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Nasal Epithelial Cells of Donor Origin after Allogeneic Hematopoietic Cell Transplantation are Generated at a Faster Rate in the First 3 Months Compared with Later Posttransplantation

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Detection of donor-type epithelial cells (ECs) after allogeneic hematopoietic cell transplantation (allo-HCT) using XY chromosome fluorescein in situ hybridization (FISH) has suggested that hematopoietic stem cells carry a degree of developmental plasticity. This is controversial, given artifacts of XY-based detection and the possibility of hematopoietic–nonhematopoietic cell fusion. Moreover, the kinetics of donor-type ECs (quantity at different time points after transplant) is unknown. Here, we document unequivocally the existence of donor-type ECs using a method obviating the artifacts of XY-FISH and study their kinetics. Nasal scrapings and blood specimens were collected from 60 allo-HCT survivors between 7 days and 22 years posttransplantation. DNA extracted from laser-captured nasal ECs (ie, CK^+CD45^- cells) and blood leukocytes was polymerase chain reaction–amplified for a panel of 16 short tandem repeat markers. The median percentage of donor-type ECs (among nasal ECs) was 0% on day 7 posttransplantation, 2.8% at 3 months posttransplantation, and 8.5% at 12-22 years posttransplantation. Cell fusion was ruled out by FISH analysis for two autosomes. We conclude that donor-type nasal ECs exist after HCT, and that their percentage rises rapidly in the first 3 months posttransplantation and more slowly thereafter.

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INTRODUCTION

The classical dogma of stem cell hierarchy, according to which the property of pluripotency is restricted to the embryonic stem cells, has recently been challenged by studies reporting the presence of donor-type nonhematopoietic cells after human allogeneic hematopoietic cell transplantation (allo-HCT) (reviewed by Spyridonidis et al. [1]). There is increasing evidence that adult hematopoietic stem cells (HSCs) can differentiate into nonhematopoietic cells, including nonmesodermal cells (cells that in normal embryogenesis originate from the ectoderm or endoderm) [1,2]. Using XY chromosome–based detection methods, donor-derived nonhematopoietic cells have been documented in the lungs [3], skin [4], intestine [5], liver [6], brain [7], conjunctiva [8], endometrium [9], and buccal mucosa [10] of female recipients of HSC grafts from male donors. Donor-type epithelial cells (ECs) have been typically identified as Y chromosome–containing cells expressing an epithelial lineage marker (eg, cytokeratin [CK]) and not expressing a hematopoietic lineage marker (eg, CD45). The presumed transdifferentiation of HSCs may be a repair response to graft-versus-host disease (GVHD)-induced epithelial tissue injury [4], although donor-derived ECs also have been reported in patients without a history of GVHD [6,10].

Despite the evidences, however, some investigators consider transdifferentiation or even the existence of donor-derived ECs contentious (reviewed by Bianchi and Fisk [11]). Initial skepticism involved methodological limitations (eg, assigning Y chromosome to ECs when in fact it should have been assigned to an intraepithelial lymphocyte). These limitations were significantly addressed in later reports [12]. However, the

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primary reason for the persistent skepticism is fetomaternal chimerism [11]; male fetal cells have been identified in 48% of healthy parous women [13] as long as 27 years after their last pregnancy with a male offspring [14]. Moreover, the presence of fetomaternal chimerism results not only from successful pregnancies, but also from abortions/miscarriages [11]. In addition, the ability of HSCs to transdifferentiate into ECs has been repudiated by investigators claiming that the fusion of donor hematopoietic cells with recipient ECs is the reason for the detection of seemingly donor-derived ECs [9,15]. Thus, it seems appropriate to reassess whether donor-type ECs in HCT recipients truly exist, using a method that is not based on Y chromosome detection and ruling out cell fusion.

Finally, assuming that the transdifferentiation of donor-type ECs is in fact real, its kinetics remain unknown. Using a method that circumvents the limitations of Y chromosome–based donor–recipient distinction, we set out to determine whether donor-type ECs are truly present in the nasal mucosa (an organ not typically involved in GVHD) and, if so, to measure their quantity at different posttransplantation time points.

METHODS

Subjects and Specimen Collection

A total of 60 subjects were accrued for the study, which includes both short-term (7-98 days; n = 32) and long-term (6-22 years; n = 28) survivors of myeloablative allo-HCT. Written informed consent was obtained from each subject. The University of Calgary's Ethics Committee approved the study design. The subjects' demographic and clinical data are summarized in Table 1.

Both peripheral blood and nasal scrapings were collected at approximately 1 week, 1 month, 2 months, 3 months, 6-10 years, or 12-22 years posttransplantation. In most subjects, sampling was done only once; in 5 subjects, samples were collected at multiple time points. Nasal scrapings were collected by first cleaning the nose with sterile saline swab and then gently but firmly twirling a sterile curette (Rhino PRO, Arlington-scientific, Inc. USA) on the inferior nasal turbinate.

Cytospinning, Immunocytochemistry, and Laser Capture Microscopy

Nasal scraping specimens were diluted with 1% PBS to maximize the possibility of obtaining single cells after cytospinning. Between 8 and 10 cytospin slides were prepared for each specimen by centrifuging 500 μL of cell suspension on each slide at 5000 RPM using a Cytospin Cytocentrifuge. The cytospun nasal cells were then fixed using xylene. All slides from each individual subject were then subjected to immunocytometry, except in 10 subjects, for whom one slide each was used for the detection of cell fusion. Dual-color immunocytochemistry was performed using the enzymes alkaline phosphatase and horseradish peroxidase and their substrates, 5-bromo-4-chloro-3'-indolyphosphate p-toluidine salt

Table 1. Demographic and Clinical Information

Characteristic	Distribution and Range	
	Short-Term Survivors	Long-Term Survivors
n	32	28
Time after transplantation, days	Median, 56 days; range, 7-98 days	Median, 10 years; range, 6-22 years
Sex	Male, n = 14; female, n = 18	Male, n = 18; female, n = 10
Age at transplantation, years	Median, 53 years; range, 19-65 years	Median, 42 years; range, 18-59 years
Age at the time of sample collection, years	Median, 52 years; range, 20-66 years	Median, 51 years; range, 31-68 years
Graft source	PBSCs, n = 30; cord blood, n = 2	Bone marrow, n = 18; PBSCs, n = 7
Disease	AML, n = 11; CML/CMML, n = 2; ALL, n = 5; CLL/lymphoma, n = 13; MF, n = 1	AML, n = 5; CML, n = 8; ALL, n = 7; CLL/lymphoma, n = 6; MM, n = 1; SAA, n = 1
Donor type*	Related, n = 16; unrelated, n = 16	Related, n = 21; unrelated, n = 7
Conditioning†	Flu + Bu + TBI, n = 18; Flu + Bu, n = 13; VP16 + TBI, n = 1	Cy + TBI, n = 6; Cy + Bu, n = 8; Cy, n = 1; Flu + Bu, n = 11; VP16 + TBI, n = 2
GVHD prophylaxis	CsA‡ + MTX§ + ATG¶, n = 32	CsA + MTX, n = 15; MTX + steroid, n = 2; CSA + MTX + ATG, n = 11
Acute GVHD	Yes, 13; no, 19	Yes, 10; no, 18
Chronic GVHD‖	NA	Yes, 18; no, 7

NA indicates not applicable; AML, acute myelogenous leukemia; ALL, acute lymphogenous leukemia; CML, chronic myelogenous leukemia; CLL, chronic lymphocytic leukemia; MM, multiple myeloma; NHL, non-Hodgkin lymphoma; SAA, severe aplastic anemia; MDS, myelodysplastic syndrome; CMML, chronic myelomonocytic leukemia; MF, myelofibrosis; Flu, fludarabine; Bu, busulfan; Cy, cyclophosphamide; VP16, etoposide; Mel, mephalan; CsA, cyclosporine A; MTX, methotrexate; ATG, antithymocyte globulin.

*Both related and unrelated donors were typically HLA-matched. This was defined as antigen-matched for HLA-A, -B and -DR for the long-term survivors and as allele-matched for HLA-A, -B, -C, -DRB1, and -DQB1 for the short-term survivors.

†Myeloablative in all subjects, except possibly the one subject with severe aplastic anemia, who received 200 mg/kg cyclophosphamide.

‡Administered for the first 3-6 months posttransplantation, longer in cases of chronic GVHD.

§Administered on days 1, 3, 6, and 11.

¶Administered on days -2, -1 and 0.

‖At any time between transplantation and sample collection. In most subjects, chronic GVHD was inactive at the time of sample collection.

and nitro blue tetrazolium chloride (BCIP-NBT) and 3,3′-diaminodbenzidine (DAB) (Lab Vision, Fremont, CA), respectively, after the cells were stained with monoclonal antibody directed against human CKs (clone AE1/AE3) and CD45/leukocyte common antigens (LCAs). Both ECs (brown CK^+ cells) and leukocytes (blue $CD45^+$ cells) were observed (Figure 1A). After the cells were hydrated, ECs—defined as large brown (CK^+CD45^-) cells clearly separated from small blue ($CD45^+$) cells—were subjected to laser capture microdissection (LCM) using an Arcuturus LaserMicrobeam System (Arcuturus, San Diego, CA) (Figure 1B). A total of 35-60 ECs were laser-captured from each slide, and at least 200 ECs were used for subsequent DNA extraction.

Short Tandem Repeat (STR) Analysis for Chimerism Evaluation

Laser capture microdissected nasal ECs were collected into the lid of a 0.5-mL reaction tube and digested in 20 μL of Tris-EDTA buffer containing proteinase K (20 mg/mL) at 56°C for 12 hours. After denaturation at 100°C for 10 minutes, the DNA was used for individual polymerase chain reaction (PCR) runs. DNA from blood leukocytes was extracted using the Qiagen QIAamp DNA Micro Kit (Qiagen, Hilden, Germany) and denatured at 100°C for 10 minutes.

DNA was PCR-amplified for a commercial panel of 15 autosomal microsatellite markers and an XY-differentiating locus (Identifiler; Applied Biosystems, Foster City, CA, USA) to quantify chimerism using the manufacturer's recommended operating procedures, reagents, and PCR conditions. The fluorochrome-labeled amplicons together with an internal size standard (500-LIZ; Applied Biosystems) were size-fractionated by denaturing capillary electrophoresis on an ABI-3130 genetic analyzer (Applied Biosystems). The allele analysis based on fluorescent peak height was done by Genemapper version 2 software (Applied Biosystems) (Figure 1B).

Stutter peaks, dye-associated peaks, blobs, and spikes were identified and excluded from the analysis. Pretransplantation DNA from donor and recipient were used to determine the informative markers (one with a distinct allelic profile for donor and recipient). Fluorescent measurements were used to estimate the quantity of DNA at an allele of the informative microsatellite marker in the posttransplantation specimen. This DNA estimate served as the basis for computing the relative proportions of the donor and recipient components (Figure 1B). Donor chimerism percentage for each run was determined by calculating the mean chimerism for all informative microsatellite markers using ChimerTrack software [16]. Each DNA sample was analyzed in 3 independent PCR runs, followed by the electrophoretic run. Other stringent parameters, including DNA measurement error, were used to gauge the performance of each microsatellite marker [17]. Our calibration experiments showed an assay sensitivity of 0.1% for detecting the minor allele when we used electrophoretic modifications, such as a 50-second injection time, as described previously [18].

In control experiments, we mixed nasal scrapings from healthy individual A with blood buffy coat cells from healthy individual B and treated this artificially mixed specimen as a nasal specimen from a transplant recipient (ie, staining for CK and CD45, LCM of CK^+CD45^- cells, DNA extraction, and chimerism analysis using STRs). In a set of 5 experiments, we detected a maximum of 0.5% DNA of individual B in the laser-captured ECs from individual A. Thus, all results of nasal EC chimerism in transplant recipients are reported as corrected percentage of donor cells (0.5% subtracted from the mean percent donor chimerism obtained after 3 runs, whenever the donor chimerism was >0.5%).

Circulating leukocytes were of >92% donor origin in all subjects studied at 2 months or later posttransplantation. In subjects studied earlier after transplantation, the median percentage of donor-type leukocytes (of total leukocytes) was 58% (range, 52%-68%) on days 7-8 and 79% (range, 72%-90%) on days 28-32.

Assessment of Hematopoietic–EC Fusion

To examine whether the detection of donor-type nasal ECs is a result of EC fusion with a hematopoietic cell, an additional cytospin slide from 10 subjects was analyzed by combining CK staining with fluorescein in situ hybridization (FISH) for two autosomal chromosomes (chromosomes 7 and 14) and examined for the number of chromosome signals present in all CK^+ cells (Supplementary Figure 1). In case of cell fusion, some CK^+ cells would be expected to have 4 signals (instead of the 2 seen in diploid cells) for each chromosome. Two chromosomes were used for FISH analysis because fusion might lead to the loss of some chromosomes, and thus the detection of two chromosomes should minimize the chance of false-negative results.

Statistical Analysis

Allo-HCT recipients were grouped based on the time after transplantation (day 7-8, day 28-32, day 54-62, day 84-98, 6-10 years, and 12-22 years). The 6-10 year group was further subdivided into a subgroup of peripheral blood stem cell transplantation (PBSCT) recipients and bone marrow transplantation (BMT) recipients. All subjects studied between days 7 and 98 posttransplantation received PBSCT, and all those studied at 12-22 years posttransplantation underwent BMT. The significance of the difference in the percentages of donor-type nasal ECs collected at different time points after HCT was tested using the Mann-Whitney-Wilcoxon rank-sum test. A P value of .05 (two-tailed) or less was considered significant.

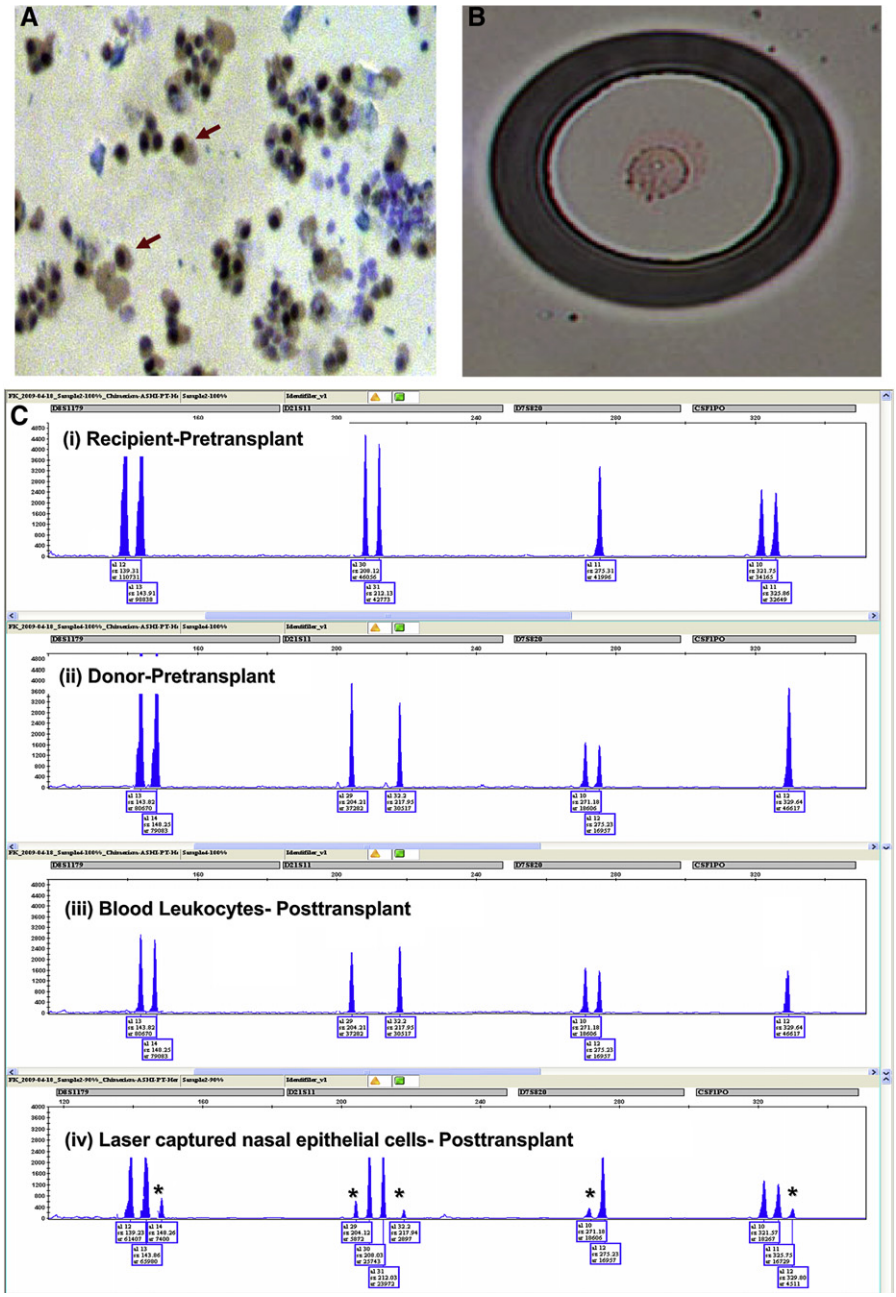

Figure 1. EC chimerism analysis. (A) Dual-color immunocytochemistry performed after staining cytospun nasal mucosal cells with monoclonal antibodies directed against human CKs and CD45. Brown cells represent CK^+ ECs; blue cells, and $CD45^+$ leukocytes. Red arrows indicate single CK^+CD45^- ECs that were used mainly for LCM. (B) A laser-captured single EC (CK^+CD45^- cell). (C) An example of STR-based chimerism analysis. PCR-amplified DNA from the following specimens was studied: (i) recipient PBMCs pretransplantation, (ii) donor peripheral blood mononuclear cells (PBMCs) pretransplantation, (iii) recipient PBMCs posttransplantation (7 years posttransplantation in this example) and (iv) Laser-captured CK^+CD45^- nasal ECs from a recipient at 7 years posttransplantation. The allelic profile is shown at 4 microsatellites (from left to right): D8S1179, D21S11 D7S820, and CSF1PO. The posttransplantation blood specimen showed 100% donor chimerism, and posttransplantation nasal ECs showed 8.2% donor chimerism in this example. An asterisk denotes donor-specific peaks detected in the CK^+CD45^- nasal ECs from the recipient posttransplantation.

RESULTS

Unequivocal Detection of Donor-Type Nasal ECs

Donor-type ECs were detected in 60 of 68 nasal mucosal specimens from allo-HCT recipients (88% of the total specimens analyzed) with the LCM/STR method that precludes artifacts of Y chromosome–based detection. To examine whether donor-type ECs detected by the LCM/STR method resulted from cell fusion, we used a combination of CK staining with FISH for two autosomal chromosomes (chromosomes 7 and 14) and examined the number of chromosome signals present in all CK^+ cells (250-300 cells per specimen) in nasal specimens from 10 allo-HCT recipients. Cells generated by cell fusion in vivo have been reported to

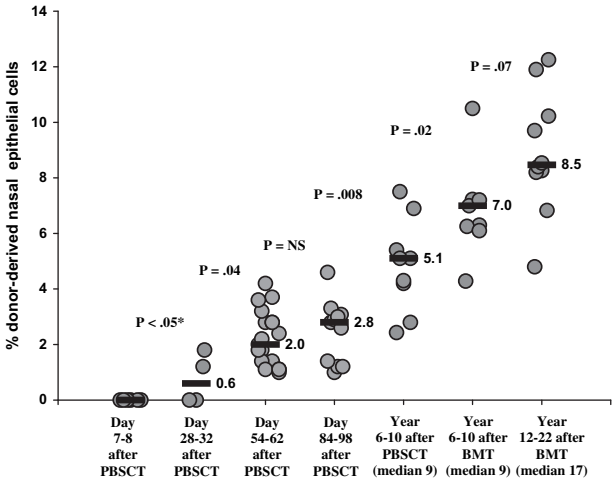

Figure 2. Percentage of donor-type nasal ECs collected from early (day 7-98) and late (6-22 years) posttransplantation recipients. Shown is the percentage of donor-type ECs detected in the nasal specimens among the following group of allo-HCT recipients (circles): day 7-8 (median, 7 days), day 28-32 (median, 30 days), day 56-62 (median, 56 days), day 84-98 (median, 88 days), 6-10 years who underwent PBSCT (median, 9 years) and 6-10 years (median, 9 years) and >10-22 years who underwent BMT (median, 17 years). The horizontal bars represent the median values. The *P* value for significance of the difference between two groups of subjects is shown in the space between the groups. *The *P* value was not calculated between day 7-8 (median, 7 days) and day 28-32 (median, 30 days), because none of the day 7-8 posttransplantation recipients showed donor-type ECs. The *P* value of .05 is an arbitrary value indicating a significant increase in donor-type ECs in day 28-32 posttransplantation recipients.

form polyploid cells known as heterokaryons [19]. If seemingly donor-type ECs were generated by cell fusion, then probes for a single somatic chromosome would target at least 4 chromosomes within the nucleus of those cells. We did not find any ECs with more than two signals for each chromosome in the nuclei of CK+ cells (Supplementary Figure 1). This indicates that the donor-type ECs were truly of donor origin.

Faster Generation of Donor-Type ECs Early Postransplantation Compared with Late Posttransplantation

As shown in Figure 2, the median percentages of donor-type nasal ECs (of all nasal ECs) were 0% on day 7, 0.6% on days 28-32, 2.0% on days 56-62, 2.8% on days 84-98, and 5.1% at 6-10 years (median, 9 years) after PBSCT, and 7.0% at 6-10 years (median, 9 years) and 8.5% at 12-22 years (median, 17 years) after BMT. The main determinant of the percentage of donor ECs was the time after transplantation; note the significant differences between most adjacent time points in Figure 2. The percentage at 6-10 years was slightly, but statistically significantly, higher post-BMT compared with post-PBSCT, perhaps because transferred marrow stromal cells might facilitate transdifferentiation [20]. Other clinical/demographic factors did not appear to play a role; exploratory analyses found no association between the percentage of donor-type nasal ECs and recipient age at the time of transplantation or the time of sampling, sex, donor type (HLA-matched sibling vs unrelated), underlying disease (myeloid vs lymphoid), type of preparative regimen (with vs without total body irradiation [TBI]), or history of acute GVHD (grade 0-I vs II-IV). The percentage of donor-type ECs increased rapidly early after transplantation; no donor-type ECs were found in the subjects evaluated on day 7, whereas 50% of the subjects studied at days 28-32 had detectable donor-type ECs (median, 0.6%), and 100% of the subjects studied between day 56 and day 98 had detectable donor-type ECs (median, 2.0% on days 56-62 and 2.8% on days 84-98). The majority of the subjects who underwent nasal mucosa sampling multiple time points early after transplantation had evidence of a rapidly increasing percentage of donor-type ECs (Figure 3). The increase was slower after 3 months posttransplantation compared with that seen during the first 3 months after transplantation (Figure 2). Specifically, the rate of increase between 1 and 3 months posttransplantation was 2.2% (2.8%-0.6%) per 2 months (ie, 13.20% per year), that between 3 months and 9 years was 2.3% (5.1%-2.8%) per 8.75 years (ie, 0.26% per year), and that between 9 and 17 years was 1.5% (8.5%-7.0%) per 8 years (ie, 0.19% per year).

DISCUSSION

The present study has demonstrated the presence of donor-type ECs in allo-HCT recipients using a method that obviates the artifacts of XY-FISH. It also has shown that the donor-type ECs are primarily generated early after transplantation. Early reports suggesting that human allo-HCT leads to chimerism of not only HSCs, but also ECs, were challenged in 1999, when Endler et al. [21] assumed that the donor DNA found in recipient buccal ECs resulted from hematopoietic "contaminants" among ECs. Nevertheless, subsequent, more carefully conducted studies reported the existence of donor-type ECs [6,22-24]. Spyridonidis et al. [12] and Murata et al. [4] used stringent methods, including hematopoietic markers and 3-dimensional histological analysis, to rule out hematopoietic contaminants. But, despite technological advances, no study was able to preclude the problems associated with Y chromosome–based detection of donor-type cells, including fetomaternal chimerism [11], blood transfusion from a male donor [25], quantitative inaccuracy of FISH in detecting Y chromosome–containing cells [26], and loss of Y chromosome, especially in elderly donors [27]. Perhaps for this latter reason, we found higher percentages of donor-type ECs compared with those reported by studies that used a combination of CK and CD45 staining with Y-FISH using confocal micrososcopy. Our relatively high percentage of ECs cannot be from hematopoietic

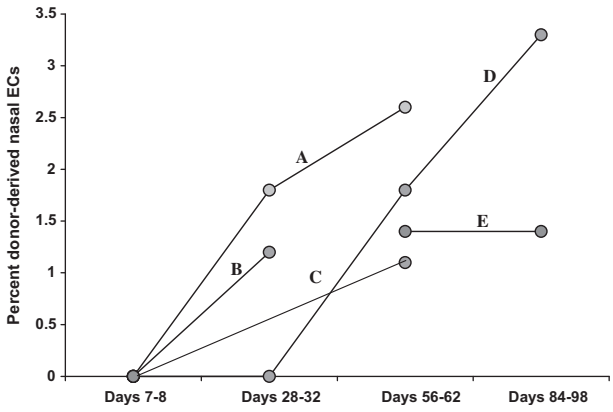

Figure 3. Percentage of donor-type ECs in the nasal epithelial specimens collected from 5 allo-HCT recipients at different time points after transplantation. Shown are the percentages of donor-type ECs detected in the nasal specimens collected from recipient A on days 8, 31, and 60 posttransplantation, from recipient B on days 7 and 32 posttransplantation, from recipient C on days 7 and 56 posttransplantation, from recipient D on days 7, 28, 62, and 95 posttransplantation, and from recipient E on days 56 and 94 posttransplantation.

contaminants, given our use of double-staining (CK and CD45) to rule out infiltrating/adhering leukocytes. Moreover, we performed LCM of only single or paired CK^+CD45^- cells, clearly separated from $CD45^+$ cells, to discount the possibility of leukocyte contamination. In addition, our relatively high percentage of ECs cannot be due to the quantitative inaccuracy of the STR technique, because we used highly stringent parameters, including average donor DNA percentage detected by 16 STR markers, measurement error to eliminate an aberrant marker profile [17], and the mean chimerism percentage from 3 independent PCR and electrophoresis runs. The 16 STR marker panel is used routinely at our center for clinical engraftment monitoring, and our laboratory has a 100% proficiency testing record in the American Society for Histocompatibility and Immunogenetics annual proficiency testing. Another reason for our relatively high percentage of ECs could be the dispersion of genetic material from leukocytes on slides during cytospinning, which might have been laser-captured along with CK^+CD45^- ECs. This is unlikely, however, given the results of our control mixing experiments of nasal cells from individual A with leukocytes from individual B (see Methods), and because no chromosome signals outside of nuclei were detected on any of the 10 cytospin slides stained by FISH for chromosomes 7 and 14 (Supplementary Figure 1).

The most salient findings of the present study are the kinetics of EC chimerism. The rapid increase early after transplantation suggests that the early posttransplantation state (mucosal repair from conditioning-induced damage?) facilitates the HCS to EC transdifferentiation. The significant increase in donor-type ECs between 3 months and 9 years post-PBSCT and the increasing trend between 9 and 17 years post-BMT suggest that the transdifferentiation is ongoing even late posttransplantation. Two earlier reports noted a similar kinetic trend for ECs of the buccal mucosa [10] and gastrointestinal tract [23]; however, the numbers of subjects were too small to allow conclusive statements on kinetics. Regarding the first month posttransplantation, we found donor-type nasal ECs in no subjects studied at 1 week after transplantation and in 50% of subjects studied at 1 month posttransplantation, whereas other investigators found donor-type ECs in skin or colon already at 7-15 days posttransplantation [10]. This discrepancy could be related to the relatively long turnover time of nasal epithelium (20-80 days [28]) compared with 7 days for skin and 5 days for colon [10], or could result from the limited sensitivity of our assay.

The present study has some limitations. First, we were not able to detect <0.5% of donor-type ECs because of the limited sensitivity of the STR-based analysis, as well as the background we found in mixing studies of nasal mucosal cells. Nevertheless, we detected donor-type cells in 82% of our allo-HCT recipients, with non-detection restricted to the very early posttransplantation period (days 7-32). Second, our ruling out cell fusion as the possible mechanism of detecting donor-type ECs might have been incomplete, given that heterokaryons rarely give rise to two euploid cells by cytoreductive division [19]. Third, apart from transdifferentiation, other mechanisms might explain the presence of donor-type ECs after allo-HCT, such as the transfer of ECs or their precursors with the HSC graft; we have found ECs or their precursors in PBSC grafts (Khan et al, unpublished data, March 2010). Another possible mechanism underlying donor-type EC detection is horizontal gene transfer (ie, ECs phagocytosing DNA from apoptotic hematopoietic cells) [29]. In vitro experiments using epithelial and T cell lines recently suggested this mechanism [29], but whether or not the transfer can occur in vivo is unknown. But even if this transfer could occur in vivo, our inability to detect donor-type ECs on day 7-8 despite the fact that a median of 58% leukocytes were of donor origin argues against horizontal gene transfer as a significant reason for our detection of donor-type ECs. Finally, it also is important to mention that mucosal sampling was done only once in most cases, and that the kinetics of the EC chimerism would have been more conclusive had there been a smaller time gap between early and late posttransplantation survivors, allthough a rapidly increasing percentage of donor-type ECs was observed in most subjects who underwent nasal mucosa sampling at multiple time points early after transplantation.

Overall, our results indicate that donor-type ECs are present in most allo-HCT recipients, are present even in organs that typically are not involved with GVHD, and increase in number rapidly in the first several months after transplantation and continue to increase slowly thereafter. In an optimistic scenario, these findings suggest that allo-HCT could be used

instead of epithelial organ transplantation to treat epithelial diseases like cystic fibrosis and thereby avoid the need for life-long pharmacologic immunosuppression. From this perspective, it will be important to determine what stimulates the generation of donor-type ECs early after transplantation; the use of that stimulus might be explored for increasing the amount of donor-type ECs to a physiologically relevant number. On the other hand, the presence of donor-type ECs also may be associated with such posttransplantation problems as donor-type carcinoma, possibly because of an association between transdifferentiation and malignant transformation [30].

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SUPPLEMENTARY DATA

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bbmt.2010.07.012.

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