Donor Origin of Circulating Endothelial Progenitors After Allogeneic Bone Marrow Transplantation

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ABSTRACT

Endothelial cell precursors circulate in blood and express antigens found on hematopoietic stem cells, suggesting that such precursors might be subject to transplantation. To investigate, we obtained adherence-depleted peripheral blood mononuclear cells from 3 individuals who had received a sex-mismatched allogeneic bone marrow transplant (BMT) and cultured the cells on fibronectin-coated plates with endothelial growth factors. The phenotype of the spindle-shaped cells that emerged in culture was characterized by immunofluorescent staining, and the origin of the cells was determined using a polymerase chain reaction (PCR)-based assay for polymorphic short tandem repeats (STRs). The cells manifested a number of endothelial characteristics—such as von Willebrand factor, CD31, and Flk-1/KDR expression; *Bandeiraea simplicifolia* lectin 1 binding; and acetylated low-density lipoprotein uptake—but lacked expression of certain markers of activation or differentiation, including intercellular adhesion molecule-1, vascular cell adhesion molecule-1, and the epitope for the anti–endothelial cell antibody P1H12. For each patient and at all time points studied (ranging from 5 to 52 months after transplantation), STR-PCR analysis showed that cultured cells and nucleated blood cells came exclusively from the bone marrow donor. These results demonstrate that circulating endothelial progenitors are both transplantable and capable of long-term repopulation of human allogeneic BMT recipients.

KEY WORDS

Endothelial progenitor cells • Allogeneic bone marrow transplantation • Chimerism

INTRODUCTION

Hematopoietic and endothelial cells are among the earliest cell types to differentiate in embryonic development. Their common site of origin in the yolk sac, multiple shared immunophenotypic markers, and concurrent involvement by several spontaneous or induced mutations in model organisms suggest that these 2 cellular lineages derive from a single precursor, the hemangioblast [1]. The subsequent differentiation of hemangioblasts into committed endothelial progenitors, or angioblasts, capable of contributing to the formation of blood vessels was considered to occur only in embryogenesis. However, recent studies have demonstrated the presence of endothelial precursor cells in umbilical cord blood [2] and the peripheral blood of adults [3].

Because these circulating endothelial progenitors apparently could be purified on the basis of their expression of membrane antigens also found on hematopoietic stem cells [2-4], we speculated that they might be transplantable in allogeneic bone marrow transplant (BMT) recipients. To investigate, we obtained peripheral blood mononuclear cells from 3 individuals 5 to 52 months after a sex-mismatched, HLA-compatible BMT. We cultured the cells on a fibronectin surface with endothelial growth factors, characterized the phenotype of the cells that emerged, and determined their origin using a polymerase chain reaction (PCR)-based assay for polymorphic short tandem repeats (STRs).

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MATERIALS AND METHODS

Experimental Subjects

Approval to conduct this study was given by the Institutional Review Board of Vanderbilt University Medical Center and the Research and Development Committee of the Nashville Veterans Affairs Medical Center, both in Nashville, Tennessee. Three men who had received an HLA-matched BMT from a sibling donor, in each case a sister, were enrolled. Subject 1, a 52-year-old man with chronic myelogenous leukemia, was studied 52 months after transplantation; subject 2, a 50-year-old man with mantle cell lymphoma, was studied 5 months after transplantation; and subject 3, a 43-year-old man with acute nonlymphocytic leukemia, was studied 38 months after transplantation. Subject 1 had mild chronic graft-versus-host disease (GVHD), and subject 3, although without clinical evidence of GVHD, was receiving a tapering course of immunosuppressive therapy at the time he was studied. Two-color fluorescence in situ hybridization (FISH) analysis of peripheral blood or bone marrow samples showed complete donor chimerism of hematopoietic cells in all 3 subjects (data not shown).

Rea gents and Antibodies

1,1′-Dioctadecyl-3,3,3′,3′-tetramethyl-indocarbocyanine–labeled acetylated low density lipoprotein (DiI-acLDL) was purchased from Molecular Probes (Eugene, OR). Biotinylated *Bandeiraea* (*Griffonia*) *simplicifolia* lectin 1 (BS-1 lectin) was purchased from Vector Laboratories (Burlingame, CA). Monoclonal antibodies 45C6A and 30Q8A to human cadherin-5 (vascular endothelial [VE] cadherin) were provided by ICOS (Bothell, WA) as hybridoma supernatants. Rabbit polyclonal antibody to zonula occludens-1 (ZO-1) was purchased from Zymed (San Francisco, CA). Mouse monoclonal antibodies JC/70A [5] to CD31 (PECAM-1), PD7/26 and 2B11 [6] to CD45, and KP1 [7] to CD68, along with rabbit polyclonal antibody to human von Willebrand factor (vWF), were purchased from Dako (Carpinteria, CA). Monoclonal antibodies CH-11 to Flk-1/KDR, P1H12 [8] to mature endothelial cells, and P2A4 to intercellular adhesion molecule-1 (ICAM-1, or CD54) were from Chemicon International (Temecula, CA). Monoclonal antibody HAE-2 to vascular cell adhesion molecule-1 (VCAM-1, or CD106) was obtained from Oncogene Research Products (Cambridge, MA). Biotinylated secondary antibodies and streptavidin–indocarbocyanine-3 (Cy3) conjugates were purchased from Jackson ImmunoResearch (West Grove, PA).

Isolation and In Vitro Culture of Endothelial Pr ogenitors

Forty milliliters of anticoagulated peripheral blood was collected from each patient and diluted 1:3 with phosphate-buffered saline (PBS) containing 5 mmol/L EDTA and 0.5% bovine serum albumin (BSA) (Life Technologies, Gaithersburg, MD). Mononuclear cells were obtained by density gradient centrifugation (Ficoll-Hypaque; ICN Biochemicals, Aurora, OH), subjected to 3 cycles of red cell lysis, washed with PBS containing 5 mmol/L EDTA and 0.5% BSA, and resuspended in 0.3 mL of this solution. The resulting suspension was diluted to a concentration of 1.3×10^6 cells/mL in Medium 199 (Life Technologies) with 20% fetal bovine serum (Life Technologies), 12 µg/mL bovine brain extract (Clonetics, San Diego, CA), 50 µg/mL gentamicin, and 50 ng/mL amphotericin B. Cells were added in a volume of 15 mL to 10-cm plastic tissue culture dishes and incubated overnight at 37°C. The cells that had not adhered to plastic were then transferred with medium to 10-cm fibronectin-coated dishes (Becton Dickinson, Bedford, MA). Every other day, cultures were fed and nonadherent cells removed by complete changes of medium. Cells used for immunophenotyping were washed with calcium- and magnesium-free PBS, trypsinized, and replated in fresh medium at a concentration of 2.0×10^5 cells/mL on fibronectin-coated 16-well slide chambers (Nunc Lab-Tek; Nalge Nunc International, Rochester, NY). In cultures terminated for DNA extraction, cells were trypsinized and processed as described below.

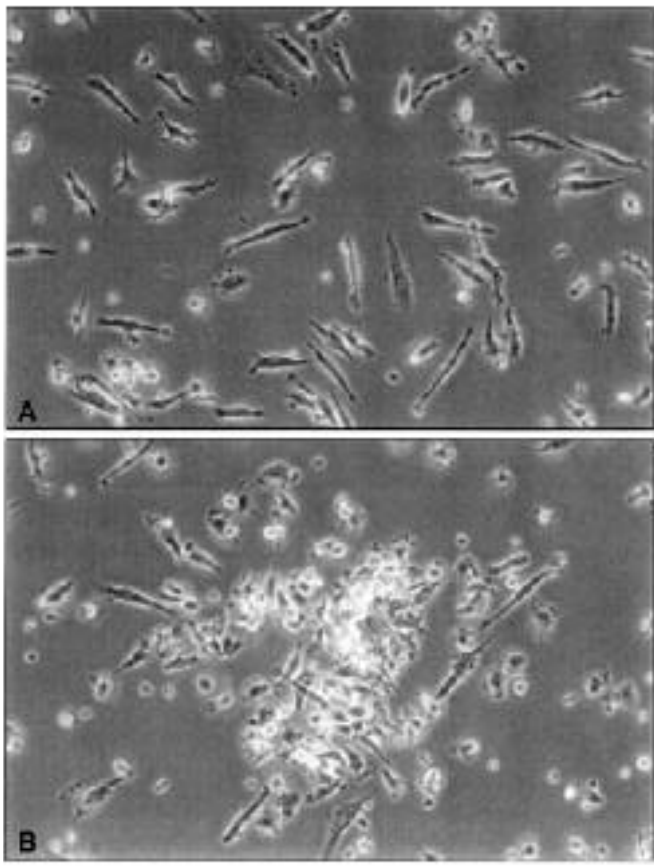
Phenotypic Characterization of Cultured Cells

The fibronectin-adherent cells that remained after 6 days in culture were trypsinized and transferred to fibronectin-coated microwell culture slides for staining. For analysis of acLDL uptake, DiI-acLDL was diluted in serum-free Medium 199 to a concentration of 1 µg/mL and added to wells in aliquots of 200 µL. Cells were incubated for 4 hours at 37°C and visualized by both phase-contrast and fluorescence microscopy.

Immunofluorescent staining was carried out on fixed slides as described [9]. Briefly, microwell cultures were washed twice with 37°C calcium- and magnesium-free PBS, incubated in prewarmed 4% paraformaldehyde for 5 minutes, and washed 3 times with calcium- and magnesium-free PBS. When indicated, cells were permeabilized with 250 mmol/L ammonium sulfate, 10 mmol/L piperazine-*N*,*N*′-bis(2-ethanesulfonic acid) at pH 6.8, 50 mmol/L NaCl, 300 mmol/L sucrose, 3 mmol/L $MgCl_2$, and 0.5% Triton X-100 and washed with calcium- and magnesium-free PBS. After permeabilization, slides were blocked with 2% goat serum in PBS for 30 minutes at room temperature, treated with an additional blocking reagent (Vector Laboratories) to minimize nonspecific binding of biotin/avidin reagents, and incubated with biotinylated BS-1 lectin diluted 1:45 in blocking solution. Cells were incubated with antibodies against cadherin-5, ZO-1, vWF, CD31, Flk-1/KDR, CD68, CD45, ICAM-1, VCAM-1, and P1H12 for 1 hour at room temperature and washed 3 times with calcium- and magnesium-free PBS. They were then incubated with a biotin-conjugated secondary antibody for 30 minutes, then with Cy3-conjugated streptavidin for 30 minutes or, for lectin binding, Cy3-conjugated streptavidin directly. Coverslips were applied with Vectashield mounting medium (Vector Laboratories), and the cells were examined under a Zeiss Axioplan microscope equipped for epifluorescence. Images were captured using a digital camera with Image-Pro Plus software (Media Cybernetics, Silver Spring, MD). Human umbilical vein endothelial cells (HUVECs) (Clonetics) were used as positive controls for expression of ICAM-1, VCAM-1, cadherin-5, and P1H12; peripheral blood mononuclear cells were used as positive controls for expression of CD45 and CD68.

Allelotyping Analysis of Cultured Cells

Genomic DNA was isolated from blood (QIAmp Blood Kit; Qiagen, Valencia, CA) and cultured cells (QIAamp Tis-



Figur e 1. *Morphology of blood-derived endothelial progenitors. Phase contrast photomicrograph of individual spindle-shaped cells (A) and a cluster of these cells (B) obtained 7 days after culturing plastic adherence–depleted peripheral blood mononuclear cells on fibronectin-coated dishes with endothelial growth factors. Original magnification ×20.*

sue Kit; Qiagen) using a silica gel method according to the manufacturer's instructions. For subject 2, DNA was obtained from nucleated peripheral blood cells before transplantation, whereas for subject 3, germline DNA was prepared from a buccal swab (MasterAmp Buccal Swab DNA Extraction Kit; Epicentre Technologies, Madison, WI). In the remaining patient (subject 1), gingival bleeding, possibly related to chronic GVHD, precluded collection of a buccal sample that was not also contaminated with peripheral blood. For all 3 BMT recipients, DNA was extracted from nucleated peripheral blood leukocytes at the time blood was collected for cell culture and also from the cells remaining after 10 days of culture. DNA concentrations were determined fluorometrically (DyNA Quant 200; Hoefer Pharmacia Biotech, San Francisco, CA) using a dye-binding method.

Fluorochrome-conjugated primers for the STR loci TH01, TPOX, CSF1PO, F13A1, FES/FPS, FGA, and D21S11 and for the X-Y homologous gene amelogenin were purchased from Perkin-Elmer (now PE Biosystems, Foster City, CA). The loci to which these primers anneal contain short (3– to 7–base pair [bp]), tandemly repeated sequences and are highly polymorphic in the number of such repeats present. Amplification of β-globin sequences was performed before STR-PCR analysis to ensure that sample DNAs could be efficiently amplified. PCR was carried out in glass capillary tubes in an air-driven thermal cycler (Rapid Cycler; Idaho Technologies, Idaho Falls, ID) using 25 ng genomic DNA in a 10-µL reaction volume containing 5.0 µL sample DNA, 1.0 µL H_2O, 1.0 µL of 10× buffer, and 1.0 µL deoxynucleoside triphosphate mixture (each at a concentration of 2 mmol/L); 1.0 µL primer mixture (each at a concentration of 10 µmol/L); and 1.5 U Taq polymerase (Life Technologies). Denaturation, annealing, and extension times were optimized for each primer pair, with a total of 30 cycles used in all reactions. After amplification, 1.5 µL reaction mixture was combined with 4.5 µL gel loading buffer (75% formamide, 5 mmol/L EDTA, and 0.1% blue dextran, pH 8.0), heated to 95°C, and chilled on ice. Samples were loaded in a volume of 1.0 µL/well on 4.25% acrylamide–8 mol/L urea gels in an ABI 377 automated DNA sequencer (Perkin-Elmer) and electrophoresed for a minimum of 8 hours. Fluorescence DNA fragment analysis was carried out with the GeneScan Analysis (version 2.1) software package (Perkin-Elmer). Dedicated pipettes with disposable, barrier-style tips were employed with all solutions used for DNA extraction or PCR, and control samples lacking DNA template were included in every PCR reaction to monitor for contamination. Under the conditions employed, the amelogenin primers were capable of detecting a subpopulation of male cells present in a frequency of ≤10% in mixtures of cells from male and female subjects (data not shown).

RESULTS

Cell Culture

Small, spindle-shaped cells possessing the phenotype of immature endothelial cells (see below) first became evident 3 to 4 days after inoculating fibronectin-coated dishes with adherence-depleted peripheral blood mononuclear cells. By 5 to 6 days, few to no cells were removed with medium changes, and after a week in culture, a homogeneous population of adherent, spindle-shaped cells representing some 0.1% to 0.3% of total mononuclear cells initially plated were present. Cells were generally distributed over the surface of the culture dish (Figure 1A), although small clusters (Figure 1B) or linear arrays (not shown) of cells were occasionally seen. The appearance of these spindle-shaped cells was identical to that described by others [3,10,11].

Phenotypic Characterization of Blood-Derived Endothelial Cells

After their transfer to fibronectin-coated microwell slides, cells were characterized for uptake of acLDL and expression of a number of immunophenotypic markers. More than 90% of cultured cells exhibited DiI-acLDL incorporation, BS-1 lectin binding, and immunofluorescent staining for vWF, ZO-1, and CD31 expression (Figure 2 and Table 1). Consistent with the apparent absence of connections between cells, the junctional protein ZO-1 was observed in an intracellular location [12]. Weak staining was also noted for vascular endothelial growth factor (VEGF) receptor-2 (Flk-1/KDR), which increased with the addition of recombinant VEGF to cultures (not shown). No staining was noted with antibodies to the monocyte/macrophage marker CD68, the leukocyte common antigen CD45, or

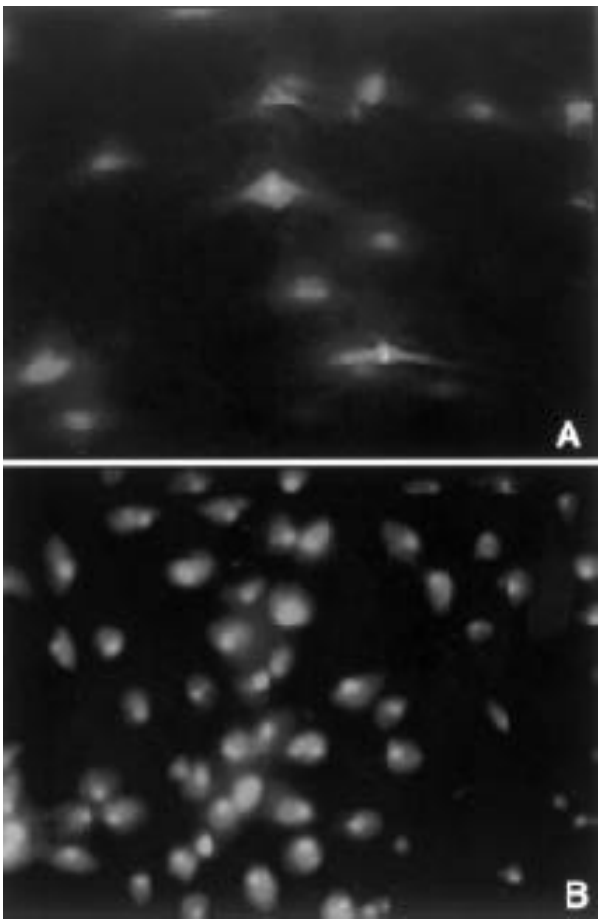


Figure 2. *Selected results of phenotyping analysis of blood-derived endothelial progenitors. Fluorescence photomicrograph of 1,1′-dioctadecyl-3,3,3′,3′-tetramethyl-indocarbocyanine–labeled acetylated low density lipoprotein uptake (A) and von Willebrand factor expression (B) by spindle-shaped cells cultured as described in text. A considerably greater number of cells were present in the well shown in B compared with the well shown in A.*

ICAM-1, VCAM-1, cadherin-5, or P1H12. All these antibodies reacted with HUVEC or peripheral blood mononuclear cell controls processed in parallel. Because of the considerable phenotypic overlap between endothelial and hematopoietic cells, none of the markers used could be considered endothelial specific. In aggregate, however, these results indicate that under the specific culture conditions employed, a homogeneous cell population with endothelial characteristics proved capable of proliferating, differentiating, or both.

Genotypic Analysis of Blood-Derived Endothelial Cells

Each patient in this study had received a sex-mismatched transplant, which assured that, at minimum, the primers for the X- and Y-linked amelogenin locus [13] would be informative in determining the origin of their cells. In subject 2, from whom DNA was isolated before transplantation, and in subject 3, from whom a buccal sample free of blood contamination was obtained, 7 primer pairs for STRs, in addition to the amelogenin primers, were tested for their ability to discriminate between donor and recipient. Two or more STR loci were identified in which one or both alleles were unique to donor or recipient (Figure 3 and Table 2). Only the primer set for the amelogenin locus was used in the 1 patient (subject 1) from whom neither pretransplantation nor germline DNA was available.

STR-PCR analysis of peripheral blood leukocyte DNA confirmed FISH results showing complete donor chimerism of hematopoietic cells (Table 2). Because both lymphoid and myeloid cells were present in the peripheral blood of these subjects at the time they were studied (ie, 5 to 52 months after transplantation), it is probable that complete lympho-hematopoietic reconstitution had occurred in all 3. PCR analysis of DNA from cultured cells similarly revealed that each patient's circulating endothelial progenitors were derived exclusively from his bone marrow donor (Figure 3 and Table 2).

Validation that these methods could have detected mixed chimerism, had it been present, was obtained by analysis of

Table 1. *Summary of Results of Phenotypic Analysis of Cultured Cells**

Antigen	Antibody or Ligand	Staining, Binding, or Uptake
von Willebrand factor	Rabbit polyclonal antibody	Strongly positive
CD31 (PECAM-1)	Monoclonal antibody JC/70A	Strongly positive
Scavenger receptor	DiI-AcLDL	Strongly positive
Endothelial α-D-galactosyl epitope	***Bandeiraea simplicifolia*** lectin	Strongly positive
ZO-1	Rabbit polyclonal antibody	Positive
Flk-1/KDR	Monoclonal antibody CH-11	Weakly positive
Activated/circulating endothelial cells	Monoclonal antibody P1H12	Negative
CD106 (VCAM-1)	Monoclonal antibody HAE-2	Negative
CD54 (ICAM-1)	Monoclonal antibody P2A4	Negative
Cadherin-5 (VE cadherin)	Monoclonal antibodies 45C6A and 30Q8A	Negative
CD45 (leukocyte common antigen)	Monoclonal antibodies PD7/26 and 2B11	Negative
CD68 (human macrophage)	Monoclonal antibody KP1	Negative

*Results of the immunophenotypic analysis carried out on cultured cells. Immunofluorescence staining with the antibodies indicated, *Bandeiraea simplicifolia* lectin binding, and 1,1′-dioctadecyl-3,3,3′,3′-tetramethyl-indocarbocyanine–labeled acetylated low density lipoprotein (DiI-acLDL) uptake are described in the text. Human umbilical vein endothelial cells (HUVECs) and Ficoll-Hypaque–purified peripheral blood mononuclear cells were processed in parallel as controls; HUVECs were used as controls for P1H12, CD106, CD54, and cadherin-5 staining; Ficoll-Hypaque–purified peripheral blood mononuclear cells were used as controls for CD45 and CD68 staining.

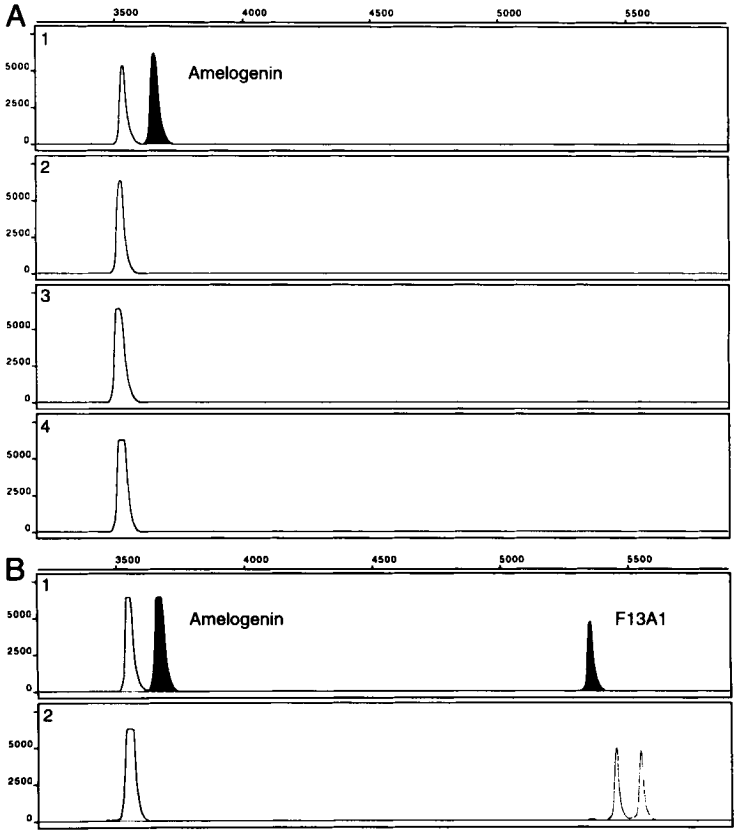


Figure 3. *Selected results of allelotyping analysis of blood-derived endothelial progenitors. A. Electrophoretogram of short tandem repeat–polymerase chain reaction (STR-PCR) results from subject 1 using primers for the X-Y homologous gene amelogenin. Panel 1 depicts allelotype of this recipient obtained with peripheral blood leukocyte DNA extracted before transplantation, with the unique 106–base pair (bp), Y chromosome–derived allele shown shaded. Fifty-two months after transplantation, only the 112-bp, X chromosome–derived allele characteristic of the female marrow donor (panel 2) was detected on PCR analysis of DNA prepared from nucleated peripheral blood cells of the marrow recipient (panel 3) and of DNA prepared from the spindle-shaped cells cultured from that subject's blood (panel 4). B. Composite electrophoretogram of STR-PCR results from subject 2 using primers for the amelogenin locus and for an STR in the factor XIII A subunit gene (F13A1). Alleles unique to the patient's cells before transplantation are shown shaded (panel 1). These alleles were undetectable 5 months after transplantation, with only the 112-bp, X chromosome–derived amelogenin allele and the 2 donor-specific F13A1 alleles detected on PCR analysis of DNA prepared from cultured spindle-shaped cells (panel 2) and nucleated peripheral blood cells (data not shown). The scale to the left of each electrophoretogram indicates fluorescence intensity. The scale at the top corresponds to the distance fragment migrated in the gel.*

defined mixtures of peripheral blood leukocyte DNA from bone marrow donors and recipients before transplantation and of nucleated blood cell DNA prepared from individual recipients at various times before hematopoietic reconstitution (data not shown). Indeed, as reported for other fluorescence-based assays of chimerism [14,15], a linear correlation was observed between the amount of a given DNA species in sample mixtures and that determined by PCR to be present (data not shown). These results indicate that the circulating hematopoietic cells and endothelial progenitors in these allogeneic BMT recipients were donor derived within 5 months and remained so for at least 4 years after transplantation.

DISCUSSION

These studies confirm that endothelial precursor cells are present in the peripheral blood of humans [3,16] and establish that such cells are transplantable in allogeneic BMT recipients. Their existence had been suspected from the observation that synthetic vascular grafts often become seeded with endothelial cells on their luminal surfaces [17,18] and from the finding that direct application of bone marrow cells to such grafts was particularly effective in promoting their endothelialization [19]. While this study was in progress, published results of experiments in canine [20] and murine [4,21,22] BMT recipients indicated that endothelial precursors that homed to intravascular grafts (canine recipients) and circulated in blood (murine recipients) similarly derived from the marrow donor, although those experiments were carried out at short intervals from transplantation. Because aspiration of peripheral blood is nearly unavoidable in human bone marrow harvests, the possibility that a circulation- rather than bone marrow–derived progenitor was transplanted cannot be excluded. At minimum, however, these results establish that the source of endothelial precursor cells in circulation is transplantable and emphasize the potential of such cells for long-term engraftment.

Because our subjects had undergone complete lymphohematopoietic reconstitution with donor-derived cells by the time of analysis, it was particularly important to distinguish

Table 2. *Summary of Results of Allelotyping Analysis of Cultured Cells*

	Subject 1	Subject 2	Subject 3
Interval from bone marrow transplantation to analysis, mo	52	5	38
Informative markers	Amelogenin	Amelogenin, TPOX, F13A1, FGA	Amelogenin, THO1
Origin of nucleated blood cells	Donor	Donor	Donor
Origin of cultured cells	Donor	Donor	Donor

the cells that we cultured from hematopoietic cells. Using the identical conditions employed by Asahara et al. [3], but with much more highly purified $CD34^+$ cell populations (data not shown), we were unsuccessful at culturing any endothelial cells. It may be significant, however, that Asahara et al. found readdition of $CD34^-$ cells to result in a much higher yield of spindle-shaped endothelial cells than could be obtained with $CD34^+$ cells alone [3]. Despite starting with a mononuclear cell preparation depleted only of cells that would adhere to plastic, the cells that ultimately populated our cultures were morphologically and immunophenotypically homogeneous. This finding suggests that the growth factors, fibronectin substratum, and possible paracrine effects of other cells initially present in culture restricted the growth and differentiation of all but endothelial cells. However, even if a small number of hematopoietic cells had persisted until the time the cultures were terminated, the only circumstance under which such contamination could have accounted for the observed complete donor chimerism of the cultured cells would have been for all such cells to have been hematopoietic.

The endothelial nature of the cultured cells was confirmed by their (1) expression of CD31 and vWF, (2) binding of a lectin (BS-1) widely recognized by endothelial cells, and (3) avid uptake of acLDL. Although the scavenger receptor [23] and vWF [24] are also expressed by macrophages and megakaryocytes, respectively, and CD31 is expressed by certain hematopoietic cells [25-27], the lack of CD68 and CD45 expression in the cells that emerged in culture distinguished them from lymphocytes and macrophages present in circulation. Furthermore, their expression of CD31 and vWF differentiated them from mesenchymal stem cells [28], which are present in bone marrow and capable of giving rise to multiple cell types, possibly including endothelial cells [29]. In addition, mesenchymal stem cells are not mobilized into peripheral blood by colony-stimulating factors [30], in contrast to mouse [4] and human endothelial progenitors [31] (our unpublished data) and, at least in patients with lysosomal and peroxisomal storage diseases, appear not to be transplantable with allogeneic bone marrow [32]. Finally, although endothelial cells have themselves been observed to circulate in blood, particularly under conditions of vascular injury [8,33-36], they are normally present in very small numbers [8,37] and express antigens (in particular the epitope recognized by the P1H12 monoclonal antibody [8]) that were not identified in the population of cells we cultured. Still, although the spindle-shaped cells we and others have cultured from plastic adherence–depleted mononuclear cell preparations [10,11] and mixtures of $CD34^+$ and $CD34^-$ cells [3] are clearly endothelial in type, they are not identical, morphologically or immunophenotypically, to mature endothelial cells. Whether they represent the adult counterparts of an embryonic Flk-1^+ $PECAM^+$ VE cadherin$^-$ endothelial precursor [38], are at an intermediate stage of differentiation because of specific culture conditions [10], or, as suggested by xenograft studies [3], are themselves angioblasts will require further study.

Three recent studies provide additional support for the existence of circulating endothelial progenitor cells in humans [10,31,39]. Although each study used different culture conditions, cells possessing the appearance and immunophenotype of endothelial cells emerged 18 days [10], 2 weeks [31], and 9 days [39] after initiation of cultures with $CD34^+$ [10], $AC133^+$ Flk-1/KDR^+ [31], and $P1H12^+$ [39] peripheral blood cells. Lin et al. [39], in fact, studied 4 recipients of sex-mismatched BMTs 5 to 20 months posttransplantation. In contrast to our results, however, endothelial cells of both donor and recipient genotype were noted in their cultures, although donor cells showed the greatest expansion in cell number at late times [39]. Previous studies by Solovey et al. [8] documented the presence of mature, P1H12-reactive endothelial cells in circulation, and although they avoided any possibility of monocyte contamination, their use of P1H12-conjugated immunomagnetic beads to isolate endothelial progenitors may account for the mixed chimerism of their endothelial cell cultures.

Our finding that circulating endothelial progenitors in human BMT recipients were completely donor derived more than 4 years after transplantation indicates that the cell from which they arose must be either long-lived or capable of self-renewal. Murray [40] posited a common precursor for hematopoietic and endothelial cells in embryonic development, and a cell with this developmental capacity was recently purified from gastrulation-stage mesoderm in chick embryos [41] and murine embryonic stem cell–derived embryoid bodies [42]. Whether the hemangioblast is also present in adults, however, awaits studies in which it is genetically marked and that marker identified in both endothelial and hematopoietic descendants. Independent of whether there are common or independent stem cells for these lineages, the endothelial precursors present in the blood of these human allogeneic BMT recipients appear to have long-term repopulating ability. Remarkably, adult bone marrow also contains progenitors for a number of mesenchymal [28,43] and even epithelial [44] cell types.

The capacity of circulating human endothelial cell precursors to contribute to blood vessel repair or assembly is unknown, although their murine and canine counterparts have been shown to traffic to sites of physiologic and pathologic neovascularization [3,4,21,22] and to be deposited on intravascular grafts [20]. Although the finding that circulating endothelial precursors are capable of long-term repopu-

lation suggests that they could be used for adoptive cell transfer, with [45] or without genetic modification, more work is needed to determine these precursors' relationship to hematopoietic stem cells, their site of production, and the vascular beds to which they are able to contribute.

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