P- and E-selectin, and improved cell rolling on P- and E-selectin under flow. Similar treatment of CB mononuclear cells enhanced engraftment of human hematopoietic cells in bone marrows of irradiated NOD/SCID mice. These observations suggest that α1-3 fucosylation of CB cells might be a simple and effective method to improve hematopoietic cell homing to and engraftment in bone marrows of patients receiving CB transplants. (Blood. 2004;104:3091-3096)

Introduction

When expressed on activated endothelial cells, P-selectin and E-selectin cooperatively mediate leukocyte rolling, the first step in leukocyte recruitment into sites of inflammation.1-3 P- and E-selectin are membrane-bound C-type lectins that bind to cell-surface glycoconjugate ligands. Physiologically relevant selectin ligands must be α1-3 fucosylated to form terminal glycan determinants such as sialyl Lewis x (sLe^a) (NeuAα2-3Galβ1-4[Fucα1-3]GkNAcβ1-R). P-selectin glycoprotein ligand-1 (PSGL-1), a mucin expressed on leukocytes, is the best-characterized selectin ligand.1,3 In vivo, PSGL-1 mediates leukocyte tethering to and rolling on P-selectin and supports tethering to E-selectin in flow.4,5 P-selectin binds to a small N-terminal region of PSGL-1 that must be modified with tyrosine sulfates and a core 2 O-glycan capped with sLe^a. E-selectin binds to one or more different sites on PSGL-1 and to other α2-3-sialylated and α1-3-fucosylated cell-surface glycoconjugates.2,3

P- and E-selectin also contribute to homing of hematopoietic stem and progenitor cells (HSPCs) to murine bone marrow, where both selectins are constitutively expressed on endothelial cells.4,6 HSPC rolling in bone marrow vessels and HSPC homing to bone marrow require cooperative interactions with P-selectin, E-selectin, and vascular cell adhesion molecule-1 (VCAM-1) on endothelial cells.7,9 In mice, E-selectin appears to play a more dominant role than P-selectin in mediating HSPC homing.8 Like leukocytes, murine HSPCs use PSGL-1 as the major ligand for P-selectin.8 HSPCs use PSGL-1 to interact with E-selectin during homing, but other less well-characterized E-selectin ligands also participate.9

Hematopoietic cell transplantation is used to treat a variety of malignancies and genetic disorders.10 The procedure requires intravenous infusion of hematopoietic cells from healthy donors into recipients conditioned by irradiation and/or chemotherapy. The infused hematopoietic cells then home to and engraft in bone marrow. Adult bone marrow or mobilized peripheral blood has been the principal source of donor hematopoietic cells, but the shortage of HLA-matched donors has prevented wider application of these treatment options. In recent years, cord blood (CB) has been increasingly used as an alternative donor source because of its greater availability and its lower risk of acute and chronic graft-versus-host disease.11-13 Furthermore, CB is enriched in primitive hematopoietic stem cells. However, hematopoietic cell engraftment following infusion of CB cells is significantly delayed compared with engraftment following infusion of hematopoietic cells from adult bone marrow or mobilized peripheral blood.11-13 This defect might result from impaired homing of CB cells to bone marrow. Consistent with this hypothesis, human CB CD34+ cells do not home as well to bone marrow of irradiated nonobese diabetic/severe combined immune deficiency (NOD/SCID) mice as do CD34+ cells from human adult bone marrow or mobilized peripheral blood.14 Selectins contribute to homing of human adult CD34+ cells, as demonstrated by their impaired homing to bone
marrow of NOD/SCID mice that lack P- and E-selectin and by diminished rolling in bone marrow microvessels of mice after injection of blocking monoclonal antibodies (mAbs) to P-selectin or PSGL-1.14 A subset (~30%) of CB CD34+ cells does not bind to P-selectin, and many of these are CD34+CD38−flow cells that constitute an enriched population of multipotent hematopoietic stem cells.14 These data imply that diminished homing of CB cells might result in part from defective interactions of CD34+ cells with P-selectin in bone marrow vessels. CD34+ CB cells express the PSGL-1 polypeptide, suggesting that defective posttranslational modification of PSGL-1 might account for defective binding to P-selectin.14 However, the putative defect in posttranslational modification has not been identified. It is also not known whether CB CD34+ cells have defective binding to E-selectin, which might further impair homing to bone marrow.

Compared with bone marrow or mobilized peripheral blood, CB cells have a higher potential to reconstitute recipient bone marrow because they contain 3 to 6 times more repopulating cells, a small subset of cells critical for long-term engraftment.15 Despite this advantage, the small number of cells obtained from each umbilical cord limits the utility of CB as a source of hematopoietic cells for transplantation. Correction of the homing defect in CB cells might overcome this limitation. In this study, we demonstrate that inadequate α-1 fucosylation of CB CD34+ cells caused defective binding to E-selectin as well as P-selectin. Transient fucosylation of CB cells with guanosine diphosphate (GDP) fucose and exogenous α-1 fucosyltransferase increased binding to both P- and E-selectin, particularly to E-selectin, and enhanced engraftment in bone marrows of irradiated NOD/SCID mice.

To measure αLe4 determinants, CB mononuclear cells were incubated with phycoerythrin (PE)-conjugated anti-human CD34 mAb or control murine IgG (IgG1; BD Biosciences) and with rat anti-αLe4 mAb HECA-452 (IgM; hybridoma from American Type Culture Collection, Manassas, VA)17 or control rat IgM (BD Biosciences). Bound HECA-452 was detected with fluorescein isothiocyanate (FITC)–labeled goat anti–rat IgM (EMD Biosciences).

For the P-selectin–binding assay, CB mononuclear cells were incubated with human P-selectin/human IgG chimera (P-selectin/IgG), which was purified from conditioned medium of stably transfected human 293 cells (a generous gift from Dr Ajit Var Ki, University of California at San Diego). The bound P-selectin was then detected by biotinylated protein A and streptavidin–FITC (BD Biosciences).14 Incubations were performed for 20 minutes at 4°C for each step. In some experiments, P-selectin isolated from human platelets was used.15 P-selectin binding was then detected with FITC-labeled S12, a nonbinding mAb to human P-selectin.19 A saturating amount of P-selectin, determined by measuring binding over a range of P-selectin concentrations, was used in all experiments. In control experiments, binding was measured in the presence of G1, a blocking mAb to P-selectin20; PL1, a blocking mAb to PSGL-1;12 or 10 mM EDTA (ethylene-diaminetetraacetic acid), which eliminates Ca2+-dependent selectin-ligand interactions. For E-selectin binding, murine soluble E-selectin/human IgM chimera (E-selectin/IgM) was used.3 CD45 human IgM chimera (CD45/IgM) was used as negative control. The chimeras were obtained from conditioned medium of COS-7 cells that were transfected, respectively, with cPDM8 vectors encoding each molecule (generous gifts from Dr John B. Lowe, University of Michigan Medical Center, Ann Arbor). E-selectin binding was detected with FITC-labeled goat anti-human IgM (Chemicon International, Temecula, CA). Incubations were performed at 4°C for 30 minutes. A saturating amount of E-selectin, determined by serial titration, was used in each experiment. In control experiments, binding was conducted in the presence of 9.9, a blocking mAb to murine E-selectin22 (a generous gift from Dr Barry Wolditzky, Roche Research Center, Hoffmann-La Roche, Basel, Switzerland), or 10 mM EDTA.

For triple-colored staining, CB mononuclear cells were incubated with PE-cyanine 5 (PE-Cy5)-conjugated anti-CD34 mAb with one of the following combinations: anti-αLe4 mAb HECA-452 followed by either PE-conjugated goat anti–rat IgM; PE-conjugated anti-CD38 mAb, plus P-selectin/IgG followed by biotinylated protein A and streptavidin–FITC; or E-selectin/IgM followed by FITC-conjugated goat anti–human IgM. Isotype-matched murine IgG, rat IgM, or CD45/IgM was used as control. Except for HECA-452, all antibodies were from BD Biosciences.

All flow cytometric analyses were carried out on a FACSCalibur (BD Biosciences). Data were collected and analyzed using the CellQuest program (BD Biosciences).

Cell adhesion under flow

Rolling of FTVI-treated and sham-treated CB CD34+ cells was measured by previously described methods.21 Briefly, P-selectin isolated from human platelets18 or recombinant human soluble E-selectin was immobilized in a parallel-plate flow chamber. Recombinant soluble E-selectin was isolated from conditioned medium of stably transfected Chinese hamster ovary cells that expressed the entire extracellular domain of human E-selectin. The site densities were 145 sites/μm2 for P-selectin or 200 sites/μm2 for E-selectin, as measured by binding of [125I]-labeled anti-P-selectin mAb G1 or anti–human E-selectin mAb ES1.24 Sham-treated or FTVI-treated CB CD34+ cells (106/mL in HBSS containing 0.5% HSA) were perfused over P-selectin– or E-selectin–coated surfaces at 0.5 dynes/cm2. For control experiments, cells were continuously maintained in buffer containing 10 mM EDTA or a blocking mAb to human P-selectin, E-selectin, or PSGL-1. The accumulated number of rolling cells was measured with a videomicroscopy system coupled to an image analysis system.

Bone marrow engraftment

Male and female pathogen-free NOD/SCID mice (The Jackson Laboratory, Bar Harbor, ME), 4 to 5 weeks of age, were used as recipients.22 The mice were irradiated with 230 cGy 137Cs γ-ray 3 hours before intravenous injections of FTVI-treated or sham-treated CB mononuclear cells (8 × 107 mouse in 300 μL saline). Each mouse in a control group received 300 μL
saline without CB mononuclear cells. Six weeks after transplantation, the mice were bled and killed. Bone marrow cells were isolated from both femora and filtered through a 100-μm mesh cell strainer to remove debris. After lysis of erythrocytes, the bone marrow nucleated cells from each mouse were resuspended in HBSS at a concentration of 1 x 10^9/mL. Engraftment of human cells was analyzed by human hematopoietic progenitor assays and by flow cytometry.

For assays of human clonogenic hematopoietic progenitor cells, 1 x 10^5 bone marrow cells were plated in duplicate on 35-mm culture dishes in MethoCult H4433 medium (Stem Cell Technologies, Vancouver, BC, Canada) and incubated at 37°C, 5% CO2 in a humidified chamber. This medium only supports the growth of human hematopoietic cells. Total colonies, erythroid burst-forming units (BFU-Es), granulocyte-macrophage colony-forming units (CFU-GMs), and granulocyte-erythroid-megakaryocyte-macrophage colony-forming units (CFU-GEMMs) were enumerated on day 14 of culture and analyzed. The human origin of the colonies was confirmed by flow cytometry of cells collected from different colonies that were stained with FITC-conjugated mAb to human CD45 (BD Biosciences) to identify leukocytes or with FITC-conjugated mAb to glycophorin A (BD Biosciences) to identify erythroid cells.

For flow cytometry, equal numbers of nucleated cells from peripheral blood or bone marrow were incubated with FITC-conjugated anti-human CD45 mAb and analyzed for the percentages of human CB-derived leukocytes.

**Results**

**CB CD34+ cells that lack sLex epitopes bind poorly to both P-selectin and E-selectin**

We asked whether CB CD34+ cells might have impaired binding to E-selectin as well as P-selectin and whether defective selectin binding might correlate with inadequate α-1,3 fucosylation of cell-surface ligands. Flow cytometric analysis demonstrated that approximately 25% of CB CD34+ cells did not express sLex as detected by binding of anti-sLex mAb HECA-452 (Figure 1A). Triple-colored staining showed that 88% ± 2% (n = 3) of CB CD34+ cells without sLex did not bind to P-selectin, whereas only 35% ± 11% of CB CD34+ cells with sLex failed to bind to P-selectin (Figure 1B). Significantly, 67% ± 9% of CB CD34+ cells without sLex also did not bind to E-selectin, whereas only 10% ± 5% of CB CD34+ cells with sLex failed to bind to E-selectin (Figure 1C). Consistent with published data,14 approximately 30% of CB CD34+ cells were CD34+CD38-low cells, which are enriched in HSPCs (Figure 1D). Triple-colored staining revealed that 76% ± 12% of CB CD34+CD38-low cells did not bind to P-selectin (Figure 1E), and that 74% ± 13% of CB CD34+CD38-low cells did not bind to E-selectin (Figure 1F). Furthermore, 63% ± 9% of CB CD34+CD38-low cells did not express sLex (data not shown). These data demonstrate that most CB CD34+CD38-low cells lack sLex epitopes and fail to bind to E-selectin as well as to P-selectin. Thus, low sLex expression strongly correlates with defective selectin binding, suggesting a causal relationship.

**Figure 1. CB CD34+ cells that lack sLex epitopes have impaired binding to P- and E-selectin and are enriched in CD34+CD38-low cells.** (A-C) Cells were incubated with PE-Cy5-conjugated anti-CD34, anti-sLex mAb HECA-452 identified with a PE-conjugated secondary antibody, and P-selectin/CD29 identified with biotinylated protein A and streptavidin-FITC or E-selectin/CD62L identified with a FITC-conjugated goat anti-IgM. Binding was measured by flow cytometry. (D-F) Cells were incubated with PE-Cy5-conjugated anti-CD34, PE-conjugated anti-CD38, and P-selectin or E-selectin identified as described in “Materials and methods”, 13% of CB CD34+ cells were gated as R1 region. (R1 region, red) and CD34+CD38-low cells did not bind to P-selectin (Figure 1E), and that 74% ± 13% of CB CD34+CD38-low cells did not bind to E-selectin (Figure 1F). Furthermore, 63% ± 9% of CB CD34+CD38-low cells did not express sLex (data not shown). These data demonstrate that most CB CD34+CD38-low cells lack sLex epitopes and fail to bind to E-selectin as well as to P-selectin. Thus, low sLex expression strongly correlates with defective selectin binding, suggesting a causal relationship.

**Figure 2. Surface α-1,3 fucosylation of CB CD34+ cells with exogenous FTVI and GDP fucose increases sLex epitopes.** CB CD34+ cells were incubated in the presence or absence of FTVI and GDP fucose. The cells were then incubated with PE-conjugated anti-CD34 mAb and with anti-sLex mAb HECA-452 identified with a FITC-conjugated secondary antibody. Binding was measured by flow cytometry. Cells incubated with isotype-matched control antibodies were used to determine fluorescence thresholds for specific binding. The results are representative of 5 independent experiments. The percentage of cells in each quadrant is indicated.

**Transient fucosylation of CB CD34+ cell surfaces augments binding to fluid-phase P-selectin and E-selectin and enhances rolling on P-selectin and E-selectin**

We previously developed a technique to add sLex epitopes to cell surfaces by treating intact cells with GDP fucose and an exogenous α-1,3 fucosyltransferase, FTVI.16 This short-term treatment transiently increases cell-surface fucosylated glycans, which then decline as glycoproteins and glycolipids turn over and as cells divide. Using the same method, we tested whether forced fucosylation would increase functional selectin ligands on CB CD34+ cells. Treatment with both FTVI and GDP fucose created many new sLex epitopes on CB CD34+ cells, as measured by binding of HECA-452 (Figure 2).

Compared with sham-treated CB CD34+ cells, more FTVI-treated CB CD34+ cells bound to P- and E-selectin, and they expressed more binding sites, especially for E-selectin (Figure 3). Binding of P-selectin and E-selectin was specific because it was eliminated by addition of a blocking mAb to the respective selectin (Figure 3) or by EDTA (data not shown). Incubation with control CD45/IgM yielded the same staining profile as with cells incubated with E-selectin/IgM in the presence of EDTA or anti–E-selectin mAb, further demonstrating specificity (data not shown). These results demonstrate that forced fucosylation of CB CD34+ cells increases binding to P-selectin and markedly increases binding to E-selectin.

To determine whether forced fucosylation of CB CD34+ cells enhanced interactions with selectins in a more physiologically relevant setting, we compared rolling of FTVI-treated and sham-treated cells on immobilized human P- or E-selectin under flow conditions. FTVI treatment increased the number of cells rolling on P-selectin by 1.9-fold (Figure 4A) and on E-selectin by 2.9-fold.
Surface fucosylation of CB mononuclear cells enhances engraftment in bone marrow of NOD/SCID mice

To examine whether transient surface fucosylation of CB cells improves engraftment in bone marrow, sublethally irradiated NOD/SCID mice received transplants of control saline or of FTVI-treated or sham-treated CB mononuclear cells. After 6 weeks, the mice were killed, and the number of human-derived hematopoietic cells in bone marrows and peripheral blood was analyzed. The bone marrows of mice that received FTVI-treated CB mononuclear cells had significantly improved engraftment of human hematopoietic progenitors of all lineages, as demonstrated by increases in BFU-Es, CFU-GMs, and CFU-GEMMs in colony-forming assays (Figure 5A). No CD45<sup>+</sup> cells were detected in marrow or blood of mice that received only control saline, documenting the specificity of the mAb for human CD45 (Figure 5B).

Discussion

We have demonstrated that insufficient α1-3 fucosylation of selectin ligands on CB CD34<sup>+</sup> cells causes defective binding to both P-selectin and E-selectin. Transient α1-3 fucosylation of CB cells increases selectin binding and augments engraftment of CB cells in bone marrows of irradiated NOD/SCID mice. These findings suggest that in vitro fucosylation of CB cells might provide a simple method to improve hematopoietic cell homing to and engraftment in bone marrows of patients receiving CB transplants.

We observed a strong correlation between impaired selectin binding and low or absent expression of sLex epitopes on CB CD34<sup>+</sup> cells. This was particularly true for CD34<sup>+</sup> CD8<sup>+</sup><sub>low</sub> cells, which are significantly enriched in multipotent stem cells. Most of these cells lacked sLex determinants, and approximately 75% of them failed to bind to P-selectin and E-selectin. Forced fucosylation of CD34<sup>+</sup> cells with exogenous GDP fucose and FTVI increased expression of sLex<sup>+</sup> and selectin ligands in parallel. The enzymes FTVI and FTVII are responsible for α1-3 fucosyltransferase activity in hematopoietic cells of mice and humans.26-31 CB CD34<sup>+</sup> cells may not express sufficient levels of these enzymes to fucosylate physiologically relevant selectin ligands or to express measurable levels of sLex<sup>+</sup> epitopes on the cell surface.

Surface fucosylation of CB mononuclear cells enhances engraftment in bone marrow of NOD/SCID mice

(Figure 4B). The FTVI-treated cells also rolled more stably, as manifested by more regular rolling motions. There was a trend toward slower mean rolling velocities on P-selectin and a significant reduction in mean rolling velocities on E-selectin (data not shown). Rolling was specific because a mAb to P-selectin (G1) or to E-selectin (ES1) reduced the number of rolling cells to the basal levels observed on surfaces coated only with HSA (Figure 4A-B). PL1, a mAb to the N-terminal P-selectin–binding region of PSGL-1, blocked rolling on P-selectin (Figure 4A) but did not impair rolling on E-selectin (Figure 4B). The latter observation is consistent with the ability of leukocytes to use ligands other than PSGL-1 to roll on E-selectin.2,3 These data demonstrate that forced fucosylation of CB CD34<sup>+</sup> cells enhances rolling on both P-selectin and E-selectin under flow. The particular improvement in cell rolling on E-selectin is consistent with the marked increase in E-selectin ligands after FTVI treatment (Figure 3B).
E-selectin (L.X., J.M.M., and R.P.M., unpublished observation, February 2004). This suggests that surface fucosylation created additional binding sites for E-selectin on other regions of PSGL-1 or on other glycoproteins or glycolipids. Indeed, surface fucosylation created many more new binding sites for E-selectin than for P-selectin as measured by flow cytometry. Forced fucosylation increased physiologically relevant rolling of CB CD34+ cells on both P-selectin and E-selectin under flow. Rolling was particularly improved on E-selectin, consistent with the large increase in E-selectin ligands. PL1 did not inhibit rolling on E-selectin, further implying that fucosylation created binding sites for E-selectin on glycoproteins other than PSGL-1. Although we did not determine whether multipotent stem cells actually rolled on selectins, fucosylation enabled virtually all CD34+ cells, including the CD34+CD38−low cells, to bind to fluid-phase P-selectin and E-selectin. This suggests that fucosylation also improved the ability of CB HSPCs to roll on P-selectin and E-selectin.

Like recruitment of mature leukocytes to sites of inflammation, homing of HSPCs to bone marrow requires the combinatorial engagements of selectins, integrins, and chemokines with their respective receptors.5,6,9,33,34 These collective interactions enable HSPCs to roll and arrest on vessel surfaces and then emigrate into the bone marrow. Forced fucosylation of CB HSPCs might improve rolling on selectins but not increase entry into the bone marrow if the cells also lack integrins, chemokine receptors, or other molecules essential for homing. In a recent preliminary description, a subset of CB CD34+ cells was independently found to express few or no sLeα determinants, in agreement with our findings.35 These workers demonstrated that forced fucosylation of CB CD34+ cells augmented rolling in bone marrow vessels of NOD/SCID mice, in agreement with our observations of enhanced rolling of treated cells on P-selectin and E-selectin in vitro. Furthermore, more treated cells transitioned from rolling adhesion to firm adhesion on bone marrow vessels, an important prerequisite for transendothelial emigration.35 Importantly, we observed that fucosylation of CB mononuclear cells significantly increased engraftment of human cells in bone marrows of irradiated NOD/SCID mice 6 weeks after cell injection, demonstrating that human hematopoietic cells could home to and function in the bone marrow microenvironment. The primitive human cells that proliferate and differentiate into multiple hematopoietic lineages in murine bone marrow are called SCID-repopulating cells (SRCs).15 SRCs are concentrated among CB CD34+ cells, especially the CD34+CD38− subpopulation, which supports the possibility that in vitro fucosylation of CB mononuclear cells enhances engraftment by improving selectin-dependent homing of CD34+ SRCs to bone marrow. However, human CD34+ cells have some SRCs as well as accessory cells that support survival and expansion of primitive hematopoietic cells.36-38 In addition, some human lineage-committed progenitors might contribute to hematopoietic recovery 6 weeks after transplantation, the end point studied in our experiments. Thus, further studies are required to determine the specific mechanisms by which in vitro fucosylation of unfractionated CB mononuclear cells improves engraftment.

In vitro, adhesion of human adult CD34+ cells to high densities of P-selectin or E-selectin induces growth inhibition and apoptosis.39,40 Although the physiologic relevance of these observations is unclear, our data suggest that surface fucosylation of CB cells did not adversely affect their ability to repopulate the bone marrow space after homing. In any event, short-term biochemical treatment with exogenous GDP fucose and FTVI only transiently increases fucosylated glycans on CB cells, which decline as glycoproteins and glycolipids turn over and as cells divide. Because the increased fucosylation is transient, it is less likely to affect the long-term functions of hematopoietic stem cells and accessory cells after they enter the bone marrows of conditioned recipients.

It is unclear whether P-selectin and E-selectin make equivalent contributions to HSPC homing to bone marrow of mice and humans. Murine hematopoietic cell lines roll cooperatively on both P- and E-selectin in murine bone marrow vessels.7 However, E-selectin appears to be used more than P-selectin for the actual bone marrow homing of murine adult hematopoietic cells.8 Adult human CB CD34+ cells apparently roll on both P- and E-selectin in bone marrow vessels of irradiated NOD/SCID mice, although the relative contributions of each selectin were not specifically tested.14 Defective P-selectin interactions account for most of the diminished rolling of CB CD34+ cells in NOD/SCID bone marrow vessels.14 Although the specific contributions of P- and E-selectin to homing of CB CD34+ cells to NOD/SCID bone marrow have not been examined, forced fucosylation of these cells might increase homing through more effective rolling interactions with P-selectin and especially with E-selectin in bone marrow vessels, as we observed with immobilized human P- and E-selectin in vitro. Human bone marrow endothelial cells constitutively express E-selectin and, like mice, they may constitutively express P-selectin.41 Thus, surface fucosylation of CB cells might also augment homing to and engraftment in human bone marrow.

CB is now a standard source of cells for pediatric hematopoietic cell transplantation.11,42 Unfortunately, the limited cell number per CB unit and the delayed engraftment after CB cell transplantation preclude wide use of CB as a donor source for adult recipients. Methods to increase cell dose by pooling multiple CB units and by ex vivo cell expansion are being pursued.11 However, multiple-unit transplantsations may complicate matching of HLA antigens, and ex vivo cell expansion may risk altering the intrinsic properties of the HSPCs. In vitro fucosylation of CB cells might provide a simple and effective means to enhance hematopoietic cell homing to and engraftment in bone marrows of both pediatric and adult patients. Furthermore, it might significantly reduce the number of CB cells that must be administered, potentially expanding the utility of CB transplantations for adult recipients.
Acknowledgments

We thank Paul Kincade and Takafumi Yokota for insightful suggestions and advice on hematopoiesis and bone marrow transplantation protocols. We are grateful to Sandy Kinman, Bonnie Stephens, Shelhy McMullin, Shelia Scott, and other staff at Renaissance Women’s Hospital of Edmond for their generous assistance in cord blood collection. We thank Juliet Butler for producing recombinant soluble human E-selectin. We also thank Samuel McGee for technical assistance.

References


13. Wang JC, Doedens M, Dick JE. Primitive human hematopoietic cells are enriched in cord blood compared with adult bone marrow or mobilized peripheral blood as measured by the quantitative in vivo SCID-repopulating cell assay. Blood. 1997;89:3919-3924.


