β-Cell Function Following Human Islet Transplantation for Type 1 Diabetes

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Islet transplantation can provide metabolic stability for patients with type 1 diabetes; however, more than one donor pancreas is usually required to achieve insulin independence. To evaluate possible mechanistic defects underlying impaired graft function, we studied five subjects at 3 months and four subjects at 12 months following intraportal islet transplantation who had received comparable islet equivalents per kilogram (12,601 ± 1,732 vs. 14,384 ± 2,379, respectively). C-peptide responses, as measures of β-cell function, were significantly impaired in both transplant groups when compared with healthy control subjects (P < 0.05) after intravenous glucose (0.3 g/kg), an orally consumed meal (600 kcal), and intravenous arginine (5 g), with the greatest impairment to intravenous glucose and a greater impairment seen in the 12-month compared with the 3-month transplant group. A glucose-potentiated arginine test, performed only in insulin-independent transplant subjects (n = 5), demonstrated significant impairments in the glucose-potentiation slope (P < 0.05) and the maximal response to arginine (ARmax; P < 0.05), a measure of β-cell secretory capacity. Because ARmax provides an estimate of the functional β-cell mass, these results suggest that a low engrafted β-cell mass may account for the functional defects observed after islet transplantation. Diabetes 54:100–106, 2005

RESEARCH DESIGN AND METHODS

Subjects were recruited from the islet transplantation program at the Hospital of the University of Pennsylvania (HUP) for participation at 3 (n = 5) or 12 months (n = 4) after their last transplant. The procedure for islet transplantation at HUP has previously been reported (3). In short, all subjects have long-standing C-peptide–negative type 1 diabetes complicated by hypoglycemia unawareness and undergo one or more intraportal islet infusions in order to achieve insulin independence. The immunosuppression regimen includes dexamethasone, tacrolimus, and sirolimus according to the Edmonton protocol (1,2). The five subjects studied at 3 months had a mean ± SE HbA1c of 6.4 ± 0.3% (Table 1), and four were insulin-independent. The four subjects studied at 12 months had an HbA1c of 7.4 ± 0.8%, and only one remained off insulin. One subject underwent testing at 3 months after a first transplantation and again 9 months later at 3 months after a second transplantation was performed to sustain insulin independence; both occurrences of testing have been included as 3-month data. Another subject is included in both the 3-month and 12-month groups, and while insulin independent at 3 months, was insulin dependent at 12 months.

Healthy nondiabetic control subjects for the AST and GPA test (n = 6) were sex-, age-, and BMI-matched to the transplant recipients. Data from healthy nondiabetic control subjects for the IVGTT (n = 10) and MMT (n = 15) were derived from previous studies (13,14). This study protocol was

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approved by the Institutional Review Board of the University of Pennsylvania, and all subjects gave their written informed consent to participate.

**Tests of β-cell function and secretory capacity.** All tests were performed in the General Clinical Research Center at HUP, where subjects fasted overnight after 2000 for 12 h before testing. Subjects who were not insulin independent held any long-acting insulin for 24 h and any short-acting insulin for 12 h before testing. If needed, intravenous insulin was administered overnight to maintain the blood glucose concentration <150 mg/dl and was discontinued at least 30 min before testing. By 0700 each morning, one catheter was placed in an antecubital vein for infusions, and one catheter was placed retrograde in a contralateral hand vein for blood sampling, with the hand placed in a thermoregulated box (25°C) to promote optimal arterIALIZation of venous blood (15). Patency of the intravenous catheters was maintained with slow infusions of 0.9% saline.

**IVGTT.** Following baseline blood sampling at −15, −10, and −5 min, 0.3 g/kg of 50% glucose was injected over a 1-min period starting at t = 0. Additional blood samples were collected at t = 2, 3, 4, 5, 6, 8, 10, 12, 14, 16, 18, and 20 min following injection (16).

**MMT.** Following baseline blood sampling at −15, −10, and −5 min, a 600-kcal meal containing 50% carbohydrate, 15% protein, and 25% fat was consumed over a 15-min period starting at t = 0. Additional blood samples were collected at t = 2, 4, 6, 8, 10, 15, 20, 30, 45, 60, 75, 90, 105, 120, 135, 150, 165, 180, 195, 210, 225, and 240 min following ingestion (14).

**AST.** Following baseline blood sampling at −5 and −1 min, 5 g of 10% arginine was injected over a 1-min period starting at t = 0. Additional blood samples were collected at t = 2, 3, 4, and 5 min following injection (17,18).

**GPA test.** GPA was performed only in insulin-independent transplant recipients (n = 5), defined as having a fasting plasma glucose (FPG) concentration <125 mg/dl without the use of exogenous insulin, and in matched control subjects (n = 6). After the baseline AST above, a hyperglycemic clamp technique (18) using a variable rate of 20 and 10% glucose solutions was performed to achieve a plasma glucose concentration of 230 mg/dl.

**Biochemical analysis.** All samples were collected on ice into tubes containing EDTA, trasylol, and leupeptin, centrifuged at 4°C, separated, and frozen at −80°C for subsequent analysis. Plasma glucose was measured in duplicate by the glucose oxidase method using an automated glucose analyzer (YSI 2300, Yellow Springs Instruments). Plasma immunoreactive C-peptide and insulin were measured in duplicate by double-antibody radioimmunoassays (Linco Research, St. Charles, MO).

**Calculations and statistics.** Intrageneric glucose tolerance during the IVGTT was evaluated by the glucose disappearance rate, $K_g = \ln(\text{glucose})/\text{min} \cdot 100$, calculated as the slope of the natural log of glucose values between 10 and 20 min with least-squares linear regression using the computer software Origin (Northampton, MA) (21). The C-peptide response to the IVGTT was evaluated by the incremental area under the curve (AUC) between 0 and 20 min postinjection, where the AUC is calculated by the trapezoidal rule with the mean of the baseline values subtracted using the computer software Origin. The C-peptide response to the MMT was evaluated by the incremental AUC between 0 and 240 min postigestion. The C-peptide response to the AST was evaluated by the incremental acute C-peptide response (ACR_{\text{AST}}), where the ACR_{\text{AST}} is calculated as the mean of the 2-, 3-, 4-, and 5-min values minus the mean of the baseline values (17,18). The ACR_{\text{AST}} during the 230 mg/dl

**TABLE 1**

Characteristics of islet transplant recipients at 3 and 12 months and control subjects

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Sex (M/F)</th>
<th>Age (years)</th>
<th>BMI (kg/m²)</th>
<th>IE/kg</th>
<th>HbA1c (%)</th>
<th>Fasting glucose (mg/dl)</th>
<th>Fasting C-peptide (ng/ml)</th>
<th>Insulin (units · kg⁻¹ · day⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 months</td>
<td>5</td>
<td>4/1</td>
<td>42 ± 6</td>
<td>23.1 ± 1.4</td>
<td>12,601 ± 1,732</td>
<td>6.4 ± 0.3</td>
<td>101 ± 7</td>
<td>0.49 ± 0.15</td>
<td>0.08 ± 0.08</td>
</tr>
<tr>
<td>12 months</td>
<td>4</td>
<td>2/2</td>
<td>41 ± 3</td>
<td>22.9 ± 2.1</td>
<td>14,384 ± 2,379</td>
<td>7.4 ± 0.8</td>
<td>123 ± 9</td>
<td>0.55 ± 0.27</td>
<td>0.16 ± 0.06</td>
</tr>
<tr>
<td>Control (AST and GPA)</td>
<td>6</td>
<td>5/1</td>
<td>41 ± 4</td>
<td>24.8 ± 0.4</td>
<td>—</td>
<td>—</td>
<td>5.35 ± 0.1²</td>
<td>79 ± 2*</td>
<td>0.66 ± 0.07</td>
</tr>
<tr>
<td>Control (IVGTT)†</td>
<td>10</td>
<td>10/0</td>
<td>26 ± 2 ² ²</td>
<td>23.6 ± 0.3</td>
<td>—</td>
<td>—</td>
<td>84 ± 2²</td>
<td>1.03 ± 0.07²</td>
<td>—</td>
</tr>
<tr>
<td>Control (MMT)§</td>
<td>15</td>
<td>11/4</td>
<td>30 ± 2²</td>
<td>25.2 ± 1.2</td>
<td>—</td>
<td>—</td>
<td>78 ± 2²</td>
<td>1.10 ± 0.11³</td>
<td>—</td>
</tr>
</tbody>
</table>

*Data are means ± SE, *P < 0.01 for comparison to the 3- and 12-month transplant group; †data are from Teff and Townsend (13); ‡P ≤ 0.05 for comparison with the 3- and 12-month transplant group; §data are from Teff and Townsend (14).

**FIG. 1.** Plasma glucose (A) and C peptide (B) responses to the intravenous administration of 0.3 g/kg glucose at t = 0 min during the IVGTT. In panel A, the glucose disposal rate ($K_g$) was significantly impaired at 12 but not 3 months posttransplant when compared with the control group ($P < 0.05$ for 12-month comparison). In panel B, first-phase C-peptide release was absent in the transplant groups, whose incremental AUCs were significantly impaired when compared with the control group ($P < 0.01$ for both comparisons).
glucose clamp allows for determination of the glucose-potentiation slope for C-peptide release, defined as the difference in the ACRarg at 230 mg/dl and fasted glucose levels, divided by the difference in plasma glucose ($ACR_{arg}/PG$) (17,18). The ACRarg during the 340 mg/dl glucose clamp allows for determination of the $\beta$-cell secretory capacity (AR max) because the acute responses to arginine are maximal at plasma glucose concentrations $\geq$315 mg/dl (10,22). The same calculations were performed for insulin. All data are expressed as mean ± SE. Comparisons of the transplant groups and control groups were performed with two-tailed, unpaired Student’s t tests using the computer software Statistica (Tulsa, OK). Significance was considered at $P < 0.05$.

RESULTS

Subject characteristics. The subjects studied at 3 ($n = 5$) and 12 months ($n = 4$) were comparable in sex, age, BMI, islet equivalents per kilogram body weight (IE/kg) transplanted, HbA1c, FPG and C-peptide concentrations, and daily insulin requirements (Table 1). Two of the five subjects at 3 months did not complete the MMT. One of the subjects at 12 months completed only the MMT, and another completed only the IVGTT and the AST.

The control group for the AST and GPA test ($n = 6$) was matched to the transplant groups for sex, age, and BMI but had significantly lower HbA1c and FPG ($P < 0.01$; Table 1). The control groups for the IVGTT ($n = 10$) and MMT ($n = 15$) were significantly younger than the transplant groups ($P < 0.05$; Table 1) and had significantly lower FPG ($P < 0.01$; Table 1) and higher fasting C-peptide ($P < 0.05$; Table 1) but were comparable for BMI.

Tests of $\beta$-cell function and secretory capacity

IVGTT. IVGTTs were performed in five subjects at 3 months (FPG $= 97 \pm 7$ mg/dl; four of five insulin independent) and three subjects at 12 months (FPG $= 123 \pm 11$ mg/dl; one of three insulin independent) posttransplant.

FIG. 2. Plasma glucose (A) and C-peptide (B) responses to an orally consumed 600-kcal mixed-nutrient meal at $t = 0$ during the MMT. In panel A, the level of glycemia was significantly greater in the groups at 3 and 12 months posttransplant when compared with the control group ($P < 0.01$ for all comparisons). In panel B, the peak C-peptide response was significantly delayed in the transplant recipients at 3 and 12 months when compared with the control group ($P < 0.05$ and $P < 0.01$, respectively). The incremental AUC for C-peptide was significantly reduced at 3 but not 12 months posttransplant when compared with the control group ($P < 0.05$ for 3-month comparison).

FIG. 3. Plasma glucose (A) and C-peptide (B) responses to the intravenous administration of 5 g arginine at $t = 0$ min during the AST. In panel A, plasma glucose values were significantly higher in both transplant groups when compared with the control group ($P < 0.05$ for all comparisons). In panel B, the incremental C-peptide response ($ACR_{arg}$) was significantly impaired in the transplant recipients at 3 and 12 months when compared with the control group ($P < 0.05$ for both comparisons).
and in 10 healthy nondiabetic control subjects. Following the intravenous glucose bolus, Fig. 1A shows a significantly impaired glucose disposal rate (K_i) at 12 but not 3 months posttransplant when compared with the control group (0.88 ± 0.40 and 1.39 ± 0.20 vs. 1.78 ± 0.16/min, respectively; P < 0.05 for 12-month comparison). Figure 1B demonstrates that first-phase C-peptide release, present as a peak in C-peptide levels between 0 and 10 min postinjection in the control group, was absent in the transplant recipients at 3 and 12 months when compared with the control group (0.38 ± 0.09 and 0.21 ± 0.14 vs. 1.20 ± 0.20 ng/ml; P < 0.05 for both comparisons).

**MMTs.** MMTs were performed in three subjects at 3 months (FPG = 121 ± 10 mg/dl; two of three insulin independent) and three subjects at 12 months (FPG = 114 ± 12 mg/dl; one of three insulin independent) posttransplant and in 15 healthy nondiabetic control subjects. Following ingestion of the meal, Fig. 2A shows a significantly greater level of glycemia in the groups at 3- and 12-months posttransplant when compared with the control group (5.4 ± 4.1 and 1.8 ± 1.7 vs. 26.2 ± 3.4 ng·ml⁻¹·min⁻¹, respectively; P < 0.01 for both comparisons). Figure 2B demonstrates that the peak C-peptide response was significantly delayed in the transplant recipients at 3 and 12 months when compared with the control group (155 ± 22 and 185 ± 13 vs. 104 ± 8 min; P < 0.05 and P < 0.01, respectively). The incremental AUC for C-peptide between 0 and 240 min postingestion was significantly reduced at both 3- and 240 mg·dl⁻¹ clamp conditions, and the AR_max (P < 0.05), defined by the ACRarg during the 340 mg·dl⁻¹ clamp condition, are apparent.

**GPA test.** The five insulin-independent subjects all completed the GPA test (FPG = 101 ± 5 mg/dl) and were compared with six healthy nondiabetic control subjects. Figure 4A shows that the ACRarg was significantly impaired when compared with the control group under fasting (0.46 ± 0.06 vs. 1.20 ± 0.20 ng/ml; P < 0.05) and 230 mg·dl⁻¹ (1.07 ± 0.23 vs. 4.54 ± 0.72 ng/ml; P < 0.05) and 340 mg·dl⁻¹ (1.37 ± 0.37 vs. 6.09 ± 1.60 ng/ml; P < 0.05) hyperglycemic clamp conditions. In Fig. 4B, the ACRarg is plotted as a function of the plasma glucose, demonstrating impairments in both the glucose-potentiation slope (0.005 ± 0.001 vs. 0.021 ± 0.004; P < 0.01) and AR_max (1.37 ± 0.37 vs. 6.09 ± 1.60 ng/ml; P < 0.05).

**Correlations of β-cell function measures to β-cell secretory capacity (AR_max) and of FPG and IE/kg to Table 2.**

<table>
<thead>
<tr>
<th>Correlation</th>
<th>r</th>
<th>n</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC_IVGTT vs. AR_max</td>
<td>0.91</td>
<td>5</td>
<td>0.03</td>
</tr>
<tr>
<td>AUC_MMT vs. AR_max</td>
<td>0.99</td>
<td>3</td>
<td>0.09</td>
</tr>
<tr>
<td>ACR_arg vs. AR_max</td>
<td>0.86</td>
<td>5</td>
<td>0.06</td>
</tr>
<tr>
<td>GPS vs. AR_max</td>
<td>0.89</td>
<td>5</td>
<td>0.04</td>
</tr>
<tr>
<td>FPG vs. AUC_IVGTT</td>
<td>-0.67</td>
<td>8</td>
<td>0.07</td>
</tr>
<tr>
<td>FPG vs. AUC_MMT</td>
<td>-0.77</td>
<td>6</td>
<td>0.07</td>
</tr>
<tr>
<td>FPG vs. ACR_arg</td>
<td>-0.80</td>
<td>8</td>
<td>0.02</td>
</tr>
<tr>
<td>FPG vs. GPS</td>
<td>-0.92</td>
<td>5</td>
<td>0.02</td>
</tr>
<tr>
<td>FPG vs. AR_max</td>
<td>-0.91</td>
<td>5</td>
<td>0.03</td>
</tr>
</tbody>
</table>

GPS, glucose-potentiation slope.
**β-CELL FUNCTION AFTER ISLET TRANSPLANTATION**

**FIG. 5.** In panel A, the glucose-potentiation slope (ΔACRarg/ΔPG) is positively correlated to the ARmax, a measure of β-cell secretory capacity. In panel B, the FPG is negatively correlated to ARmax.

**β-cell function measures and ARmax.** In the five insulin-independent subjects, the incremental C-peptide responses to the IVGTT, MMT, and AST correlated with ARmax (ACRarg during the 340 mg/dl hyperglycemic clamp) (Table 2), as did the glucose-potentiation slope (ΔACRarg/ΔPG) (Table 2 and Fig. 5A). The FPG correlated inversely with all measures of β-cell function (Table 2) and with the ARmax (Table 2 and Fig. 5B). There were no significant correlations of IE/kg transplanted with the β-cell function measures (data not shown) or ARmax (r = 0.45, n = 5, P = 0.45).

**Insulin responses.** Insulin responses were reduced similarly as the C-peptide responses in the transplant recipients when compared with normal for all four tests above (data not shown).

**DISCUSSION**

These studies provide new insights into the β-cell function that follows human islet transplantation. We found a markedly impaired first-phase secretory response to intravenous glucose and a blunted secretory response to intravenous arginine, confirming previous reports of absent first-phase insulin secretion in response to glucose (7,8) and a relatively greater β-cell secretory response to arginine compared with glucose (9) following islet transplantation. We also found a blunted and delayed secretory response to an orally consumed meal. Finally, these results are the first to demonstrate that in insulin-independent transplant recipients, there is a blunted secretory response to glucose-potentiated arginine with a markedly impaired glucose-potentiation slope and ARmax. While the number of subjects evaluated by each test of β-cell function is small, the results are remarkably consistent across the four tests of β-cell function performed. By including subjects who required a reduced dose of insulin to maintain normoglycemia, the results may be more generalizable to a larger population undergoing islet transplantation than a study of only insulin-independent transplant recipients.

The ARmax, a measure of β-cell secretory capacity, has been reported to correlate with β-cell mass in animal studies of β-cell reduction (23–25) and in human studies of auto-islet (11) and pancreas (12) transplantation; thus, the ARmax can be used to estimate the mass of islets surviving intrahepatic transplantation in humans (11). The markedly impaired ARmax reported here suggests that there is an insufficient β-cell mass following even “successful” islet transplantation (i.e., insulin-independent islet transplantation). Furthermore, the strong correlations of the AUCIVGTT, AUCMT, ACRarg, and glucose-potentiation slope to the ARmax in the insulin-independent subjects suggest that the blunted responses to all the tests conducted may be attributable to a low engrafted β-cell mass. That these blunted responses may be explained by a reduced β-cell mass is consistent with animal models of β-cell reduction, including rats treated by either streptozotocin administration or partial pancreatectomy (26). In addition, streptozotocin-treated baboons demonstrate impairment in both glucose-mediated insulin secretion and the ability of glucose to potentiate the insulin response to arginine (27). That the IE/kg, an estimate of transplanted islet mass, correlated poorly with all measures of β-cell function and ARmax suggests that this measure may overestimate the number of islets actually surviving intrahepatic transplantation.

Further support for a marginal β-cell mass comes from the greater impairments in intravenous and oral glucose tolerance and β-cell secretory responses to glucose and arginine in the 12- compared with 3-month transplant group. While the incremental C-peptide response to a mixed-nutrient meal was comparable in the 12- and 3-month groups, there was greater hyperglycemia in the 12-month group, which would supply a greater stimulus for C-peptide secretion in that group. Nevertheless, the timing of the C-peptide response was significantly delayed, and that delay was greater in the 12- compared with the 3-month group. Alternatively, the greater reduction in β-cell secretory responses in the 12- compared with the 3-month group may be attributable to the higher FPG in the 12-month group because even modest glucose intolerance has been associated with profoundly decreased β-cell function (28). In this study, increasing FPG correlated negatively with all measures of β-cell function, including
AR\text{max}. A proposed decline in β-cell mass over time will require confirmation by longitudinal study.

Similar to the defects reported here following islet transplantation, data obtained from type 2 diabetic subjects demonstrate impaired first-phase secretory responses to intravenous glucose (28), a preserved secretory response to arginine (29), and a markedly impaired glucose-potentiation slope and AR\text{max} (17). Despite autopsy studies that have reported a β-cell mass ~60% of normal in type 2 diabetes (30), the AR\text{max} was 18% of normal in type 2 diabetic subjects (17), an inconsistency possibly explained by the presence of both a decreased β-cell mass and a functional β-cell defect. Recently, islet isolation studies using type 2 diabetic organ donors demonstrated both a reduced β-cell mass and a functional defect in glucose-mediated insulin secretion from the existing β-cells (31). These data suggest that the similarly reduced AR\text{max} in islet transplantation reported here may be due to both a quantitative defect in the mass of engrafted β-cells and a qualitative defect in the function of those β-cells.

While a reduced β-cell mass may explain the observed impairments in glucose-mediated insulin secretion (26), these effects can also occur independently of the β-cell mass because both the intrahepatic transplant site (32) and the immunosuppressive drugs tacrolimus, a calcineurin inhibitor (33–35), and sirolimus (36,37) have been reported to impair glucose-mediated insulin secretion. In fact, the calcineurin inhibitor cyclosporine has been reported to decrease β-cell secretory capacity in pancreas and nondiabetic kidney transplant recipients (22), and tacrolimus has been reported to have a similar effect in liver transplant recipients (38). Thus, generalized β-cell dysfunction can mimic a reduced β-cell mass and may partially explain the impairments in β-cell function and secretory capacity following islet transplantation. Furthermore, islet transplant recipients may be at greater risk for diabetogenicity caused by the immunosuppression regimen because portal blood concentrations of these drugs are twice that present peripherally (39,40) and are delivered directly to the intrahepatic islets (41).

In conclusion, these studies demonstrate impaired β-cell responsiveness of transplanted islets consistent with both an insufficient β-cell mass and a functional defect in glucose-mediated insulin secretion. It has been proposed that a minimum transplantation of 9,000 IE/kg is necessary to achieve insulin independence, yet an increasing proportion of subjects return to insulin after each year of transplantation (9). While eight of the nine subjects studied here were initially insulin independent, only one of four studied at 12 months remained so, despite the mean IE/kg transplant being >12,000. Thus, advances in islet isolation techniques and immunosuppressive drug regimens should be considered in order to improve the engraftment and survival of transplanted islets (42), which should improve β-cell function and secretory capacity and lead to a more sustained independence from exogenous insulin for transplant recipients. In addition, efforts should be directed to enhance the function of the existing β-cell mass. Immunosuppressive strategies that minimize the use of diabetogenic drugs such as tacrolimus may also contribute to improvements in β-cell function and secretory capacity following islet transplantation.

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