Transplanted Beta Cell Response to Increased Metabolic Demand

Changes in Beta Cell Replication and Mass

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Abstract

We determined the capacity of transplanted beta cells to modify their replication and mass when stimulated by changes in metabolic demand. Five groups of Lewis rats were studied: group 1 (Tx-Px) had a 95% pancreatectomy 14 d after transplantation of 500 islets; group 2 (Px-Tx) had a 95% pancreatectomy 14 d before transplantation of 500 islets; group 3 (Tx) was transplanted with 500 islets; group 4 (Px) had a 95% pancreatectomy; and group 5 (normal) was neither transplanted nor pancreatectomized. Blood glucose was normal in Tx-Px and Tx groups at all times. Px-Tx and Px groups developed severe hyperglycemia after pancreatectomy that was corrected in Px-Tx group in 83% of rats 28 d after transplantation. Replication of transplanted beta cells increased in Tx-Px ($1.15\pm0.12\%$) and Px-Tx ($0.85\pm0.12\%$) groups, but not in Tx group ($0.64\pm0.07\%$) compared with normal pancreatic beta cells ($0.38\pm0.05\%$) ($P < 0.001$). Mean beta cell size increased in Tx-Px ($311\pm14\ \mu m^2$) and Px-Tx ($328\pm13\ \mu m^2$) groups compared with Tx ($252\pm12\ \mu m^2$) and normal ($239\pm9\ \mu m^2$) groups ($P < 0.001$). Transplanted beta cell mass increased in Tx-Px (1.87 ± 0.51 mg) and Px-Tx (1.55 ± 0.21 mg) groups compared with Tx group (0.78 ± 0.17 mg) ($P < 0.05$). In summary, changes in transplanted beta cells prevented the development of hyperglycemia in Tx-Px rats. Transplanted beta cells responded to increased metabolic demand increasing their beta cell mass. (*J. Clin. Invest.* 1994. 93:1577–1582.) Key words: diabetes • islet transplantation • beta cell growth • beta cell mass • pancreatectomy

Introduction

The first successful transplants of islet cells in diabetic patients have been reported recently (1–4). However, restoration of normoglycemia is often not achieved or has limited duration. The causes of the limited survival of transplanted islets in diabetic patients and in large animals (5) are not well understood. In addition to rejection, nonimmunological factors could play a role in these poor outcomes. For example, impaired growth capacity of transplanted islets could lead to a decline in beta cell mass and contribute to graft failure.

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A major problem in islet transplantation is obtaining sufficient mass of islet tissue for transplantation. A critical islet mass must be transplanted to achieve normoglycemia, with continued success being dependent upon the number of initially transplanted islets (6–8). Moreover, the islet mass needed for islet transplantation is higher that predicted on the basis of beta cell measurements in diabetes (9). An unknown factor in these considerations in the growth capacity of transplanted islets. Although some information about growth of transplanted islets has been obtained over the past decade (10–16), neither specific beta cell replication nor beta cell mass have been determined. The implications of changes in beta cell replication can only be understood when beta cell mass is also taken into account.

We have shown that, in short-term successful islet transplants, basal nonstimulated replication of transplanted beta cells is similar to that of endogenous pancreatic beta cells (17). The aim of this study was to determine the capacity of transplanted beta cells to modify their replication and mass when stimulated by changes in metabolic demand. We have used the partial pancreatectomy model (18–21) to create a situation of increased metabolic demand in rats with islet transplants in order to determine the beta cell replicative capacity and the changes of the transplanted beta cell mass.

Methods

Male Lewis rats (Harlan Sprague Dawley, Inc., Indianapolis, IN) were obtained at 5 wk of age. Rats were transplanted with 500 syngeneic islets under the kidney capsule, and/or underwent a 95% pancreatectomy according to the protocol described below. Animals were bled and weighed on the day of transplantation, day of pancreatectomy, and at least on days 2, 5, 7, 10, 14, 18, 21, 25, and 28 after transplantation and/or pancreatectomy. Blood glucose, determined between 9 and 11 a.m. in nonfasting conditions, was obtained from the snipped tail with a heparinized microcapillary tube, and glucose was measured with a portable glucose meter (Accu-Check II; Boehringer Mannheim Biochemicals, Indianapolis, IN). Animals were kept under conventional conditions in climatized rooms with free access to tap water and standard pelleted food.

Animal groups. Five experimental groups were studied (Table I). Group 1 ($n = 7$): rats were transplanted and 14 d later underwent a 95% pancreatectomy (Tx-Px group); grafts and pancreatic remnants were harvested 28 d after transplantation. Group 2 ($n = 12$): a 95% pancreatectomy was performed and rats were transplanted 14 d later (Px-Tx group); grafts and pancreatic remnants were harvested 14 ($n = 6$) or 28 ($n = 6$) d after transplantation. Group 3 ($n = 13$): rats were transplanted and grafts and pancreas were harvested 14 ($n = 6$) or 28 ($n = 6$) d later (Tx group). Group 4 ($n = 6$): rats underwent 95% pancreatectomy and pancreatic remnants were harvested 14 d later (Px group). Group 5 ($n = 6$): normal animals that had their pancreases removed (normal group). The beta cell mass of 7 groups of 500 islets isolated on different days was also determined. Since beta cell replication decreases with age (22), pancreatectomized groups were matched for age at pancreatectomy (groups 1, 2, and 4) (7–8 wk old), and

Table I. Experimental Groups

Group	n	Day 0	Day 14	Day 28	Day 42
1 (Tx − Px)	7	Transplantation	Pancreatectomy	Harvest	—
2 (Px − Tx)	12	Pancreatectomy	Transplantation	Harvest (6)	Harvest (6)
3 (Tx)	13	Transplantation	Harvest (6)	Harvest (7)	—
4 (Px)	6	Pancreatectomy	Harvest	—	—
5 (Normal)	6	Harvest	—	—	—

Day 0 denotes the day when the first experimental procedure was performed in each group. Groups 1, 2, and 4 were matched for age at pancreatectomy and groups 3 and 5 had were matched for age at harvest with groups 1 and 4, and group 2 at day 28. Figures in parentheses in groups 2 and 3 show number of animals harvested at each time point.

groups 3 and 5 had the same age at harvest as groups 1 and 4. Rats were transplanted with islets from 5–7-wk-old animals.

Islet isolation and transplantation. The method for islet isolation and transplantation has been previously described for mice (17). Briefly, under sodium amobarbital anesthesia, pancreases were distended with 6 ml of cold (4°C) M-199 medium (GIBCO BRL, Gaithersburg, MD) containing 2 mg/ml of collagenase (Collagenase from *Clostridium Histoliticum;* Serva, Heidelberg, Germany), excised, and incubated in a stationary bath at 37°C. The islets were separated by a density gradient (Histopaque-1077; Sigma Chemical Co., St. Louis, MO) and isolated islets were hand picked under a stereomicroscope two or three times until a population of pure islets was obtained. Only islets > 75 and < 250 µm in diameter were collected. With this restriction the final yield was 200–250 islets per pancreas. The islets were transplanted under the kidney capsule of the left kidney on the day of the isolation using a 200-µl pipette tip previously plugged with Gelfoam® (Upjohn, Kalamazoo, MI).

Pancreatectomy. Partial pancreatectomy (95%) was performed in 7–8-wk-old rats as previously described (18). In brief, animals were anesthetized with sodium amobarbital and 95% of the pancreas was removed by gentle abrasion with cotton applicators, being careful to leave major blood vessels intact. The pancreatic remnant was the tissue between the common bile duct and the first loop of the duodenum. Particular attention was placed on removing the small flap of pancreas attached to the pylorus. Nonpancreatectomized animals did not undergo any sham operation.

Graft and pancreas removal. On the day of graft removal rats were injected with 5-bromo-2'deoxyuridine (BrdU)[1] (Sigma Chemical Co.), 100 mg/kg i.p. body weight, and 6 h later the graft was removed. The kidney capsule surrounding the graft was incised and removed with the graft. Usually all the graft was removed with the capsule; whenever the grafted islets were infiltrating the kidney cortex a second piece of tissue was also taken to ensure that the entire graft was harvested. The grafts were fixed in Bouin's solution and processed for plastic embedding. The weight of the graft was determined on a balance reading to 0.01 mg (A240; Mettler Instrument Corp., Hightstown, NJ) as described (17). Immediately after graft removal, rats were killed and the pancreas or the pancreatic remnants were excised, blotted, weighed, and fixed in Bouin's solution. Subsequently, the pancreatic tissue was embedded in paraffin.

Immunocytochemistry. Sections of graft and pancreas were double stained for BrdU and for the endocrine nonbeta cells of the islets with immunoperoxidase. Immunostaining for BrdU used a cell proliferation kit (Amersham International, Amersham, UK). Staining for the endocrine nonbeta cells used a cocktail of antibodies: rabbit anti-bovine glucagon (final dilution, 1:3,000; gift of Dr. M. Appel, University of Massachusetts Medical School), rabbit antisynthetic somatostatin (final dilution, 1:300; made in our own laboratory), and rabbit anti-bovine pancreatic polypeptide, final dilution, 1:3,000; gift of Dr. R. Chance, Eli Lilly & Co.).

1. *Abbreviation used in this paper:* BrdU, 5-bromo-2'deoxyuridine.

Beta cell replication, individual beta cell area, and beta cell mass. Methods used for measurement of beta cell replication, area and mass have been described in detail (17). For beta cell replication, beta cells and BrdU-positive beta cells were counted using microscope (BH-2; Olympus Corp., Lake Success, NY) connected to a video camera with a black and white monitor. Results were expressed as the percentage of BrdU-positive beta cells. At least 1,200 cells were counted per graft or pancreas.

The mean cross sectional area of individual beta cells, a measure of beta cell size, was determined on immunoperoxidase-stained sections of grafts and isolated islets. For both grafts and isolated islets the beta cell nuclei on a random field were counted and the area of the beta cell tissue in that field measured with an electronic planimetry program (Sigma Scan; Jandel Scientific, Corte Madera, CA). The beta cell area was divided by the number of beta cell nuclei to calculate the area of the individual beta cells. As pointed out previously (17), the actual number of beta cells was higher than the number counted, since not all beta cells were sectioned across their nuclei and, therefore, the size of the beta cells was overestimated.

Beta cell mass was measured by point counting morphometry on immunoperoxidase-stained sections of graft and endogenous pancreas. Each section was covered systematically using a 48-point grid to obtain the number of intercepts over beta cells, over endocrine nonbeta cells, and over other tissue. In pancreatic sections intercepts over exocrine tissue and over nonpancreatic tissue were counted separately. The beta cell relative volume was calculated by dividing the intercepts over beta cells by intercepts over total tissue; then the beta cell mass was estimated by multiplying beta cell relative volume by graft weight. Endocrine nonbeta cell mass and pancreatic exocrine mass were obtained in the same way. The beta cell mass of the islets at the time of the transplantation was determined in 7 groups of 500 islets isolated on different days. Beta cell mass was obtained by multiplying the weight of the islets by the percentage of beta cell volume as derived from our data (92.7%).

Statistical analysis. Results were expressed as mean and standard error of the mean ($\bar{x}\pm$SEM). For comparisons between two groups the unpaired Student's t test (two tailed) was used and for multiple comparisons the one-way analysis of variance (ANOVA) was used. A P value of < 0.05 was considered significant.

Results

Metabolic evolution after transplantation and pancreatectomy

The body weight and blood glucose at critical time points (transplantation, pancreatectomy, and harvesting) are shown in Table II. The evolution of blood glucose throughout the experiment is summarized in Fig. 1. Pancreatectomy induced severe hyperglycemia in Px-Tx and Px groups. In contrast, in Tx-Px group hyperglycemia was prevented by the previous transplantation of 500 islets. Blood glucose in this group was not different from glucose in normal group or Tx group during

Table II. Metabolic Characteristics of Experimental Groups at Different Time Points

Group	Day of transplantation		Day of pancreatectomy		Day of harvesting	
	Body weight	Blood glucose	Body weight	Blood glucose	Body weight	Blood glucose
	g	*mmol/liter*	*g*	*mmol/liter*	*g*	*mmol/liter*
1 (Tx + Px)	130±7	6.11±0.3	228±6	6.11±0.3	269±10	6.44±0.3
2 (Px + Tx)	255±3	18.8±0.9	218±3	6.22±0.2	308±6	9.78±1.8
3 (Tx)	117±5	5.17±0.2	—	—	245±10	5.83±0.2
4 (Px)	—	—	202±4	6.50±0.2	254±9	14.5±0.2
5 (normal)	—	—	—	—	279±11	6.28±0.2

For groups 2 and 3 the values show mean body weight and blood glucose of animals killed 14 and 28 d after transplantation. Results are expressed as mean±SEM.

the follow up. One rat, however, developed transient moderate hyperglycemia (blood glucose, 10.2 mM/liter). Transplantation in already pancreatectomized rats (Px-Tx group) restored euglycemia (blood glucose, < 7.2 mM/liter; mean plus 2 SD of blood glucose in normal group) in 7 of 12 rats 14 d after Tx and in 5 of 6 rats 28 d after transplantation.

Beta cell replication

Transplanted beta cells. As shown in Fig. 2, beta cell replication was increased in transplanted islets from Tx-Px group (1.15±0.12%) compared with pancreatic islets from normal group (0.38±0.05%) and with transplanted beta cells from Px-Tx group (0.56±0.09%) and Tx group (0.64±0.07%) (*P* < 0.001). Replication was not statistically different in Px-Tx, Tx, and normal groups. However, in Px-Tx groups, when day 28 and 42 grafts were considered separately, replication was increased at day 28 (0.81±0.12%) compared with day 42 (0.33±0.04%) and with normal pancreatic islets (*P* < 0.001). Replication was similar in Tx group at days 14 and 28 after transplantation.

Endogenous pancreas. As shown in Fig. 3, in Tx-Px group beta cell replication in the pancreatic remnant was increased (1.01±0.25%) compared with Px-Tx group (0.49±0.11%), Tx group (0.40±0.11%), Px group (0.65±0.18%), and normal group (0.38±0.05%) (*P* < 0.05). When experimental animals were analyzed individually, it was apparent that replication in transplanted beta cells and in pancreatic beta cells was similar

and the presence of a positive correlation between replication in both locations was established (*r* = 0.55, *P* = 0.003). However, in Px-Tx group replication was increased on day 28 in transplanted beta cells (0.81±0.12%) but not in pancreatic beta cells (0.55±0.18%). The discrepancy between replication in endogenous and transplanted islets in this group could reflect the different exposure to hyperglycemia, ≤ 14 d in transplanted islets and > 21 d in the endogenous islets (17).

Beta cell area

As shown in Fig. 4, the individual cross sectional area of beta cells in isolated islets was 239±10 μm^2. Beta cell size was increased in transplanted islets from Tx-Px group (311±14 μm^2) and Px-Tx group (328±13 μm^2) compared with nonpancreatectomized group (Tx group: 252±12 μm^2) or with beta cell area in isolated islets (*P* = 0.001).

Beta cell mass

Transplanted islets. As shown in Fig. 5, transplanted beta cell mass increased in Tx-Px (1.81±0.51 mg) and Px-Tx (1.55±0.21 mg) rats compared with nonpancreatectomized animals (Tx group: 0.78±0.17 mg) (*P* < 0.05). In Tx group one outlying value (higher than mean plus 2.5 SD) was ex-
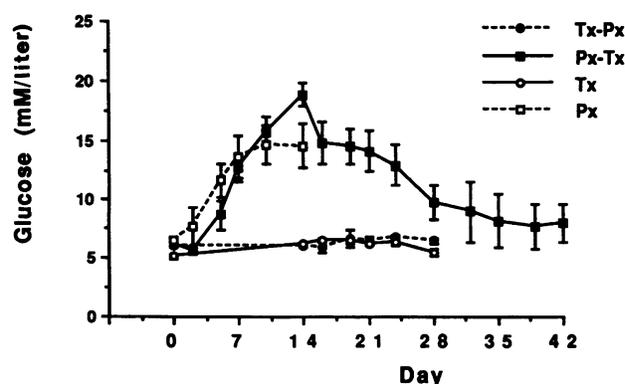
Figure 1. Evolution of fed blood glucose in experimental groups. Groups are described in Table I. Values are mean±SEM.
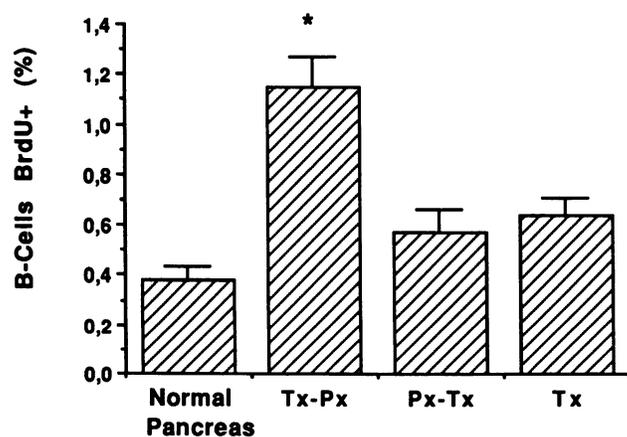
Figure 2. Beta cell replication in normal pancreas and in transplanted islets. Replication is expressed as percentage of BrdU-positive beta cells. Names on the *x*-axis correspond to groups shown in Table I. Values are mean±SEM. **P* < 0.001 between Tx-Px group and all other groups.
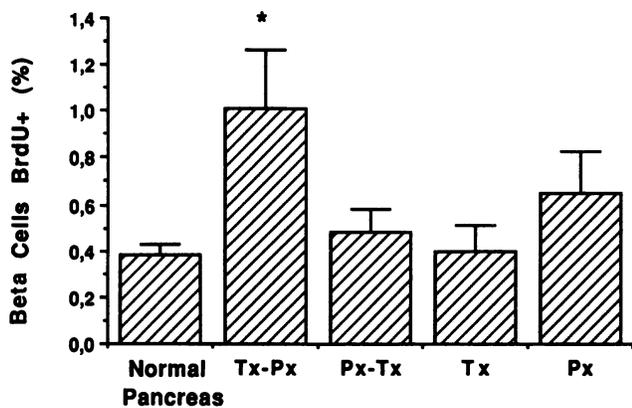
Figure 3. Beta cell replication in endogenous pancreatic beta cells. Replication is expressed as percentage of BrdU-positive beta cells. Names on the x-axis correspond to groups shown in Table I. Values are mean±SEM. *$P < 0.001$ between Tx-Px group and all other groups.

cluded. Beta cell mass in Tx group grafts was reduced to 74% of the initially transplanted beta cell mass (1.05±0.07 mg, P = NS).

Endogenous pancreas. Beta cell mass was not significantly different among the remnants of the three pancreatectomized groups or between the two nonpancreatectomized groups, despite the different protocols for transplantation (Table III). Beta cell mass in pancreatic remnants of Tx-Px and Px-Tx groups increased to 23 and 19%, respectively, of the total beta cell mass in normal pancreases. In Px group the value represented 42% of the beta cell mass in normal pancreases. Beta cell mass in Tx group was similar to that of normal rats.

To compare the beta cell volume in the pancreas of all five groups beta cell mass was expressed per gram of pancreas (Table III). Beta cell relative volume was increased in Px group compared with all other groups.

Endocrine nonbeta cells and exocrine pancreas mass

Differences in endocrine nonbeta cell mass of transplanted islets among groups did not reach statistical significance (results
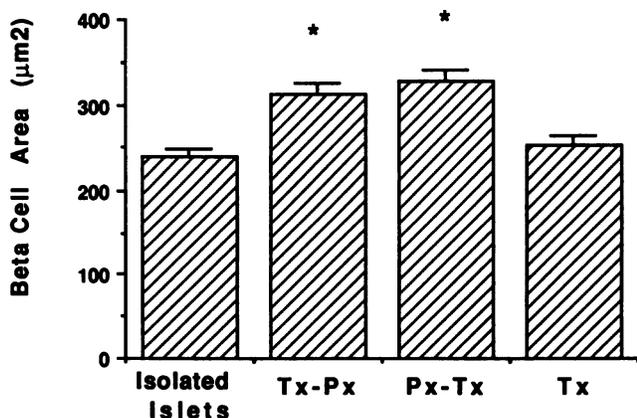
Figure 4. Area of individual beta cells from isolated and transplanted islets. Groups on the x-axis correspond to those described in Table I. Isolated islets show beta cell area on the transplantation day. Values are mean±SEM. *$P < 0.001$ between Tx-Px and Px-Tx groups and isolated islets and Tx groups.
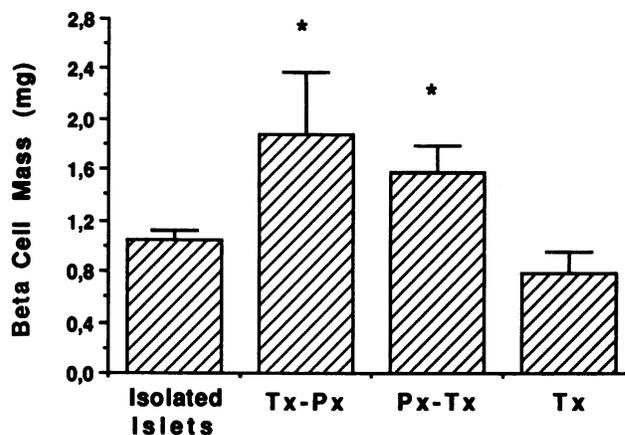
Figure 5. Transplanted beta cell mass. Groups on the x-axis correspond to those described in Table I. Isolated islets show the initially transplanted beta cell mass. Values are mean±SEM. *$P < 0.05$, between Tx-Px and Px-Tx groups and Tx group.

not shown). Endocrine nonbeta cell mass in Tx-Px and Px-Tx groups was ~ 10% that of normal pancreas. No differences were seen between Tx and normal groups.

The exocrine tissue showed a significant regeneration in pancreatectomized groups (Table III), attaining 15–17% of the exocrine mass in the normal group instead of the 5% remaining after surgery. The mass of exocrine tissue in the pancreatic remnant was similar in all three pancreatectomized groups, confirming that the extent of pancreatectomy was similar in all groups.

Discussion

Beta cells transplanted into normal rats increased their replication and size when a 95% pancreatectomy was performed (Tx-Px group), and total beta cell mass almost tripled. Similar results were obtained when islets were transplanted into already pancreatectomized rats (Px-Tx group). However, although normoglycemia was maintained in Tx-Px rats after pancreatectomy, it took several days to restore it in Px-Tx group and in some cases this was not achieved. Beta cell response to pancreatectomy in the grafted islets was similar to that of islets in the endogenous pancreas, indicating that normal growth capacity was preserved in beta cells after transplantation.

Table III. Endocrine and Exocrine Mass in Pancreas and Pancreatic Remnants

Group	Beta cell	Nonbeta cell	Exocrine pancreas	Relative beta cell volume
	mg	mg	mg	
1 (Tx − Px)	1.08±0.26	0.15±0.05	152±12	6.95±1.70
2 (Px − Tx)	0.87±0.25	0.13±0.04	171±10	4.83±1.30
3 (Tx)	5.09±0.59	1.29±0.16	913±30	5.55±0.58
4 (Px)	1.99±0.75	0.22±0.05	150±14	12.3±3.84*
5 (normal)	4.68±0.65	1.48±0.26	977±30	4.81±0.65

Relative beta cell volume is beta cell mass per gram of pancreas. Values are mean±SEM. *$P < 0.05$ compared with all other groups.

The increase in replication and size of transplanted beta cells after pancreatectomy in Tx-Px rats resulted in increased beta cell mass. The severe hyperglycemia that followed pancreatectomy in Px and Px-Tx groups was prevented in Tx-Px rats by the transplantation of 500 islets. Thus, transplanted beta cells were able to compensate for the increased metabolic demand placed upon them by the surgical reduction of endogenous beta cell mass. It is not clear, however, how beta cells sensed this increased metabolic demand. The concept of adaptive beta cell proliferation has been used to suggest that functional demand is a mechanism of control of beta cell replication (23). Glucose is a well-known stimulus for beta cell replication both in vitro (22, 24) and in vivo (25), and hyperglycemia is present in most of the conditions in which adaptive beta cell proliferation occurs. However, hyperglycemia was not detected in Tx-Px group. The results resemble the islet growth described in other situations of increased metabolic demand where hyperglycemia is not detected, such as 40 and 60% pancreatectomies (20, 21) or pregnancy (26). Subtle elevations in blood glucose could have been responsible for increased beta cell replication in Tx-Px rats. Moreover, in addition to glucose, other mediators may also have contributed to increase beta cell proliferation. The identification of these mediators could open the possibility for interventions to modulate the growth of transplanted beta cells.

When islets were transplanted into already pancreatectomized rats (Px-Tx group) beta cell replication and size increased and beta cell mass doubled 14 d after transplantation. However, although transplanted and endogenous beta cell mass were similar in Tx-Px and Px-Tx groups, normoglycemia was sustained in Tx-Px rats throughout the follow up while its restoration was delayed, and in some case even not achieved, in Px-Tx rats. Since pancreatectomy in Tx-Px group was performed 14 d after transplantation, one could wonder whether an increase in grafted beta cell mass during that period was responsible for the maintenance of normoglycemia after pancreatectomy. The question was addressed by the measurement of beta cell mass in Tx group 14 d after transplantation. Beta cell mass in this group was found to be 74% of that initially transplanted, ruling out the possibility of an increased beta cell mass in Tx-Px rats at the time of pancreatectomy. The reduction confirms our previous results in mice showing that transplantation of islets into normal recipients results in a decreased beta cell mass in the graft after transplantation (17). Endogenous beta cell mass in the pancreas remnant was also similar in Tx-Px and Px-Tx rats. The extent of pancreatectomies was equivalent in both groups, as confirmed by the amount of exocrine tissue found at the end of follow up. Therefore, factors other than the absolute beta cell mass contributed to differences in blood glucose in these two groups. The previous observation that maintenance of normoglycemia may require a lower beta cell mass than its restoration (27) agrees with our current results in that a similar beta cell mass was able to sustain normoglycemia in Tx-Px rats but not to restore it in Px-Tx rats. Hyperglycemia, which is associated with insulin resistance, could have contributed to the differences in beta cell mass requirement. In addition, vascularization could have also played a role in the different evolution. It has been shown that transplanted islets are fully vascularized 10 d after transplantation (28, 29), and therefore islets transplanted into Tx-Px rats were well vascularized at the time of pancreatectomy. In con-trast, the islets transplanted into Px-Tx rats were confronted with hyperglycemia at the time of transplantation, when they had deficient vascularization. Impaired vascularization could reduce beta cell capacity to sense and respond to increased metabolic demand, and therefore decrease the functional beta cell mass. In a situation such our experimental groups, where only a marginal islet mass was transplanted (30, 31), vascularization of islets could have been crucial for an appropriate response to changes in metabolic conditions. Therefore, the delayed but eventual achievement of normoglycemia in most rats of Px-Tx group may have resulted from the combination of progressive increase in beta cell mass after transplantation and restoration of islet vascularization.

Beta cell replication and beta cell mass were also increased in the pancreatic remnant of Tx-Px and Px-Tx groups. Replication in transplanted and endogenous beta cells showed a good correlation in all three transplanted groups, suggesting that the responsiveness of transplanted beta cells was normal. The one exception was Px-Tx group 14 d after transplantation when beta cell replication was increased in the graft but not in the pancreatic remnant. The discrepancy could be due to different exposure times to severe hyperglycemia: > 21 d for endogenous pancreatic beta cells and < 14 d for transplanted beta cells. We have previously reported that the increased beta cell replication accompanying hyperglycemia did not persist when exposure to severe hyperglycemia became chronic (17). Similarly, replication was not significantly increased in Px rats in a situation of chronic severe hyperglycemia, suggesting again a limitation of beta cell replication.

Both beta cells and exocrine pancreas underwent significant regeneration after pancreatectomy. Beta cell mass and exocrine mass increased to a similar extent in Tx-Px and Px-Tx groups as shown by the preservation of the ratio between beta cell mass and pancreatic mass. The increase in exocrine tissue in Px group was equivalent to that in the other pancreatectomized groups, but beta cell mass increased more markedly in this nontransplanted group. Perhaps the presence of transplanted beta cells restrained the increase of endogenous beta cell mass in Tx-Px and Px-Tx groups.

The capacity of transplanted beta cells to respond to changes in metabolic demand and the similarity between their replicative response and that of endogenous nontransplanted beta cells have important implications for islet transplantation. In clinical transplantation, different factors may modify the metabolic demand placed on transplanted beta cells. For instance, episodes of acute rejection decrease the transplanted beta cell mass and higher doses of immunosuppressive drugs can increase insulin resistance and impair beta cell function or islet cell DNA replication (32, 33). It is therefore encouraging to find that transplanted beta cells are able to respond effectively to severe reductions in beta cell mass. Moreover, if the transplantation method could be optimized it may be possible to obtain a successful result with a smaller number of islets, thus alleviating the islet shortage that limits current transplantation efforts.

Acknowledgments

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