Original Article

Normal Relationship of β- and Non–β-Cells Not Needed for Successful Islet Transplantation

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Islets are composed mostly of β-cells, and therefore stem cell research has concentrated on generating purified β-cells, neglecting the other endocrine cell types in the islet. We investigated the presence of endocrine non–β-cells after islet transplantation. In addition, we studied whether the transplantation of pure β-cells, in volumes similar to that used in islet transplantation, would suffice to reverse hyperglycemia in diabetic mice. Rat islets were dispersed and β-cells were purified by fluorescence-activated cell sorting according to their endogenous fluorescence. After reaggregation, 600 islet equivalents of the purified β-cell aggregates were implanted into diabetic SCID mice. In mice implanted with β-cell–enriched aggregates, the hyperglycemia was reversed and good graft function over a 12-week period was observed with regard to glucose and insulin levels, glucose tolerance tests, and graft insulin content. The endocrine cell composition of the β-cell–enriched aggregates remained constant; before and 12 weeks after transplantation, the β-cell–enriched aggregates comprised 95% β-cells and 5% endocrine non–β-cells. However, islet grafts, despite originally having comprised 75% β-cells and 25% endocrine non–β-cells, comprised just 5% endocrine non–β-cells after transplantation, indicating a loss of these cells. β-Cell–enriched aggregates can effectively reverse hyperglycemia in mice, and transplanted intact islets are depleted in non–β-cells. It is therefore likely that islet non–β-cells are not essential for successful islet transplantation. *Diabetes* 56:2312–2318, 2007

Islets of Langerhans consist of four main endocrine cell types: insulin-producing β-cells, glucagon-producing α-cells, somatostatin-producing δ-cells, and pancreatic polypeptide–producing PP cells. The most abundant cell is the β-cell, which makes up ~60–80% of islet cells in most mammalian species (1). The replacement of the β-cells by islet or pancreas transplantation is presently the only treatment of type 1 diabetes that can render the patient insulin independent (2,3). However, the supply of donor pancreases is severely limited, and the demand is far greater than the supply. To overcome this problem, much effort has been made in trying to derive β-cells from embryonic stem cells (4–7) and other putative adult stem cells, such as pancreatic duct cells (8), bone marrow cells (9), and acinar cells (10). This would potentially create an unlimited supply of β-cells for transplantation purposes. Moreover, it can be envisaged that the β-cells generated in vitro could be genetically manipulated to confer protection against damage after transplantation (11). It may be that stem cell technology will find a way to produce β-cells but not islet non–β-cells. Therefore, it is important to know whether a pure β-cell graft can effectively reverse hyperglycemia. The importance of the non–β-cells in the physiology of maintaining normoglycemia is well known (12). However, it is not clear that the non–β-cells of islets have a meaningful local influence on β-cell function. In fact, the microvasculature of the islet and functional studies indicate that non–β-cells are downstream from β-cell secretion, suggesting that most β-cells see very little intraislet glucagon or somatostatin (13,14). Also, there are nonendocrine cell types present in the islets, including endothelial cells, which could potentially have an impact on islet transplantation outcome (15,16). To obtain a purified population of β-cells, the islets had to be dispersed into single cells. Our initial study therefore investigated the effect of dispersion and reaggregation of islet cells on the outcome of transplantation. We then studied the outcome of β-cell–enriched transplants and investigated further non–β-cells in normal islet transplantation.

RESEARCH DESIGN AND METHODS

Male Sprague-Dawley rats were used as islet donors. Male ICR/SCID mice (Taconic, Germantown, NY) aged 6–10 weeks were used as recipients of grafts. Recipient mice were made diabetic with a single intraperitoneal injection of streptozocin (Sigma, St. Louis, MO) 180 mg/kg body wt, freshly dissolved in citrate buffer (pH 4.5). Only those mice with a blood glucose concentration >350 mg/dl at 5 days were used as recipients. Blood glucose concentrations were determined using a glucose meter (Precision QID; Abbott Labs, Bedford, MA) with blood obtained from a snipped tail. All animal experiments were approved by the Joslin Animal Care Committee.

Islet isolation and culture. Rat islets were isolated by using a collagenase digestion followed by separation using a density gradient as previously described in detail (17). Briefly, under anesthesia, a laparotomy was performed, and the pancreas was exposed. After ligation at the ampulla of Vater, 9 ml collagenase solution (Liberase RI; Roche, Indianapolis, IN) was injected into the pancreas via the common bile duct. The pancreas was removed and incubated in a stationary water bath for ~25 min at 37°C. The islets were separated by a density gradient (Histopaque-1077; Sigma) and centrifuged at 1,750*g* for 20 min. After washing, islets were handpicked and cultured

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IE, islet equivalent; IPGTT, intraperitoneal glucose tolerance test.

overnight in RPMI-1640 plus 10% FCS before islet transplantation or dispersion.

Dispersion. The rat islets were washed two times in PBS. As previously described (18), a solution of 1 mg/ml trypsin and 30 μg/ml DNase was then added to the islets, which were then incubated for 15 min in a 37°C incubator. During the digestion, the islets were vortexed every 5 min for 10 s. Cold media with serum was then added to stop the digestion, and the cells were washed two times. The cells were then counted and either sorted or aliquoted for either transplantation or reaggregation.

β-Cell sorting. Before sorting, cells were filtered through a 35-μm mesh to exclude cell clumps. Cells were sorted using a DakoCytomation MoFlo Cytometer (Dako, Ft. Collins, CO), where cells were gated according to forward scatter (19,20), and then sorted on the basis of endogenous fluorescence (21). Sorted cells were collected on ice and put into culture for reaggregation as described below.

Reaggregation. Cells were cultured in RPMI-1640 and 10% FCS for a period of 72 h in Corning Ultra Low Attachment plates (Corning/Costar, Ithaca, NY). During this time, the cells aggregated to islet-like clusters ~50–150 μm in size. These reaggregates were counted and calculated as islet equivalents (IEs) (22).

Transplantation. Cells were counted and aliquoted into groups of 1.2×10^6 or 2.5×10^6 cells. Islets or clusters were counted and aliquoted into groups of 600 IEs. The cells, cell clusters, or islets were then centrifuged in PE50 polyethylene tubing (Becton Dickinson, Sparks, MD) to form pellets. Male ICR/SCID mice were anesthetized using Avertin (Sigma) and placed on a heated plate for surgery. The left kidney was exposed through a lumbar incision, and the kidney capsule was incised. Using a Hamilton syringe (Fisher, Pittsburg, PA) and the PE50 polyethylene tubing, rat islets, aggregates or dispersed cells were placed under the kidney capsule as previously described (23). The incision in the kidney capsule was then cauterized, and the peritoneum was sutured. The skin was then closed using staples, and mice were allowed to recover under a warm lamp.

Graft function. The blood glucose and weights of the ICR/SCID recipient mice implanted with dispersed rat islet cells or reaggregated rat islet cells were monitored weekly for 12 weeks. Intraperitoneal glucose tolerance tests (IPGTTs) were carried out 12 weeks after transplantation in recipient mice and weight-matched controls to assess graft function. In the IPGTTs, 2 g/kg glucose was injected into mice with a fasted blood glucose concentration of <200 mg/dl. Blood glucose concentrations were measured before the injection (time 0) and then at 15, 30, 60, 90, and 120 min. At the end of the experiment, a nephrectomy was then carried out to remove the graft.

In a second set of experiments, diabetic mice were implanted with either 600 IEs whole rat islets or 600 IEs rat β-cell–enriched aggregates for 12 weeks. Blood glucose concentrations were measured, weights were monitored weekly, and IPGTTs were carried out 4 and 12 weeks after transplantation in recipient mice and age-matched controls as described above. In addition, at 4 and 12 weeks after transplantation, serum samples were collected at the 0-, 30-, 60-, and 120-min time points during the IPGTT. Serum insulin was then quantified using enzyme-linked immunosorbent assay. The grafts were removed by nephrectomy. Some were used to measure graft insulin content, whereas other grafts were processed for histology, and their composition studied as described below. Furthermore, additional diabetic mice were implanted with 600 IEs intact rat islets for a period of 1 or 2 weeks to study graft composition.

Quantification of β-cells versus non–β-cells. Cell aggregates and graft-bearing kidneys were fixed in 4% paraformaldehyde and embedded in paraffin. Sections were stained for insulin and other islet endocrine cells (glucagon, somatostatin, and pancreatic polypeptide). Briefly, sections were hydrated, and donkey serum (1:50) was added for 30 min. An antibody cocktail was added that contained the following antibodies: rabbit anti-glucagon (1:3,000), rabbit anti-somatostatin (1:200), and rabbit anti–pancreatic polypeptide (1:200). Alternatively, some slides were incubated with the rabbit anti-glucagon antibody alone (1:3,000). The slides were stored at 4°C in a humidity chamber overnight. After washing, donkey biotin anti-rabbit (1:400) was added for 1 h before washing and the addition of Streptavidin Alexofluor (1:400) for 1 additional h. The slides were then washed, guinea pig anti-bovine insulin antibody (1:200) was added to the sections for 1 h before washing, and a Texas red–conjugated anti–guinea pig antibody (1:200) was added for 1 h. Complete grafts were photographed with the confocal mode of an LSM410 microscope. The number of insulin-positive cells (red) and endocrine non–β-cells (green) was quantified on each image of each graft by independent counting by two individuals who were blinded to the origin of the slide. On average, 1,200 cells were counted in each preparation. Eight of each (islet and β-cell-enriched) of the grafts were analyzed. In addition, the percentage of insulin-positive cells that was >50 μm from any endocrine non–β-cells was calculated. This was done to calculate how many β-cells were so far from any endocrine non–β-cell that paracrine interactions were unlikely. To do this, circles with a radius of 50 μm were drawn around each non–β endocrine cell, and the number of insulin-positive cells not falling within any circle was counted and expressed as a percentage of total insulin-positive cells.

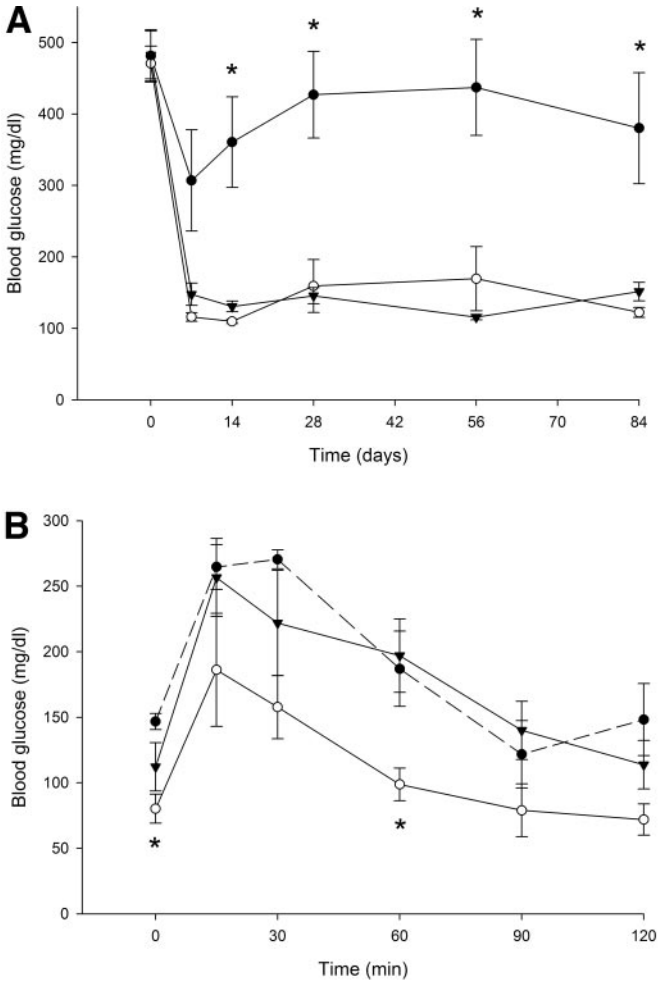

FIG. 1. *A*: Blood glucose after transplantation of 1.2×10^6 dispersed rat islet cells (●) or 2.5×10^6 dispersed rat islet cells (○) or 600 reaggregated rat IEs (~$0.9–1.2 \times 10^6$ cells) (▲). *$P < 0.05$ vs. 2.5×10^6 dispersed rat islet cells and 600 reaggregated rat IE (ANOVA with Bonferroni post hoc test, $n = 5–8$). *B*: IPGTT (2 g/kg body wt) 12 weeks after transplantation in cured mice transplanted with 2.5×10^6 dispersed rat islet cells (○) or 600 reaggregated rat IEs (▲). Mice transplanted with 1.2×10^6 dispersed rat islet cells are excluded from the graph because of too few mice that were cured. Controls are weight-matched, nontransplanted, nondiabetic, control mice (●, broken line). *$P < 0.05$ vs. nondiabetic nontransplanted controls at time 0 and 600 reaggregated IEs at time 60 min (ANOVA with Bonferroni post hoc test, $n = 4–5$).

Statistics. The Student's *t* test was used to compare two groups. When the data were not normally distributed or did not have equal variance, Mann Whitney rank sum test was used. When more than two groups were compared, an ANOVA was carried out, with a Bonferroni post hoc test. In cases in which the data were not normally distributed or did not have equal variance, an ANOVA on ranks was carried out. The statistical test used is specified for each dataset. All analysis was carried out using Sigmastat (Systat Software, Point Richmond, CA).

RESULTS

Transplantation of nonsorted dispersed and reaggregated islet cells. Transplantation of reaggregated nonpurified islet cells (600 IEs, which contain $0.9–1.2 \times 10^6$ cells) produced better outcomes than 1.2×10^6 dispersed islet cells, showing the benefit of reaggregation (Fig. 1*A*). However, a larger number of transplanted nonpurified dispersed islet cells, 2.5×10^6, had the same success as the

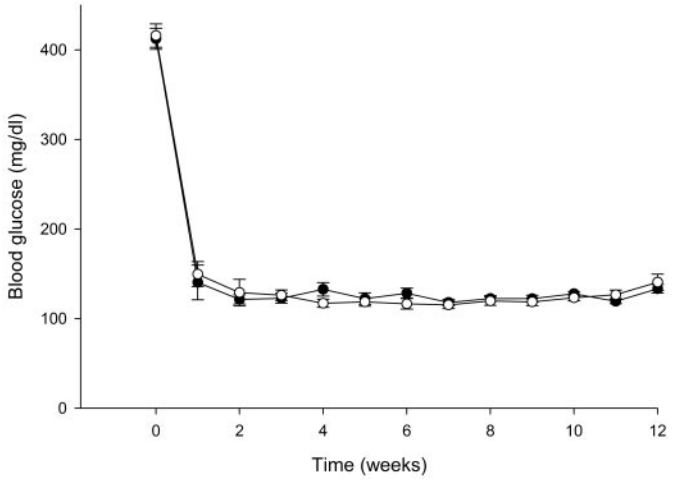

FIG. 2. Blood glucose of diabetic ICR/SCID mice after transplantation of 600 intact rat IEs (●, n = 14) or 600 rat β-cell–enriched reaggregates (○, n = 13).

aggregates, which had one-half the number of cells. In terms of how many animals in each group had been cured, at 84 days, 100% of mice transplanted with 2.5×10^6 cells (n = 8) or 600 IE reaggregated clusters (n = 5) had been cured, compared with just 33% of mice transplanted with 1.2×10^6 cells (n = 6). Twelve weeks after transplantation, an IPGTT was carried out, using only the animals that had nonfasted blood glucose levels of <200 mg/dl (Fig. 1*B*). Because of the small number of animals that had a nonfasting blood glucose of <200 mg/dl in the group transplanted with 1.2×10^6 cells (n = 2), this group was excluded from the statistical analysis. At time point 0 (i.e., before glucose injection), mice transplanted with 2.5×10^6 cells had significantly lower blood glucose than weight-matched nondiabetic nontransplanted control mice (ANOVA with Bonferroni post hoc test, $P < 0.05$; n = 4–5). In addition, at 60 min after glucose injection, the mice transplanted with 2.5×10^6 cells had lower blood glucose levels than mice transplanted with islet cell aggregates (ANOVA with Bonferroni post hoc test, $P < 0.05$; n = 5). Of the two cured mice that had received 1.2×10^6 cells, one was glucose intolerant (blood glucose 60 min after glucose injection, 296 mg/dl), and the other had quite low blood glucose levels 60 min after injection (67 mg/dl), which rose to 76 mg/dl at the 2-h time point.

Transplantation of β-cell–enriched aggregates. No difference was seen in the nonfasted blood glucose of recipients of intact islets (600 IEs) and recipients of β-cell–enriched aggregates (600 IEs) (Fig. 2). During an IPGTT 4 weeks after transplantation, blood glucose levels were lower at 60 and 90 min after injection in mice transplanted with β-cell–enriched aggregates than in mice transplanted with islets (Fig. 3*A*; $P < 0.05$, ANOVA with Bonferroni post hoc test, n = 13–14). Age-matched nontransplanted controls had higher blood glucose levels 90 and 120 min after glucose injection compared with islet recipients. Moreover, age-matched controls had higher fasting blood glucose levels (87.0 ± 5.3 mg/dl) than mice that had received an islet transplant ($P < 0.05$, ANOVA with Bonferroni post hoc test; n = 6). There was no difference in fasting blood glucose levels in mice that had received an islet transplant versus an enriched β-cell aggregate transplant (68.7 ± 3.7 vs. 64.5 ± 4.9 mg/dl, respectively). At the 4-week

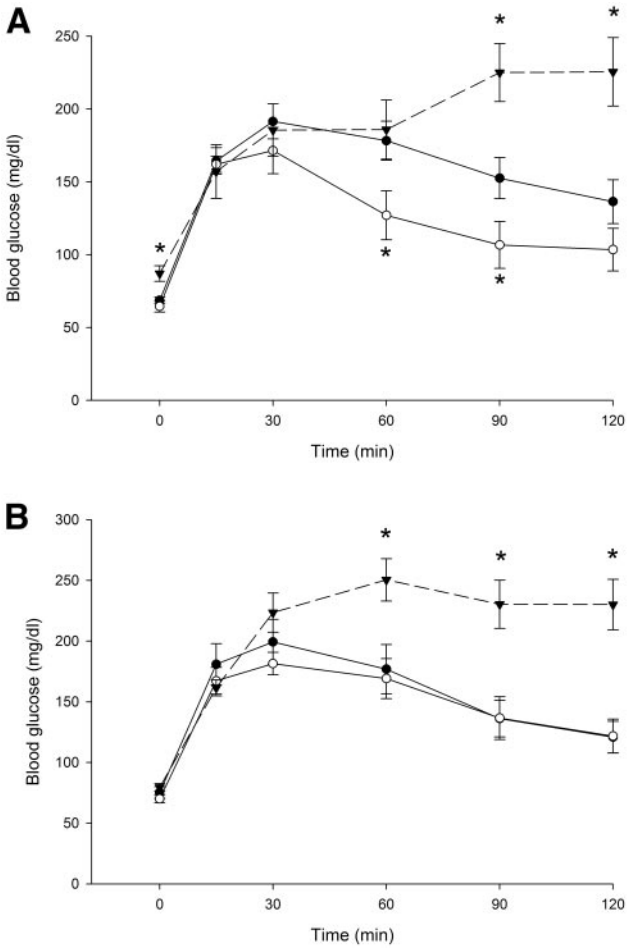

FIG. 3. Blood glucose after an IPGTT (2 g/kg body wt) in mouse recipients of 600 intact rat β-cell–enriched reaggregates (○) and 600 rat IEs (●) 4 or 12 weeks after transplantation (*B*) or age-matched controls (broken line, ▲). *P < 0.05 vs. mice receiving islets (ANOVA with Bonferroni post hoc test).

IPGTT, the weights of the mice that had a received a transplant of enriched β-cell aggregates were similar to those that had received islets (29.9 ± 0.83 vs. 30.2 ± 0.85 g). However, the age-matched controls weighed on average 35.4 ± 1.6 g. When glucose tolerance was tested 12 weeks after transplantation, there were no differences between mice implanted with islets or β-cell–enriched cell aggregates (Fig. 3*B*). Age-matched controls had higher blood glucose levels than islet transplant recipients 60, 90, and 120 min after transplantation ($P < 0.05$, ANOVA with Bonferroni post hoc test; n = 8–14). The weight of the age-matched controls at this time point was 39.9 ± 1.8 g compared with 30.3 ± 0.9 g and 32.8 ± 1.2 g in mice that had received enriched β-cell aggregate grafts and islet grafts, respectively.

Four weeks after transplantation, there were no significant differences in serum insulin levels in mice implanted with islets or β-cell–enriched aggregates before and after a glucose tolerance test. However, 60 min after glucose injection, age-matched control mice had higher serum insulin levels than in the mice transplanted with islets (Table 1; $P < 0.05$, ANOVA with Bonferroni post hoc test; n = 6). Twelve weeks after transplantation, serum insulin levels after an IPGTT did not differ among any of the groups ($P > 0.05$, ANOVA; n = 6).

Twelve weeks after transplantation, the mice were

TABLE 1
Serum insulin levels in mice after intraperitoneal injection of 2 g/kg glucose

	Time after glucose injection (min)					
	0		30		60	
	4 weeks	12 weeks	4 weeks	12 weeks	4 weeks	12 weeks
Nontransplanted, nondiabetic control mice (ng/ml)	0.69 ± 0.19	0.61 ± 0.11	0.65 ± 0.11	0.51 ± 0.07	0.96 ± 0.11	0.72 ± 0.10
Islet recipients (ng/ml)	0.39 ± 0.06	0.35 ± 0.11	0.38 ± 0.09	0.40 ± 0.08	0.53 ± 0.11	0.48 ± 0.10
β-Cell aggregates recipients (ng/ml)	0.48 ± 0.06	0.34 ± 0.03	0.63 ± 0.07	0.42 ± 0.07	0.72 ± 0.06	0.42 ± 0.07

Data are means ± SE.

nephrectomized, after which all animals reverted to hyperglycemia. In some mice, graft composition was quantified (see below). In others, the insulin content of the graft was measured. No differences could be detected in graft insulin content when comparing islet grafts with β-cell–enriched aggregate grafts (5.7 ± 1.7 μg in islet grafts vs. 3.4 ± 1.0 μg in β-cell aggregates grafts; $P = 0.28$, t test; $n = 5$).

Graft composition. As shown in Table 2, the β-cell–enriched aggregates contained a significantly higher percentage of β-cells than islets before transplantation (Table 2; Fig. 4*A* and *B*). However, 12 weeks after transplantation, the islets contained a similar percentage of β-cells as the islet grafts (Table 2; Fig. 4*C* and *D*), with ranges between 92.7 and 97.4% β-cells in the islet grafts and 93.2 and 98.5% β-cells in the β-cell–enriched aggregate grafts. The decrease in percentage of non–β-cells in islet grafts compared with islets before transplantation was highly significant (t test, $P < 0.001$). Similar results were seen when a glucagon antibody alone was used rather than the cocktail of glucagon, somatostatin, and PP antibodies, with the percentage of glucagon-positive cells falling from 26.1 to 4.4% after transplantation. In addition, in the grafts, the majority of β-cells were >50 μm from any non–β-cell (Table 2; Fig. 5). In 1- and 2-week-old grafts, the percentage of endocrine cells that were β-cells were 93.7 ± 0.7 and 98.0 ± 0.6%, respectively (average cells counted per graft, 931 ± 102; $n = 4$ in each group).

DISCUSSION

This study provides evidence that β-cells can function well in a graft site without nearby non–β-cells. To study β-cell–enriched grafts, islets had to be dispersed into single cells to allow fluorescence-activated cell sorting. Our initial studies therefore investigated the effect of islet dispersion and reaggregation on transplantation outcome. When the dispersed cells were reaggregated before transplantation, fewer cells were needed to reverse hyperglycemia. After IPGTTs, reaggregated islets had a glucose profile similar to weight-matched nondiabetic, nontransplanted control mice. However, mice transplanted with 2.5 million dispersed islet cells had even lower blood glucose levels after the glucose tolerance test. This finding indicates that transplantation of dispersed islet cells can be efficient, providing that sufficient numbers are transplanted. However, if islet cells are reaggregated before transplantation, one-half the number of cells can restore normoglycemia. These results are in line with several previous studies that have shown that insulin secretory responses are decreased in dispersed islet cells and are improved by reaggregation of the cells (24–27). This superior result may be due to a positive influence of cell communication upon insulin secretion and raises questions about whether aggregated cells are more resistant to apoptosis. It is likely that the dispersed cells formed clusters in vivo after transplantation, but this may occur after many cells are lost in the first days after transplantation. In summary, it is evident that larger numbers of dispersed cells are initially required to reverse hyperglycemia.

In our next study, dispersed islet cells were enriched for β-cells by use of fluorescent-activated cell sorting. β-Cells were sorted according to their endogenous fluorescence and forward scatter (size), as has been previously described (19–21). Using this technique, we were able to obtain cell populations of ~95% β-cells. These β-cell–enriched fractions were then reaggregated before transplantation. Mice that had been transplanted with β-cell–enriched aggregates had a rapid reversal of hyperglycemia in a similar manner to mice transplanted with a similar volume of whole islets. It should be noted that the number of β-cells would have been lower in the islet transplants compared with the β-cell transplants. It is likely that the β-cell transplants, with 95% β-cell purity, contained ~20% more β-cells than in the islets. However, the aim of the study was to establish whether purified β-cells, transplanted in volumes similar to those used in islet transplantation, could reverse diabetes and to establish whether β-cells derived from stem cells could ever be useful for transplantation to diabetic patients. In glucose tolerance tests 4 weeks after transplantation, mice transplanted with β-cell–enriched ag-

TABLE 2
Composition of islets and β-cell aggregates before and after transplantation

Graft characteristics	Islets		β-Cell aggregates	
	Before transplantation	12 weeks after transplantation	Before transplantation	12 weeks after transplantation
n	7	8	4	8
β-Cells (%)	75.3 ± 1.5	95.1 ± 0.6	96.7 ± 1.0	94.3 ± 0.8
β-Cells >50 μm from an endocrine non–β-cell (%)	23.4 ± 6.2	70.3 ± 1.8	63.8 ± 2.1	67.2 ± 5.1

Data are means ± SE.

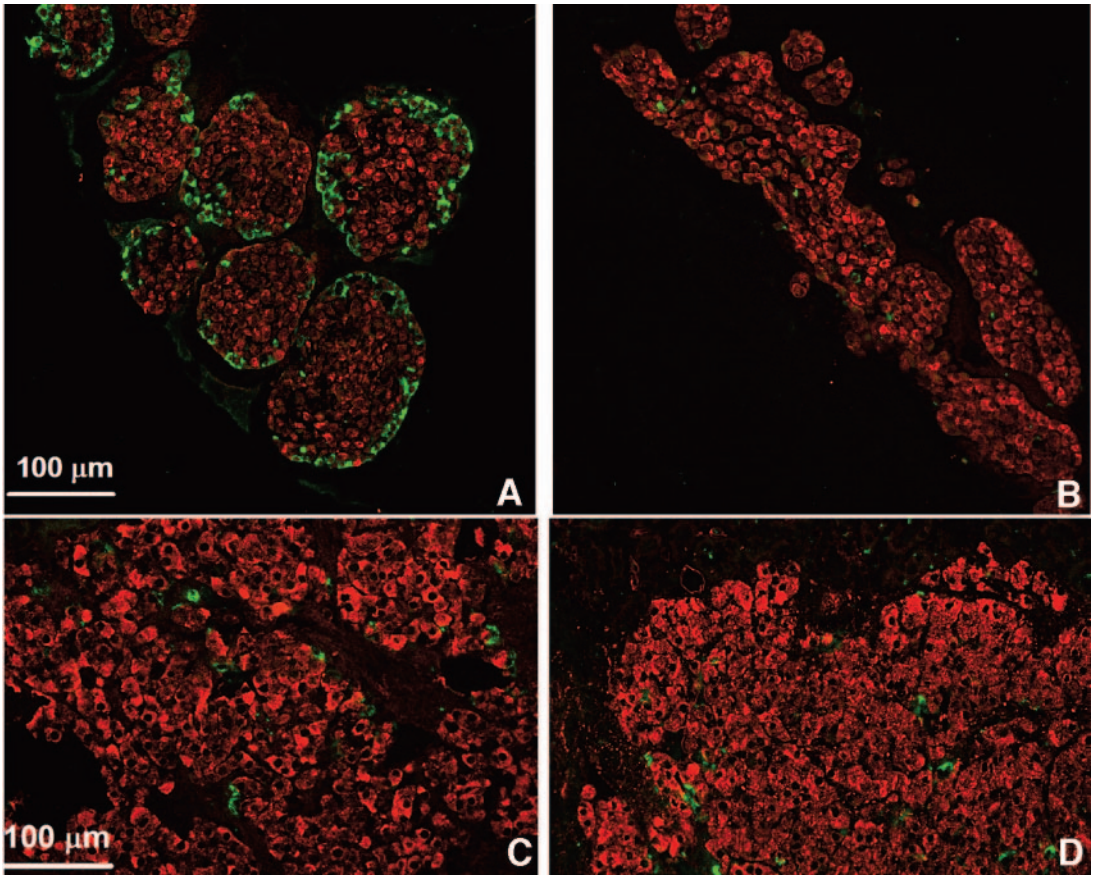

FIG. 4. Representative section of rat islets (*A*) and β-cell–enriched aggregates (*B*) before transplantation and 12 weeks after transplantation (*C* and *D*, respectively). Insulin is stained red; glucagon, somatostatin, and PP are stained green.

gregates had slightly lower blood glucose levels and tended to have slightly higher insulin levels than those transplanted with islets. However, by 12 weeks there was no difference between these groups with regard to glucose tolerance, insulin levels, or graft insulin content. In this experimental series, the nondiabetic, nontransplanted control mice were age matched rather than weight matched. Whereas the weights of the transplanted mice had remained stable or dropped during their period of diabetes, the control mice continued to gain weight throughout the study. Their poor glucose tolerance and higher insulin levels suggest that these control mice had become insulin resistant. In addition, the lower glucose levels of the transplanted mice may be explained by the fact that rat islets were implanted, because rats typically have lower glucose levels than mice and may have a lower set point for glucose-stimulated insulin secretion.

These results show that the transplantation outcome of β-cell–enriched aggregates is similar to that of islets. Transplantation of a purified population of β-cells has been previously shown by Keymeulen et al. (28) to be successful, although it was suggested in this study that mixing endocrine non–β-cells with β-cells before transplantation improved graft function. In the Keymeulen study, the grafts were studied for 64 weeks rather than the 12 weeks in this study. Their rat β-cell grafts and mixed grafts of rat β-cells plus rat endocrine non–β-cells functioned well in rats until ~20 weeks, when some of the grafts started to fail. By 64 weeks, all grafts, regardless of whether they contained purified β-cells or β-cells mixed with other endocrine non–β-cells, had partially or fully failed. However, they implanted about 1.2–1.7 million β-cells into rats, which could be regarded as a minimal mass model. This thorough study also showed that β-cells from older rat donors do not function as well as β-cells from younger rat donors. Thus, the Keymeulen study complements the findings of the present study in that both show impressive function of grafts consisting of purified β-cells. Another cell type within islets that could potentially contribute to the outcome of transplantation is the endothelial cell, which has been reported to contribute to the revascularization of transplanted islets (15,16). However, it is not clear that there are enough of these cells to make a meaningful contribution. Nonetheless, it is evident from the current studies that although endocrine non–β-cells and donor endothelium may be beneficial to the function of transplanted islet grafts, they do not seem to be essential for successful transplantation. This finding indicates that β-cells derived in vitro from stem cells or some other source could be very useful for transplantation purposes.

When graft composition was studied 12 weeks after transplantation, the β-cell–enriched aggregates had virtually the same 5% of non–β-cells in the grafts as was in the initial preparations. Although this was not surprising, it was remarkable to find that islets that initially had 25% non–β-cells had only 5% within the grafts. The vast majority of these cells are glucagon-containing α-cells. If the β-cell–enriched grafts had lost non–β-cells to the same degree as the islet grafts, one would have expected them to have only 1% of non–β-cells after transplantation. It is

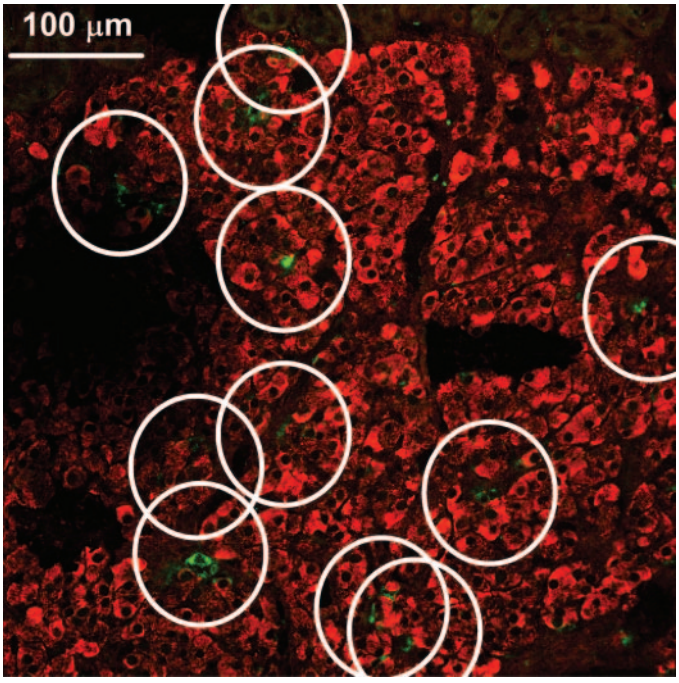

FIG. 5. To calculate how many β-cells were >50 μm from any endocrine non–β-cell, circles with a radius of 50 μm were placed around each endocrine non–β-cell (stained green for glucagon, somatostatin, and PP). The number of insulin-positive cells (stained red) that were outside any circle was then expressed as a percentage of total β-cells.

also intriguing that the loss of non–β-cells in the islet grafts seemed to stop at 5%, which was the same percentage of non–β-cells found in the β-cell–enriched grafts. In agreement with these findings, we also found that when immature neonatal porcine pancreatic cell clusters were transplanted into nude mice, the grafts many months later were ~95% β-cells (29). This phenomenon of the present experiments with rat islets might be explained by preferential death of the non–β-cells as described before (30–33). These studies and our own conclude that the decrease is mainly accounted for by glucagon-producing cells (30–33). We do not know whether δ-cells and PP cells also disappear. The peripheral localization of α-cells in rodent islets could potentially make them more vulnerable to damage during the isolation. Although we have not studied this in detail in the present study, previous studies have indicated that this is not the major reason for α-cell loss after transplantation. In a study by Lau et al. (33), islets implanted in the liver had lost α-cells at 4 weeks after transplantation, whereas islets implanted in kidney from the same isolation had not, indicating that isolation-related events had not caused the loss of these peripheral cells (33). In that study, islets implanted intraportally showed loss of α-cells, whereas islets implanted under the kidney capsule showed no loss of α-cells. In the present study, we saw loss of non–β-cells in islets under the kidney capsule 1 week after implantation. One difference between their study and this study is that they implanted islets into normoglycemic rats, whereas in the present study, the recipients were hyperglycemic mice. It is possible that differences in metabolic load on the islet graft may affect the α-cell survival. It cannot be ruled out that α-cell loss is more rapid in the liver site, as Lau et al. (33) reported. Gunther et al. (31) reported the loss of α-cells as early as 2 days after implantation into the liver of diabetic rats (31). Because normal levels of glucagon and other non–β-cell–derived peptides are still produced by the endogenous pancreas, it is possible that the non–β-cells in the grafts disappear because of involution to prevent hyperglucagonemia.

The hypothetical question, which may one day be of practical importance, is whether a graft of pure β-cells would provide an adequate transplant result. In terms of whole-body metabolic effects of glucagon secretion from grafts, it appears that secretion from grafts provides minimal protection against acute hypoglycemia (34), yet human islet transplants can maintain near normal glucose levels. The persistent glucagon secretion from the pancreas may make some contribution to glucose homeostasis but, again, little protection against acute hypoglycemia. These deficient glucagon responses could make recipients more prone to hypoglycemia during exercise (35,36), but this is unlikely to outweigh the benefits. The next question is whether there is some important local influence of islet non–β-cells on β-cell function. Considering the islet microvascular anatomy and the physiological experiments showing that non–β-cells are downstream of β-cells (13,14), it seems likely that most β-cells are not influenced by non–β-cells. Some insight may be provided by our measurements that determined how far β-cells were away from non–β-cells. When we quantified the number of β-cells that were in close proximity to a non–β-cell, it was found that 65–75% of β-cells were 50 μm from a non–β-cell. It seems unlikely that local secretion from non–β-cells could have much paracrine influence at that distance. Moreover, from studies of others (37,38), we know that the microvasculature of transplanted islets is very different from normal. Thus, the anatomy of islet grafts is very different from islets in the pancreas, yet these grafts are still efficacious in reversing hyperglycemia in diabetic recipients.

In conclusion, we found that β-cell–enriched aggregates can effectively reverse hyperglycemia in mice and that transplanted intact islets are depleted in non–β-cells. It is therefore likely that islet non–β-cells are not essential for successful islet transplantation.

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