No Evidence for Significant Transdifferentiation of Bone Marrow Into Pancreatic β-Cells In Vivo

Andreas Lechner,[1] Yong-Guang Yang,[2] Robyn A. Blacken,[1] Lan Wang,[2] Anna L. Nolan,[1] and Joel F. Habener[1]

Several recent studies have suggested that the adult bone marrow harbors cells that can differentiate into tissues from all three germ layers. Other reports have contradicted these findings or attributed them to cell fusion. In this study, we investigated whether bone marrow−derived cells contribute to the renewal of adult pancreatic endocrine cells, in particular insulin-producing β-cells, in vivo. To address this issue, we studied mice transplanted with green fluorescent protein (GFP)−positive, sex-mismatched bone marrow. We also extended our studies to pancreatic injury models (partial pancreatectomy and streptozotocin administration). All animals showed stable full donor chimerism in the peripheral blood and microscopic analysis at 4–6 weeks and 3 months after transplantation, indicating that the GFP$^+$ and Y chromosome−positive donor bone marrow contributed substantially to blood, lymphatic, and interstitial cells in the pancreas. However, after examining >100,000 β-cells, we found only 2 β-cells positive for GFP, both of which were in control animals without pancreatic injury. Thus our study results did not support the concept that bone marrow contributes significantly to adult pancreatic β-cell renewal. *Diabetes* 53:616–623, 2004

Transplantation of pancreatic islets can now be applied successfully to treat diabetes (1), but its widespread use is hampered by a shortage of donor organs (2). Because insulin-producing β-cells cannot be expanded significantly in vitro (3), efforts are under way to identify stem or progenitor cells that potentially could be grown and differentiated into β-cells in vitro. Such cells could provide an ample supply of transplantable tissue. Current research in this field focuses mainly on pluripotential embryonic stem cells (4,5) and on pancreas-specific adult progenitor cells (6,7).

However, several recent reports have suggested that adult bone marrow harbors cells with broad developmental plasticity that extends beyond the well-established differentiation potential of bone marrow stem cells (i.e., hematopoietic lineages, vascular endothelial cells [8,9], and connective tissue [10]). Studies in bone marrow−transplanted mice have found that marrow-derived cells can assume neuronal (11), muscular (12), and hepatic phenotypes (13). In addition, pluripotential cells (multipotent adult progenitor cells [MAPCs]) have been derived from human and mouse bone marrow after extensive passaging in vitro and differentiated into endo-, meso-, and ectodermal cells (14,15). Yet other reports have contradicted some of these findings (16,17) or attributed the differentiation results to cell fusion (18,19).

In light of these conflicting results, it seemed warranted that we explore whether bone marrow cells can also differentiate into pancreatic β-cells, thus providing an alternate tissue source for diabetes treatment. Indeed, it was recently reported that bone marrow contributes significantly to adult β-cell renewal in mice and that 4–6 weeks after transplantation of genetically marked bone marrow, 1.7–3% of islet β-cells were of marrow origin (20).

Here we investigated whether or not bone marrow contributes to the in vivo turnover of adult pancreatic endocrine cells in bone marrow−transplanted mice. We also extended our studies to determine whether bone marrow has a role in islet cell regeneration after pancreatic injury. To address this issue, we used two established rodent models of pancreatic injury: partial pancreatectomy (21) and streptozotocin administration (22,23).

RESEARCH DESIGN AND METHODS

Bone marrow transplantation. Animals were maintained under conditions approved by the institutional committee on research animal care. Female C57BL/6 (B6, H-2^b; age 10 weeks) and male actin green fluorescent protein (GFP)-transgenic B6 mice (C57BL/6-TgN[ACEbEGFP]10sb; age 6 weeks) were purchased from Jackson Laboratory (Bar Harbor, ME) and used as the recipients and donors, respectively. In two independent experiments, recipient mice were lethally irradiated (10 and 11 Gy, respectively; ^{137}Cs source; 0.8 Gy/min) and reconstituted within 4–8 h by a single intravenous injection of 10 × 10^6 bone marrow cells from GFP$^+$ donors. Tissue analysis was performed 3 months after (experiment 1) or 4–6 weeks (experiment 2) after bone marrow transplantation (BMT). The percentage of GFP$^+$ cells in the recipient blood was determined by flow cytometric analysis 4 weeks and 3 months (experiment 2 only at 4 weeks) after BMT and levels of donor chimerism were calculated by the following formula: donor chimerism (%) = (% GFP$^+$ cells of the BMT recipient/% GFP$^+$ cells of normal GFP-transgenic mice) × 100. At the end of the study, the mice were killed by CO_2 asphyxiation.

Partial pancreatectomy. Mice were anesthetized with a combination of isoflurane and pentobarbital. After an abdominal midline incision, the pancreas was exposed and 60–70% of pancreatic tissue was gently removed with a cotton swab. Only tissue in the immediate vicinity of the duodenum was left
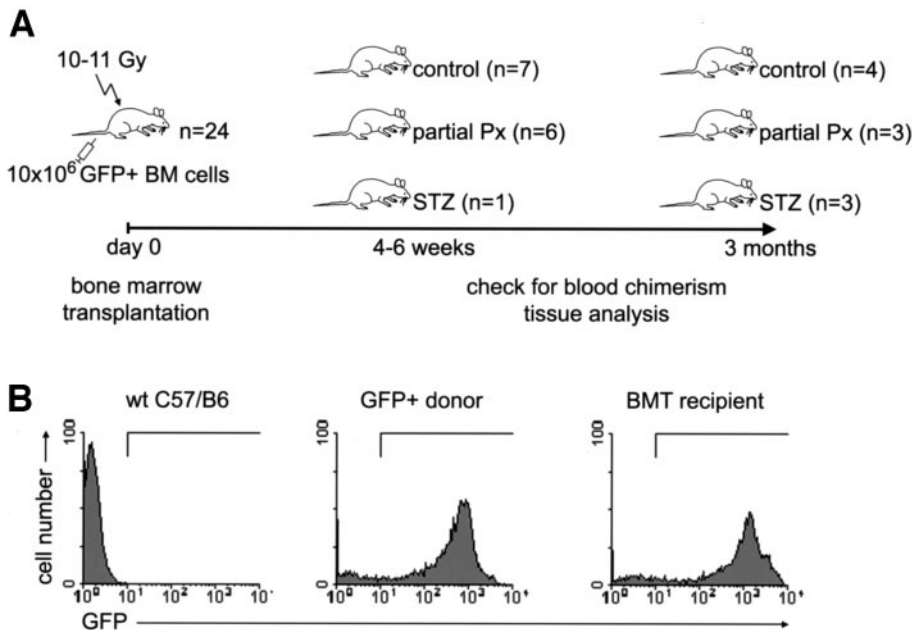
FIG. 1. Diagram of experimental design and fluorescence-activated cell sorter (FACS) analysis of peripheral blood chimerism. *A*: In two independent experiments, female mice were lethally irradiated, transplanted with 10×10^6 unfractioned, syngeneic, male, GFP$^+$ bone marrow cells, and separated into three experimental groups. Tissue analysis was done 4–6 weeks and 3 months after BMT. *B*: Representative FACS analysis of peripheral blood chimerism (percent GFP$^+$ nucleated blood cells) from wild-type, actin GFP-transgenic donor and BMT recipient mice. Px, pancreatectomy.

behind. The abdominal wall was closed with 5.0 silk, and wound clips were applied to the skin. Animals were killed 7–14 days after the procedure.

Streptozotocin/insulin administration. Streptozotocin (STZ)/insulin was administered as previously described by Guz et al. (23). Mice were injected with 200 mg/kg STZ i.p. after an overnight fast. Blood glucose was measured 24 h later to confirm hyperglycemia >200 mg/dl. The animals then received a subcutaneous implant of two LinBit sustained insulin release pellets (LinShin, Scarborough, CA), given according to the manufacturer's instructions. Animals were killed 12 days after STZ injection.

Immunohistochemistry. Pancreatic tissue was fixed overnight in 10% buffered formalin, embedded in paraffin, and cut into 4-μm sections. Sections were dewaxed in xylene, hydrated, and boiled for 10 min in a microwave oven in 10 mmol/l sodium citrate (pH 6) for antigen retrieval. Sections were blocked with PBS/normal donkey serum, incubated with first antibodies at 4°C overnight, washed, incubated with Cy2- or Cy3-labeled secondary antibodies (Jackson ImmunoResearch, West Grove, PA), washed again, and mounted with fluorescence mounting medium. Antibodies used were rabbit and mouse anti-GFP (1:300; both from Chemicon, Temecula, CA), goat anti-amylase (1:100; Santa Cruz, Santa Cruz, CA), guinea pig anti-insulin (1:5,000), guinea pig anti-glucagon (1:2,000), rabbit anti-somatostatin (1:2,000), and guinea pig anti-pancreatic polypeptide (1:2,000; all from Linco, St. Charles, MO).

For immunostaining of platelet endothelial cell adhesion molecules (PECAM; 1:100; goat; Santa Cruz) and CD45 (1:50; rat; BD Pharmigen), pancreatic tissue was fixed for 3–5 h in 10% buffered formalin, cryoprotected in 30% sucrose/PBS overnight at 4°C, and frozen in optimal cutting temperature compound. Next, 5-μm cryosections were stained as described above, but without antigen retrieval and using PBS/0.1% Triton instead of pure PBS.

Images were acquired with a Spot digital camera (Diagnostic Instruments, Sterling Heights, MI). Digital overlay for multicolor images was done with Adobe Photoshop 6.0.

Bromo-deoxyuridine labeling. After partial pancreatectomy, some animals were injected intraperitoneally with bromo-deoxyuridine (BrdU) (0.1-ml cell proliferation labeling reagent; Amersham Biosciences, Little Chalfont, U.K.) 12, 36, and 60 h and 4, 6, 9, and 12 days after the intervention. To visualize BrdU staining, paraffin-embedded tissue sections were hydrated and incubated in 2 mol/l HCl and in 0.1 mol/l HCl plus 0.05 mg/ml pepsin (Sigma, St. Louis, MO), each kept at 37°C for 20 min. Immunostaining was done with a mouse monoclonal anti-BrdU antibody (1:300; Sigma) overnight at 4°C and the mouse-on-mouse detection kit (Vector, Burlingame, CA) using NovaRed (Vector) as a peroxidase substrate.

Calculation of the number of analyzed cells. To approximate the number of β-cells that we microscopically examined in this study, we immunostained several pancreas sections for insulin, measured the surface area of the insulin-stained β-cell core of randomly selected islets, and counted the number of β-cell nuclei (nuclear holes clearly surrounded by insulin-positive cytoplasm) in these islets. We next calculated the average number of β-cell nuclei per 1,000 μm^2 of islet area (4.76). We then measured the surface area of all the insulin-stained islets on one representative section each from all study animals and used these measurements to calculate the average β-cell

number per tissue section for each animal. For STZ-administered animals, all four major endocrine islet cells were included in this calculation instead of β-cells. The numbers in Table 2 represent the average number of β-cells per tissue section multiplied by the number of sections that were examined. The number of analyzed amylase-positive exocrine cells was estimated in a similar fashion (1.49 cells/1,000 μm^2; average number of cells per tissue section: 42,000).

Fluorescent in situ hybridization. After being immunostained and captured as digital images, selected tissue sections were further processed for Y chromosome fluorescent in situ hybridization (FISH). The coverslip was removed and slides washed once in 2$\times$ sodium chloride−sodium citrate (SSC) and then incubated in 1 mol/l sodium thiocyanate for 30 min at 80°C. Slides were then washed twice in 2$\times$ SSC and digested in proteinase K (10 μg/ml in 2$\times$ SSC; Sigma, St. Louis, MO) for 90 min at 37°C, washed again in 2$\times$ SSC, air dried, and treated with methanol/acetic acid 3:1 for 5 min at room temperature. After being air dried, slides were denatured at 72°C for 10 min in 70% deionized formamide/2$\times$ SSC. Denaturation was stopped for 2 min in cold (−20°C) 70% ethanol. Slides were dehydrated in 90 and 100% ethanol for 2 min each and air dried. Next, 12 μl of the biotinylated mouse Y chromosome probe (Cambio, Cambridge, U.K.) were applied under a sealed coverslip overnight at 37°C. Probe denaturation, posthybridization washes, and signal detection with the Biotin Texas Red Amplification Painting Kit (Cambio) were done following the manufacturer's instructions. Sections were mounted in fluorescence mounting media containing 4′,6-diamidino-2-phenylindole (DAPI) as a nuclear counterstain.

RESULTS

Bone marrow transplantation, analysis of peripheral blood chimerism, and study design. To address the question of whether bone marrow cells can give rise to pancreatic endocrine cells, in particular β-cells, we lethally irradiated female wild-type C57BL/6 mice and then transplanted them with unfractioned bone marrow from syngeneic, male, actin GFP-transgenic mice. In these animals, GFP is expressed under the control of the chicken β-actin promoter plus the cytomegalovirus enhancer, and all tissues with the exception of erythrocytes and hair are GFP$^+$ (24). We performed two independent experiments with tissue analysis 3 months (experiment 1) and 4–6 weeks (experiment 2) after BMT (Fig. 1*A*). At 4 weeks after BMT, all animals in both experiments showed high donor chimerism of peripheral blood nucleated cells (mean 89.5%; range 84.8–92.9%), which was further increased at 3 months after BMT in experiment 1 (mean 96.6%; range 91.2–99.9%) (Fig. 1*B*; Table 1).

TABLE 1
Peripheral blood chimerism in study animals

	n	GFP$^+$ cells	Chimerism
GFP$^+$ donors	3	90.9 (89.4–92.2)	100
Wild-type C57BL/6	3	0.28 (0.22–0.35)	NA
BMT recipients at 4 weeks	24	81.4 (77.1–84.5)	89.5 (84.8–92.9)
BMT recipients at 3 months	10	87.8 (82.9–90.8)	96.6 (91.2–99.9)

Data are percent and are expressed as mean (range). The percentage of GFP$^+$ nucleated cell in the recipient blood was determined by flow cytometric analysis 4 weeks and 3 months (experiment 1) after BMT. Levels of donor chimerism were calculated by the following formula: donor chimerism (%) = (% GFP$^+$ cells of the BMT recipient/ % GFP$^+$ cells of actin GFP-transgenic mice) $\times$ 100.

At 2 weeks before the end of each experiment, we separated the mice into three groups. One group underwent partial pancreatectomy, one group received STZ and subcutaneously implanted insulin pellets, and the third group (control) went without any further intervention (Fig. 1A). **Evaluation of tissue analysis methods.** We next established that pancreatic tissue, including β-cells, in the actin-GFP donor strain of mice was homogenously GFP$^+$ and that we could clearly distinguish positive from negative tissue by immunofluorescent staining (Fig. 2A and B). In addition, we confirmed that all cells in freshly isolated islets from the donor strain were robustly GFP$^+$ (data not shown). We also tested FISH for the mouse Y chromosome on male and female control tissue (Fig. 2C). Using 4-μm paraffin sections, we detected a Y chromosome in ~60% of male nuclei. This percentage is explained by the fact that a single tissue section often contains only part of an individual nucleus, which may or may not contain the Y chromosome.

Pancreatic donor chimerism under steady-state conditions. To investigate whether bone marrow contributes to β-cell turnover under steady-state conditions, we examined pancreatic tissue from mice that were killed 4–6 weeks or 3 months after BMT without any further intervention. We analyzed multiple tissue sections from all animals by fluorescent doublestaining with antibodies against GFP and insulin. We inspected >30,000 individual β-cells in each of the two experiments. In the animals killed after 4–6 weeks, we found no cells that were co-stained for insulin and GFP. After 3 months, we detected two such cells, one each in two different animals (Fig. 3A; Table 2). Co-localization of the two proteins in the same cell was confirmed by confocal microscopy (Fig. 3E); however, Y chromosome staining of both cells was negative (data not shown). We also detected three GFP$^+$ pancreatic exocrine cells after 3 months (Fig. 3B and F), but no co-localization of GFP and the other major islet hormones (i.e., glucagon, somatostatin, and pancreatic polypeptide; data not shown).

In contrast to the low donor contribution to endocrine and exocrine cells, the pancreases of all animals contained large numbers of donor-derived hematopoietic (CD45$^+$) and interstitial cells (Fig. 3C and D, G–M). Most interstitial GFP$^+$ cells did not co-stain with PECAM, a marker of vascular endothelial cells (Fig. 3L). We only occasionally observed donor-derived vascular endothelial cells (Fig. 3M).

We also examined several tissue sections by Y chromosome FISH as a transgene-independent technique to detect donor tissue; we found no GFP-negative/Y chromosome-positive β-cells. By digital overlay of FISH and immunostaining images, we were, however, able to demonstrate a Y chromosome in the majority of GFP$^+$ cells (Fig. 3I and J).
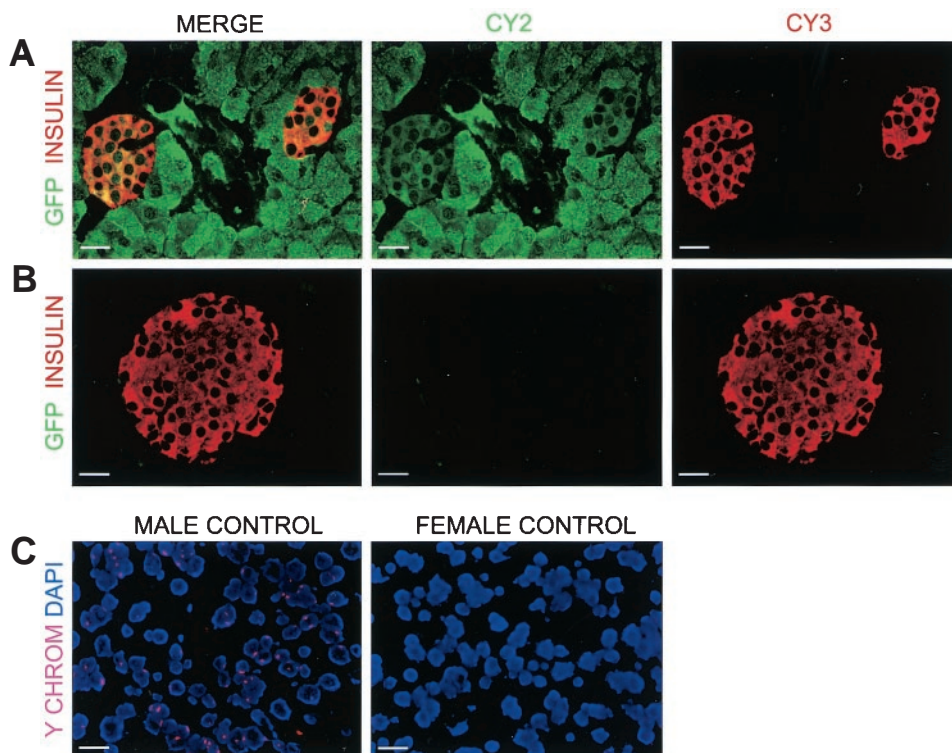
FIG. 2. **Positive and negative controls for GFP immunostaining and Y chromosome FISH.** *A*: GFP/insulin doublestaining of a pancreatic section from an actin GFP-transgenic donor mouse (positive control). *B*: GFP/insulin doublestaining of a wild-type mouse (negative control). *A* and *B* were done in parallel and with the same exposure times for image acquisition. *C*: Positive and negative control for Y chromosome FISH. Scale bars are 20 μm.
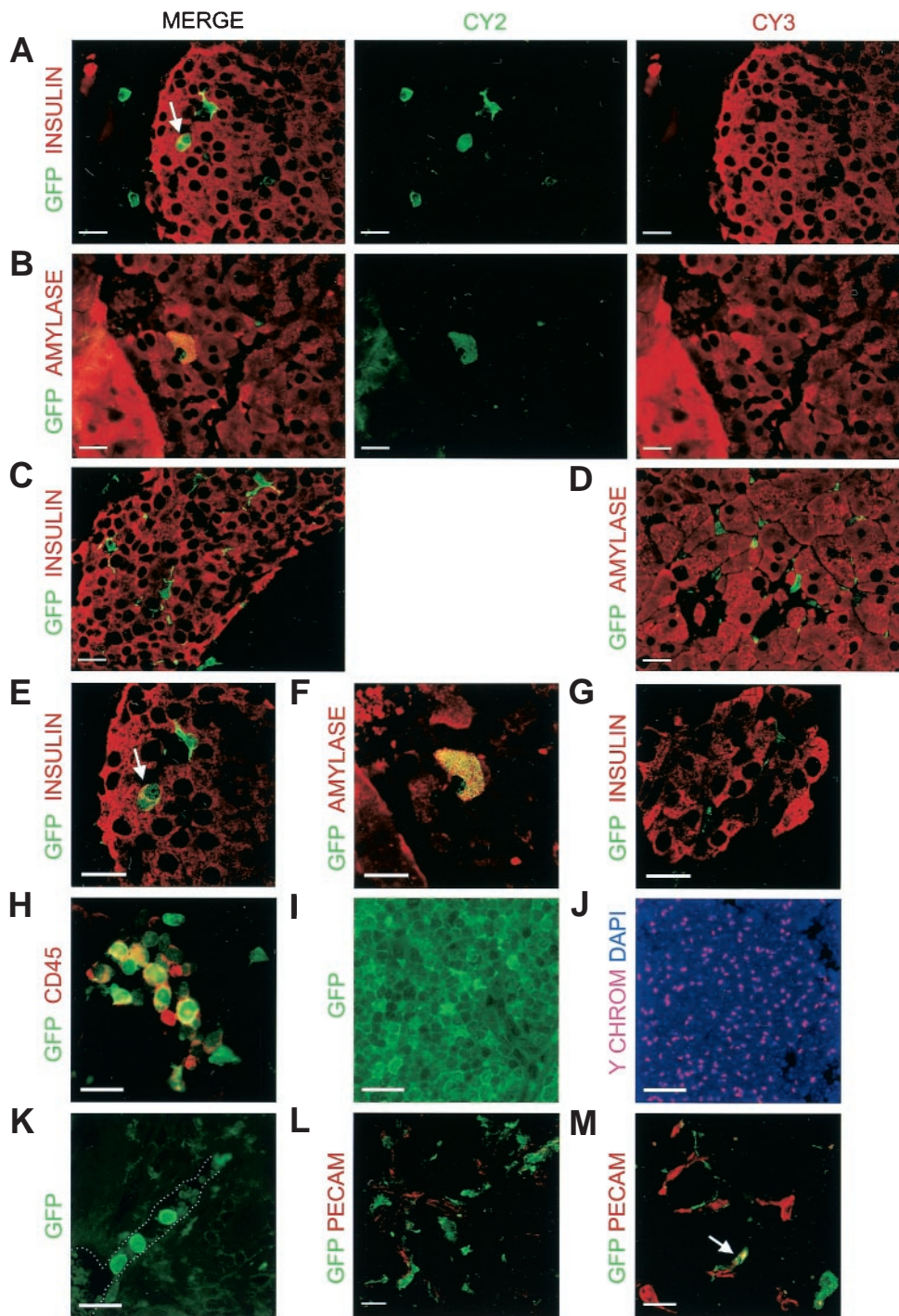
FIG. 3. Tissue analysis in animals 4–6 weeks and 3 months after BMT: control group/steady-state turnover. *A*: One out of the two GFP⁺ β-cells detected in this study. *B*: One out of the five GFP⁺ pancreatic exocrine cells. *C* and *D*: Numerous GFP⁺ interstitial cells were seen in all animals, both between islet β-cells (*C*) and exocrine cells (*D*). *E*: Confocal 1-μm optical section showing the same GFP⁺ β-cell as in *A*. *F*: Confocal image of the GFP⁺ exocrine cell shown in *B*. *G*: Confocal image of two GFP⁺ interstitial cells in a small islet. *H*: GFP/CD45 double-staining of donor derived hematopoietic cells in the pancreas. *I*: Part of a lymph node with the majority of cells GFP⁺. *J*: Y chromosome FISH of the same lymph node. *K*: Intravascular GFP⁺ white blood cells in a pancreatic tissue section. *L* and *M*: Most GFP⁺ cells do not co-stain for the vascular endothelial maker PECAM, but occasional double-positive cells are seen (*arrow* in *M*). Scale bars are all 20 μm.

Pancreatic donor chimerism after partial pancreatectomy. We next determined if pancreatic injury would increase the number of bone marrow–derived cells that adopted an endocrine phenotype. We therefore performed a 60–70% pancreatectomy in several mice 7–14 days before killing the animals. BrdU labeling demonstrated the proliferation of ductal and islet cells after pancreatectomy (Fig. 4*A* and *B*). We also observed typical regenerative foci (21), with multiple small ducts and islets surrounded by interstitial tissue. We found that in these foci a high fraction of interstitial cells, but no islet or duct cells, were of bone marrow origin (Fig. 4*C* and *D*). Analysis of pancreatic tissue sections spaced throughout the depth of the pancreatic remnants revealed no GFP⁺ or Y chromosome–positive β-cells or other endocrine cells in any of the pancreatectomized animals (Table 2). We found one GFP⁺ exocrine cell in one mouse 3 months after BMT.

Pancreatic donor chimerism after STZ/insulin administration. As a second model of islet cell injury, we administered 200 mg/kg STZ i.p. to bone marrow–transplanted mice, then subcutaneously implanted insulin-releasing pellets. Insulin treatment has previously been shown to support the partial restoration of β-cell mass in STZ-diabetic CD1 mice (22,23). We found in pilot studies with female C57BL/6 mice, similar to those used in this BMT project, that the proliferation of endocrine non–β-cells was a prominent fea-

TABLE 2
Bone marrow contribution to islet β-cells and pancreatic exocrine cells

	n	Donor-derived β-cells		Donor-derived exocrine cells	
		4–6 weeks	3 months	4–6 weeks	3 months
Control	11	0/30,000	2/30,000	0/>100,000	3/>100,000
Partial pancreatectomy	9	0/30,000	0/20,000	0/>100,000	1/>100,000
STZ	4	0/1,500*	0/2,500*	0/40,000	1/>100,000
All	24	0/60,000 (NA)	2/50,000 (0.004)	0/>240,000 (NA)	5/>300,000 (<0.002)

Data represent number of cells co-stained for GFP and insulin/total number of cells examined; percent is given in parentheses for "All" cells. Numbers of analyzed cells were approximated as described in RESEARCH DESIGN AND METHODS. *Because of the high portion of non–β-cells in the islets of STZ-administered mice, a cocktail of the four major islet hormones was used to stain all endocrine cells in these animals. The number of analyzed cells in this field therefore includes all endocrine cells, not only β-cells.

ture of islet remodeling after STZ in this mouse strain. The same was true for the bone marrow−transplanted mice in this study. When the animals were killed 12 days after STZ administration, the absolute number of α- and δ-cells and PP cells per islet cross section was significantly increased over that of control mouse islets (Fig. 4E). We also found that a subgroup of cells co-expressed pancreatic polypeptide and another islet hormone such as insulin or glucagon, a type of cell not seen in normal adult mouse islets (Fig. 4F). Because many islet cells after STZ administration were non–β-cells, we used doublestaining with anti-GFP and an antibody cocktail of the four major islet hormones to look for donor-derived endocrine cells. However, no donor-derived endocrine cells were found in any of these animals, whereas the number of interstitial cells of donor origin was comparable with that of control animals (Fig. 4G, Table 2).
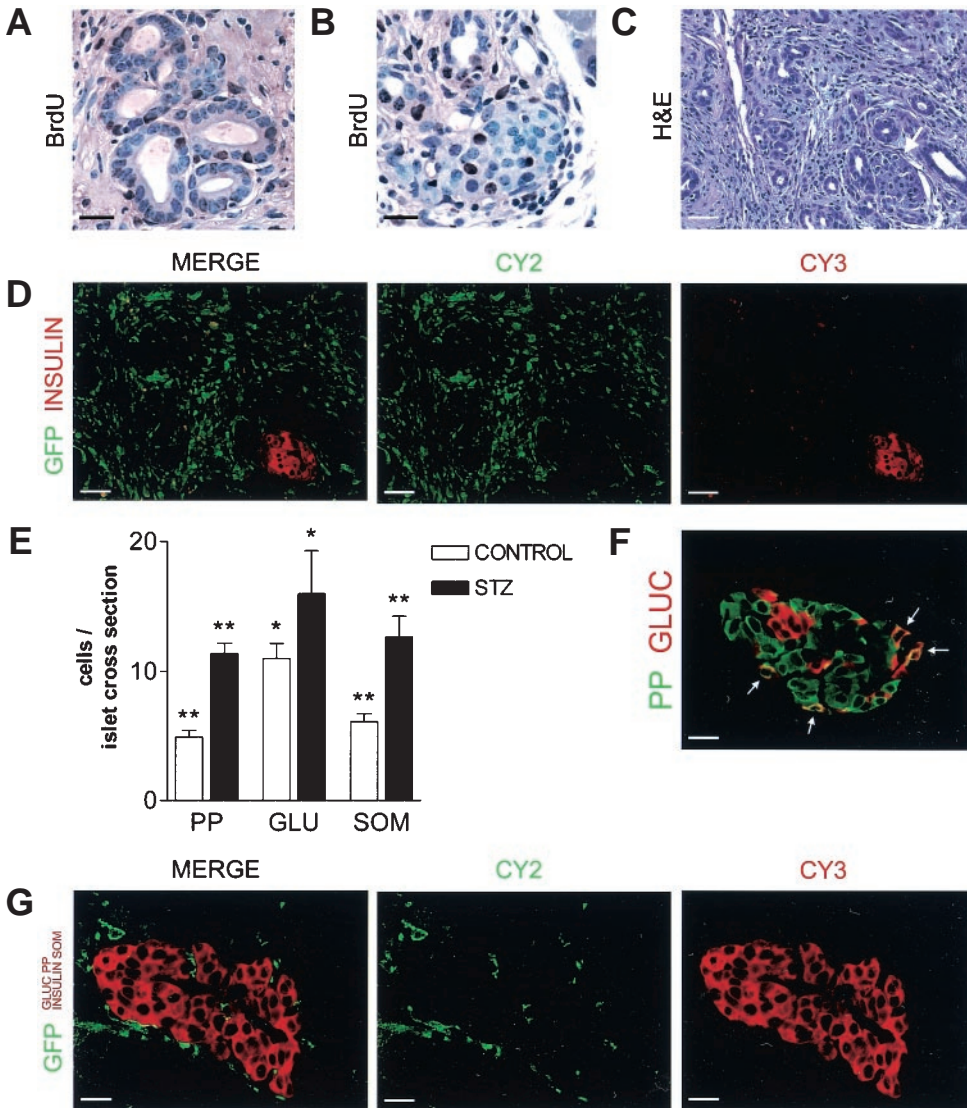

FIG. 4. Tissue analysis in bone marrow−transplanted animals after partial pancreatectomy and STZ/insulin administration. A and B: BrdU labeling of pancreatic ducts (A) and a small islet (B) during tissue regeneration after partial pancreatectomy. C: Hematoxylin and eosin (H&E)−stained section showing a regenerative focus with multiple small pancreatic ducts and a budding islet (arrow) after partial pancreatectomy. D: GFP/insulin immunostaining of an adjacent tissue section showing multiple GFP+ interstitial cells, but no positive ductal or endocrine cells. (The small yellow dots are autofluorescent erythrocytes). E: Mean numbers of PP-, glucagon (GLU)-, and somatostatin (SOM)-positive cells in islet cross sections from control ($n = 4$) and STZ/insulin-administered mice ($n = 4$). *$P < 0.05$, **$P < 0.0001$ Mann-Whitney test. F: Glucagon/PP immunostaining of a pancreatic islet after STZ/insulin administration. Some cells co-expressed both hormones (arrows). G: Immunostaining with GFP and a cocktail of antibodies against the four major islet hormones. A representative islet of a mouse administered STZ/insulin is shown. Scale bars are 20 μm, except for C and D, in which they are 80 μm.

DISCUSSION

In this study, we found no significant in vivo differentiation of bone marrow into pancreatic islet β-cells in adult mice, neither under steady-state conditions nor after tissue injury through partial pancreatectomy or STZ administration. We analyzed pancreatic tissue 4–6 weeks and 3 months after BMT. No donor marker−positive β-cells were found at 4–6 weeks, whereas we detected two positive cells at 3 months (0.004% of all β-cells examined). Both of the cells were in control mice that had not undergone additional pancreatic injury.

In contrast, all animals exhibited stable and almost complete hematopoietic donor chimerism. Moreover, tissue analysis of the pancreas revealed a high contribution of transplanted bone marrow cells to cells in the pancreatic interstitium and to blood and lymphatic cells. These findings indicated that the host bone marrow was efficiently repopulated with donor cells and that the lack of donor-derived β-cells was not caused by incomplete chimerism.

The analysis of pancreatic tissue sections as well as isolated islets from the donor strain demonstrated robust GFP expression in β-cells. Therefore, it seems unlikely that GFP would be specifically downregulated in donor-derived β-cells after BMT. We also applied Y chromosome FISH as a transgene-independent method to identify donor-derived cells. Using this method, we detected no additional bone marrow−derived β-cells. We are therefore confident that GFP immunostaining did not significantly underestimate the number of marrow-derived β-cells in our study, even taking into account that the sensitivity of Y chromosome FISH on the 4-μm tissue sections used in this study is only about 60%. We were unable to detect a Y chromosome in the two GFP⁺ β-cells found and thus could not confirm the donor origin of these cells with complete certainty. However, based on a 60% sensitivity, the chance of missing two subsequent Y chromosomes is 16%. Lacking an alternative explanation for GFP expression in these cells, we therefore still believe that donor cells contributed to the genome of the GFP⁺ β-cells by either transdifferentiation or fusion (see below).

We also studied two established models of pancreatic injury repair. Tissue regeneration after partial pancreatectomy has been investigated by several groups over more than two decades and is believed to involve duct-derived progenitor cells (21,25–27). Regeneration after partial pancreatectomy is rapid, with the main proliferative response occurring during the first week (21). Islet regeneration in mice after STZ administration has been demonstrated more recently by two groups (22,23,28); further, insulin supplementation has been shown to have a positive effect on regeneration (23). Using these models, we did not observe any bone marrow contribution to pancreatic epithelial tissue. We have provided evidence for duct and islet cell proliferation after partial pancreatectomy and for islet remodeling after STZ administration, but because of the limited number of bone marrow−transplanted animals, we did not quantify the extent of tissue regeneration in this study. Therefore, it remains possible that other experimental models can induce more efficient pancreas regeneration with a bone marrow contribution that was not seen in our work.

Our results differ from those of a recent study by Ianus et al. (20), who found that 1.7–3% of islet β-cells were of bone marrow origin 4–6 weeks after bone marrow transplantation in mice. The reasons for these different outcomes are unclear at this point.

Both our study and the one reported by Ianus et al. (20) were done in the same strain of mouse. The dosages of radiation used for host preconditioning (10/11 Gy in our study vs. 10.5–11 Gy in the Ianus et al. study) were similar, but the number of transplanted bone marrow cells was 10 times higher in our study (10^7 vs. 10^6 cells). Peripheral blood donor chimerism was also stronger in our study. Tissue analysis was done 4–6 weeks after BMT in both studies and also 3 months after BMT in ours.

Ianus et al. (20) used an insulin promoter−based Cre-Lox/GFP system to quantify the number of donor-derived β-cells in contrast to the constitutively GFP-expressing bone marrow used in our investigations. Whether this difference could explain the contrasting results remains to be determined. Another possible explanation could be unrecognized differences in the preconditioning of the mice for BMT or the preparation of the donor cells. Hypothetically, a yet-unknown type of stem cell that can give rise to pancreatic β-cells but that is only inconsistently replaced by donor cells with the current BMT regimens, could exist within the bone marrow. Even in the presence of full hematopoietic donor chimerism, such a cell type might then still be of host origin and its progeny would not be recognized as being derived from bone marrow.

In another recent report, Hess et al. (29) found that the transplantation of bone marrow cells after diabetes induction with STZ supports the recovery of pancreatic β-cell mass and partial reversal of hyperglycemia. This study noted that after STZ administration, up to 2.5% of insulin-positive cells were also donor marker positive. Yet these cells did not express the transcription factor PDX1, usually seen as an essential feature of functional β-cells. Donor-derived, insulin-positive cells also did not account for the functional recovery seen in the study. Instead, BMT augmented the regeneration of host β-cells by a yet-unknown mechanism. This could involve bone marrow−derived endothelial progenitor cells, as up to 9% of donor cells in the pancreases of STZ-administered animals in this study stained positive for the vascular marker PE-CAM. We did not observe a similarly high frequency of donor-derived vascular cells, but only occasional cells that co-stained for PECAM. However, the two experimental approaches are not entirely comparable: we established stable bone marrow chimerism first and then inflicted pancreatic injury, whereas Hess et al. gave bone marrow after STZ as a therapeutic intervention. This approach could bring a number of vascular progenitor cells directly to the injured pancreas, more than what the bone marrow provides regularly.

Our study did not address the question of whether the small number of donor marker−positive β-cells (two) was the result of true transdifferentiation or cell fusion. Cell fusion was recently found to be the primary mechanism by which bone marrow cells rescue mice with fumarylaceto-acetate hydrolase deficiency, a hereditary defect in liver metabolism (18,19). Our understanding of in vivo cell fusion is still minimal. However, one prerequisite seems to

be that the resulting tetraploid cell can be functional and is not forced to undergo apoptosis. The naturally high frequency of tetraploid hepatocytes might therefore support the successful fusion of some bone marrow−derived cells with liver cells. Most pancreatic β-cells in vivo are diploid, but β-cells with more than two sets of chromosomes are present in human and rodent islets (30,31). Thus it seems possible that a low rate of fusion between bone marrow−derived cells and β-cells occurs in vivo. This could be an alternative explanation for the very low numbers of pancreatic β-cells that expressed bone marrow markers. The fact that we were able to find two GFP[+] β-cells 3 months, but not 4–6 weeks, after BMT could indicate that the low level contribution of bone marrow to pancreatic tissue is a slow process. Yet, given the overall small number of positive cells, a sampling error can also not be excluded.

Differing results on the differentiation potential of bone marrow stem cells are not unique to the field of diabetes; although two groups have reported the in vivo differentiation of central nervous system neurons from transplanted bone marrow (11,32), another study using a similar experimental approach had a negative outcome (17). Krause et al. (33) described how transplantation with a single bone marrow cell could lead to engraftment in multiple different tissues in addition to full hematopoietic reconstitution, yet others were unable to reproduce these findings (16). Finally, as mentioned above, the differentiation of bone marrow cells into hepatocytes (13) has recently been identified as cell fusion, not independent differentiation (18,19).

The results of present study support the notion that the differentiation capacity of adult bone marrow stem cells is limited and that pancreatic β-cells are not substantially derived from bone marrow in vivo. However, at this point our results are only applicable to the mouse models used in this study. Whether β-cells are derived from bone marrow in other species or experimental settings needs to be determined. Our studies also did not exclude the possibility that β-cells could be derived from bone marrow stem cells in vitro. Results with marrow-derived MAPCs (15) and brain-derived oligodendrocyte precursors (34) suggest that the differentiation capacity of cells in vitro can be broader than in vivo. On the other hand, a recent report that insulin uptake can mimic β-cell differentiation in vitro cautions that such experiments need to be done carefully (35).

The conflicting results in other areas of adult stem cell biology remind us that this field of investigation is still at an early stage. Further studies are necessary to establish definitive answers on the versatility of different adult stem cell populations and their utility for the treatment of disease. In particular, new techniques to trace the progeny of adult stem cells in vivo have to be developed along with clonal in vitro assays that test the differentiation capacity of various cell types.

ACKNOWLEDGMENTS

NOTE ADDED IN PROOF

During revisions of this manuscript, a report was published by Choi et al. (36) also describing the lack of significant bone marrow–to–pancreatic β-cell transdifferentiation in bone marrow−transplanted mice.

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