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Non-fucosylated CB CD34$^+$ cells represent a good target for enforced fucosylation to improve engraftment following cord blood transplantation

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Abstract

Background aims. Despite ethnic diversity and ready availability of cryopreserved, human leukocyte antigen–typed cord blood (CB), delayed engraftment remains a significant hurdle to successful CB transplantation. Suboptimal homing of CB hematopoietic stem and progenitor cells (HSPCs) to the hematopoietic microenvironment (HM) is thought to be responsible and due to low levels of HSPC fucosylation. Fucosylation (decoration with sialyl-Lewis^X) may improve HSPC homing to HM by increasing the strength of HSPC/E-selectin interactions, where E-selectin is constitutively expressed by HM microvasculature. Enforced fucosylation of CB HSPCs using fucosyltransferases, increases the rate and magnitude of engraftment in xenogeneic transplant models. However, it is unclear whether endogenously fucosylated and non-fucosylated CB HSPC are qualitatively identical or whether endogenous fucosylation marks a qualitative difference between CB HSPC. If qualitatively identical, non-fucosylated CB HSPCs represent a good target for enforced fucosylation with improved engraftment conferred on an increased number of otherwise qualitatively identical HSPC. If qualitatively different, then conferring engraftment upon a majority, possibly lower "quality," non-fucosylated HSPCs by enforced fucosylation might inadvertently compromise engraftment. *Methods.* Functional (xenogeneic engraftment, colony-forming unit and selectin-binding assays) and phenotypic analyses of fluorescence-activated cell sorting–isolated, endogenously fucosylated and non-fucosylated CB CD34$^+$ cells were performed. *Results.* Endogenous fucosylation of CB HSPCs exists as a continuum. Endogenously fucosylated HSPCs engrafted more efficiently in a xenogeneic transplantation model than non-fucosylated HSPCs. Outside of the differences in endogenous fucosylation, no other qualitative (functional and/or phenotypic) differences were identified. *Discussion.* The majority of endogenously non-fucosylated CB HSPCs represent a good target for enforced fucosylation with the goal of improving engraftment following CB transplantation.

Key Words: *cord blood (CB), engraftment, E-selectin, fucosylation, hematopoietic stem and progenitor cell (HSPC)*

Despite the ethnic diversity and ready availability of human leukocyte antigen–typed, cryopreserved cord blood (CB) units as a source of hematopoietic tissue for transplantation [1–15], delayed engraftment remains an important clinical barrier to its use compared with the use of bone marrow (BM) or mobilized peripheral blood (mPB) [16–18]. The delayed engraftment associated with CB transplantation (CBT) may, at least in part, be a consequence of suboptimal homing of CB hematopoietic stem and progenitor cells (HSPCs) to the hematopoietic microenvironment. For effective homing and engraftment to the hematopoietic microenvironment cell surface ligands expressed by HSPCs need to interact with specific receptors expressed by the endothelial cells lining the blood vessels of the hematopoietic microvasculature. Although the expression of certain cell surface glycoproteins alone might be sufficient for homing to hematopoietic tissues [19–27], there is evidence that the binding affinity of some classes of cell surface glycoproteins, particularly selectin ligands, can be enhanced when they are fucosylated. Fucosylation is a fucosyltransferase (FT)-driven process that decorates specific sites on cell surface molecules with sialyl-Lewis^X (sLe^X) moieties [28–35]. Fucosylation has been shown to play a key role in the selectin-associated

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homing of lymphocytes and other leukocytes [28,31]. P- and E-selectins are constitutively expressed by the microvasculature of the hematopoietic system [36], and the hypothesis that fucosylation may also play a key role in the homing of HSPCs to the hematopoietic microenvironment has been proposed.

Relative levels of cell surface fucosylation can be revealed by the use of the HECA-452 antibody (also known as anti-human cutaneous lymphocyte associated antigen). Comparative studies have revealed that the proportion of fucosylated HSPCs present in CB is markedly lower than that found in BM or mPB [37]. This led to the hypothesis that the delayed engraftment associated with CBT is due, at least in part, to this relatively low proportion of endogenously fucosylated CB HSPCs. This hypothesis was supported by the demonstration that enforced fucosylation of CB HSPCs using recombinant FT-VI [32,37] or FT-VII [38] markedly improved the rate and magnitude of xenogeneic engraftment in the NOD-SCID IL-2Rγ^{null} (NSG) mouse model. These data provided the rationale for the use of *ex vivo* enforced fucosylation as a means to increase the proportion of fucosylated HSPCs in CB products with the goal of improving engraftment following CBT in the clinic. A clinical trial to assess the impact of *ex vivo* enforced fucosylation in a double CB transplant (DCBT) setting is currently underway at the University of Texas M.D. Anderson Cancer Center (ClinicalTrials.gov identifier NCT01471067). Preliminary data from this ongoing trial, albeit in a more complex double CBT and cancer therapy setting, show improved neutrophil and platelet engraftment in patients where the second and smaller of the two CB units transplanted was subject to *ex vivo* enforced fucosylation before transplantation [39].

These findings suggest that because the use of *ex vivo* enforced fucosylation as a means to improve the proportion of fucosylated CB HSPCs is building on an existing and therefore physiologically relevant mechanism, it is an effective strategy to pursue in the clinic. However, it is unclear whether endogenously fucosylated and non-fucosylated CB HSPCs are "qualitatively" identical or whether endogenous fucosylation marks a "qualitative" difference between CB HSPCs. If endogenously fucosylated and non-fucosylated CB HSPCs are qualitatively identical, then non-fucosylated CB HSPCs represent a good target for enforced fucosylation with improved engraftment conferred on an increased number of otherwise qualitatively identical HSPCs. However, if endogenous fucosylation represents a qualitative difference between CB HSPCs, then conferring engraftment on a majority of possibly lower "quality" non-fucosylated HSPCs by enforced fucosylation might inadvertently compromise engraftment. This concern provided the rationale for this study.

Methods

Hematopoietic cells

Fresh CB units and animals were used under University of Texas M.D. Anderson Cancer Center Institutional Review Board and Institutional Animal Care and Use Committee–approved protocols, respectively. Mononuclear cells were isolated from eight fresh CB units by Ficoll density separation and $CD34^+$ cells enriched by magnetic-activated cell sorting (MACS) (CD34 Reagent, Miltenyi Biotec). Pooled $CD34^+$ cells were stained with FITC-HECA-452 and APC-CD34 (BD Biosciences) for fluorescence-activated cell sorting (FACS; Beckman Coulter MoFlo Astrios) and $CD34^+HECA^+$ (fucosylated) and $CD34^+HECA^-$ (non-fucosylated) cells collected (Figure 1).

Human engraftment in the NSG mouse

NSG mice (n = 5 per group) were sublethally irradiated (3 Gy, ^{137}Cs source, 3 Gy/min, J. L. Shepherd and Associates Mark I-25 Irradiator) and received 10^4 $CD34^+HECA^+$ (fucosylated), or 10^4 $CD34^+HECA^-$ (non-fucosylated) cells intravenously. Only 10^4 $CD34^+$ cells were transplanted in an attempt to better accentuate qualitative differences between the $HECA^+$ and $HECA^-$ groups. Human engraftment was determined in serial (twice weekly) 40-μL bleeds and in BM and spleen (>12 weeks after transplant). PB, BM and spleen were assessed for human and murine $CD45^+$ cells by flow cytometry (BD FACSCalibur) using PE-rat anti-mouse CD45 and APC-mouse anti-human CD45 (both BD Biosciences). Analysis was performed using BD CellQuest Pro software.

The pattern of multi-tissue (PB, BM and spleen), multi-lineage, human engraftment was determined: myeloid (CD33, CD14, CD16), T-lymphocyte (CD3, CD4, CD8), B-lymphocyte (CD19, CD20) and platelet (CD41a, CD61) (all antibodies from BD Biosciences). Human platelets were identified by their forward and side scatter profile (log-scale) and expression of human CD41a and CD61.

Phenotypic analyses of FACS isolated $CD34^+HECA^+$ (fucosylated) and $CD34^+HECA^-$ (non-fucosylated) fractions

FACS isolated $CD34^+HECA^+$ (fucosylated) and $CD34^+HECA^-$ (non-fucosylated) fractions were stained with antibodies against CD133, CD90 (Thy-1), CD117 (c-kit), BB9 (CD143), CD33, CD14, CD38, CD3 and CD20 (all antibodies from BD Biosciences) to determine whether phenotypic differences between the two groups could be identified.

In addition, levels of HECA reactivity (fucosylation) were measured for relatively more primitive ($CD34^+38^-$) and relatively more mature ($CD34^+38^+$) subpopulations