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Direct Comparison of Umbilical Cord Blood versus Bone Marrow–Derived Endothelial Precursor Cells in Mediating Neovascularization in Response to Vascular Ischemia

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ABSTRACT

Endothelial precursor cells (EPCs) cultured from adult bone marrow (BM) have been shown to mediate neovasculogenesis in murine models of vascular injury. We sought to directly compare umbilical cord blood (UCB)- and BM-derived EPC surface phenotypes and in vivo functional capacity. UCB and BM EPCs derived from mononuclear cells (MNC) were phenotyped by surface staining for expression of stromal (Stro-1, CXCR4, CD105, and CD73), endothelial (CD31, CD146, and vascular endothelial [VE]-cadherin), stem cell (CD34 and CD133), and monocyte (CD14) surface markers and analyzed by flow cytometry. The nonobese diabetic/severe combined immunodeficiency murine model of hind-limb ischemia was used to analyze the potential of MNCs and culture-derived EPCs from UCB and BM to mediate neovasculogenesis. Histologic evaluation of the in vivo studies included capillary density as a measure of neovascularization. Surface CXCR4 expression was notably higher on UCB-derived EPCs (64.29% ± 7.41%) compared with BM (19.69% ± 5.49%; $P = .021$). Although the 2 sources of EPCs were comparable in expression of endothelial and monocyte markers, BM-derived EPCs contained higher proportions of cells expressing stromal cell markers (CD105 and CD73). Injection of UCB- or BM-derived EPCs resulted in significantly improved perfusion as measured by laser Doppler imaging at days 7 and 14 after femoral artery ligation in nonobese diabetic/severe combined immunodeficiency mice compared with controls ($P < .05$). Injection of uncultured MNCs from BM or UCB showed no significant difference from control mice ($P = .119$; $P = .177$). Tissue samples harvested from the lower calf muscle at day 28 demonstrated increased capillary densities in mice receiving BM- or UCB-derived EPCs. In conclusion, we found that UCB and BM-derived EPCs differ in CXCR4 expression and stromal surface markers but mediate equivalent neovasculogenesis in vivo as measured by Doppler flow and histologic analyses.

KEY WORDS

Umbilical cord blood • Endothelial precursor cells • Neovascularization • Vascular ischemia

INTRODUCTION

Atherosclerotic cardiovascular disease is the leading cause of morbidity and mortality in the United States and accounts for approximately 1 million deaths per year. Coronary artery disease, a pathologic process of arterial luminal narrowing by atherosclerotic plaque that results in obstruction of blood flow to the myocardium, accounts for approximately half of these deaths. In 2004, approximately 1 million percutaneous coronary interventions and 500 000 coronary artery bypass grafting procedures were performed; these restore blood flow to ischemic myocardium. Characteristics of vessels inadequately treated include small caliber, calcification, and lesions in the distal segments. Not surprisingly, patients who receive complete revascularization have improved rates of survival when compared with those incompletely revascularized [1]. Therefore, treatment limitations may be significant in patients who possess areas of viable myocardium jeopardized by the impaired perfusion supplied by vessels that are poor targets for conventional revascularization techniques. Prior studies, primarily using adult bone marrow (BM)–derived mononuclear cells (MNCs), point to the efficacy of cultured endothelial precursor cells (EPCs) in mediating neovascularization in vascular ischemic animal models [2-4]. Initial animals studies suggest that such cells could potentially also be used to repair ischemic or damaged cardiac tissue [5-8]. Clinical trials using autologous BM- or circulating blood–derived progenitor cells have illustrated the safety of cell infusions in human cardiac patients [9-12].

The cellular mechanisms underlying vasculogenesis, a process in which EPCs differentiate in situ into mature endothelial cells [13], has been further clarified over the past 7 years with the identification of BM-derived circulating EPCs shown to migrate to areas of ischemia and participate in neovascularization [2,14-21]. Although vasculogenesis was previously considered to occur only in the embryo [22], transplantation of cultured marrow-derived EPCs from human adults into rodent models of both myocardial and peripheral ischemia resulted in evident postnatal vasculogenesis [2,3].

Umbilical cord blood (UCB), a robust source of hematopoietic stem cells, has been extensively studied in hematology clinical applications and is capable of regenerating the entire hematopoietic system in adult patients [23]. Several qualities of UCB render it potentially advantageous for human therapeutic EPC applications for vascular ischemia when compared with patient-derived BM, including a higher concentration of stem cells, greater proliferative capacity, longer telomeres, a broad HLA repertoire, immune tolerance, and a lack of viral contamination [24,25]. Murohara et al. [26] have recently derived EPCs from UCB and shown enhanced perfusion and neovascularization in rodent models of vascular ischemia. However, to date, no direct comparison of cellular characteristics and functionality of UCB- and BM-derived EPCs in mediating vasculogenesis in response to ischemia has been performed. In this study, we directly compared EPCs derived from UCB and BM and analyzed their surface phenotype and functionality in neovascularization in a nonobese diabetic/severe combined immunodeficiency (NOD/SCID) vascular injury model.

MATERIALS AND METHODS

Cell Culture

Human UCB and adult BM from volunteer healthy donors were collected according to institutional review board protocols, including informed consent. MNCs were isolated by density gradient centrifugation from fresh UCB or BM. MNCs were plated on fibronectin-coated tissue culture flasks at a density of 4 to 6 $\times$ 10^6/mL (UCB MNC) or 1 to 2 $\times$ 10^6/mL (BM MNC) in EBM2 medium (Clonetics, Walkersville, MD) with 5% fetal bovine serum and standard SingleQuot additives that included vascular endothelial growth factor, fibroblast growth factor, insulin-like growth factor, hydrocortisone, ascorbic acid, and heparin, as previously described [3,27]. Nonadherent cells were discarded after 4 days of culture, and medium was replaced. EPCs were harvested at day 7 of culture for characterization before injection in study animals.

Assessment of Acetylated Low-Density Lipoprotein Uptake and *Ulex europaeus*–Lectin Binding

After 7 days of culture in endothelial conditions, cells were tested for uptake of 1,1'-dioctadecyl-3,3,3',3'-tetramethylindocarbocyanine perchlorate-labeled acetylated low-density lipoprotein (Molecular Probes, Eugene, OR) and adherence of fluorescein isothiocyanate–conjugated *Ulex europaeus* lectin (UEA-lectin; Sigma Chemical Companies, St. Louis, MO) and were visualized by confocal microscopy. After 7 days of culture, adherent cells were incubated for 4 hours with 1,1'-dioctadecyl-3,3,3',3'-tetramethylindocarbocyanine perchlorate-labeled acetylated low-density lipoprotein at 37°C. The cultures were washed, fixed with 1% formaldehyde for 10 minutes, and washed again with phosphate-buffered saline (PBS). UEA-lectin was added at a concentration of 10 μg/mL and incubated for 1 hour at 25°C. For nuclear detection, Hoechst 33258 (Sigma Chemical Companies) was added at 3 μg/mL for 10 minutes. Cultures were then washed twice with PBS and visualized with confocal microscopy. Thereafter, culture plates were washed twice with PBS and incubated with 2 mmol/L ethylenediaminetetraacetic acid to remove cells. The cells were washed and reconstituted in 1% formaldehyde and analyzed by flow cytometry for quantification of fluorescence.

Assessment of Surface Phenotype by Flow Cytometry of MNCs and EPCs

MNCs were characterized immediately after isolation from UCB and BM. After 7 days of culture, adherent cells were incubated with trypsin for 5 minutes at 37°C. Cells were then washed with PBS and counted. The surface phenotype was evaluated by incubation for 20 minutes at 4°C with fluorochrome-conjugated monoclonal antibodies: CD14, CD34, CD31, CD73 (Becton Dickinson, San Jose, CA), CD105 (Serotec, Raleigh, NC), CD133 (Miltenyi, Auburn, CA), CXCR4, Stro-1 (R&D, Minneapolis, MN), CD146 (P1H12), and vascular endothelial (VE)-cadherin (Chemicon, Temecula, CA). Nonviable cells were excluded on the basis of forward/side scatter, and no additional gating was applied. Positive-staining quadrants were set on nonstained samples as a result of significant autofluorescence of cultured cells. An LSR flow cytometer (Coulter, Miami, FL) was used that acquired >5000 fluorescence events per sample. Compensation and data analysis were performed with WinList software (Verity Software House Inc., Topsham, ME).

Experimental Animals

Female NOD/SCID mice (Jackson Laboratories, Bar Harbor, ME) were anesthetized with intraperitoneal injection of a combination of ketamine, acepromazine, and xylazine. To reduce immune-mediated rejection of the injected cells by murine endogenous natural killer cells, the mice were irradiated with a sublethal dose (2.5 Gy) from a cesium 137 source before human cell or control injections [28]. Blood flow of the hind limbs was measured with laser Doppler imaging (Laser flow-meter ALF21D; Advance Company Ltd., Tokyo, Japan) for baseline measurements. Under sterile conditions, a small skin incision was made in the right groin area. The right femoral artery was exposed, ligated along with adjacent branches, and transected. The skin incision was then closed with a continuous suture. Study animals then survived for 4 weeks. Blood flow measurements on both feet were repeated at 30 minutes after femoral artery ligation and at 7, 14, and 28 days after femoral ligation. All procedures were performed in accordance with the policies of the Case Western Reserve University Institutional Animal Care and Use Committee.

Transplantation of Cells

After femoral artery ligation, the mice were randomized into 1 of 4 study groups. Group 1 (control) was treated with intracardiac injection of culture medium or normal saline (0.3 mL), group 2 was treated with intracardiac injection of cultured EPCs (10^6 in 0.3 mL of culture medium) from UCB, and group 3 received intracardiac injection of cultured EPCs (10^6 in 0.3 mL of culture medium) from adult BM. Group 4 mice were injected with the starting material, BM or UCB MNC (10^6 in 0.3 mL of culture medium). Intracardiac injection was performed to avoid first-pass uptake in lung and liver resulting from an intravenous tail vein injection.

Histologic Analysis

After 28 days, samples from the lower gastrocnemius in both ischemic and healthy hind limbs were harvested for capillary density determination and staining for human CD31. Capillary density was used as a measure of neovascularization in addition to blood flow [3,26,29-31]. For this, fresh-frozen gastrocnemius transverse sections were stained for alkaline phosphatase to detect capillary endothelial cells, as previously described [29]. Briefly, 12 fresh-frozen sections were cut (10-μm thickness) from each specimen and stained for alkaline phosphatase by using the indoxyl-tetrazolium method and counterstained with eosin to detect capillary endothelial cells. Capillaries were counted with a ×40 objective by 2 independent investigators blinded to the study groups. Capillaries were counted in 20 fields per study animal, and 3 animals were evaluated for each study group.

Formalin-fixed sections were mounted on saline-coated glass slides and stained with anti-human CD31 antibody (Dako, Glastrup, Denmark) to identify incorporation of human EPCs by staining. Cells positive for human CD31 staining were counted in 5 high-power fields by 2 blinded investigators. Sections from control animals were also stained with primary CD31 antibody; no positive staining was noted, and this indicated an absence of cross-reactivity with murine endothelial cells.

Statistical Analysis

Data are summarized by the mean ± SEM. Statistical significance was determined between the indicated values by 2-tailed, nonpaired, unequal variance Student *t* tests to determine differences between control and study group mean values. A value of $P < .05$ was considered significant. The Kruskal-Wallis test, a nonparametric test based on Wilcoxon scores, was used if the normality assumption was violated.

RESULTS

In Vitro Characterization of UCB- and BM-Derived EPCs

After 1 week of culture, adherent cell yields from UCB cultures were on average 2.4% ± 0.3% of the initial MNC input, compared with 18.6% ± 2.2% obtained from BM MNCs (Figure 1A; n = 14). A median of 18.7×10^7 MNCs (range, 2.80-68.7) were plated from single UCB units, and a median of 4.65×10^7 MNCs (range, 6.0-14.3) were plated from 20 mL of marrow aspirate cells. Comparable numbers of EPCs

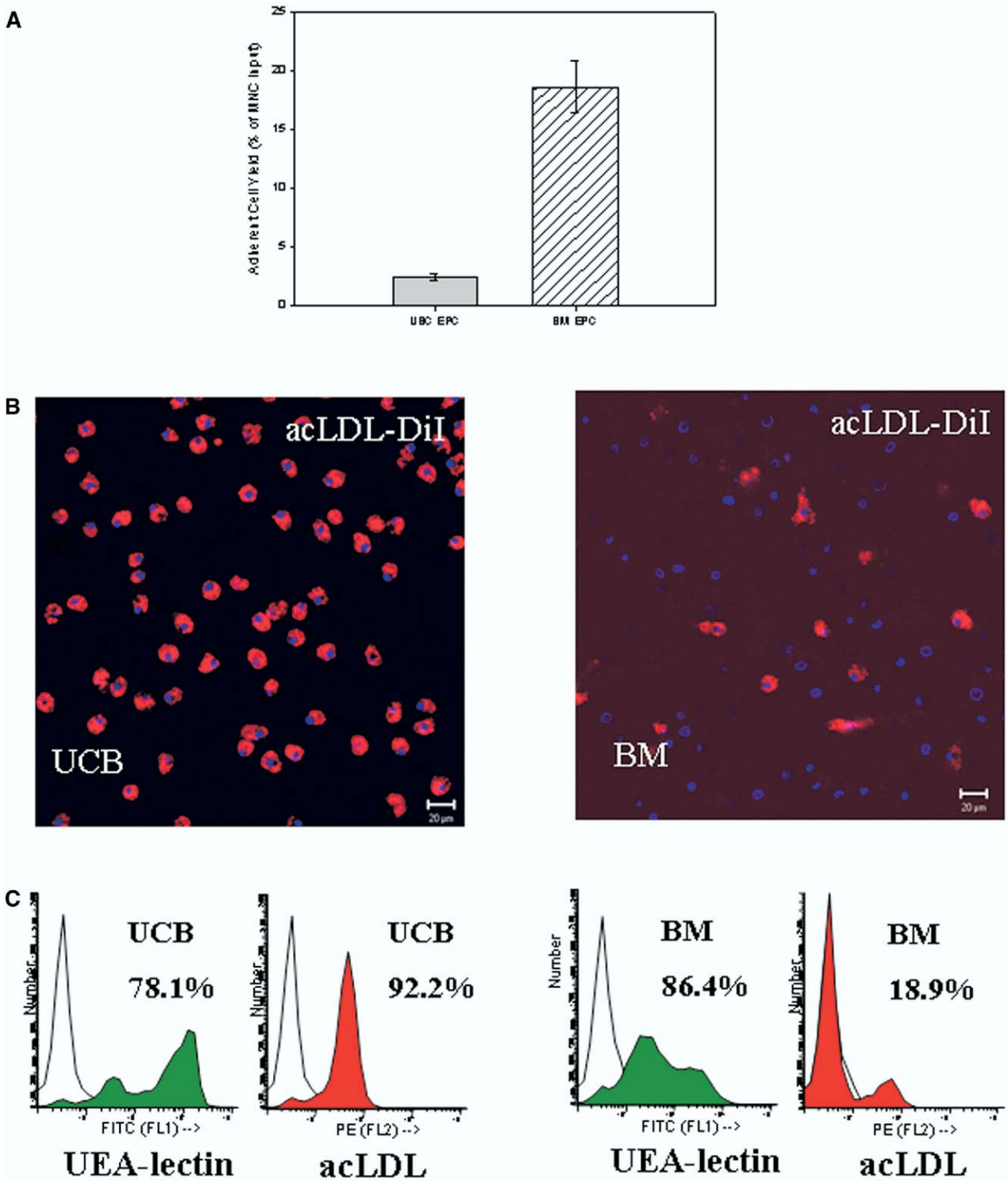

Figure 1. Characteristics and yield of UCB- and BM-derived EPCs. Mononuclear cells (MNCs) from fresh UCB or BM were isolated and cultured under endothelial conditions for 7 days [13]. Adherent cell yields at day 7 from UCB cultures were on average 2.4% ± 0.3% of the initial MNC input, compared with 18.6% ± 2.2% obtained from BM MNCs (A). Fluorescent microscopy of adherent cells was used to assess the uptake of acetylated low-density lipoprotein (acLDL; red) and nuclear staining with Hoechst 33258 (blue) after 7 days of culture (B). Representative images shown here were taken at ×40 with a Zeiss (Jena, Germany) LSM510 confocal microscope. For flow analysis, cultured cells were dissociated (2 mmol/L ethylenediaminetetraacetic acid), and the fluorescence of acetylated low-density lipoprotein and UEA-lectin was quantified (C).

were harvested at day 7 comparing 1 UCB unit (3.0 ± 0.4 $\times 10^6$) and a 20-mL BM aspirate (4.9 ± 0.9 $\times$ 10^6).

Fluorescent microscopy was used to evaluate the EPC cell cultures (Figure 1B), and flow cytometry was used to quantify fluorescent microscopy results (Figure 1C). UCB- and BM-cultured cells demonstrated a spin-

dle shape indicative of endothelial cell morphology. A representative sample is shown in Figure 1. Binding of UEA-lectin, a marker of endothelial lineage, was similar in cells cultured from UCB (71.9%) compared with BM-derived cells (81.9%). Most (92.4%) UCB-derived EPCs stained positive for uptake of acetylated low-density lipoprotein, whereas a smaller proportion (28.5%) of BM-derived EPCs were positive for acetylated low-density lipoprotein uptake (n = 3). Double staining, indicative of endothelial cell lineage [32], made up 60.7% of the UCB-derived cells and 20.8% of the BM-derived cells.

Surface Phenotyping of UCB and BM MNCs and Cultured EPCs

Freshly isolated MNCs from UCB and BM and cells cultured for 7 days were analyzed for expression of endothelial (CD146, CD31, and VE-cadherin), stromal (Stro-1, CXCR4, CD105, and CD73), stem cell (CD34 and CD133), and monocyte (CD14) markers (n $\geq$ 3).

Expression of CD31 was comparable in both sources of starting material: 26% in BM MNCs and 18% in UCB MNCs. The MNCs contained 8% positive CD14 in UCB and 9% in BM. UCB MNCs had a low expression of CD73 (5%) compared with BM MNCs (10%). A small percentage of the MNCs from UCB and BM exhibited CD105 surface expression: 6% and 7%, respectively. The expression of the stromal-derived factor-1 (SDF-1) receptor CXCR4 on the UCB- MNCs was 28%, and in BM MNCs it was 11%.

The data for the UCB- and BM-derived EPCs are shown in Figure 2. Adherent cells were trypsinized and washed. Cells were stained for the endothelial-specific markers CD146 (P1H12, MUC18, or melanoma adhesion molecule [MCAM]), CD31, and VE-cadherin. Similar proportions of UCB and BM-derived EPCs expressed endothelial cell surface markers: 37% of the cultured adherent cells from UCB- and 57% of BM-derived EPCs stained positive for CD146 (P = .229). After the 7-day culture CD31 expression was present in UCB (30%) and BM (29%), but there was not a significant difference between the 2 populations (P = .77). VE-cadherin was expressed in 15% of cells from BM, compared with 8% of cells from UCB (P = .732). As in the MNCs, after 7 days of culture, expression of CD14 remained low in UCB (2%) and BM (6%). Cultures from UCB showed greater expression of Stro-1, with 31% of UCB and 17% of BM having positive staining (P = .034). BM-derived EPCs exhibited a higher expression of CD73 (44%) after culture compared with UCB (4%; P = .05). However, the expression of CD105 in BM EPCs increased to 40% after 7 days of culture, and this was significantly greater than the UCB EPCs (7%; P = .034). EPCs derived from UCB also showed a significantly higher expression of CXCR4 (64%) after 7 days of culture compared with BM-derived EPCs (20%; P = .021).

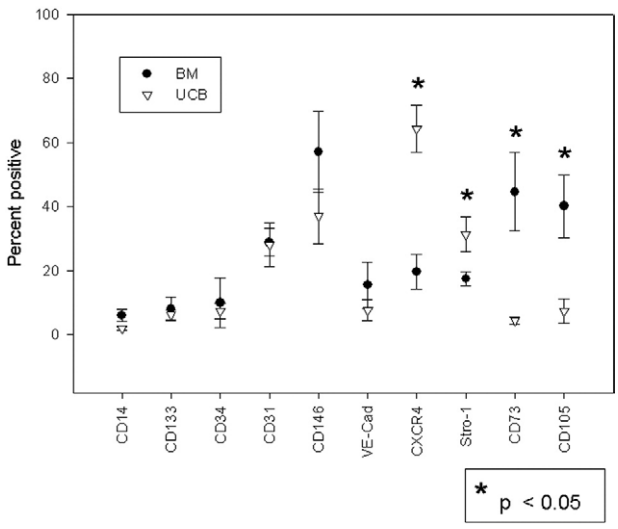

Figure 2. Flow cytometric analysis of EPC cells derived from UCB versus BM. UCB and BM MNCs were cultured for 7 days, and adherent cells were trypsinized, washed, and stained for stromal (Stro-1, CXCR4, CD105, and CD73), endothelial (CD31, CD146, and VE-cadherin [VE-Cad]), stem cell (CD34 and CD133), and monocyte (CD14) surface markers and analyzed by flow cytometry.

Transplantation of UCB- and BM-Derived EPCs in an In Vivo Model

In vivo studies of neovascularization in the NOD/SCID hind-limb ischemia model were performed: freshly isolated UCB- or BM MNCs and UCB- or BM-derived EPCs (adherent cells only) were harvested at day 7 and given via an intracardiac injection (1×10^6 cells per mouse) after femoral artery ligation. Weekly laser Doppler blood-flow measurements were taken of both the injured and uninjured legs of all study animals. The ratio of perfusion in the ischemic/healthy limb was used as an index of neovascularization in the study groups. Baseline perfusion ratios were 0.994 $\pm$ 0.019 (n = 17) before ligation (data not shown). Immediately after femoral ligation, the perfusion ratios decreased to 0.057 $\pm$ 0.011 (control group), 0.031 $\pm$ 0.008 (UCB MNCs), 0.039 $\pm$ 0.008 (BM MNCs), 0.029 $\pm$ 0.007 (UCB-derived EPCs), and 0.020 $\pm$ 0.003 (BM-derived EPCs), thus indicating reduced perfusion in all groups. As shown in Figure 3, after 7 days, significantly higher perfusion ratios were noted when mice injected with EPCs were compared with control mice (P < .05). Higher perfusion ratios were measured in animals injected with BM-derived EPCs compared with UCB-derived EPCs (P = .045) at this early time point. A sustained and significant increase in blood flow at day 14 was measured in mice injected with either human BM- or UCB-derived EPCs compared with the control mice. Perfusion ratios in the control and starting material MNC groups remained low at day 14: 0.24 $\pm$ 0.032 (n = 14; control), 0.25 $\pm$ 0.032 (n = 6; BM MNC), and 0.27 $\pm$ 0.035 (n = 8; UCB MNC) com-

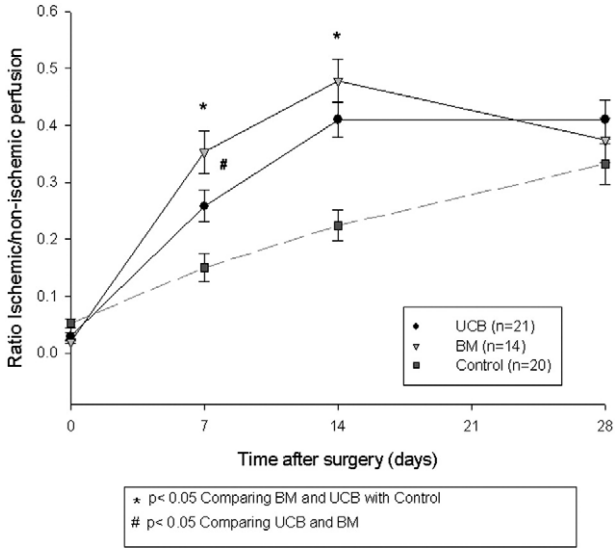

Figure 3. Perfusion ratios after injection of EPCs into NOD/SCID mice with induced hind-limb ischemia. NOD/SCID mice underwent femoral artery ligation and excision followed by intracardiac injection of EPCs. Control animals were injected with saline or complete EBM2 media. Adherent UCB or BM EPCs on the day of injection (day 7 of culture) were removed with trypsin, and 1×10^6 cells per mouse were injected. Doppler measurements were taken immediately after ligation and then on days 7, 14, and 28. Perfusion ratios were determined between the ischemic and nonischemic leg of each study animal.

pared with 0.41 ± 0.031 (n = 21) in the group that received UCB-derived EPCs (P = .0008) and 0.48 ± 0.039 (n = 14) in the group that received BM-derived EPCs. It is important to note that at day 14 there was no significant difference in blood flow between the 2 sources of EPCs (P = .18). No limb loss was observed in any study animal during the 28 days of observation. After 28 days, perfusion ratios in control animals improved to levels that approached those measured in animals treated with human cells.

Histologic Analysis

Tissue from the lower calf muscles of both hind limbs was harvested at day 28 for histologic evaluation. Capillary density was used as an additional evaluation of neovascularization. Data from the 28-day time point are shown in Figure 4. In the animals treated with EPCs from UCB, the density was significantly increased: 103 ± 6 capillaries per square millimeter (20 fields per animal; 3 animals per group) versus 87 ± 5 capillaries per square millimeter in control animals (P = .035). Animals that received BM-derived EPCs also showed increased capillary density compared with control mice (117 ± 8 capillaries per square millimeter; P = .001). Figure 5 depicts the anatomic localization of EPCs derived from human cells in 28-day samples as identified by staining with anti-human CD31 antibody. Sections from mice injected with human EPCs (UCB or BM) showed cells expressing human CD31 localized within the microvasculature, whereas control femoral artery–ligated mice demonstrated no cells that expressed human CD31.

DISCUSSION

Previous studies have demonstrated recovery of EPCs from UCB [26,33-36]. However, no direct comparison of UCB- and BM-derived EPCs, including cellular characteristics and functionality, has been performed to date. Because UCB is a more readily available allogeneic stem cell source than BM, it is important to address whether UCB-derived EPCs have equivalent

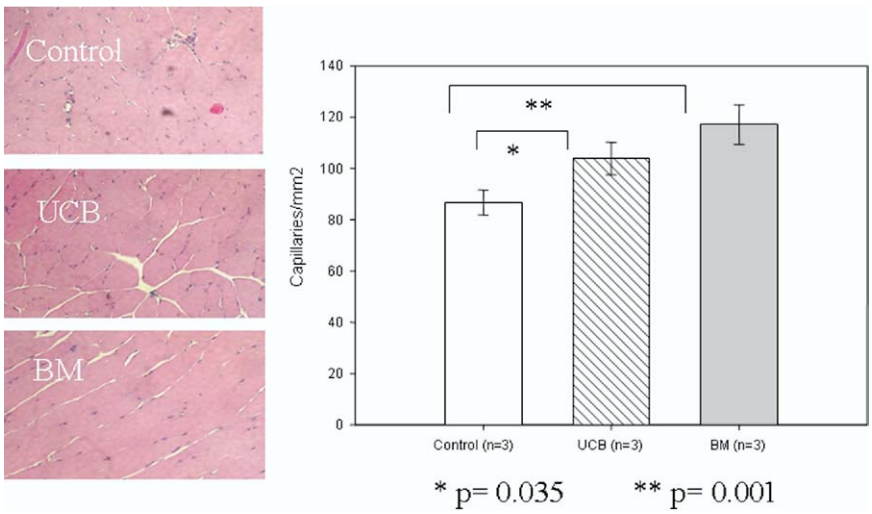

Figure 4. Comparison of capillary density in treated and nontreated mice. At 28 days after isolation and ligation of the right femoral artery, tissue was harvested from the lower calf muscle of study and control mice. Tissue was fresh-frozen and stained for alkaline phosphatase by the indoxyl-tetrazolium method. Two blinded investigators counted 20 fields per sample, and capillary density was expressed as capillaries per square millimeter.

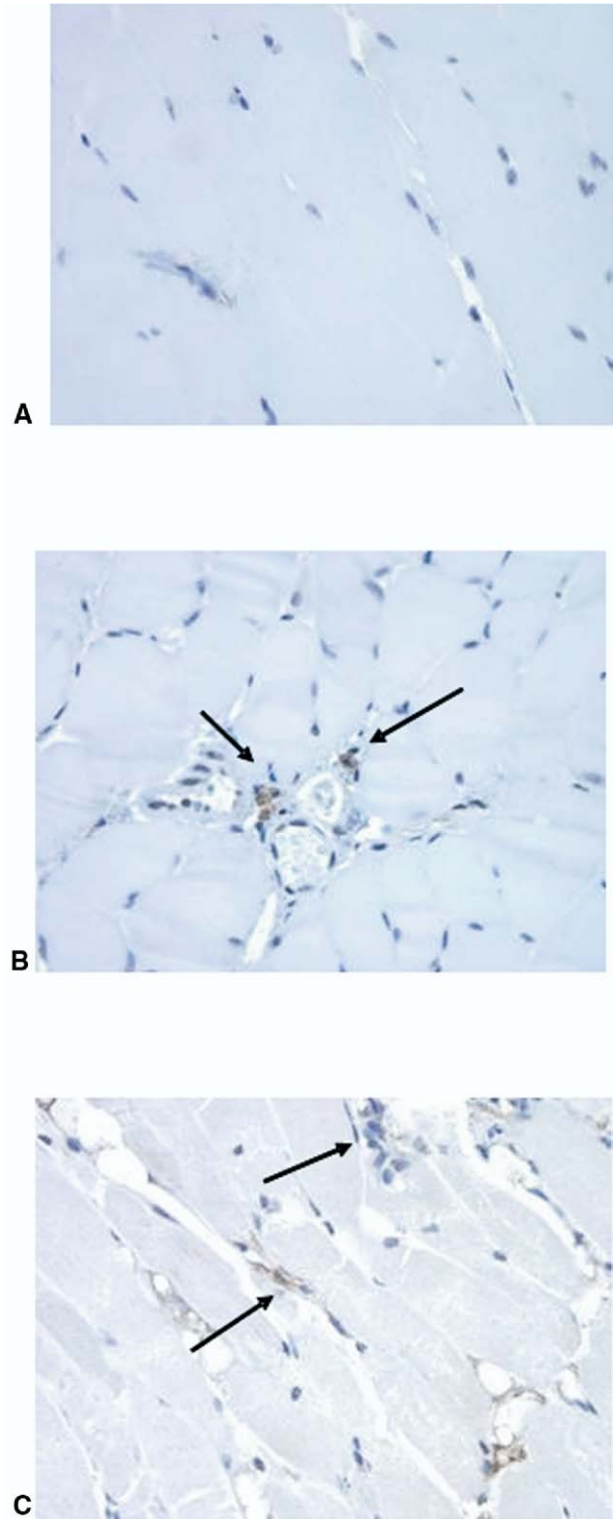

Figure 5. Histologic assessment of ischemic hind limbs with CD31. Tissue from the lower calf muscles of both hind limbs was harvested at day 28 for histologic evaluation. The samples were fixed in formalin. Sections (6-μm thickness) were mounted on saline-coated glass slides and stained with anti-human CD31 antibody to identify EPCs derived from human cells. Control mice (A) were negative for CD31 staining. Specimens from mice that were injected with UCB-derived EPCs (B) or BM-derived EPCs (C) showed positive staining for CD31 expression by human cells within the microvasculature.

functionality compared with BM-derived EPCs. We therefore cultured EPCs from UCB and BM with standard conditions used for BM-derived EPCs and directly compared their potency in an in vivo murine model of hind-limb ischemia.

Our studies directly comparing infusions of EPCs cultured from nonselected UCB or BM MNCs revealed equivalent biologic effects of increased blood flow and microvascular density in the NOD/SCID study model of hind-limb vascular injury. We observed that both UCB- and BM-derived EPC cell injections significantly increased blood flow in the ischemic leg by days 7 and 14 after injury/cell injection.

To confirm the neovascularization seen with blood-flow recovery, we evaluated the capillary density in histologic sections from treated animals. At day 28, capillary density was significantly increased in animals that received UCB- or BM-derived EPCs. Although we observed significantly increased capillary density in study animals treated with human cells, only a few detectable human $CD31^+$ cells were found anatomically incorporated in tissue distal to the site of injury. These observations suggest possible paracrine effects mediated by injected human cells which contribute to augmentation of microvascular density. This interpretation is consistent with observations made by Kamihata et al. [7] that showed paracrine effects of BM MNCs implanted into ischemic myocardium. A complex sequence of steps of paracrine mechanisms are coordinated in the development of the adult vascular system [37]. Paracrine signaling pathways regulated by hypoxia elicit vascular endothelial cell responses and vascular remodeling in ischemic tissue repair [38,39].

Despite equivalent biological effects of UCB- and BM-derived EPCs, we observed significant differences in the surface phenotype of injected cells. Whereas the expression of classic endothelial markers such as CD31, VE-cadherin, and CD146 was similar, the expression of CXCR4, the receptor for SDF-1, was markedly higher in UCB-derived cells. Expression of this chemokine receptor may have implications in the homing capacity of this cell population [40]. SDF-1 supports vasculogenesis, contributes to neovascularization in vivo by augmenting cellular recruitment in ischemic tissues [30,40,41], and regulates endothelial cell branching morphogenesis [42], thereby serving a critical role in vascular remodeling in ischemic tissue [43]. In the context of local tissue ischemia in the NOD/SCID hind-limb ligation model, increased migration of intracardiac-injected UCB-derived cells homing to sites of hind-limb vascular injury may be expected to be facilitated because 64% of UCB-derived cells express CXCR4, compared with only 20% of BM-derived cells.

In contrast, expression of stromal markers (CD105 and CD73) was significantly higher in BM-derived cells

as compared with UCB-derived EPCs. Stromal cells contribute to the recruitment and proliferation of vascular network formation by promoting vascular growth by direct cell-to-cell contact [44,45]. Enhanced expression of stromal cell markers on BM-derived EPCs could allow these cells to contribute to angiogenesis events in which new vessels arise by the migration and proliferation of endogenous endothelial cells from preexisting vessels [37]. Taken together, despite differences in the surface phenotypes of injected cells, the observed equivalent improvements in hind-limb blood flow comparing UCB- and BM-derived EPCs may stem from the enhanced expression of the CXCR-4 chemokine receptor on EPCs derived from UCB on the one hand and increased expression of stromal cell markers on BM-derived EPCs on the other.

It is important to note that our studies directly comparing UCB- and BM-derived EPCs indicate that the cells have equivalent functionality and demonstrate that a simple isolation technique, the collection of adherent cells after 7 days of culture of whole MNCs, produces a cell population with a functional vasculogenesis efficacy equivalent to that of BM-derived EPCs in the NOD/SCID vascular injury model. UCB has several advantages over BM in potential applications, including UCB collection at no risk to the donor, greater accessibility for storage, immediate availability in a bank, wider availability of diverse HLA genotypes, and, importantly, a lower pathogen content. Particularly attractive is the wide availability of UCB, with >4 million live births every year in the United States alone; this would allow realistic clinical approaches using this stem cell source to treat ischemic cardiac disease, still a leading cause of death in the United States [46].

Moreover, prior reports indicate that adult stem cells, including hematopoietic, neural, mesenchymal, and cardiac stem cells, diminish in number and function with increasing age [47-50]. EPCs grown from the BM of older patients may not yield consistently satisfactory cell numbers for transplantation and neovascularization for individual patient treatment. UCB has been shown to contain hematopoietic stem cells of higher proliferative capacity than those from the BM of healthy donors [51] and has been used successfully in the treatment of hematologic disorders [52]. UCB may be considered an alternative to BM in the cardiac clinical setting. Further studies are ongoing to determine the specific cell subpopulations with the highest EPC biologic function, the relative contributions of direct cell-mediated versus paracrine effects underlying neovascularization in response to vascular injury, and the immunogenicity of cells derived from UCB.

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