Original Article

Failure of Transplanted Bone Marrow Cells to Adopt a Pancreatic β-Cell Fate

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Recent studies in normal mice have suggested that transplanted bone marrow cells can transdifferentiate into pancreatic β-cells at relatively high efficiency. Herein, adopting the same and alternative approaches to deliver and fate map-transplanted bone marrow cells in the pancreas of normal as well as diabetic mice, we further investigated the potential of bone marrow transplantation as an alternative approach for β-cell replacement. In contrast to previous studies, transplanted bone marrow cells expressing green fluorescence protein (GFP) under the control of the mouse insulin promoter failed to express GFP in the pancreas of normal as well as diabetic mice. Although bone marrow cells expressing GFP under the ubiquitously expressed β-actin promoter efficiently engrafted the pancreas of normal and hyperglycemic mice, virtually all expressed CD45 and Mac-1/Gr-1, demonstrating that they adopt a hematopoietic rather than β-cell fate, a finding further substantiated by the complete absence of GFP$^+$ cells expressing insulin and the β-cell transcription factors pancreatic duodenal homeobox factor-1 and homeodomain protein. Thus, transplanted bone marrow cells demonstrated little, if any, capacity to adopt a β-cell fate. *Diabetes* 55:290–296, 2006

A number of recent studies (1–8) have suggested that bone marrow transplantation may give rise to nonhematopoietic cell lineages in multiple organs by a process termed transdifferentiation. However, these data are controversial, as other studies (9–14) have failed to document such transdifferentiation, although in some cases bone marrow–derived hematopoietic cells have been demonstrated to contribute at very low frequencies to other cell lineages through cell fusion (12,15–19). In parallel with this work, other studies (20–24) have extended the concept of developmental plasticity of transplanted bone marrow cells. One of the most surprising and compelling claims of cell plasticity following bone marrow transplantation suggests that transplanted bone marrow cells can give rise to fully competent insulin-producing pancreatic β-cells (25). Whereas most other reports (13,26,27) of bone marrow plasticity have indicated that transdifferentiation occurs at a very low rate, the studies of Ianus et al. (25) suggested that as much as 1.7–3% of the insulin-expressing cells in the islets of transplanted mice were derived from reconstituting bone marrow cells. If these findings can be confirmed, bone marrow cells might clearly play a role in the adaptive increase in β-cell mass that occurs physiologically during, for example, obesity and pregnancy. Moreover, bone marrow cells may also represent an attractive source for cell replacement therapy of type 1 and type 2 diabetes, not least in view of the limited supply of human islets and ethical issues surrounding embryonic stem cell research (28–31). Although some subsequent studies (32,33) have obtained results compatible with the generation of insulin-expressing pancreatic cells from transplanted bone marrow cells, the reported efficiency has been several orders of magnitude lower than initially reported, and other studies have failed to detect any transdifferentiation into β-cells at all (34). Even when insulin-expressing cells were obtained following bone marrow transplantation, these did not express any of the transcription factors characterizing the mature β-cell (35). Thus, the capacity of transplanted bone marrow cells to contribute to β-cell regeneration remains controversial. However, until this controversy has been resolved, bone marrow cells continue to be promoted as a candidate source for cell replacement therapy for diabetes (31). A problem in this context is the different transplantation, conditioning, and transgenic fate-mapping approaches used in the previous studies (25,32,34,35). Here, we have adopted different experimental strategies to follow the fate of transplanted bone marrow cells in the pancreas of normal as well as hyperglycemic mice. Although we found evidence for extensive bone marrow cell engraftment in control and damaged islets, the engraftment proved to be almost exclusively of the hematopoietic lineage, and no evidence was obtained for bone marrow cells giving rise to cells expressing insulin or the essential β-cell transcription factors pancreatic duodenal homeobox factor-1 (Pdx-1) and homeodomain protein (Nkx6.1).

RESEARCH DESIGN AND METHODS

Two mouse strains on a C57BL/6 background, transgenic for green fluorescence protein (GFP) and driven by mouse insulin I gene promoter (MIP/GFP; kindly provided by Dr. Manami Hara, University of Chicago, Chicago, IL) (36) or the β-actin promoter (β/GFP; kindly provided by Dr. M. Okabe, Osaka

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GFP, green fluorescence protein; Nkx6.1, homeodomain protein; Pdx-1, pancreatic duodenal homeobox factor-1.

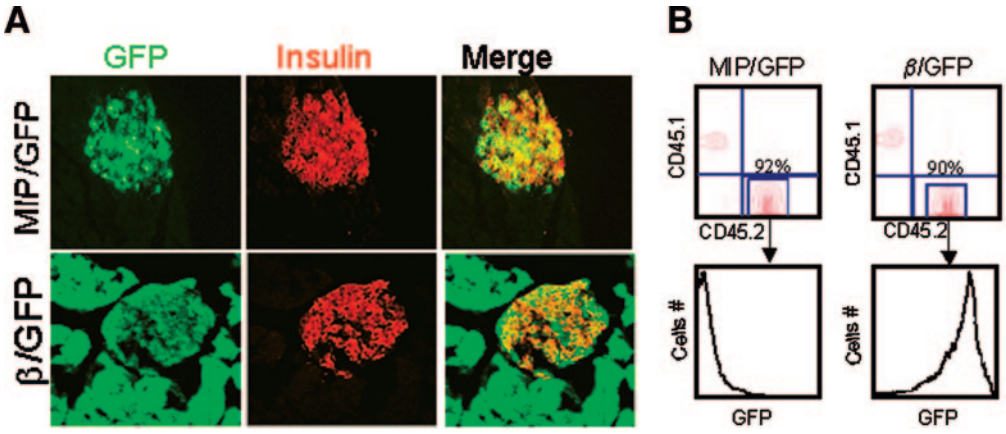

FIG. 1. Hematopoietic reconstitution in peripheral blood of bone marrow–transplanted mice. *A*: Double staining of GFP (green) and insulin (red) in pancreatic sections (10 μm) from MIP/GFP and β/GFP transgenic mice showing cellular colocalization (Merge) of GFP and insulin signals. Images were acquired using conventional fluorescence (*upper panels*) and confocal (*lower panels*) microscopy. Magnification: ×200. *B*: Contribution of transplanted MIP/GFP and β/GFP bone marrow cells (CD45.2) to blood cell reconstitution in irradiated wild-type recipient mice (CD45.1) 6 weeks after transplantation. Also shown is the absence of GFP expression in CD45.2 blood cells derived from MIP/GFP bone marrow cells and the ubiquitous GFP expression in CD45.2 blood cells in mice transplanted with β/GFP bone marrow cells. Results are representative of analyses performed in 21 mice for each transgenic mouse strain in three independent experiments.

University, Suita, Japan) (37), were used as donors of bone marrow cells. Both of these strains express the CD45.2 isoform of the panhematopoietic cell surface antigen CD45. As recipient mice, we used wild-type C57BL/6 mice expressing CD45.1. All mice were given sterile food and autoclaved acidified water and housed under pathogen-free conditions. All procedures were performed with consent from the local ethical committee at Lund University.

Generation of bone marrow chimeric mice. Whole bone marrow cells were collected from femurs and tibias of 10- to 14-week-old CD45.2 MIP/GFP or β/GFP donor mice. Femurs and tibias were gently crushed in a mortar, and the cell suspension was filtered through a 70-μm mesh filter (Becton Dickinson Falcon, Erembodegem, Belgium). The fraction of dead cells in the suspension was determined with Trypan blue (Sigma-Aldrich, St. Louis, MO), and the volume used for transplantation was adjusted accordingly. MIP/GFP or β/GFP (both CD45.2) bone marrow cells were transplanted into lethally irradiated (975 cGy of γ irradiator from a Cs137 source) wild-type C57BL/6 (CD45.1) recipient mice. Hematopoietic chimerism and blood lineage distribution was evaluated in peripheral blood of recipient mice 4–6 weeks after transplantation by flow cytometry (FACSCalibur; Becton Dickinson, San Jose, CA) as previously described (12). Data were analyzed using FlowJo software (TreeStar, San Carlos, CA).

Alloxan-induced diabetes and cytokine mobilization of bone marrow chimeric mice. Wild-type mice with high levels of GFP^+ multilineage hematopoietic reconstitution underwent cytokine mobilization and alloxan treatment (Sigma) 8 weeks after bone marrow transplantation (Fig. 4*A*). Starting 2 days before alloxan injection, bone marrow cells were, as previously described (12), mobilized to peripheral blood by five daily injections (5 $\mu g \cdot mouse^{-1} \cdot day^{-1}$) with recombinant human fms-like tyrosine kinase-3 ligand and recombinant murine granulocyte macrophage colony–stimulating factor (kind gifts of Amgen, Thousand Oaks, CA). Diabetes was induced by a single intravenous injection of alloxan monohydrate at 80 mg/kg body wt as previously described (38,39). Blood insulin and glucose levels were evaluated 5 days later, and 4–6 weeks after alloxan administration, mice were killed and pancreata harvested and fixed for immunohistochemistry.

Exendin-4 treatment. Six weeks after MIP/GFP bone marrow transplantation, mice were treated with 10 daily intraperitoneal injections of exendin-4, an analog of glucagon-like peptide-1 (24 nmol/kg) (Sigma-Aldrich). Mice were killed for immunohistochemical analysis 4 weeks after the last injection of exendin-4 treatment.

Antibodies. The following primary antibodies were used: rabbit anti–GST-STF1 (Pdx-1), rabbit anti–GST-Nkx6.1 (both from Hagedorn Research Institute, Copenhagen, Denmark), guinea-pig anti-insulin/proinsulin (B65-1) (Euro-Diagnostica, Malmö, Sweden), chicken anti-GFP (Chemicon, Temecula, CA), rat anti-mouse CD45 (NeoMarkers, Fermont, CA), and rat anti-mouse CD4 (145–2C11), CD8 (53-6.7), B220 (RA3-6B2), Mac-1 (M1/70), Gr-1 (RB6-8C5), CD45.2 (104), and CD45.1 (A20) antibodies (all from PharMingen, San Diego, CA). Secondary antibodies included fluorescein isothiocyanate– and Texas red–conjugated donkey anti-chicken IgG, Cy5-conjugated donkey anti-rat IgG, Texas red–conjugated donkey anti–guinea pig IgG (all from Jackson Immuno Research Laboratories, West Grove, PA), and Alexa Fluor 555 conjugated with goat anti-rat IgG or goat anti-rabbit IgG (Molecular Probes, Eugene, OR).

Phenotypic and morphological evaluations of pancreatic sections. Dissected pancreata as well as livers were fixed with Stefanini fixative solution overnight at 4°C followed by equilibration overnight in 20% sucrose at 4°C, embedded in Tissue-Tek O.C.T. (Sakura Finetek Europe B.V., Zoeterwoude, the Netherlands), cryosectioned (8–12 μm), and stored at −80°C until examination. For immunohistochemistry, the sections were permeabilized with 0.25% Triton X100 (Sigma) for 10 min, washed three times with PBS, and blocked with 5% donkey or goat serum (Sigma) for 1 h at room temperature. Primary antibodies were diluted with 5% donkey or goat serum and incubated overnight at 4°C. After washing with PBS three times, appropriate secondary antibodies were incubated for 45 min at room temperature. Finally, to visualize the cell nuclei, sections were washed and incubated with Hoechst 33342 for nuclear staining (Sigma). Staining with control antibodies, as well as staining of negative and positive control tissues, was always performed to control the specificity of the antibodies. β-Cell–specific antibodies were titrated on sections of viable pancreatic islets from wild-type and β/GFP transgenic mice. Hematopoietic marker-specific antibodies were titrated on samples from bone marrow and spleen. Immunoreactivity was visualized and images taken using conventional fluorescence (Olympus, London, U.K.) or confocal (LSM 510 Meta; Zeiss, Jena, Germany) microscopy. In nondiabetic mice, an average of 60 pancreatic sections as well as 30 liver sections were analyzed per mouse, and in alloxan-treated mice an average of 30–40 pancreatic sections were examined per mouse, equally distributed between the pancreatic head and the tail. For all experimental groups, a minimum of 14 mice (except for the exendin-treated cohort, in which only 5 mice were used) were examined, for a total of 420–840 pancreatic sections representing 3×10^4 to 7.5×10^5 β-cells. All datasets were independently confirmed by at least two investigators in two different laboratories involved in the studies.

RESULTS

Bone marrow cells engrafted into the pancreas adopt hematopoietic, but not β-cell, fates in nondiabetic mice. We first used the same experimental strategy as Ianus et al. (25), in which bone marrow cells were reported to acquire a β-cell fate in vivo upon transplantation into nondiabetic mice. Lethally irradiated wild-type mice were first transplanted intravenously with a high number (1×10^7) of unfractionated bone marrow cells from mice in which GFP is expressed under control of the mouse insulin promoter (MIP/GFP) (36). As expected, GFP expression in MIP/GFP mice was restricted to pancreatic β-cells (Fig. 1*A*), and no peripheral blood cells derived from MIP/GFP bone marrow cells expressed GFP (Fig. 1*B*). Transplanted mice were killed, as in the studies of Ianus et al. (25), 4–6 weeks posttransplantation, at which time transplanted bone marrow cells contributed to >80% of peripheral blood cells (Fig. 1*B*, Table 1). Examination

TABLE 1
Hematopoietic and pancreatic engraftment of bone marrow–derived cells in transplanted nondiabetic mice

Source	Transgene	*n**	Percent hematopoietic chimerism	Pancreatic GFP$^+$cells (*n* sections†; positive sections‡)	Percent of pancreatic GFP$^+$ cells (insulin, Pdx1.1, Nkx6.1, CD45, CD4/CD8, B220, and Mac-1/Gr-1)
Whole bone marrow	MIP/GFP	14	81 ± 9	800; 0%	NA, NA, NA, NA, NA, NA, and NA
Whole bone marrow	β/GFP	14	82 ± 8	420; 100%	0, 0, 0, >99.9, 0, 0, and >99

Data are means ± SD, unless otherwise indicated. *Lethally irradiated nondiabetic mice were transplanted with 1×10^7 whole bone marrow cells from MIP/GFP or β/GFP mice. Transplanted mice were analyzed for hematopoietic and pancreatic reconstitution at 4–6 weeks posttransplantation. †Number of pancreatic sections analyzed for GFP (by epifluorescence and/or anti-GFP antibodies) as well as insulin, Pdx-1, and Nkx6.1 expression. Forty-four fields of pancreatic sections of mice transplanted with β-GFP bone marrow cells were analyzed (using ×40 objects) for coexpression of GFP and CD45 as well as the blood lineage markers CD4/CD8, B220, and Mac-1/Gr-1. ‡Frequency of sections containing GFP$^+$ cells. Specificity of each marker was confirmed using positive and negative control tissues (see RESEARCH DESIGN AND METHODS). NA, not applicable.

by direct fluorescence microscopy of as much as 800 pancreatic islet sections (representing ~7.5×10^5 β-cells) from 14 MIP/GFP bone marrow–transplanted mice failed to reveal pancreatic engraftment of any GFP$^+$ cells even when using an anti-GFP antibody to enhance sensitivity (Fig. 2*A*, Table 1). We also failed to detect any GFP expression in the liver of transplanted mice (420 liver sections from 14 transplanted mice analyzed). The same negative picture was obtained when the pancreata were analyzed 3 months posttransplantation (J.T., S.E.W.J., unpublished observation). Although the use of MIP/GFP bone marrow cells allows specific and high-resolution tracking of bone marrow–derived cells expressing insulin, such cells cannot be used to determine their level of pancreatic engraftment and fate determination. Thus, we next performed the same experimental bone marrow transplantation protocol in nondiabetic mice, using bone marrow cells in which GFP was instead expressed under the ubiquitously expressed β-actin promoter (β/GFP) (37) (Fig. 1*A* and *B*). In contrast to MIP/GFP bone marrow–transplanted mice, high levels of engrafted GFP$^+$ cells were observed in the pancreas of all mice transplanted with β/GFP bone marrow cells (Fig. 2, Table 1). However, in 420 sections (containing 4.0×10^5 β-cells), we found no

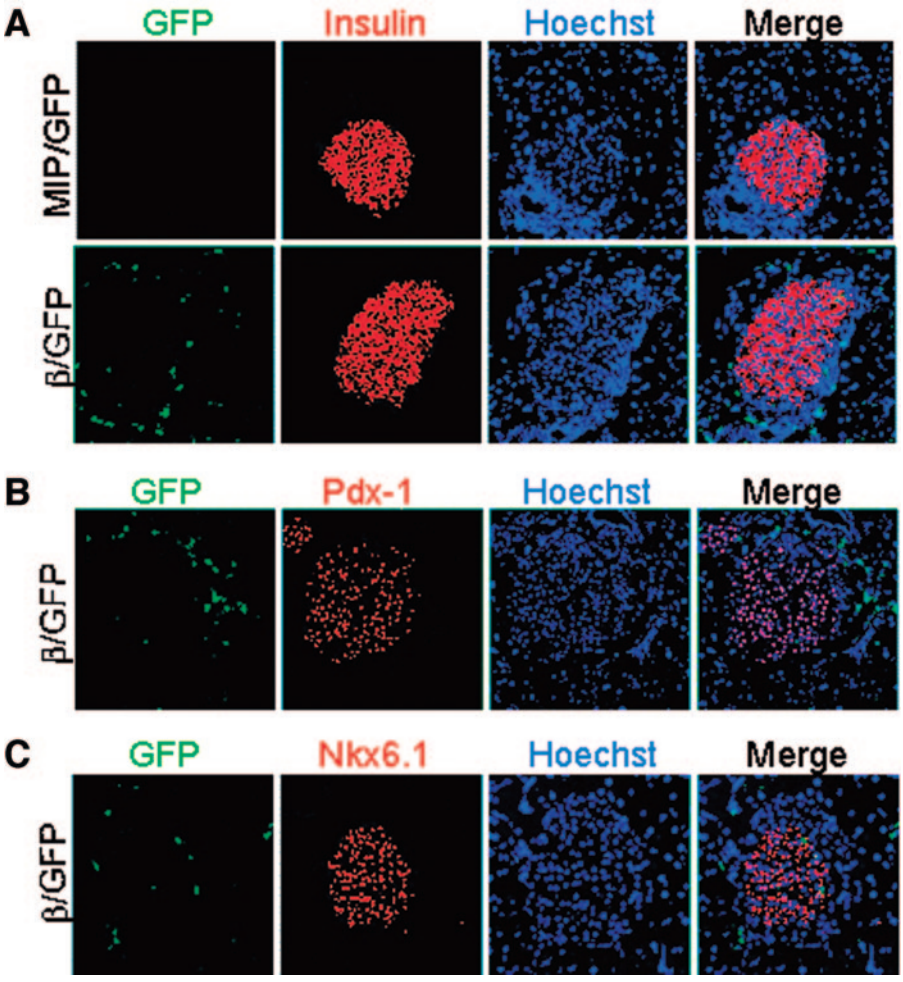

FIG. 2. Pancreatic engraftment of bone marrow–derived GFP$^+$ cells in nondiabetic mice. *A*: Triple analysis of GFP (green), insulin (red), and Hoechst 33442 (blue-nuclear stain) staining of pancreatic sections from nondiabetic mice transplanted with MIP/GFP and β/GFP transgenic bone marrow cells. *B* and *C*: Triple analysis of pancreatic sections in β/GFP bone marrow–transplanted mice of GFP, Pdx-1, and Hoechst 33442 (*B*) or GFP, Nkx6.1, and Hoechst 33442 (*C*). For all datasets, sections are from representative mice out of 14 mice transplanted with MIP/GFP and 14 with β/GFP bone marrow cells. Images were acquired using fluorescence microscopy. Magnification: ×200.

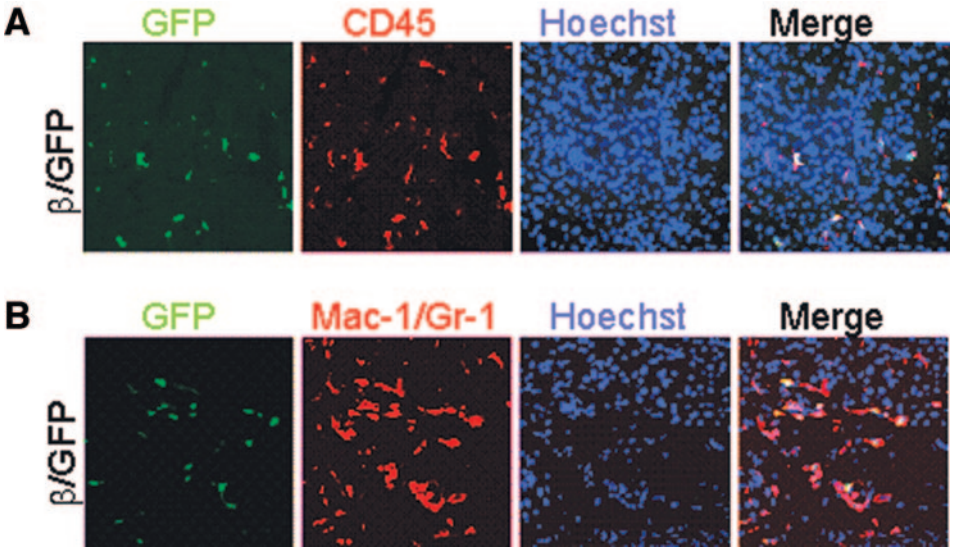

FIG. 3. Hematopoietic engraftment of bone marrow–derived GFP$^+$ cells in nondiabetic mice. Triple analysis of pancreatic sections in β/GFP bone marrow–transplanted mice of GFP, CD45, and Hoechst 33442 (*A*) or GFP, Mac-1/Gr-1, and Hoechst 33442 (*B*). Images were acquired using fluorescence microscopy. Magnification: ×200.

cells coexpressing GFP and insulin (Fig. 2*A*, Table 1). Furthermore, no bone marrow–derived GFP$^+$ cells coexpressed the β-cell transcription factors Pdx-1 or Nkx6.1 (Fig. 2*B* and *C*, Table 1). By contrast, virtually every GFP$^+$ cell engrafted into the pancreas expressed the panhematopoietic marker CD45 (>99.9%; Fig. 3*A*, Table 1) as well as myeloid antigens Mac-1/Gr-1, whereas they were negative for T-cell (CD4, CD8) and B-cell (B220) markers (Fig. 3*B*, Table 1). Thus, transplanted bone marrow cells have the capacity to engraft into the pancreas of nondiabetic recipients but then exclusively adopt a hematopoietic rather than a β-cell fate.

Lack of evidence for bone marrow–derived β-cells in diabetic mice. A number of studies (12,15,17,18) have suggested that the ability of bone marrow–derived cells to adapt nonhematopoietic fates in vivo, primarily through cell fusion, is dependent on or enhanced by organ-specific insults. In addition, it has been suggested that cytokine-induced mobilization of bone marrow cells to peripheral blood can promote their migration to nonhematopoietic tissues and nonhematopoietic fate conversion (12,40). Thus, we next explored the possibility that mobilized bone marrow cells might adapt a β-cell fate in mice rendered diabetic following treatment with the β-cell–specific toxin alloxan (38,39). Wild-type mice reconstituted with MIP/GFP or β/GFP bone marrow cells were cytokine mobilized before and after alloxan treatment (Fig. 4*A*). To verify the extent of alloxan-induced β-cell injury, blood insulin and glucose levels were measured 5 days later. Alloxan-treated mice had reduced blood insulin levels (4 ± 2 μU/ml)

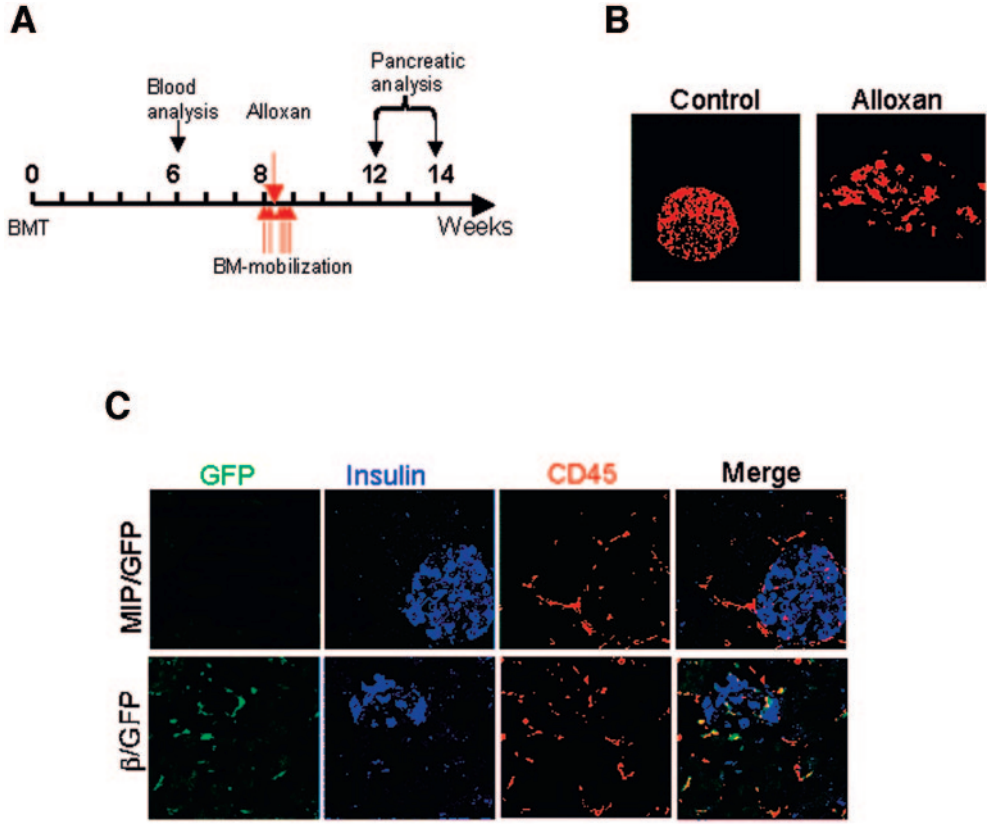

FIG. 4. Pancreatic engraftment of cytokine-mobilized bone marrow cells in mice with alloxan-induced diabetes. *A*: Experimental design. Lethally irradiated wild-type (CD45.1) mice were transplanted with bone marrow cells from MIP/GFP or β/GFP transgenic (both CD45.2) mice (time 0). Analysis of contribution of transplanted bone marrow cells to peripheral blood of recipient mice was performed at week 6. Arrows indicate time points for cytokine treatment to promote bone marrow mobilization and for alloxan treatment to induce diabetes. Mice were killed and pancreatic sections were prepared for analysis 4–6 weeks after alloxan treatment (12–14 weeks posttransplantation). *B*: Insulin immunoreactivity (red) of pancreatic islets from untreated control and alloxan-treated mice, demonstrating typical alloxan-induced reduction in the number of insulin-expressing β-cells. *C*: Triple analysis of pancreatic sections for GFP (green), insulin (blue), and CD45 (red) in alloxan-treated mice transplanted with MIP/GFP or β/GFP transgenic bone marrow cells. Sections are representative for data obtained in 21 MIP/GFP (three experiments) and 21 β/GFP (three experiments) bone marrow–transplanted mice. Images were acquired using fluorescence microscopy. Magnification: ×200.

TABLE 2
Hematopoietic and pancreatic engraftment of bone marrow–derived cells in transplanted diabetic mice

Source	Transgene	*n**	Percent hematopoietic chimerism	Pancreatic GFP$^+$cells (*n* sections†; positive sections‡)	Percent of pancreatic GFP$^+$ cells (insulin, Pdx1.1, Nkx6.1, CD45, CD4/CD8, B220, and Mac-1/Gr-1)
Whole bone marrow	MIP/GFP	21	94 ± 1	840; 0%	NA, NA, NA, NA, NA, NA, and NA
Whole bone marrow	β/GFP	21	93 ± 1	630;100%	0, 0, 0, >99.9, 0, 0, and >99

Data are means ± SD, unless otherwise indicated. *Lethally irradiated mice were transplanted with 4.0×10^6 whole bone marrow cells from MIP/GFP or β/GFP mice. Transplanted mice were analyzed for hematopoietic contribution of transplanted bone marrow cells 6 weeks posttransplantation and for pancreatic reconstitution at 4–6 weeks after alloxan treatment (12–14 weeks posttransplantation). †Number of pancreatic sections analyzed for GFP (by epifluorescence and/or anti-GFP antibodies) as well as insulin, Pdx-1, and Nkx6.1 expression. Thirty fields of pancreatic sections of mice transplanted with β-GFP bone marrow cells were analyzed (using × 40 objects) for coexpression of GFP and CD45 with the blood lineage markers CD4/CD8, B220, and Mac-1/Gr-1. ‡Frequency of sections containing GFP$^+$ cells. Specificity of each marker was confirmed using positive and negative control tissues (see RESEARCH DESIGN AND METHODS). NA, not applicable.

compared with untreated controls (25 ± 7 μU/ml), and blood glucose was elevated 2.5-fold from 6 ± 2 mmol/l in untreated mice to 15 ± 4 mmol/l. Furthermore, immunohistochemical analysis of pancreatic islets from alloxan-treated mice revealed extensive pancreatic islets damage (Fig. 4*B*). However, in accordance with the data from nondiabetic mice, no GFP$^+$ cells were observed in the pancreata of alloxan-treated mice reconstituted with MIP/GFP bone marrow cells (Fig. 4*C*, Table 2). In total, we investigated sections representing ~50,000 pancreatic β-cells in 21 mice. Alloxan-treated mice reconstituted with β/GFP cells consistently exhibited pancreatic engraftment with GFP$^+$ bone marrow–derived cells (Fig. 4*C*, Table 2). However, investigation of sections representing >30,000 pancreatic β-cells demonstrated that >99% of the bone marrow–derived GFP$^+$ cells coexpressed the hematopoietic markers CD45 and Mac-1/Gr-1, whereas not a single GFP$^+$ cell was found to express insulin, Pdx-1, or Nkx6.1 (Fig. 4*C*, Table 2).

Finally, mice reconstituted with MIP/GFP bone marrow cells were treated with exendin-4, a glucagon-like peptide-1 analog that, in addition to its insulin-releasing action, stimulates pancreatic β-cell growth with resultant increase in pancreatic β-cell mass (41,42). However, exendin-4 had no detectable effect when this cohort (alloxan-treated mice) was analyzed 4 weeks after exendin-4 treatment and no GFP$^+$ cells were observed in investigated pancreatic or liver sections (not shown).

DISCUSSION

The recent report (25) of bone marrow cells giving rise to up to 3% insulin-expressing cells when transplanted in vivo has raised the exciting possibility that bone marrow cells could be used for cellular replacement therapy in diabetes (28,31). Because they are so readily accessible and can be delivered by autologous transplantation, bone marrow cells would represent a particularly attractive source of β-cells for cell replacement therapy. However, before embarking on a route for clinical development, it is essential to first unequivocally establish that bone marrow–derived β-cell transdifferentiation does indeed occur reproducibly. Many claims of bone marrow transdifferentiation have proven to be difficult to reproduce (10,12,14, 32,43). Therefore, it has been proposed that a key criterion for demonstration of transdifferentation of bone marrow cells is that the finding should be independently reproduced in more than one laboratory (26,44). Unfortunately, several recent studies have partially (32) or fully (34) failed to reproduce the initial findings of bone marrow–derived β-cell generation (25). However, because alternative procedures were used for tracing transplanted bone marrow cells and/or conditioning the recipients, it remains possible that bone marrow transdifferentiation to insulin-expressing cells only occurs under certain conditions. Furthermore, the cellular fate(s) of a large fraction of bone marrow–derived cells engrafted in the pancreas was not established in these studies, leaving open the possibility that they might represent bone marrow cells that have partially embarked on a differentiation path toward β-cells (32,34). Here, we systematically compared multiple approaches to promote and investigate the β-cell potential of the transplanted bone marrow cells. One of these was the same as that used by Ianus et al. (25) and involved analysis of pancreatic sections for presence of GFP$^+$ cells 4–6 weeks after bone marrow transplantation into lethally irradiated mice of the same background as in the original study. To enhance resolution, we used bone marrow cells in which GFP was under the control of an insulin-specific promoter. This was essential to allow potentially very low levels of transdifferentiation to be detected. Although the levels of hematopoietic reconstitution obtained with bone marrow transplantation were high, we failed to detect any GFP$^+$ cells among the high number of transplanted mice and β-cells investigated. Importantly, if the levels of transdifferentiation had been similar to Ianus et al. (25) (1.7–3%), we would have expected to find at least 1×10^4 GFP$^+$ cells in the investigated sections. Performing similar experiments with bone marrow cells in which GFP was instead under the control of the ubiquitous β-actin promoter, we were able to demonstrate considerable GFP$^+$ engraftment in the pancreas of bone marrow–transplanted mice. However, none of the engrafted cells expressed insulin or the transcription factors Pdx-1 and Nkx6.1 (45,46). Most importantly, in contrast to Choi et al. (34) and Lechner et al. (32), who used a different anti-CD45 antibody than us, we were able to demonstrate that >99.9% of the bone marrow–derived GFP$^+$ cells expressed the panhematopoietic marker CD45 as well as the myeloid antigen Mac-1 (12). Thus, our data clearly demonstrate that β/GFP bone marrow–derived cells engraft efficiently in the pancreas but adopt almost exclusively a hematopoietic cell fate. As we failed to detect any bone marrow–derived β-cells in steady state, we also investigated the ability of MIP/GFP as well as β/GFP bone marrow cells to generate β-cells in mice rendered diabetic by alloxan treatment. However, not a single bone marrow–derived cell was found to express insulin, Pdx-1, or Nkx6.1, whereas close to 100% of them expressed the blood cell antigens CD45 and Mac-1/Gr-1. It is unclear why we were unable to confirm the observations of Ianus et al.

If this is attributable to subtle differences in the experimental protocols, the implication is that the phenomenon of in vivo bone marrow–derived β-cell differentiation is not robust. Consequently, the requisite conditions must be much better defined before even considering any clinical applications. However, as already emphasized by others (44), it is also necessary to reinvestigate the experimental approaches, as cells can show considerable autofluorescence and unspecific binding of antibodies. Although our studies provided no support of the hypothesis that fresh bone marrow cells can transdifferentiate into β-cells upon in vivo transplantation, they do not exclude that cells with such potential might be derived from cultured bone marrow cells as recently described (47,48). Furthermore, recent studies have suggested that bone marrow transplantation might promote endogenous pancreatic β-cell regeneration (35,49).

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