

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

Lijun Xia, J. Michael McDaniel, Tadayuki Yago, Andrea Doeden, and Rodger P. McEver

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 α 1-3 fucosylated to form glycan determinants such as sialyl Lewis x (sLe^x). We found that inadequate α 1-3 fucosylation of CB CD34⁺ cells, particularly CD34⁺CD38^{-low} 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 α 1-3 fucosyltransferase VI increased cell-surface sLe^x 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 α 1-3 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)

© 2004 by The American Society of Hematology

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 α 1-3 fucosylated to form terminal glycan determinants such as sialyl Lewis x (sLe^x) (NeuAc α 2-3Gal β 1-4[Fuc α 1-3]GlcNAc β 1-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^x. E-selectin binds to one or more different sites on PSGL-1 and to other α 2-3-sialylated and α 1-3-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.⁶⁻⁸ 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.⁸

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

From the Cardiovascular Biology Research Program, Oklahoma Medical Research Foundation, Oklahoma City, OK; Renaissance Women's Hospital of Edmond, Edmond, OK; and the Department of Biochemistry and Molecular Biology and Oklahoma Center for Medical Glycobiology, University of Oklahoma Health Sciences Center, Oklahoma City, OK.

Submitted February 20, 2004; accepted June 10, 2004. Prepublished online as *Blood* First Edition Paper, July 27, 2004; DOI 10.1182/blood-2004-02-0650.

Supported by National Institutes of Health grants RR 018758 (R.P.M. and L.X.) and HL 54502 (R.P.M.), a National Science Foundation grant EPSCoR

0132534 (L.X.), and a Scientist Development Grant from the American Heart Association (L.X.).

An Inside *Blood* analysis of this article appears in the front of this issue.

Reprints: Lijun Xia, Cardiovascular Biology Research Program, Oklahoma Medical Research Foundation, 825 NE 13th Street, Oklahoma City, OK 73104; e-mail: lijun-xia@omrf.ouhsc.edu.

The publication costs of this article were defrayed in part by page charge payment. Therefore, and solely to indicate this fact, this article is hereby marked "advertisement" in accordance with 18 U.S.C. section 1734.

© 2004 by The American Society of Hematology

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^{-low} 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 1-3 fucosylation of CB CD34⁺ cells caused defective binding to E-selectin as well as to P-selectin. Transient fucosylation of CB cells with guanosine diphosphate (GDP) fucose and exogenous α 1-3 fucosyltransferase increased binding to both P- and E-selectin, particularly to E-selectin, and enhanced engraftment in bone marrow of irradiated NOD/SCID mice.

Materials and methods

CB cells

CB was obtained from umbilical cords from healthy full-term newborns. Informed consent was obtained according to a protocol approved by the Institutional Review Board of the Oklahoma Medical Research Foundation. Sodium citrate or heparin was used as anticoagulant. CB was first mixed with an equal volume of 6% dextran 70 in 0.9% sodium chloride (McGraw, Irvine, CA). After sedimentation, nucleated cells were collected and washed once in Hanks balanced salt solution (HBSS; Mediatech, Herndon, VA). Mononuclear cells were isolated after centrifugation at 400g over Ficoll-Hypaque (density [d] = 1.077 g/mL; Mediatech). For some experiments, CD34⁺ cells were purified from the mononuclear cell fraction using the CD34-isolation mini-MACS kit following the manufacturer's instructions (Miltenyi Biotec, Bergisch Gladbach, Germany). The purity of the isolated CD34⁺ cells was approximately 96% as examined by flow cytometry.

Surface fucosylation

To introduce α 1-3-linked fucose to cell-surface glycans, 10⁷ CB mononuclear cells were treated with 1 mM GDP fucose (EMD Biosciences, San Diego, CA), 20 mU/mL α 1-3 fucosyltransferase VI (FTVI; EMD Biosciences), and 10 mM MnCl₂ in 0.5 mL HBSS containing 1% human serum albumin (HSA) for 30 minutes at 37°C in a humidified atmosphere containing 5% CO₂.¹⁶ These conditions resulted in minimum toxicity to CD34⁺ cells as tested by propidium iodide staining measured by flow cytometry (data not shown). Sham-treated cells were treated identically except that GDP fucose was not added.

Flow cytometry

For all flow cytometry analyses, Fc receptors on CB cells were first blocked with human immunoglobulin G (IgG; BD Biosciences, San Diego, CA).

To measure sLe^x 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-sLe^x 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 Varki, 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.¹⁸ P-selectin binding was then detected with FITC-labeled S12, a nonblocking 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-1²¹; or 10 mM EDTA (ethylenediaminetetraacetic acid), which eliminates Ca²⁺-dependent selectin-ligand interactions. For E-selectin binding, murine soluble E-selectin/human IgM chimera (E-selectin/IgM) was used.⁵ 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 pCDM8 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 9A9, a blocking mAb to murine E-selectin²² (a generous gift from Dr Barry Wolitzky, 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-sLe^x 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.²³ 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/ μ m² for P-selectin or 200 sites/ μ m² 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 dyne/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.²⁵ 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 \times 10⁶/mouse in 300 μ L saline). Each mouse in a control group received 300 μ L

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- μ m 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 1×10^6 /mL. Engraftment of human cells was analyzed by human hematopoietic progenitor assays and by flow cytometry.

For assays of human clonogenic hematopoietic progenitor cells, 1×10^5 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 sLe^x 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 sLe^x as detected by binding of anti-sLe^x mAb HECA-452 (Figure 1A). Triple-colored staining showed that 88% \pm 2% (n = 3) of CB CD34⁺ cells without sLe^x did not bind to

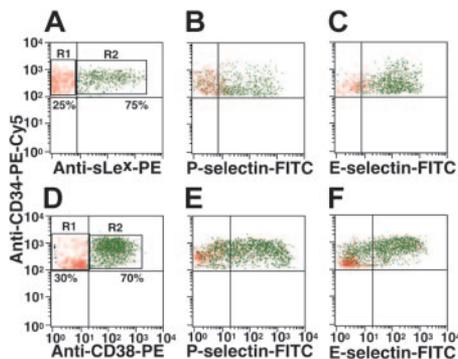


Figure 1. CB CD34⁺ cells that lack sLe^x epitopes have impaired binding to P- and E-selectin and are enriched in CD34⁺CD38^{-low} cells. (A-C) Cells were incubated with PE-Cy5-conjugated anti-CD34, anti-sLe^x mAb HECA-452 identified with a PE-conjugated second antibody, and P-selectin/IgG identified with biotinylated protein A and streptavidin-FITC or E-selectin/IgM identified with a FITC-conjugated goat anti-IgM. Binding was measured by flow cytometry. The CB CD34⁺ cells were classified as sLe^x negative (R1 region, red) or sLe^x positive (R2 region, green) as shown in panel A. Cells in the gated R1 and R2 regions were examined for interactions with P-selectin and E-selectin as shown in panels B and C. (D-F) Cells were gated as CD34⁺CD38^{-low} (R1 region, red) and CD34⁺CD38⁺ (R2 region, green) as shown in panel D. Cells from the 2 gated regions were examined for interactions with P-selectin and E-selectin as shown in panels E and F. Cells incubated with isotype-matched control IgG or IgM were used to determine fluorescence thresholds for specific binding. The results are representative of 3 independent experiments.

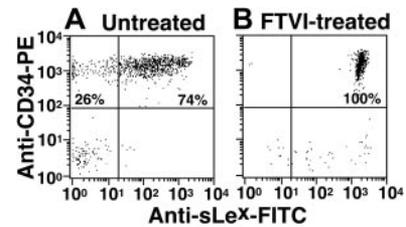


Figure 2. Surface α -1-3 fucosylation of CB CD34⁺ cells with exogenous FTVI and GDP fucose increases sLe^x epitopes. CB CD34⁺ cells were incubated in the presence or absence of FTVI and GDP fucose. The cells were then incubated with PE-conjugated anti-CD34 mAb and with anti-sLe^x mAb HECA-452 identified with a FITC-conjugated second 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.

P-selectin, whereas only 35% \pm 11% of CB CD34⁺ cells with sLe^x failed to bind to P-selectin (Figure 1B). Significantly, 67% \pm 9% of CB CD34⁺ cells without sLe^x also did not bind to E-selectin, whereas only 10% \pm 5% of CB CD34⁺ cells with sLe^x failed to bind to E-selectin (Figure 1C). Consistent with published data,¹⁴ approximately 30% of CD34⁺ cells were CD34⁺CD38^{-low} cells, which are enriched in HSPCs (Figure 1D). Triple-colored staining revealed that 76% \pm 12% of CB CD34⁺CD38^{-low} cells did not bind to P-selectin (Figure 1E), and that 74% \pm 13% of CB CD34⁺CD38^{-low} cells did not bind to E-selectin (Figure 1F). Furthermore, 63% \pm 9% of CB CD34⁺CD38^{-low} cells did not express sLe^x (data not shown). These data demonstrate that most CB CD34⁺CD38^{-low} cells lack sLe^x epitopes and fail to bind to E-selectin as well as to P-selectin. Thus, low sLe^x expression strongly correlates with defective selectin binding, suggesting a causal relationship.

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 sLe^x 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 sLe^x 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

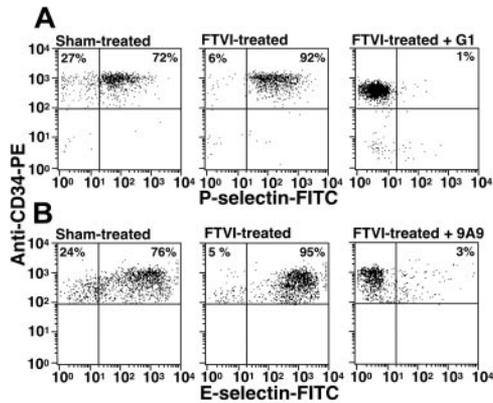


Figure 3. Surface α 1-3 fucosylation of CB CD34⁺ cells increases binding to fluid-phase P-selectin and E-selectin. (A) Sham-treated or FTVI-treated CB mononuclear cells were stained with PE-conjugated anti-CD34 mAb and with P-selectin/IgG detected by biotinylated protein A and streptavidin-FITC in the presence or absence of a blocking mAb to P-selectin (G1). (B) Sham-treated or FTVI-treated CB mononuclear cells were stained with PE-conjugated anti-CD34 mAb and with E-selectin/IgM detected by FITC-conjugated goat anti-human IgM in the presence or absence of a blocking mAb to E-selectin (9A9). Binding was measured by flow cytometry. The percentage of cells in each quadrant is indicated. The results are representative of 3 independent experiments.

(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.^{2,3,5} These data demonstrate that forced fucosylation of CB CD34⁺ 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).

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 marrow 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 colonies formed in bone marrow from irradiated mice that received only control saline, confirming the specificity of the colony-forming assay for human cells (Figure 5A). Of note, the sizes of the colonies were not significantly different in either recipient group (data not shown), indicating that short-term fucosylation did not change the growth rates of the CB hematopoietic cells. Consistent with the increased numbers of engrafted human cells, the mice that received FTVI-treated CB mononuclear cells had significantly more human CD45⁺ cells in bone marrow and peripheral blood than mice that received sham-treated CB mononuclear cells (Figure 5B).

No CD45⁺ 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 α 1-3 fucosylation of selectin ligands on CB CD34⁺ cells causes defective binding to both P-selectin and E-selectin. Transient α 1-3 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 sLe^x determinants on CB CD34⁺ cells. This was particularly true for CD34⁺CD38^{-low} cells, which are significantly enriched in multipotent stem cells. Most of these cells lacked sLe^x determinants, and approximately 75% of them failed to bind to P-selectin and E-selectin. Forced fucosylation of CD34⁺ cells with exogenous GDP fucose and FTVI increased expression of sLe^x and selectin ligands in parallel. The enzymes FTIV and FTVII are responsible for α 1-3 fucosyltransferase activity in hematopoietic cells of mice and humans.²⁶⁻³¹ CB CD34⁺ cells may not express sufficient levels of these enzymes to fucosylate physiologically relevant selectin ligands or to express measurable levels of sLe^x epitopes on the cell surface. There is also a correlation between expression of sLe^x determinants and selectin ligands on mature human leukocytes, particularly on T cells, which alter expression of FTVII to regulate the parallel display of selectin ligands and sLe^x epitopes.^{29,32} Thus, expression of sLe^x determinants is a useful tool to assess the fucosylation of selectin ligands on human hematopoietic cells.

Fucosylation of CB CD34⁺ cells augmented binding to P-selectin in a PSGL-1-dependent manner. This is consistent with previous observations that CB CD34⁺ cells express a form of PSGL-1 that does not bind to P-selectin.¹⁴ The current data suggest that this defective binding is due to inadequate fucosylation of PSGL-1, most likely at an essential core 2 O-glycan linked to a specific threonine in the N-terminal P-selectin-binding site.^{2,3} PL1, a mAb to the P-selectin-binding region of PSGL-1, did not inhibit fucosylation-augmented binding of CB CD34⁺ cells to fluid-phase

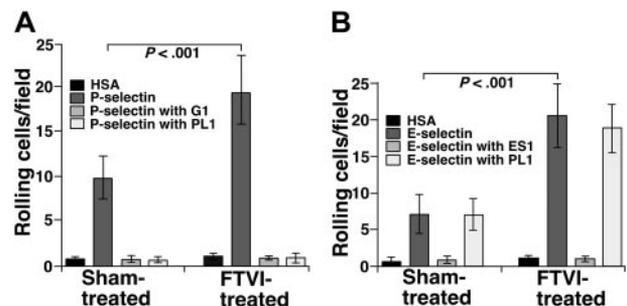
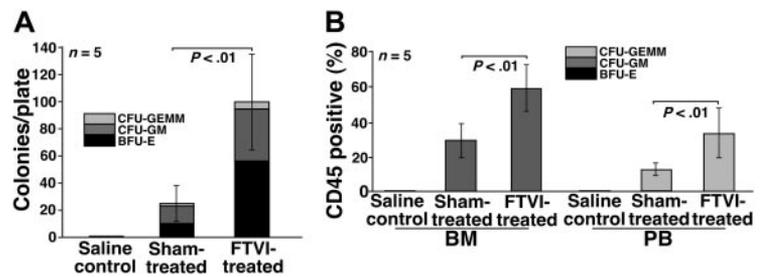


Figure 4. Surface α 1-3 fucosylation of CB CD34⁺ cells augments rolling on P-selectin and E-selectin under flow. (A) Sham-treated or FTVI-treated CB CD34⁺ cells were perfused over immobilized human P-selectin or over a control surface coated only with HSA in the presence or absence of a blocking mAb to P-selectin (G1) or PSGL-1 (PL1). (B) Sham-treated or FTVI-treated CB CD34⁺ cells were perfused over immobilized human E-selectin or over a control surface coated only with HSA in the presence or absence of a blocking mAb to E-selectin (ES1) or PSGL-1 (PL1). The wall shear stress was 0.5 dyne/cm². After 4 minutes, the number of rolling cells was quantified. The data represent the mean \pm SEM of 3 independent experiments.

Figure 5. Surface α 1-3 fucosylation of CB mononuclear cells enhances engraftment in bone marrows of sublethally irradiated NOD/SCID mice. Sham-treated or FTVI-treated CB mononuclear cells were injected intravenously into sublethally irradiated NOD/SCID mice. Control mice were injected only with saline. After 6 weeks, the mice were killed and hematopoietic cells isolated from bone marrow and peripheral blood were analyzed for engraftment of human-derived hematopoietic cells. (A) The number of human clonogenic hematopoietic progenitor cells in bone marrow was determined. (B) Bone marrow (BM) and peripheral blood (PB) cells were incubated with a FITC-conjugated mAb to human CD45 or with a FITC-conjugated isotope-matched control mAb and analyzed by flow cytometry. The percentage of cells that expressed human CD45 is shown. The data represent the mean \pm SD of measurements from 5 mice in each experimental group.



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.^{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 sLe^x 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 the CB cells were injected, 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 transplantations 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 proto-

cols. We are grateful to Sandy Kinman, Bonnie Stephens, Shelly McMullin, Shiela 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

- Vestweber D, Blanks JE. Mechanisms that regulate the function of the selectins and their ligands. *Physiol Rev*. 1999;79:181-213.
- McEver RP. Adhesive interactions of leukocytes, platelets, and the vessel wall during hemostasis and inflammation. *Thromb Haemost*. 2001;86:746-756.
- McEver RP. Selectins: lectins that initiate cell adhesion under flow. *Curr Opin Cell Biol*. 2002;14:581-586.
- Yang J, Hirata T, Croce K, et al. Targeted gene disruption demonstrates that P-selectin glycoprotein ligand 1 (PSGL-1) is required for P-selectin-mediated but not E-selectin-mediated neutrophil rolling and migration. *J Exp Med*. 1999;190:1769-1782.
- Xia L, Sperandio M, Yago T, et al. P-selectin glycoprotein ligand-1-deficient mice have impaired leukocyte tethering to E-selectin under flow. *J Clin Invest*. 2002;109:939-950.
- Frenette PS, Subbarao S, Mazo IB, Von Andrian UH, Wagner DD. Endothelial selectins and vascular cell adhesion molecule-1 promote hematopoietic progenitor homing to bone marrow. *Proc Natl Acad Sci U S A*. 1998;95:14423-14428.
- Mazo IB, Gutierrez-Ramos JC, Frenette PS, Hynes RO, Wagner DD, von Andrian UH. Hematopoietic progenitor cell rolling in bone marrow microvessels: parallel contributions by endothelial selectins and vascular cell adhesion molecule 1. *J Exp Med*. 1998;188:465-474.
- Katayama Y, Hidalgo A, Furie BC, Vestweber D, Furie B, Frenette PS. PSGL-1 participates in E-selectin-mediated progenitor homing to bone marrow: evidence for cooperation between E-selectin ligands and $\alpha 4$ integrin. *Blood*. 2003;102:2060-2067.
- Papayannopoulou T, Priestley GV, Nakamoto B, Zafiroopoulos V, Scott LM. Molecular pathways in bone marrow homing: dominant role of $\alpha 4\beta 1$ over $\beta 2$ -integrins and selectins. *Blood*. 2001;98:2403-2411.
- Thomas ED. History, current results, and research in marrow transplantation. *Perspect Biol Med*. 1995;38:230-237.
- Barker JN, Wagner JE. Umbilical cord blood transplantation: current practice and future innovations. *Crit Rev Oncol Hematol*. 2003;48:35-43.
- Rubinstein P, Carrier C, Scaradavou A, et al. Outcomes among 562 recipients of placental-blood transplants from unrelated donors. *N Engl J Med*. 1998;339:1565-1577.
- Grewal SS, Barker JN, Davies SM, Wagner JE. Unrelated donor hematopoietic cell transplantation: marrow or umbilical cord blood? *Blood*. 2003;101:4233-4244.
- Hidalgo A, Weiss LA, Frenette PS. Functional selectin ligands mediating human CD34⁺ cell interactions with bone marrow endothelium are enhanced postnatally. *J Clin Invest*. 2002;110:559-569.
- 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.
- Kobzdej MMA, Leppänen A, Ramachandran V, Cummings RD, McEver RP. Discordant expression of selectin ligands and sialyl Lewis x-related epitopes on murine myeloid cells. *Blood*. 2002;100:485-494.
- Berg EL, Robinson MK, Mansson O, Butcher EC, Magnani JL. A carbohydrate domain common to both sialyl Le^x and sialyl Le^x is recognized by the endothelial cell leukocyte adhesion molecule ELAM-1. *J Biol Chem*. 1991;266:14869-14872.
- Moore KL. Purification of P-selectin (CD62P) from human platelets. *J Tissue Culture Methods*. 1994;16:255-257.
- McEver RP, Martin MN. A monoclonal antibody to a membrane glycoprotein binds only to activated platelets. *J Biol Chem*. 1984;259:9799-9804.
- Geng J-G, Bevilacqua MP, Moore KL, et al. Rapid neutrophil adhesion to activated endothelium mediated by GMP-140. *Nature*. 1990;343:757-760.
- Moore KL, Patel KD, Bruehl RE, et al. P-selectin glycoprotein ligand-1 mediates rolling of human neutrophils on P-selectin. *J Cell Biol*. 1995;128:661-671.
- Norton CR, Rumberger JM, Burns DK, Wolitzky BA. Characterization of murine E-selectin expression in vitro using novel anti-mouse E-selectin monoclonal antibodies. *Biochem Biophys Res Commun*. 1993;195:250-258.
- Xia L, Ramachandran V, McDaniel JM, Nguyen KN, Cummings RD, McEver RP. N-terminal residues in murine P-selectin glycoprotein ligand-1 required for binding to murine P-selectin. *Blood*. 2003;101:552-559.
- Patel KD, Moore KL, Nollert MU, McEver RP. Neutrophils use both shared and distinct mechanisms to adhere to selectins under static and flow conditions. *J Clin Invest*. 1995;96:1887-1896.
- McCune JM, Namikawa R, Kaneshima H, Shultz LD, Lieberman M, Weissman IL. The SCID-hu mouse: murine model for the analysis of human hematolymphoid differentiation and function. *Science*. 1988;241:1632-1639.
- Weninger W, Ulfman LH, Cheng G, et al. Specialized contributions by $\alpha (1,3)$ -fucosyltransferase-IV and FucT-VII during leukocyte rolling in dermal microvessels. *Immunity*. 2000;12:665-676.
- Maly P, Thall AD, Petryniak B, et al. The $\alpha (1,3)$ -Fucosyltransferase Fuc-TVII controls leukocyte trafficking through an essential role in L-, E-, and P-selectin ligand biosynthesis. *Cell*. 1996;86:643-653.
- Bengtson P, Lundblad A, Larson G, Pålsson P. Polymorphonuclear leukocytes from individuals carrying the G329A mutation in the $\alpha 1,3$ -fucosyltransferase VII gene (FUT7) roll on E- and P-selectins. *J Immunol*. 2002;169:3940-3946.
- Wagers AJ, Waters CM, Stoolman LM, Kansas GS. Interleukin 12 and interleukin 4 control cell adhesion to endothelial selectins through opo-
- site effects on $\alpha 1,3$ -fucosyltransferase VII gene expression. *J Exp Med*. 1998;188:2225-2231.
- Lim YC, Henault L, Wagers AJ, Kansas GS, Lusinskas FW, Lichtman AH. Expression of functional selectin ligands on Th cells is differentially regulated by IL-12 and IL-4. *J Immunol*. 1999;162:3193-3201.
- Blander JM, Visintin I, Janeway CA Jr, Medzhitov R. $\alpha (1,3)$ -fucosyltransferase VII and $\alpha (2,3)$ -sialyltransferase IV are up-regulated in activated CD4 T cells and maintained after their differentiation into Th1 and migration into inflammatory sites. *J Immunol*. 1999;163:3746-3752.
- Fuhlbrigge RC, Kieffer JD, Armerding D, Kupper TS. Cutaneous lymphocyte antigen is a specialized form of PSGL-1 expressed on skin-homing T cells. *Nature*. 1997;389:978-981.
- Wright DE, Bowman EP, Wagers AJ, Butcher EC, Weissman IL. Hematopoietic stem cells are uniquely selective in their migratory response to chemokines. *J Exp Med*. 2002;195:1145-1154.
- Naiyer AJ, Jo DY, Ahn J, et al. Stromal derived factor-1-induced chemokinesis of cord blood CD34(+) cells (long-term culture-initiating cells) through endothelial cells is mediated by E-selectin. *Blood*. 1999;94:4011-4019.
- Hidalgo A, Frenette PS. Reduced interactions of cord blood CD34⁺ cells with bone marrow microvessels result from hypofucosylated selectin ligands: implications for bone marrow transplantation [abstract 202]. *Blood*. 2003;102:202a.
- Bhatia M, Bonnet D, Murdoch B, Gan OI, Dick JE. A newly discovered class of human hematopoietic cells with SCID-repopulating activity. *Nat Med*. 1998;4:1038-1045.
- Kim DK, Fujiki Y, Fukushima T, Ema H, Shibuya A, Nakauchi H. Comparison of hematopoietic activities of human bone marrow and umbilical cord blood CD34 positive and negative cells. *Stem Cells*. 1999;17:286-294.
- Verstegen MM, van Hennik PB, Terpstra W, et al. Transplantation of human umbilical cord blood cells in macrophage-depleted SCID mice: evidence for accessory cell involvement in expansion of immature CD34⁺CD38⁻ cells. *Blood*. 1998;91:1966-1976.
- Winkler IG, Snapp KR, Simmons PJ, Levesque JP. Adhesion to E-selectin promotes growth inhibition and apoptosis of human and murine hematopoietic progenitor cells independent of PSGL-1. *Blood*. 2004;103:1685-1692.
- Lévesque JP, Zannettino ACW, Pudney M, et al. PSGL-1-mediated adhesion of human hematopoietic progenitors to P-selectin results in suppression of hematopoiesis. *Immunity*. 1999;11:369-378.
- Schweitzer KM, Dräger AM, Van der Valk P, et al. Constitutive expression of E-selectin and vascular cell adhesion molecule-1 on endothelial cells of hematopoietic tissues. *Am J Pathol*. 1996;148:165-175.
- Rocha V, Cornish J, Sievers EL, et al. Comparison of outcomes of unrelated bone marrow and umbilical cord blood transplants in children with acute leukemia. *Blood*. 2001;97:2962-2971.