Original Article

Hematopoietic Stem Cells Derived From Adult Donors Are Not a Source of Pancreatic β-Cells in Adult Nondiabetic Humans

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OBJECTIVE—Type 1 and type 2 diabetes are characterized by an ~98 and ~65% loss of pancreatic β-cells, respectively. Efforts to reverse either form of diabetes increasingly focus on the possibility of promoting β-cell replacement and/or regeneration. Islet transplantation has been explored, but it does not provide long-term insulin independence. One possible source of β-cell regeneration is hematopoietic stem cells. In mice, there are conflicting data as to whether hematopoietic stem cells contribute to pancreatic β-cells. We sought to establish whether hematopoietic stem cells (derived from adult donors) transdifferentiate into pancreatic β-cells in adult humans.

RESEARCH DESIGN AND METHODS—We addressed this in 31 human pancreata obtained at autopsy from hematopoietic stem cell transplant recipients who had received their transplant from a donor of the opposite sex.

RESULTS—Whereas some donor-derived cells were observed in the nonendocrine pancreata, no pancreatic β-cells were identified that were derived from donor hematopoietic stem cells, including two cases with type 2 diabetes.

CONCLUSIONS—We conclude that hematopoietic stem cells derived from adult donors contribute minimally to pancreatic β-cells in nondiabetic adult humans. These data do not rule out the possibility that hematopoietic stem cells contribute to pancreatic β-cells in childhood or in individuals with type 1 diabetes.
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Type 1 and type 2 diabetes are characterized by an ~98 and ~65% defect in β-cells, respectively. There is evidence of ongoing β-cell turnover in human subjects with long-standing type 1 diabetes (1). This raises the question of the origin of these newly derived β-cells and whether they might be sufficient in number to be a source of β-cell regeneration if the mechanisms leading to β-cell destruction could be overcome. Bone marrow stem cells and their splenic derivatives have been proposed as a potential source of pancreatic β-cells. In mice, both bone marrow stem cells and their splenic stem cell derivatives have been reported to generate pancreatic β-cells (2), although these findings have subsequently been challenged (3,4).

In the present study, we sought to establish whether hematopoietic stem cells give rise to pancreatic β-cells in adult humans. To accomplish this, we examined human pancreata obtained at autopsy from 31 recipients of hematopoietic transplants from opposite-sex donors. Survival of hematopoietic recipients ranged from 13 to 1,235 days after hematopoietic stem cell transplant. In all cases, as a positive control, spleen from each hematopoietic stem cell transplant recipient was examined to verify that engraftment had occurred.

RESEARCH DESIGN AND METHODS

Institutional review board approval for this study was obtained from the Mayo Clinic. The Mayo Clinic blood and marrow transplant database was searched to identify patients who, during the course of their life, had received a hematopoietic stem cell transplant from a sex-mismatched donor (W.H.). Cases were included only if a full autopsy had been performed within 12 h of death and whether there was stored pancreatic tissue of adequate size and quality. Of the 31 patients, 26 received a bone marrow–derived graft, 4 received a peripheral blood–derived graft, and 1 patient received a combination of both peripheral blood– and bone marrow–derived stem cells. There were 23 matched related sibling donors, 3 mismatched family donors, and 5 unrelated donors matched at the antigen level for HLA A, B, and DR. One patient who initially received a bone marrow graft from an identical HLA sibling received a second peripheral blood-derived graft from the same donor without conditioning 3 years later for aplasia in the context of graft-versus-host disease. Patients were treated according to institutional guidelines, with regard to graft-versus-host disease prophylaxis and infectious disease prophylaxis.

The present human study also included two patients with documented diabetes (fasting plasma glucose >126 mg/dl) and one patient with impaired fasting glucose (fasting plasma glucose 110–125 mg/dl) before hematopoietic stem cell transplant. The patients with diabetes survived 90 and 94 days post–hematopoietic stem cell transplant, while the patient with impaired fasting glucose survived 29 days post–hematopoietic stem cell transplant.

Pancreatic tissue processing. Tail of pancreata and a random sample of spleen were obtained at autopsy, and, after overnight fixation in 10% formaldehyde, were embedded in paraffin. For the purpose of the present study, two 4 μmol/l sections from paraffin blocks containing pancreata and spleen from 32 cases were obtained and mounted on Fisher brand Ink Jet White IJL-6109-Plus-600621 charged slides (Fisher Cat no. 12550109; Fisher Scientific, Pittsburgh, PA). One section from each case was stained with hematoxylin and eosin and examined (A.E.B.) to exclude autolysis, with none being excluded on this basis. The second section from each case was first analyzed by fluorescent in situ hybridization (FISH) to detect X and Y

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FISH, fluorescent in situ hybridization; FTIC, fluorescein isothiocyanate.

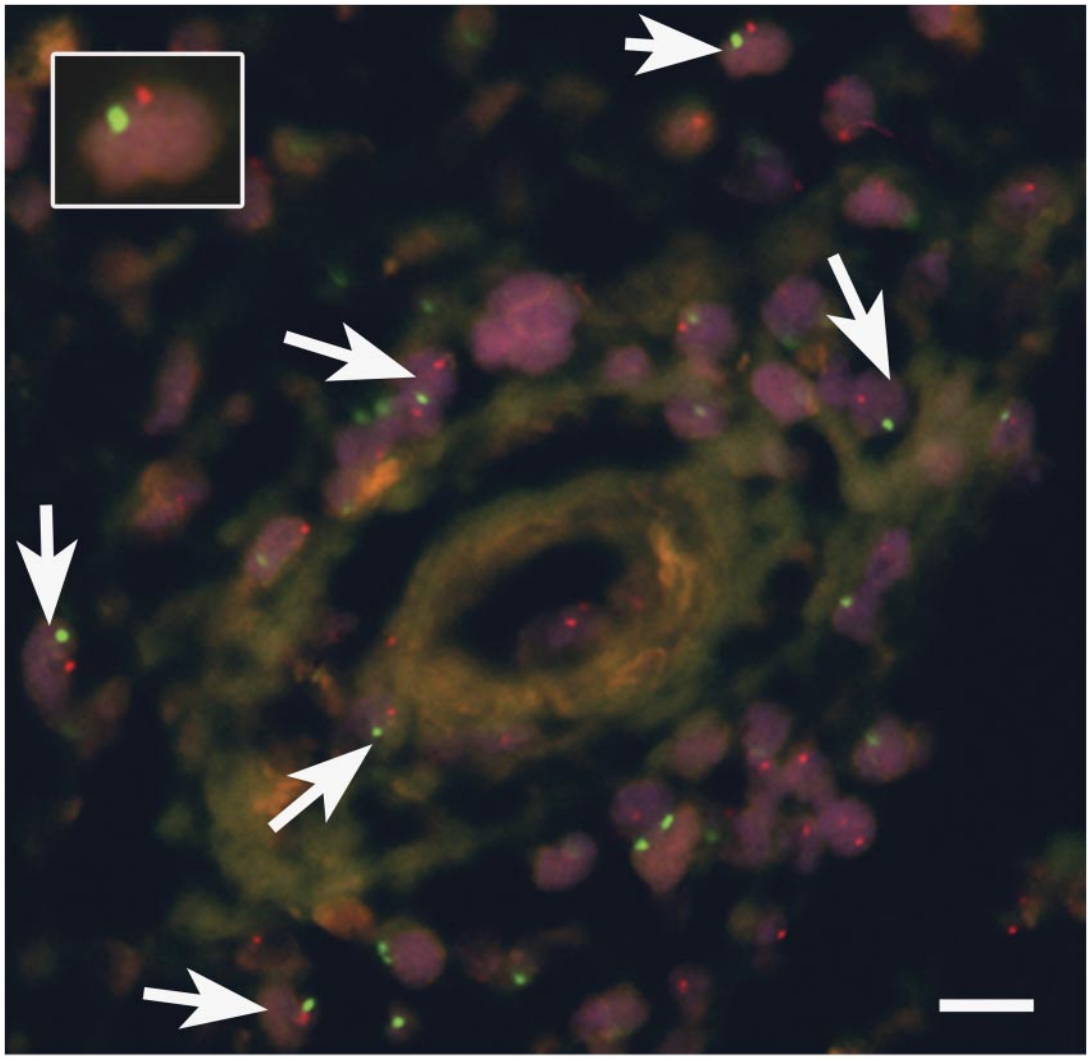

FIG. 1. Human spleen from a female patient who received a hematopoietic stem cell transplant from a male donor, demonstrating multiple FISH XY (male donor) cells present in the spleen (arrows). X chromosome, red; Y chromosome, green. Scale bar 10 μmol. (Please see http://dx.doi.org/10.2337/db06-1385 for a high-quality digital representation of this figure.)

chromosomes, as previously described (5), and subsequently for insulin, as previously described (1). In brief, sections were deparaffinized, and denaturation was carried out at 73°C for 3 min before hybridization with a Spectrum Orange–labeled centromere specific probe for chromosome X and a Spectrum Green–labeled centromeric specific probe for chromosome Y (no. 32-131051 dual-color probes; Abbott-Vysis, Downers Grove, IL). Slides were incubated overnight in a humidified box for hybridization of probes at 42°C. After a formamide wash procedure, slides were air dried and sealed with mounting medium. The mounting medium and cover slip were removed by soaking the slides in warmed PBS buffer containing citric acid (10 mmol/l, pH 6.0), before the next staining. The same slides were then fluorescent stained with insulin, using guinea pig anti-porcine insulin (dilution 1:400; Dako, Carpinteria, CA) as the primary antibody and anti–guinea pig secondary antibodies conjugated to fluorescein isothiocyanate (FITC) or Cy3 (dilution 1:100) (Jackson ImmunoResearch Laboratories, West Grove, PA). All slides were mounted with Vectashield (Vector Laboratories, Burlingame, CA) with 4′,6-diamidino-2-phenylindole (DAPI) and cover slipped. In 1 case of the original 32 cases available, FISH XY hybridization was not successful (defined by X and Y staining being present in at least 50% of cells); therefore, this case was excluded from the study, leaving a total of 31 cases. One limitation of this consecutive staining technique was that it was necessary to identify four distinct entities (X chromosomes [red], Y chromosomes [green], cytoplasmic insulin [green or red], and the 4′,6-diamidino-2-phenylindole-stained nuclei [blue]), and we were limited by having only three color cubes available on the fluorescent microscope. Therefore, in a limited number of cases where sections were available, we repeated the procedure two times, using a different secondary antibody conjugated to insulin (FITC or Cy3) on each occasion. Of note, the X and Y chromosomes present as localized signals within the nucleus. Therefore, they can be distinguished from cytoplasmic insulin granules; the size of the fluorescent probe for the Y chromosome is usually larger than that of the X, and therefore we preferred the combination of Y chromosome and insulin both fluorescing green.

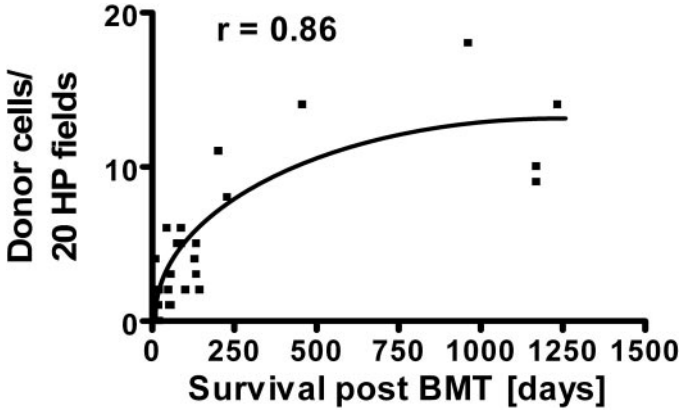

FIG. 2. The density of donor cells detected in pancreas in 20 high-power (×63) fields plotted against the survival time post–hematopoietic stem cell transplant (in days).

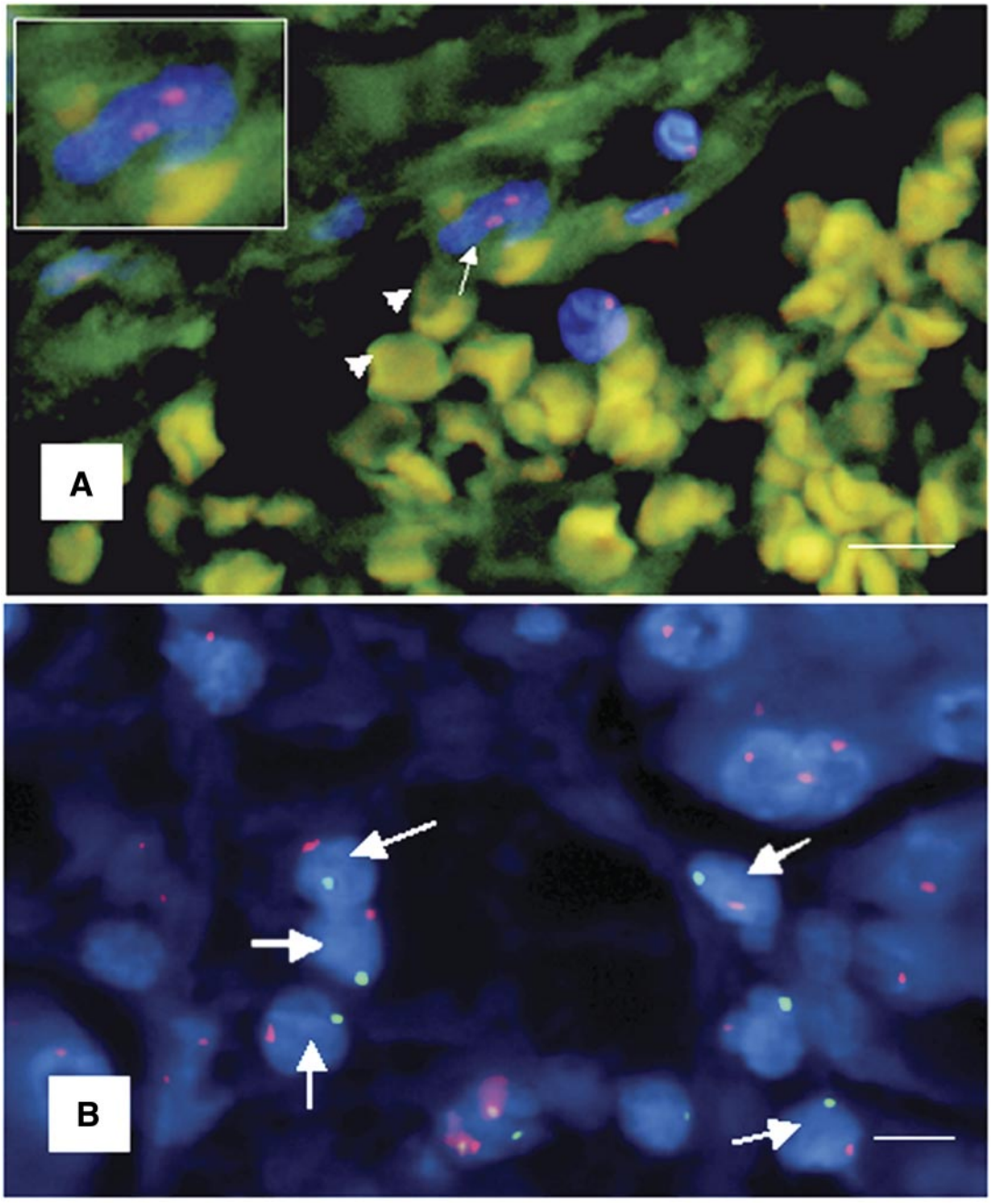

FIG. 3. Nonendocrine pancreas stained by the FISH technique (X chromosome, red; Y chromosome, green) and 4′,6-diamidino-2-phenylindole (nuclei blue). *A*: Pancreas from a male patient who received a hematopoietic stem cell transplant from a female donor, demonstrating a donor-derived female cell in the vascular endothelium (arrow). Arrowheads indicate erythrocytes within the vessel. *B*: Pancreas from a female patient who received a hematopoietic stem cell transplant from a male donor, demonstrating donor-derived cells (arrows) in the connective tissue and pancreatic acinar tissue. Scale bar 10 μmol. (Please see http://dx.doi.org/10.2337/db06-1385 for a high-quality digital representation of this figure.)

Morphological analysis. Initial screening of all slides was accomplished by analysis at high power (×63) using a Leica 6000 microscope (Leica Microsystems, Wetzlar, Germany) connected to a Macintosh computer loaded with Openlab software (Improvision, Lexington, MA). A mean of 131 islets were examined per case. Screening at high power was necessary to clearly visualize X and Y chromosomes within nuclei. In all cases where donor chromosomes were seen in close approximation with insulin stained cytoplasm, further examination was performed using confocal microscopy (described below) to determine whether the nuclei of the insulin-positive cells were of host or donor origin. To accomplish this, FISH and insulin costained slides were examined using an inverted microscope (DM-IRE2; Leica, Dearfield, IL) with a digital camera (ORCA, Hamamatsu City, Japan), attached to a confocal laser-scanning unit equipped with argon, green, and red helium neon lasers and a ×60 oil objective. FITC and Cy5 were excited by the argon laser at 488 nm, and red fluorescence was excited by the helium-neon laser at 633 nm. Scanning was done using the ×63 oil objective.

RESULTS

Positive control in spleen. In all cases, the majority of donor hematopoietic transplant–derived cells (defined by donor sex chromosomes) were present in the spleen (Fig. 1), assuring donor hematopoietic stem cell engraftment in each case included in this study.

Hematopoietic stem cell–derived cells in pancreata. There were scattered donor hematopoietic–derived cells present throughout the pancreas, with a density that increased with duration of survival from hematopoietic

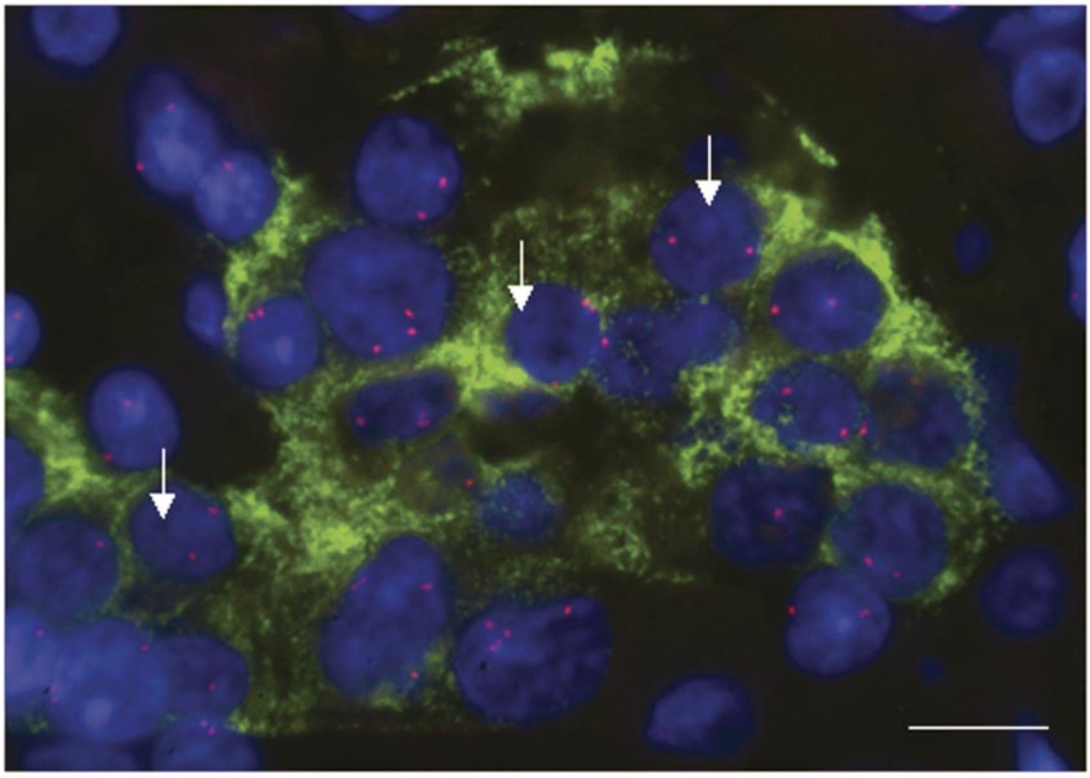

FIG. 4. A pancreatic islet stained for insulin (green) in a female patient who received a hematopoietic stem cell transplant from a male donor, demonstrating that the islet contains all female cells. Stained by FISH, with nuclei blue by 4′,6-diamidino-2-phenylindole. X chromosome, red; Y chromosome, green. Scale bar 10 μmol. (Please see http://dx.doi.org/10.2337/db06-1385 for a high-quality digital representation of this figure.)

stem cell transplant, reaching a plateau at ~500 days (Fig. 2). These cells were present in vascular endothelium, exocrine pancreatic ducts, and interlobular connective tissue (Fig. 3). Endothelial cells were identified by their elongated nuclei and their location within vascular tissue. Similarly, connective tissue could be identified based on microscopic appearance. The cells seen within the exocrine pancreata could not be definitively identified based on morphology alone; therefore, they could represent tissue macrophages and/or pancreatic acinar cells. Due to the limited number of pancreatic sections available to us, we were unable to further characterize these cells using specific antibody staining. Confocal imaging assured that the donor hematopoietic–derived cells in the pancreas were not a consequence of cell fusion because mosaicism was very rare (6).

Islets. We then examined multiple sections of pancreata that had been stained by both FISH for the sex chromosomes and insulin by immunofluorescence to identify β-cells. Despite an extensive survey of 4,192 islets, no β-cells (defined by immunostaining of cytoplasm for insulin) were identified as having nuclei that contained donor sex chromosomes (Figs. 4 and 5). Occasional donor-derived cells were present within islets, but when examined by confocal microscopy, these were not β-cells, although they were occasionally adjacent to β-cells (Fig. 5). Furthermore, by morphological appearance, these cells were most consistent with vascular and connective tissue components of the islet. Of note, there were also no donor-derived β-cells identified in the two cases of type 2 diabetes.

DISCUSSION

In the present study, we addressed the question of whether hematopoietic stem cells give rise to pancreatic β-cells in adult humans. Whereas examination of spleen in each case assured successful donor hematopoietic engraftment, we were unable to identify any β-cells with donor sex chromosomes. The present data, in a relatively large cohort of cases and with examination of >4,000 islets, imply that if there is any capacity for hematopoietic-derived cells to generate pancreatic β-cells in adult humans, it must be quantitatively negligible.

Prior studies have examined the possible transdifferentiation of hematopoietic-derived stem (2–4,7–10) or spleen (11–14) cells in pancreatic β-cells in vivo. Other studies have examined the possible transdifferentiation of hematopoietic stem cells in insulin secreting cells in vitro (15,16). These studies have all been in rodents and have provided conflicting conclusions. Initial reports favored transdifferentiation of hematopoietic (2,15,16) or spleen (11) cells to pancreatic β-cells, but subsequent studies have been negative (8–10,12–14). The current study is the first report in human subjects. We did observe engraftment of a small number of hematopoietic-derived cells in human pancreata increasing to a plateau after ~700 days. Whereas some of these cells were doubtless tissue macrophages, many had the appearance of epithelial cells, consistent with prior reports in mice (7–10). They were consistent with the time taken to accomplish steady-state engraftment since macrophages alone would presumably have reached steady state more rapidly. Because we restricted the precious sections available in this study to focus on combined insulin and FISH analysis, we did not have spare sections to explore with specific cell markers the nature of these nonendocrine pancreatic cells. On a technical note of caution, donor hematopoietic–derived cells were present in and around islets that had the

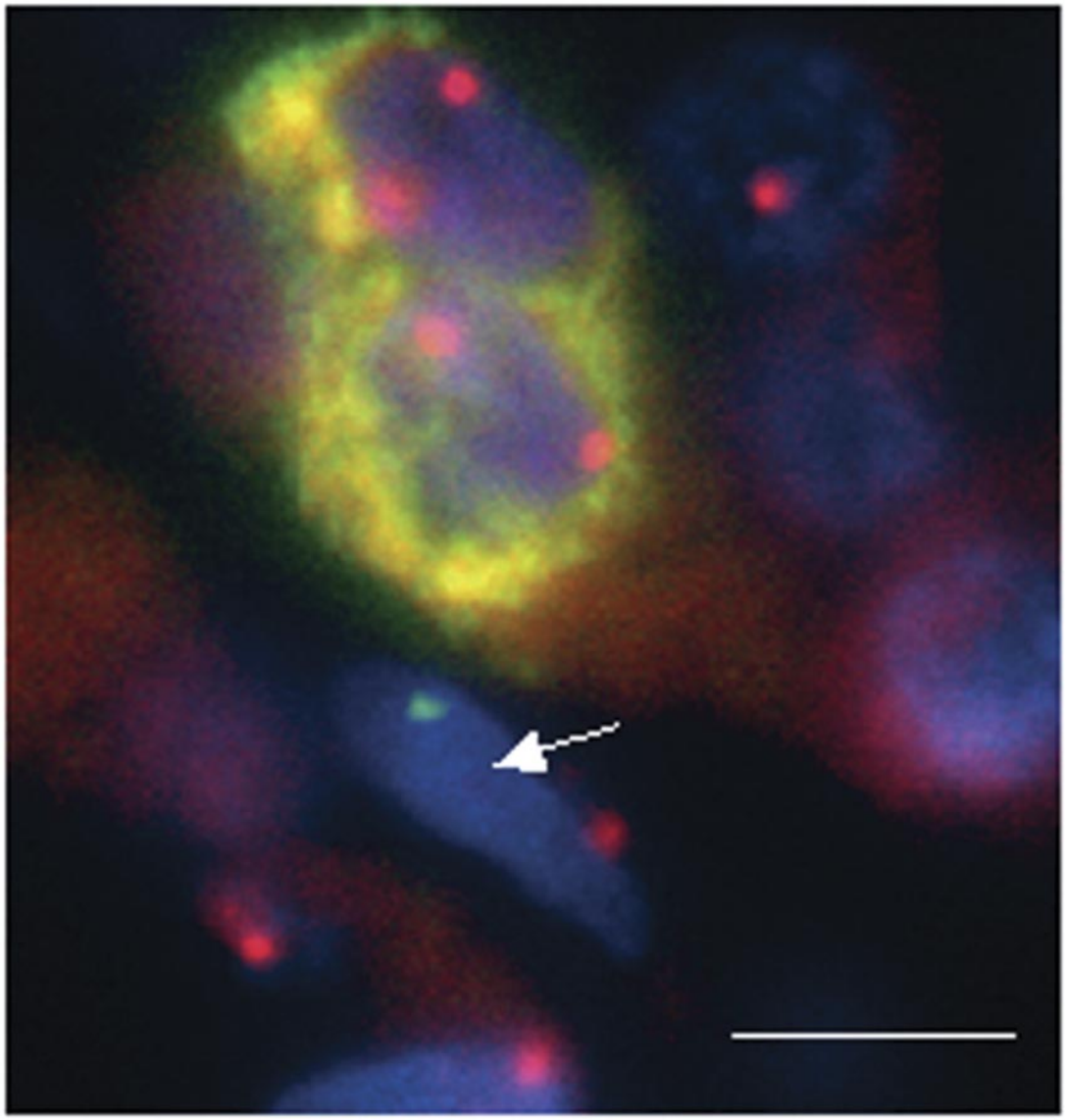

FIG. 5. Two isolated insulin-positive cells in a female recipient of a male hematopoietic stem cell transplant stained for insulin (green), sex chromosomes by FISH (X chromosome, red; Y chromosome, green), and nuclei blue by 4′,6-diamidino-2-phenylindole. The insulin-positive cells are from female subjects (XX). There is an adjacent insulin-negative cell is from a male subject (XY) (arrow). Scale bar 10 μmol. (Please see http://dx.doi.org/10.2337/db06-1385 for a high-quality digital representation of this figure.)

appearance of tissue macrophages. If careful confocal microscopy is not used to match nuclei with immunofluorescent staining for insulin, a false impression of occasional β-cells derived from hematopoietic donor cells might arise. In the present study, we benefited from the ability to directly examine nuclei sex by FISH and adjacent cytoplasmic staining for insulin in the same sections and by confocal microscopy. When the approach of examining adjacent sections for insulin and FISH was used, given the occasional hematopoietic-derived non–β-cells observed within islets, it is apparent how a false impression of transdifferentiation might arise.

The present data in human subjects are consistent with the concept that regeneration of β-cell numbers arise through β-cell replication, rather than through transdifferentiation of β-cells from adult stem cells (12–14,17–19). Alternatively, it has been suggested that hematopoietic-derived stem cells may act to support β-cell regeneration indirectly, perhaps through local signals within the islets provided by hematopoietic-derived stem cells engrafted within the islet (9,10). Our own observation of the occasional hematopoietic-derived stem cells within islets in humans is not inconsistent with such a role. One study reports that spleen cells transdifferentiated to β-cells to reverse diabetes in a murine model of autoimmune type 1 diabetes (11). However, subsequent studies reveal that this effect was accomplished through unknown mechanisms to suppress immune-mediated β-cell destruction and not through transdifferentiation (12–14), a conclusion the prior group challenged (6). It is unknown whether such a benefit might accrue in humans with type 1 diabetes treated by hematopoietic or spleen cell transplantation. However, given the morbidity associated with hematopoietic transplantation, this is not a realistic therapeutic approach for humans.

Several lines of evidence reveal that type 2 diabetes is primarily an islet disease, not the least of which, like type 1 diabetes, is reversed by pancreatic transplantation (20). Furthermore, the islet in type 2 diabetes is characterized by a loss of β-cell mass with increased β-cell apoptosis

TABLE 1
Clinical characteristics

Case	Age at transplant (years)	Sex	Diagnosis	Donor sex	Survival (days)
1	35	Male	CGL	Female	90
2	29	Male	MDS	Female	46
3	39	Male	Myeloma	Female	1,235
4	40	Female	AA	Male	13
5	20	Female	AA	Male	59
6	35	Female	AML	Male	146
7	50	Female	Myeloma	Male	27
8	57	Female	MDS	Male	116
9*	13	Male	AA	Female	29
10	26	Male	AML	Female	459
11	29	Male	MDS	Female	78
12	45	Male	AML	Female	21
13†	30	Female	AMF	Male	90
14	41	Female	MDS	Male	204
15	43	Male	MDS	Female	136
16	33	Female	CGL	Male	41
17	33	Female	AML	Male	25
18	35	Female	AML	Male	13
19	44	Male	Myeloma	Female	24
20	28	Female	CGL	Male	24
21	39	Male	MDS	Female	1,171
22	16	Male	AML	Female	51
23	44	Male	ALL	Female	230
24	42	Female	PLL	Male	964
25	33	Female	ALL	Male	94
26†	45	Male	AML	Female	131
27	48	Male	AML	Female	103
28	35	Female	NHL	Male	88
29	53	Male	AML	Female	54
30	53	Male	NHL	Female	39
31	42	Male	AML	Female	23

*Type 2 diabetes; †impaired fasting glucose. AA, aplastic anemia; ALL, acute lymphocytic leukemia; AMF, acute myelofibrosis; AML, acute myeloid leukemia; CGL, chronic granulocytic leukemia; MDS, myelodisplastic syndrome; NHL, non-Hodgkin's lymphoma; PLL, prolymphocytic leukemia.

(21,22), implying that there likely is an underlying islet inflammatory component to the pathophysiology of type 2 diabetes. Because it has been suggested that hematopoietic-derived stem cells are attracted to the islet under conditions of islet inflammation, it is of note that we still did not see evidence of β-cells derived by transdifferentiation of hematopoietic-derived stem cells in the type 2 diabetes cases examined in the present study. Another caution with regard to the present study is the fact that inevitably all the cases studied here had life-threatening diseases (Table 1). It is possible that the illness, particularly to the extent that it compromised myeloproliferation, may have influenced the capacity of the hematopoietic stem cells to transdifferentiate into pancreatic β-cells, although it is of note that vascular endothelial cells accumulated at a rate consistent with prior studies (Fig. 2).

The hematopoietic-derived stem cells used in the present study were derived from adult bone marrow and peripheral blood. Another source of blood-derived stem cells that are actively considered to be a potential source of β-cell progenitors are cord blood stem cells. It is plausible that this relatively abundant source of hematopoietic stem cells have a greater propensity to transdifferentiate into pancreatic β-cells than those derived from adults. Indeed, it has recently been reported that ~0.5% of pancreatic β-cells in pancreata obtained at autopsy from three nondiabetic children (aged 4 weeks to 14 years) had maternal microchimerism and therefore were presumed to be derived from cord blood (23). This finding suggests that hematopoietic cord blood stem cells may have the capacity to transdifferentiate into pancreatic β-cells, even in the absence of diabetes. In the same report, the frequency of maternal microchimerism in β-cells identified in the autopsy of the pancreas of one 11-year-old child with diabetic ketoacidosis was 0.96%. Caution has to be exercised in interpreting a single case of type 1 diabetes versus three nondiabetic cases. That said, the relatively modest increase (~0.4 fold) in maternal-derived pancreatic β-cells in the case of type 1 diabetes was much lower that the ~30-fold higher proportion of circulating blood cells with maternal microchimerism in individuals with type 1 diabetes compared with nondiabetic control subjects reported in the same study. This seems to argue against active replenishment of β-cells from circulating cells in the child with diabetes. An area of active interest in stem cell biology is what the signals are that attract stem cells to migrate to and participate in tissue repair. To date, no signals are known in the context of pancreatic islets. At present, it is only possible to speculate that should such signals exist, they are likely to be released by injured β-cells, and, as such, might be most evident in the context of type 1 diabetes.

We did note that other cell types in the pancreas were derived from donor cells. This finding is not surprising given prior reports that hematopoietic stem cells are capable of differentiating into a wide variety of tissues: the hematopoietic lineages, vascular endothelial cells (24,25), and connective tissue (26). More recently, studies in mice with bone marrow transplants have shown that hematopoietic-derived cells can assume neuronal (27), muscular (28), and hepatic (29) phenotypes. Multipotent adult progenitor cells have been derived from both murine and human hematopoietic cells in vitro and differentiated into endo-, meso-, and ectodermal cells (30,31). Other studies in humans have shown that bone marrow–derived cells are present in adult organs, such as cardiomyocytes in the heart (32). Conversely, other studies have contradicted these findings (33,34) or concluded that the perceived differentiation resulted from cell fusion (35,36).

In conclusion, we find no evidence of transdifferentiation of adult hematopoietic donor cells in pancreatic β-cells in adult humans studied for up to 3 years after stem cell transplantation. These findings imply that adult hematopoietic-derived stem cells contribute minimally to the replenishment of the β-cell population in adult humans. It is possible that such engraftment might occur from cord blood–derived stem cells and/or in the context of a markedly inflamed islet such as occurs in early type 1 diabetes in childhood.

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