

Five-Year Follow-Up After Clinical Islet Transplantation

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Islet transplantation can restore endogenous β -cell function to subjects with type 1 diabetes. Sixty-five patients received an islet transplant in Edmonton as of 1 November 2004. Their mean age was 42.9 ± 1.2 years, their mean duration of diabetes was 27.1 ± 1.3 years, and 57% were women. The main indication was problematic hypoglycemia. Forty-four patients completed the islet transplant as defined by insulin independence, and three further patients received $>16,000$ islet equivalents (IE)/kg but remained on insulin and are deemed complete. Those who became insulin independent received a total of $799,912 \pm 30,220$ IE ($11,910 \pm 469$ IE/kg). Five subjects became insulin independent after one transplant. Fifty-two patients had two transplants, and 11 subjects had three transplants. In the completed patients, 5-year follow-up reveals that the majority (~80%) have C-peptide present post-islet transplant, but only a minority (~10%) maintain insulin independence. The median duration of insulin independence was 15 months (interquartile range 6.2–25.5). The HbA_{1c} (A1C) level was well controlled in those off insulin (6.4% [6.1–6.7]) and in those back on insulin but C-peptide positive (6.7% [5.9–7.5]) and higher in those who lost all graft function (9.0% [6.7–9.3]) ($P < 0.05$). Those who resumed insulin therapy did not appear more insulin resistant compared with those off insulin and required half their pretransplant daily dose of insulin but had a lower increment of C-peptide to a standard meal challenge (0.44 ± 0.06 vs. 0.76 ± 0.06 nmol/l, $P < 0.001$). The Hypoglycemic score and lability index both improved significantly posttransplant. In the 128 procedures performed, bleeding occurred in 15 and branch portal vein thrombosis in 5 subjects. Complications of immunosuppressive therapy included mouth ulcers, diarrhea, anemia, and ovarian cysts. Of the 47 completed patients, 4 required retinal laser photocoagulation or vitrectomy and 5 patients with microalbuminuria developed macroproteinuria. The need for multiple antihypertensive medications increased from 6% pretransplant to 42% posttransplant, while the use of statin therapy increased from 23 to 83% posttransplant. There was no change in the neurothesiometer scores

pre- versus posttransplant. In conclusion, islet transplantation can relieve glucose instability and problems with hypoglycemia. C-peptide secretion was maintained in the majority of subjects for up to 5 years, although most reverted to using some insulin. The results, though promising, still point to the need for further progress in the availability of transplantable islets, improving islet engraftment, preserving islet function, and reducing toxic immunosuppression. *Diabetes* 54:2060–2069, 2005

Sustained C-peptide production and successful insulin independence after pancreatic islet transplantation in type 1 diabetic patients was reported over 4 years ago by the Edmonton group (1). This reality became possible with the use of newer, more potent immunosuppressive agents, the avoidance of corticosteroids, and high-quality islet preparations, although typically two islet infusions were necessary to attain insulin independence. Over this period, other centers have been able to replicate the initial success of the Edmonton Protocol with further refinements in technique (2–5), and islet transplantation is increasingly being used (6–8).

However, the need for ongoing immunosuppressive therapy and the scarcity of donor islets have precluded the widespread adoption of islet transplantation. The main indications for solitary islet transplantation have been frequent recurrent hypoglycemia or labile glucose values that have defied optimization of medical therapy. An additional hoped for, but unproven, benefit has been stabilization or improvement of diabetes complications with the achievement of stable good glycemic control.

Now, 5 years after the first islet transplant was performed with the Edmonton Protocol, we have had the opportunity to review the outcomes in terms of C-peptide secretion, insulin independence, correction of hypoglycemia and lability, acute complications encountered, chronic problems related to immunosuppressive therapy, and some assessment of the effect on diabetes complications.

RESEARCH DESIGN AND METHODS

As of 1 November 2004, 65 patients have received islet transplants at the University of Alberta. Four other subjects were transplanted as part of the Immune Tolerance Network trial of islet transplantation and will be reported independently. One further subject was transplanted with a preparation from a pediatric donor that had many trapped islets. This subject had primary nonfunction of the graft, and the data from this patient are not included in this report. At the time of the transplant, the mean age of the 65 patients was 42.9 ± 1.2 years, their duration of diabetes was 27.1 ± 1.3 years, and 57% were women. Their median weight was 68.5 kg (interquartile [IQ] range 62.8–78.1), and the units of insulin used per day pretransplant was 45 (35–55). Problematic hypoglycemia was present in 80% of subjects and labile diabetes in 60%, often with overlap of these two indications. Four patients were transplanted because of progressive complications of diabetes. Problematic hypoglycemia

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CBC, complete blood count; CMV, cytomegalovirus; HOMA, homeostasis model assessment; HYPO score, Hypoglycemic score; LI, lability index; LFT, liver function test; PRA, panel reactive antibody.

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was defined as frequent recurrent episodes of hypoglycemia, usually associated with hypoglycemia unawareness and more recently quantified with a Hypoglycemic score (HYPO score) of $\geq 1,047$ (9). Labile diabetes was defined as frequent wide swings in blood glucose that interfere with the patient's lifestyle and was characterized by a mean amplitude of glycemic excursion >11.1 mmol/L (10) and more recently by a lability index (LI) of ≥ 433 mmol/L² · h⁻¹ · week⁻¹ (9). Typically now a patient must have either a HYPO score or LI above the 90th centile derived from the general type 1 diabetic population (1,047 and 433 mmol/L² · h⁻¹ · week⁻¹, respectively) or have both scores above the 75th centiles (423 and 329 mmol/L² · h⁻¹ · week⁻¹, respectively). Pretransplant assessment of diabetes complications revealed the presence of retinopathy in 74% (proliferative in 46%), microalbuminuria in 35% with macroproteinuria (>0.2 g/day) in 25%, symptomatic coronary artery disease in 9%, clinical peripheral vascular disease in 8%, autonomic neuropathy in 15%, and peripheral neuropathy in 32%. All subjects gave written informed consent. Diabetes-related exclusion criteria included unstable coronary artery disease, the presence of active proliferative retinopathy, or macroproteinuria ≥ 1 g/day; subjects with macroproteinuria <1 g/day were considered on a case-by-case basis and more recently for renal sparing immunosuppressive protocols.

Sixty-five patients had at least one transplant, 52 patients had two transplants, and 11 patients had three transplants. At the first transplant, islets combined from two donors were used on eight occasions, and on both the second and third transplants, islets from two donors were combined on two occasions. Of the 128 total procedures, 124 were performed by the percutaneous route and the remainder via a mini-laparotomy with cannulation of a mesenteric venous portal tributary. The latter approach was used if a hepatic hemangioma was present precluding a percutaneous approach or more recently if aspirin could not be discontinued pretransplant.

Transplant procedures. Islets were prepared as previously described (1,11–13). Briefly, human cadaveric pancreases were removed from brain dead multi-organ donors following in situ vascular flushing with cold University of Wisconsin solution and transported to the clinical islet isolation laboratory using a two-layer (University of Wisconsin/perfluorochemical) cold-storage method where possible (14). Upon arrival at the laboratory, the pancreatic duct was cannulated and liberase enzyme (Roche Diagnostics, Indianapolis, IN) (11) perfused. The pancreas was enzymatically and mechanically dissociated before the islets were separated on a refrigerated Cobe 2991 centrifuge (Cobe BCT, Lakewood, CO). The majority of the islet preparations, 97 of 140, were placed in culture (median 13.0 h [IQ range 6.4–23.0]) before infusion to facilitate timing of islet infusion or as part of the immunosuppressive protocol. Islet numbers were quantified in duplicate with the use of an islet standard diameter of 150 μ m (15). Once the islets were obtained, the patient was admitted and subjected to the following tests under the Edmonton Protocol: complete blood count (CBC), chest X-ray, liver function tests (LFTs), and coagulation screen. The patient was then brought to the Department of Radiology, and portal vein cannulation was performed. When the portal vein was cannulated, the islets in 250 ml of medium in an intravenous fluid bag were allowed to infuse under gravity pressure (16); before 2001 a syringe was used. Portal pressure was monitored during and after infusion of 5 ml of islet tissue, after each subsequent milliliter of tissue, and again when the transplant was completed. To minimize the risk of bleeding, the catheter tract was plugged with coils and Tisseel. The glucose was monitored hourly initially and insulin therapy withheld until the capillary glucose increased to >6.0 mmol/L premeal or >8.0 mmol/L 2-h postmeal.

Patients were usually discharged the following day when an ultrasound had confirmed the absence of any portal vein thrombosis or intraperitoneal bleed and that the CBC and LFTs were acceptable. Aspirin (81 mg/day for 14 days) and enoxaparin (30 mg b.i.d. s.c. for 10 days) was prescribed once major bleeding had been excluded. Immunosuppressive therapy consisted of daclizumab (2 mg/kg) at transplant and at 5 days' posttransplant, sirolimus with a loading dose of 0.2 mg/kg followed by 0.1 mg/kg with target trough levels of 12–15 ng/ml, and tacrolimus at a dose of 2–4 mg twice daily with a target trough level of 3–6 ng/ml. Before 2003, daclizumab was given at a dose of 1 mg/kg every 2 weeks for five doses, but the change in daclizumab therapy was made for patient convenience together with the evidence for efficacy of the simpler regimen in other solid organ transplantation (17). At 3 months' posttransplant, the target dose of sirolimus was reduced to 8–10 ng/ml. *Pneumocystis carinii* prophylaxis with sulfamethoxazole/trimethoprim was used for 6 months. Ganciclovir 1,000 mg t.i.d. was given for cytomegalovirus (CMV) prophylaxis for 3 months in subjects who were CMV negative and receiving islets from CMV-positive donors. Complete blood count, drug levels, and basic laboratory parameters (LFTs, electrolytes, calcium, and magnesium) were measured three times a week for the 1st 2 weeks, twice a week for the next 2 weeks, weekly for the next month, and then every 2 weeks for a month, depending on the clinical need. Ten subjects were transplanted with a

modification of the standard protocol using infliximab (10 mg/kg) given at the time of transplant, and a further nine subjects were transplanted using a lymphocyte depletion protocol (Campath-1H, ultra low-dose tacrolimus and higher-dose sirolimus) and will be the subject of a separate report.

For longer-term posttransplant follow-up, the transplant subjects were seen every 1–6 months depending on how near they lived to the transplant center. At these visits, glucose control and any adverse events were reviewed and weight and blood pressure were assessed. Lipids, LFTs, electrolytes, calcium, magnesium, and CBC were measured together with fasting glucose, insulin, and A1C. Islet cell and insulin antibodies were determined in collaboration with Dr. George Eisenbarth. Every 6 months, a full physical examination was performed, neuropathy was assessed with a neurothesiometer (Horwell, Nottingham, U.K.) applied at the big toe on each side, and the mean of six readings was taken (three on each side). Every 6 months a meal tolerance test was performed in the fasting state, with blood drawn for glucose and C-peptide at baseline and then at 90 min after drinking Ensure High Protein (6 ml/kg to a maximum of 360 ml, providing 391 kcal with 8.5 g fat, 44 g carbohydrate, and 17 g protein). Yearly determinations of the HYPO score and LI were made (9), as was a composite measure of graft function, the β -score (18). Homeostasis model assessment (HOMA) was calculated for an estimation of insulin sensitivity (19,20).

Patients were deemed to have completed the islet transplant procedure once they gained insulin independence as defined by the use of no exogenous insulin for 4 weeks and no more than two values per week >10.0 mmol/L on their capillary glucose testing records. Patients who received $>15,000$ islet equivalents (IE)/kg were deemed complete even if they were not insulin independent. On longer-term follow-up, insulin therapy was recommenced if the fasting capillary glucose was >8.0 mmol/L, the 2-h postprandial glucose was >10.0 mmol/L, and/or the A1C was $>7\%$ consistently. Patients were judged to have completely lost islet graft function when two stimulation tests showed C-peptide levels below the level of detectability of the assay (0.1 nmol/L) or if the fasting glucose was >15.0 mmol/L with no measurable C-peptide present. As of 1 November 2004, 36 patients were complete using the Edmonton protocol, 7 using the infliximab protocol, and 4 using the Campath-1H protocol.

Assays. Plasma glucose concentrations were determined by the glucose oxidase method. C-peptide was measured using a commercial assay (Diagnostic Systems Laboratories, Webster, TX). The lower limit of sensitivity for C-peptide was 0.1 nmol/L in our laboratory, the intra- and interassay coefficients of variations were $<9.5\%$, and the normal range was 0.3–1.32 nmol/L. Panel reactive antibodies (PRAs) were measured with anti-human globulin and more recently by flow cytometry.

Statistics. Results are expressed as means \pm SE or the median (25th–75th IQ range) as appropriate. Comparisons were made with a two-tailed Student's *t* test, paired or unpaired as appropriate. For group comparisons, one-way repeated-measures ANOVA was used and the Holm-Sidak or Dunn test used when normality tests failed. All statistical analyses including Kaplan-Meier survival curves were performed using Sigma Stat for Windows (v. 3.0; SPSS, Chicago, IL). Significance was taken at the $P < 0.05$.

RESULTS

The mean number of islets given per procedure was $393,554 \pm 10,528$ IE ($5,783 \pm 142$ IE/kg) in a mean packed cell volume of 4.4 ± 0.2 ml. The median hospital stay was 2 days (IQ range 1–3). Forty-four patients were considered to have completed the islet transplant with insulin independence. Three further patients who received $>16,000$ IE/kg remained insulin dependent. Those who have completed the islet transplant and became insulin independent received a total of $799,912 \pm 30,220$ IE ($11,910 \pm 469$ IE/kg). The median time between the first and second transplant was 2.5 months (IQ range 1.0–4.4) and between the second and third transplant was 1.8 months (1.4–11.7). The median wait time from time of listing to the first transplant was 5.4 months (2.6–11.1), 3.9 months (1.6–7.0), and 3.9 months (2.7–6.8) for subjects with blood groups O, A, or B, respectively.

Acute complications. Fifteen subjects had evidence of a major bleed related to the procedure as defined by a drop in hemoglobin of >25 g/l or a drop in hemoglobin requiring intervention following percutaneous islet infusion, and a

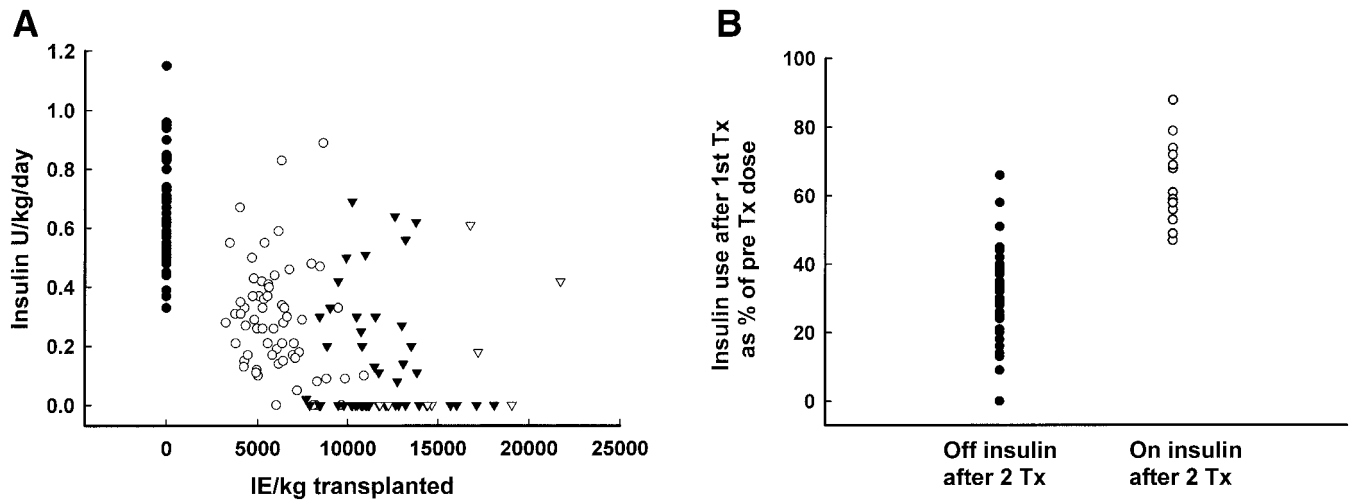


FIG. 1. A: Exogenous insulin use in relation to the number of islets transplanted. Shown are pretransplant (●), after the first transplant (○), after the second transplant (▼), or after the third transplant (▽). Insulin requirements dropped after the first transplant, and many became insulin independent after the second transplant. Three patients still required exogenous insulin after three transplants. **B:** The amount of exogenous insulin required 1 month after the first transplant (Tx) expressed as a percentage of the pretransplant insulin requirement. Shown are the groups of patients who became insulin independent after the second transplant (●) and those who did not achieve insulin independence after two transplants (○). If the insulin requirements did not fall by 50% or more, it was unlikely that the subject would become insulin independent with one further transplant.

blood transfusion was given on seven occasions with two subjects requiring a laparotomy. The risk of bleeding has recently been resolved by effective sealing of the portal catheter tract using coils and Tisseel and by discontinuation of aspirin 2 weeks before transplantation (unpublished data). Five patients had evidence of a thrombus in segmental branches of the portal vein and were treated with anticoagulation, and none of these patients has developed clinical sequelae of portal hypertension. The gall bladder was punctured in two subjects, but both resolved with conservative management. Mean portal pressure at the start of the procedure was 11.0 mmHg (IQ range 8–13) and increased by the end of the transplant to 13 mmHg (10–17) ($P < 0.001$). Liver transaminases (aspartate aminotransferase) increased to >2.5 times the upper limit of normal in 55% of procedures and to >5 times the upper limit of the normal range in 23% of procedures. These abnormalities usually resolved over 4 weeks (median 23 days [IQ range 17–35]). In the longer term, changes consistent with fatty liver were seen in 8 of 36 subjects who had magnetic resonance imaging post-transplantation.

Short-term outcomes. Five subjects became insulin independent with a single infusion of islets, having received $502,211 \pm 79,770$ IE ($6,713 \pm 944$ IE/kg provided); 33 came off insulin with two infusions, having received $792,396 \pm 27,867$ IE ($11,951 \pm 398$ IE/kg provided); and 6 required three infusions of islets for insulin independence, having received $987,820 \pm 47,463$ IE ($14,443 \pm 1,052$ IE/kg provided). Two subjects became insulin independent for a brief period after one transplant but then required a second transplant and thus are considered as having had two transplants to be complete. Insulin use in relationship to the transplant is shown in Fig. 1A. Those patients who became insulin independent with two transplants had a greater fall in insulin requirements after the first transplant compared with those who did not become insulin independent after two transplants (Fig. 1B), despite receiving a

similar number of islet equivalents ($11,791 \pm 395$ vs. $11,059 \pm 479$ IE/kg, respectively, $P = 0.272$).

Long-term outcomes. The median follow-up as of 1 November 2004 in the subjects who completed transplants was 35.5 months (range 4.1–67.8) (IQ range 23.0–50.3). Insulin independence for at least 1 month was achieved in 44 of 47 patients (94%) and has persisted to date for a median of 15 months (IQ range 6.2–25.5); C-peptide secretion has persisted to date for a median of 25.2 months (15.8–38.1). Three subjects did not become insulin independent despite receiving $1,360,343 \pm 56,319$ IE ($18,542 \pm 1,602$ IE/kg). Graft survival as measured by C-peptide positivity is shown in Fig. 2A and insulin independence in Fig. 2B. Despite persistent graft survival, the majority of subjects had to resume insulin therapy in order to maintain good glycemic control. Shown in Fig. 2C are the insulin independence survival curves based on the number of transplants required for insulin independence; there is no statistical difference among the three groups.

The A1C over the follow-up period in the subjects is shown in Fig. 3. The A1C rose once graft function was lost. The most recent A1C was well controlled in those subjects who remained off insulin (6.4% [IQ range 6.1–6.7]), similar to the level in those who resumed insulin but who were C-peptide positive (6.7% [5.9–7.5]), and lower than in those who lost all graft function (9.0% [6.7–9.3]) ($P = 0.025$). Those back on exogenous insulin who lost all graft function required 0.80 ± 0.08 units \cdot kg $^{-1}$ \cdot day $^{-1}$ of insulin, which is more than that used pretransplant (0.69 ± 0.08 , $P = 0.03$), while those who resumed insulin but had persisting C-peptide secretion required only 0.34 ± 0.04 units \cdot kg $^{-1}$ \cdot day $^{-1}$, which is significantly less than that used pretransplant (0.66 ± 0.03 units \cdot kg $^{-1}$ \cdot day $^{-1}$, $P < 0.001$).

The response to the mixed meal challenge is shown in Fig. 4. In all cases, glucose and C-peptide responses were better post- than pretransplant. Once insulin independence was maintained, the postchallenge glucose remained <11.0 mmol/l with a brisk C-peptide response

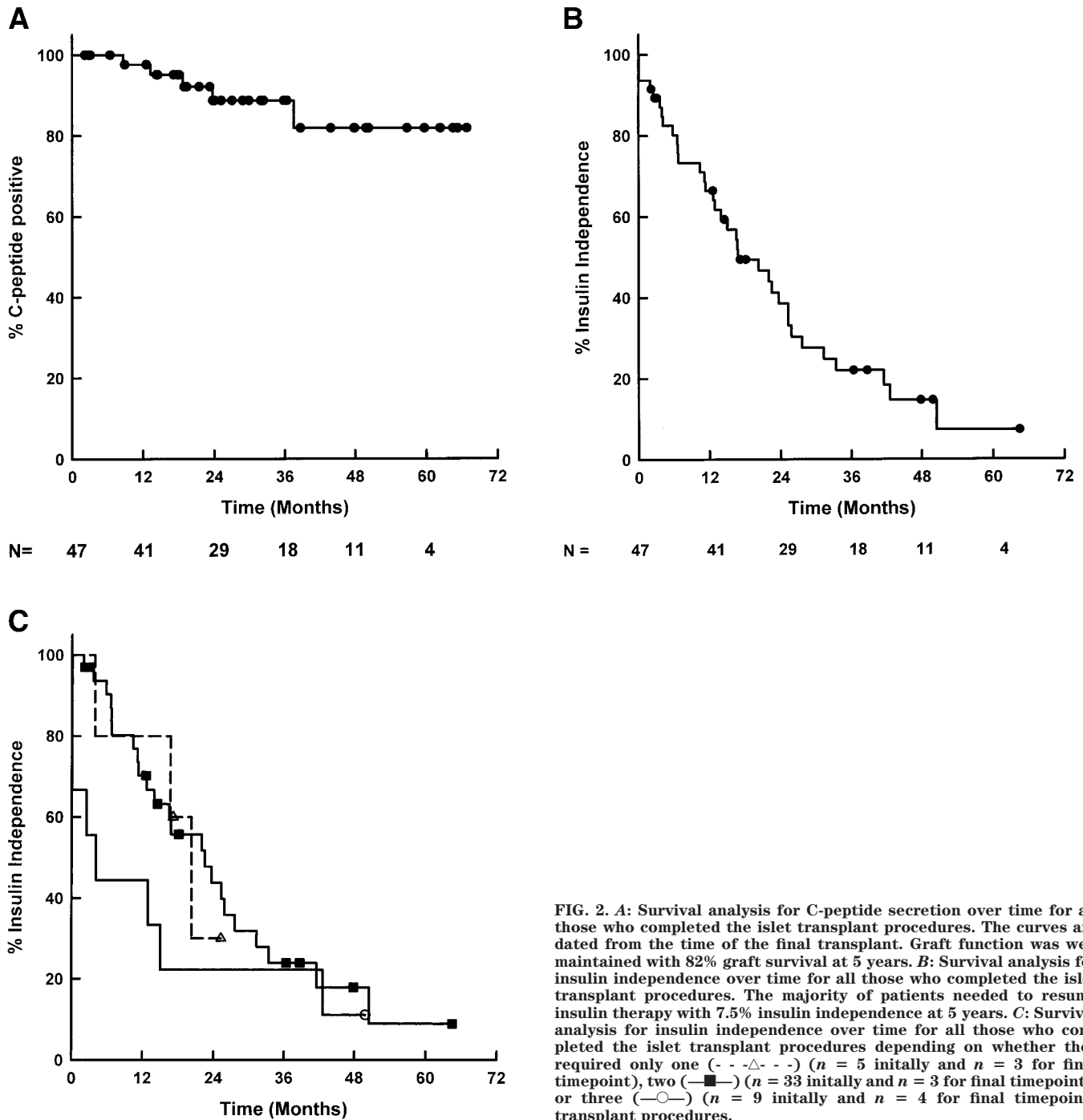


FIG. 2. *A*: Survival analysis for C-peptide secretion over time for all those who completed the islet transplant procedures. The curves are dated from the time of the final transplant. Graft function was well maintained with 82% graft survival at 5 years. *B*: Survival analysis for insulin independence over time for all those who completed the islet transplant procedures. The majority of patients needed to resume insulin therapy with 7.5% insulin independence at 5 years. *C*: Survival analysis for insulin independence over time for all those who completed the islet transplant procedures depending on whether they required only one (---△---) ($n = 5$ initially and $n = 3$ for final timepoint), two (—■—) ($n = 33$ initially and $n = 3$ for final timepoint), or three (—○—) ($n = 9$ initially and $n = 4$ for final timepoint) transplant procedures.

(Figs. 4A and B). However, in the subjects who had to resume exogenous insulin compared with those remaining insulin independent, glucose levels during the meal tolerance test were elevated both basally (7.6 ± 0.4 vs. 6.4 ± 0.2 mmol/l, $P = 0.02$) and postchallenge (13.2 ± 0.5 vs. 8.3 ± 0.3 mmol/l, $P < 0.001$). Conversely, the C-peptide levels in patients who resumed insulin compared with those remaining off insulin therapy were lower both basally (0.49 ± 0.05 vs. 0.86 ± 0.05 nmol/l, $P < 0.001$) and poststimulation (0.93 ± 0.08 vs. 1.62 ± 0.07 , $P < 0.001$). The increment in C-peptide in those subjects using exogenous insulin was lower (0.44 ± 0.06 vs. 0.76 ± 0.06 nmol/l, $P < 0.001$) in those off insulin. Finally, the increment in C-peptide with the mixed meal challenge just before

restarting insulin (within 3 months) was less than the increment when the subject first became insulin independent (0.64 ± 0.08 vs. 0.80 ± 0.08 nmol/l, $P = 0.039$).

The composite β -score (18) was 0 pretransplant, increased to 5.6 ± 0.3 at 1 year, and was 4.3 ± 0.3 at the most recent follow-up, confirming overall graft function maintenance. The HOMA score at 1 year posttransplant in the subjects remaining insulin independent was 2.3 (IQ range 1.8–5.6). In the subjects who resumed insulin therapy, the HOMA score 1 month before restarting insulin was 2.7 (2.0–4.8), and in these subjects a year earlier was 3.0 (2.2–3.6) with no statistical difference between the values. Metformin was tried in 23 patients, but 19 discontinued its use because of lack of efficacy in this setting and because

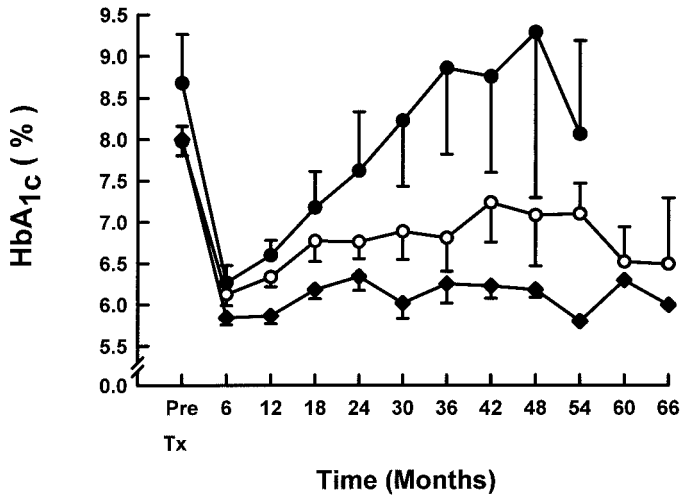


FIG. 3. The HbA_{1c} mean \pm SE, over time posttransplantation in those whose transplant failed (—●—), those whose graft remained functioning but had to resume insulin (—○—), and those who remained insulin independent (—◆—). Loss of graft function was associated with an increase of the HbA_{1c}. The group off insulin was significantly different from the others. Tx, transplant.

it was typically associated with diarrhea. Thiazolidinediones were tried in 22 patients, but only 1 patient remained on them because of a lack of efficacy in the post-islet transplant setting and problematic edema. The β -score at 1 year was similar in the infliximab, Campath 1-H, and Edmonton protocol groups, but very few of the Campath-1H group completed the protocol and are the subject of unpublished data. The HYPO score and LI show marked improvement posttransplant (Fig. 5A and B). With the use of insulin there have been some episodes of hypoglycemia and more lability, but the scores remain significantly improved for up to 4 years compared with values pretransplant.

To examine why graft function in some subjects fared

less well, we examined the β -score in terms of donor characteristics. There was no relationship of the β -score to donor age, BMI, family history of diabetes, sex, use of cultured islets, cold ischemia time, or purity of islets; the number of β -cells transplanted; or recipient characteristics, including blood group, age, sex, duration of diabetes, and BMI. Those with a low β -score at 1 year (0–4, $n = 12$) had higher pretransplant insulin requirements (0.77 ± 0.05 units \cdot kg⁻¹ \cdot day⁻¹) compared with those who had a higher β -score at 1 year (5–8, $n = 31$) (0.59 ± 0.02 units \cdot kg⁻¹ \cdot day⁻¹, $P < 0.001$). The presence of a positive PRA was associated with a lower β -score, but auto-antibody positivity had no significant influence on the β -score (Table 1). The percentage of PRA increased in 8 of 63 patients from levels $<15\%$ to those $\geq 15\%$. Three of these eight have lost graft function. Both islet cell antibodies and GAD antibodies were negative in 37 of 63 patients, and 35 of these remained negative posttransplant. Neither of the two subjects who became antibody positive have lost graft function.

Immunosuppressive therapy complications. Mouth ulcers occurred in 89% of subjects. Most responded to simple antiseptic measures or topical triamcinolone ointment together with a reduction in the dose of sirolimus. On two occasions the ulcers were severe and required surgical debridement or hospitalization. Diarrhea was a frequent (60%) problem, and acne was noted in 52%. Forty-three percent of subjects complained of edema, and in 12% it was severe enough to necessitate a change in the immunosuppressive regimen (usually conversion of sirolimus to mycophenolate mofetil). Ovarian cysts were very frequent in premenopausal women and were sometimes associated with menorrhagia. The mean hemoglobin value pretransplant was 137 ± 2 g/l, at 1 year post-first transplant was 121 ± 3 g/l, and most recently was 126 ± 2 g/l. Erythropoietin therapy was used in 8% of subjects. There was no change in the platelet count posttransplant. The

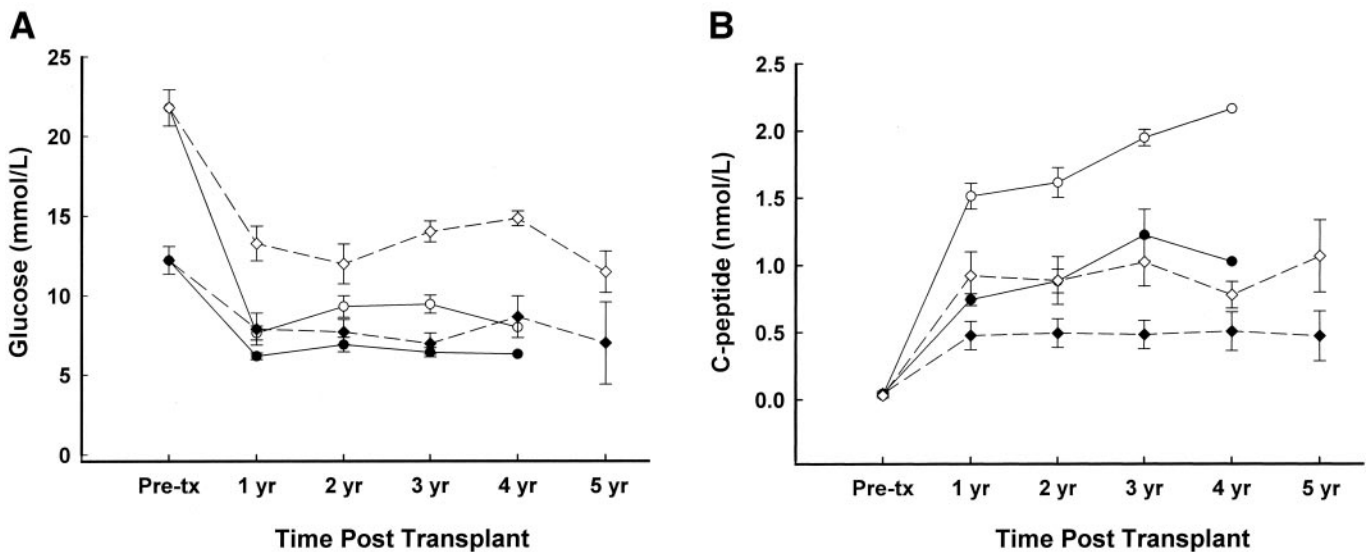


FIG. 4. Plasma glucose (A) and C-peptide (B) values, mean \pm SE, before (—●—) and 90 min after (—○—) consuming a standard meal in those subjects who remained off insulin. The number of subjects studied were 36 pretransplant, 28 at 1 year, 12 at 2 years, 7 at 3 years, and 1 at 4 years. All values posttransplant were significantly different from the pretransplant values. Also shown are the plasma glucose (A) and C-peptide values (B), mean \pm SE, before (---◆---) and 90 min after (---◇---) consuming a standard meal in those subjects who resumed insulin therapy. The number of subjects studied were 36 pretransplant, 12 at 1 year, 13 at 2 years, 12 at 3 years, 5 at 4 years, and 2 at 5 years. All values posttransplant were significantly different from the pretransplant values. Tx, transplant.

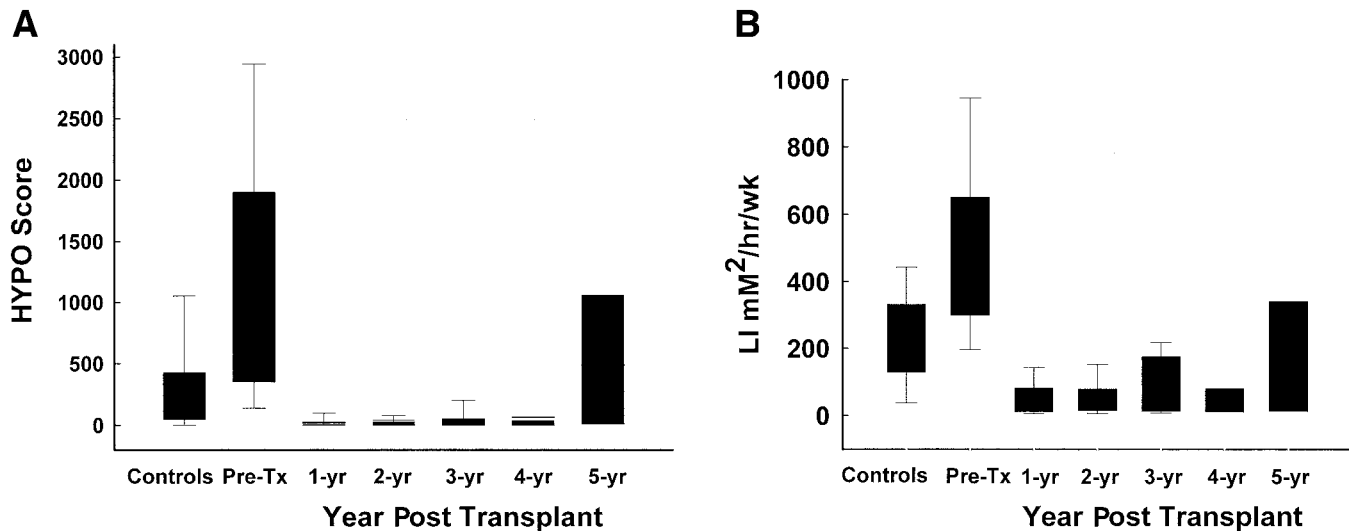


FIG. 5. The HYPO score (A) and LI (B) pre- and posttransplant in those who remained C-peptide positive. The box plots show the 25th–75th IQ range, and the bars show the 10th–90th IQ range. The controls have been described previously (9). For the HYPO score pretransplant, $n = 31$; at 1 year, $n = 40$; at 2 years, $n = 29$; at 3 years, $n = 15$; at 4 years, $n = 7$; and at 5 years, $n = 5$. For the LI pretransplant, $n = 42$; at 1 year, $n = 41$; at 2 years, $n = 28$; at 3 years, $n = 15$; at 4 years, $n = 7$; and at 5 years, $n = 5$. Posttransplant the scores for years 1–4 are significantly better than the pretransplant values. Tx, transplant.

white cell count pretransplant was $5.9 \pm 0.2 \times 10^9/l$, at 1 year was $4.7 \pm 0.2 \times 10^9/l$ ($P < 0.001$), and most recently was $5.2 \pm 0.2 \times 10^9/l$ ($P < 0.001$). Weight loss was common; the pretransplant weight was 70.5 ± 1.7 , dropped at 1 year to 65.1 ± 1.6 ($P < 0.001$), and most recently was 67.3 ± 1.7 kg ($P < 0.001$ vs. pretransplant and $P = 0.003$ vs. weight at 1 year).

Three patients had pneumonia, one of which was thought to be fungal in etiology. One patient was found to have two small foci of papillary carcinoma of the thyroid. Of 43 subjects who were CMV negative but had transplants from donor-positive subjects, 2 had seroconversion (6%) but no overt CMV disease. The titer was indeterminate pretransplant in one of these subjects, and the remaining subject was felt to have a community-acquired CMV. Of the 43 patients who used sirolimus and tacrolimus as initial immunosuppression, 33 (77%) remained on these drugs as the main immunosuppressive regimen. Five pa-

TABLE 1
Positive antibodies and the β -score

	<i>n</i>	β -score
Allo-		
% PRA range		
0–9	42	6.0 (5.0–7.0)
10–39	7	3.0 (1.0–5.5)*
≥ 40	7	2.0 (0.0–4.0)*
Auto-		
Antibody status (either pre- or posttransplant)		
GAD or ICA both negative	26	5.0 (3.0–6.0)
Either GAD or ICA positive	22	6.0 (4.0–7.0)
Both GAD and ICA positive	9	3.0 (2.0–7.0)

Data are median (IQ range). PRA was measured to both class 1 and 2 antigens. Allo: the β -score and %PRA were determined 1 year after the initial transplant. *The difference in the β -score for the PRA groups was significantly different from the 0–9% group, $P < 0.05$. Auto: autoantibodies and the β -score were determined at 1 year, and values among the antibody status groups were similar. ICA, islet cell antibody.

tients were changed to tacrolimus and mycophenolate mofetil, three to sirolimus and mycophenolate mofetil, and two to low-dose sirolimus, tacrolimus, and mycophenolate mofetil.

Diabetes complications. In the 47 completed subjects, 4 had a deterioration of eye disease and required photocoagulation or vitrectomy within 5 months of transplant. The median serum creatinine pretransplant was $80 \mu\text{mol/l}$ (IQ range 69–90) and at 1 year posttransplant was $84 \mu\text{mol/l}$ (70–106). The most recent serum creatinine determination was $92 \mu\text{mol/l}$ (77–115) ($P < 0.001$ vs. pretransplant and $P < 0.05$ vs. 1 year posttransplant). The creatinine clearance pretransplant was $1.8 \text{ ml/s} \cdot 1.73 \text{ m}^2$ (1.4–2.0) pretransplant, at 1 year posttransplant was $1.6 \text{ ml/s} \cdot 1.73 \text{ m}^2$ (1.3–1.8), and most recently was $1.4 \text{ ml/s} \cdot 1.73 \text{ m}^2$ (1.1–1.7) ($P = \text{NS}$). The albumin excretion rate was $11 \mu\text{g/min}$ (6–20), at 1 year posttransplant was $16 \mu\text{g/min}$ (8–39), and most recently was $19 \mu\text{g/min}$ (9–59) ($P = \text{NS}$). The 24-h urine protein excretion rate was 0.2 g/day (0.1–0.2) pretransplant, 0.1 g/day (0.1–0.2) at 1 year posttransplant, and 0.1 (0.1–0.2) most recently ($P = \text{NS}$). Five of 11 subjects had progression of microalbuminuria to macroproteinuria, and 3 of 30 subjects with no microalbuminuria pretransplant progressed to macroproteinuria. Mean systolic and diastolic blood pressure was unchanged pre- and posttransplant, but this was only achieved with the increased use of antihypertensive medications posttransplantation. Pretransplant, 36% of subjects were on no antihypertensives and 6% were on more than one medication for hypertension. Posttransplant, the current respective percentages are 15 and 42.

The LDL cholesterol level was $2.6 \pm 0.1 \text{ mmol/l}$ pretransplant, 2.6 ± 0.1 at 1 year posttransplant, and $2.2 \pm 0.1 \text{ mmol/l}$ most recently ($P = \text{NS}$). However, pretransplant, 23% of subjects were on lipid-lowering medications, and most recently 83% were requiring therapy. The triglyceride level pretransplant was $0.87 \pm 0.07 \text{ mmol/l}$, increased posttransplant to 1.32 ± 0.11 at 1 year, and was $1.23 \pm 0.10 \text{ mmol/l}$ most recently ($P < 0.002$). The neurothesiometer

score did not change significantly pretransplant (6.2 V [IQ range 4.5–12.1]), at 1 year posttransplant was 7.6 V (5.3–12.4), and most recently was 8.9 V (6.0–14.2) ($P = \text{NS}$). One patient with functioning islets died suddenly 22.5 months' posttransplant of an accidental cause.

DISCUSSION

This review of our experience shows a 5-year post-islet transplantation graft survival of ~80% as measured by C-peptide positivity, while insulin independence was more difficult to maintain, with a rate close to 10% at 5 years. Glucose control and problems with glycemic lability and hypoglycemia were improved with the provision of endogenous insulin secretion. The acute complication rate was low, and long-term problems related to the immunosuppressive therapy were manageable.

The divergence of persisting insulin secretion and the need for exogenous insulin is indicative of inadequate insulin reserve. The defects in insulin secretion may be due to inadequate islet mass or impaired function. Although nearly 800,000 IE were transplanted per patient, it is likely that many islets were lost at the time of engraftment, perhaps due to the instant blood-mediated inflammatory reaction (21). Islet transplantation is associated with activation of the coagulation cascade and an increase in serum cross-linked fibrin degradation products (21,22). Certainly the acute insulin response to secretagogues is well below normal when studied shortly after transplantation (23,24), suggesting that the surviving islet mass is marginal. In addition, even with excellent function it is rare to have a normal fasting glucose after islet transplantation, likely reflecting some problem with islet mass or function from the outset (18). However, there was a deterioration of glycemic control over time that may reflect a further loss of islets or problems with function. Either auto- or alloimmune destruction may be occurring. The fact that the survival curve for insulin use is different from the curve representing persisting C-peptide secretion in most subjects may suggest that the problem is not simply due to loss of cells from immune destruction. If the β -cells were being destroyed on an immune basis, one might expect that the survival curves would be similar, with the C-peptide survival curve falling steeply and simply shifted to the right. Finally, an increase in insulin resistance could also explain these changes, but the comparable HOMA values in those on and off insulin and the lack of success of thiazolidinediones argue against a prominent role for insulin resistance.

The finding of a lower increment in C-peptide just before recommencing insulin therapy compared with that a year earlier supports the conclusion that islet function declined and indicates that the lower increment in C-peptide post-meal challenge seen in Fig. 4B was not primarily due to taking exogenous insulin. The reason for this decline is not readily apparent. Perhaps the normal cycle of neogenesis and apoptosis is lost, but the reports of long-term survival of both allo- and autoislet transplants belie this (25,26). Allografted islets are also exposed to the first pass of immunosuppressive drugs, agents that are toxic to β -cells at higher concentrations (27–29). Whether over time these toxic effects lead to dysfunction and the need for exogenous insulin is unknown. The finding of more sustained

long-term insulin independence with autoislet transplants (26) may point to the deleterious effects of the immunosuppressive drugs on function, although clearly allo- or autoimmune roles cannot be discounted. In some subjects, alloimmunity appeared to be involved as evidenced by an increase in the percentage of PRA and the relationship of the β -score to this activation at 1 year posttransplant. However, the percentage of PRA reflects the reactivity to a panel of antibodies and examination of donor-specific antibody positivity on a case-by-case basis will be important. Other measures of graft rejection, especially granzyme B, may become useful as measures of graft loss (30). The blood supply that the transplanted islets develop in the liver over the first 4 weeks of engraftment does not follow the pattern of the native islet. Normally an islet arteriole delivers blood centrally that then flows to the periphery. The vessels are also fenestrated (31,32). The vasculature that develops posttransplant appears to grow in from the periphery (33). This may contribute to impaired function over time. A further consideration is that the final mass of islet tissue engrafted may be only 20% of normal, and such a limited mass may not be able to cope in the long term with the metabolic demands. Lastly, the liver may not be an optimal site for islets in terms of function in that the insulin release may be into the hepatic vein, and the islets are exposed to high concentrations of nutrients entering from the portal vein (34).

The long-term function of the islets could not be predicted by any of the simple donor characteristics, including age, sex, or weight, other than a higher insulin requirement pretransplant being associated with poorer outcome as evidenced by the lower β -score. Likewise, islet numbers transplanted (based on the accuracy of counting islets, which is problematic) did not predict insulin independence. In fact, the patients who required three islet transplants and had more islets than those with two transplants appeared to fare less well (Fig. 2C), but limited numbers prevent definitive conclusions. Some patients did well with just one transplant, having obtained a number of islet equivalents far less than the mean for those who had two transplants. In addition, if someone showed more than a 50% decline in insulin requirements following the first transplant, then they were more likely to become insulin independent with a further transplant, but if there was only a modest decline after the first transplant, then it was likely they would need more than two transplants to become insulin independent (Fig. 1B). A favorable response to the first transplant appeared more predictive of future insulin independence than the total numbers of cells provided. In our previous report (23), 10,000 IE/kg was usually enough for insulin independence, but this is less clear cut as more patients are transplanted (Fig. 1A). More than 20% of patients required in excess of 15,000 IE/kg to become insulin independent, and one subject did not become insulin independent despite having >20,000 IE/kg. Clearly islet quality, viability, engraftment, and/or function is as important as the numbers transplanted. If there is minimal response to the first and especially a subsequent transplant, then further transplantation without a new immunosuppressive regimen may not make sense.

The recent report from the Minnesota group (5) of insulin independence with a single donor is of note. Many

differences are evident, including their selection of recipients who were lighter (maximum weight 67.2 kg) and had a pretransplant insulin requirement of <41 units/day. We have been less selective in our program, as evidenced by a median weight in our recipients of 68.5 kg and insulin requirement of 45 units/day. The donor age of <50 years, BMI >27 kg/m², peritransplant use of intravenous insulin and heparin, potent induction therapy, and after 1 month posttransplant changing tacrolimus to mycophenolate mofetil were also factors that differed between our two programs. Additionally, the Minnesota Group used etanercept as anti-tumor necrosis factor therapy in place of infliximab. Which one or combination of these factors contributed to their success is not clear, but at 1 year the insulin independence rate was 62.5%.

One benefit that was clearly gained from islet transplantation was the amelioration of the problems with glycemic lability and hypoglycemia. Both the LI and the HYPO score improved posttransplant, and correction of the problems with lability and hypoglycemia, the primary indication for transplant, was achieved (Fig. 5A and B). It is notable that the glucagon response to hypoglycemia is not normalized by the intrahepatic islet transplantation (35). Thus, when insulin is recommenced, the risk of hypoglycemia increases, as demonstrated in Fig. 5A, and this was in part related to the effort of the subjects to maintain excellent glycemic control. The LI also increased as insulin was resumed.

The major acute complications of the percutaneous transhepatic approach are serious bleeding or portal vein thrombosis, as seen in this summary and reported by others (36–38). The risk of acute bleeding has been markedly reduced with the avoidance of aspirin and the use of coils and Tisseel at the time of the transplant (unpublished data). The risk of a portal thrombus remains but appears to be best abrogated by minimizing the packed cell volume and thrombogenicity of the preparation. For islet autotransplants, much higher packed cell volumes are used with unpurified preparations, but these are administered under direct vision at the time of surgery and with the protection of therapeutic heparinization (39,40). The continued risk of portal vein thrombosis together with the blunted glucagon response to hypoglycemia and the loss of function over time prompts consideration of using alternative sites for the islets. Animal studies demonstrate that the glucagon response to hypoglycemia is normal when islets are placed intraperitoneally (41), but it remains to be determined how initial islet engraftment in the intraperitoneal site compares with intraportal delivery.

The chronic complications of the immunosuppressive therapy must also be considered in the light of freedom from hypoglycemia and glycemic lability. The mouth ulcers were managed most effectively with the use of lower doses of sirolimus and triamcinolone ointment. They only became severe if the sirolimus was not reduced quickly enough. Acne was surprisingly common, as were ovarian cysts in premenopausal women, with the latter recently reported by the Miami group (42). Edema was resistant to all standard measures in 12% and was severe enough to warrant changes in immunosuppressive therapy, as has been reported (43). Also reported has been the occurrence of benign perinephric edema in a small percentage of patients (44). The finding of posttransplant fatty liver is of

unknown significance at this time (45,46). The gastrointestinal upset associated with the immunosuppressive regimen was usually diarrhea and again typically settled as the immunosuppressive dose was reduced after 3 months. The only case of neoplasm found so far involved two tiny foci of papillary cancer of the thyroid that have been resected. One case of presumed fungal pneumonia required discontinuation of the sirolimus. Finally, the finding that 13% of patients with a pretransplant percentage of PRA <15% by flow cytometry had an increase in the percentage posttransplant is a potential concern, as it may render future transplantation more problematic in terms of matching appropriate donors. The fact that 23% of subjects transplanted with the standard protocol are currently on alternative combinations points to the need for further improvements in immunosuppressive regimens.

An evident drawback is that there is no control group of subjects with type 1 diabetes followed over time for comparison, although we have started such a prospective study. For the present, however, no clear advantages for the chronic complications of diabetes are yet evident. Peripheral neuropathy remained unchanged. Subjects with autonomic gastroparesis generally found that glucose levels were much more easily controlled, especially if vomiting occurred, but maintenance of immunosuppressive drug levels was sometimes more difficult because of erratic absorption. The cholesterol levels were no different from pretransplant, but many more subjects were placed on statin therapy posttransplant. Some of this was related to the increased awareness for the need for LDL cholesterol lowering in diabetic subjects, but as reported previously, the immunosuppressive regimen we used is associated with an increase in cholesterol (47). A slight rise in triglycerides was noted, but this was not unexpected (48). Any rise in blood pressure was contained by the increased use of antihypertensive medications. More concerning was the rise in serum creatinine and the trend for a decline in the creatinine clearance. In a few patients there was a marked increase in urine protein that improved with discontinuation of the sirolimus. There was one sudden death, which was not related to the islet transplant.

In conclusion, successful islet transplantation is still a relatively new procedure. It provides clear benefits for a subset of type 1 diabetic patients in terms of improving variations in blood glucose and alleviating problematic hypoglycemia, while achieving a better A1C. The problems encountered after islet transplant are becoming more delineated. Balancing the risk-to-benefit ratio remains central to selecting appropriate candidates for transplantation, and informed consent is crucial. If a subject has severe hypoglycemic unawareness or glycemic lability that is causing a major disruption of their life, then islet transplantation can be of value. However, such a person will likely not remain insulin independent in the long-term and must accept the risks of immunosuppression so that he/she may have the endogenous insulin production to facilitate more stable glucose control. Once stable glucose control is attained, serious consideration needs to be given as to whether a further transplant will achieve any more than transient insulin independence. These results make clear that safer immunosuppression associated with fewer

side effects is needed. Further sources of islets and better engraftment remain obvious needs in order to build on the continuing islet function and to translate it into higher rates of insulin independence.

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REFERENCES

- Shapiro AMJ, Lakey JRT, Ryan EA, Korbitt GS, Toth EL, Warnock GL, Kneteman NM, Rajotte RV: Islet transplantation in seven patients with type 1 diabetes mellitus using a glucocorticoid-free immunosuppressive regimen. *N Engl J Med* 343:230–238, 2000
- Markmann JF, Deng S, Huang X, Desai NM, Velidedeoglu EH, Lui C, Frank A, Markmann E, Palanjian M, Brayman K, Wolf B, Bell E, Vitamianiuk M, Doliba N, Matschinsky F, Barker CF, Najj A: Insulin independence following isolated islet transplantation and single islet infusions. *Ann Surg* 237:741–750, 2003
- Hering BJ, Kandaswamy R, Harmon JV, Ansite JD, Clemmings SM, Sakai T, Paraskevas S, Eckman PM, Sageshima J, Nakano M, Sawada T, Matsumoto I, Zhang HJ, Sutherland DER, Bluestone JA: Transplantation of cultured islets from two-layer preserved pancreases in type 1 diabetes with anti-CD3 antibody. *Am J Transplant* 4:390–401, 2004
- Shapiro AMJ, Hering BJ, Ricordi C: Edmonton's success has indeed been replicated elsewhere (Letter). *Lancet* 362:1242, 2003
- Hering BJ, Kandaswamy R, Ansite JD, Eckman PM, Nakano M, Sawada T, Matsumoto I, Ihm S-H, Zhang H-J, Parkey J, Hunter DW, Sutherland DER: Single-donor, marginal-dose islet transplantation in patients with type 1 diabetes. *JAMA* 293:830–835, 2005
- Goss JA, Goodpastor SE, Bruncardi FC, Barth MH, Soltes GD, Garber AJ, Hamilton DJ, Alejandro R, Ricordi C: Development of a human pancreatic islet-transplant program through a collaborative relationship with a remote islet-isolator center. *Transplantation* 77:462–466, 2004
- Kessler L, Bucher P, Milliat-Guittard L, Benhamou PY, Berney T, Penfornis A, Badet L, Thivolet C, Bayle F, Oberholzer J, Renoult E, Brun MJ, Riffle G, Atlan C, Colin C, Morel P, GRAGIL Group: Influence of islet transportation on pancreatic islet allotransplantation in type 1 diabetic patients with the Swiss-French GRAGIL network. *Transplantation* 77:1301–1304, 2004
- Sutherland DER, Gruessner A, Hering BJ: B-cell replacement therapy (pancreas and islet transplantation) for treatment of diabetes mellitus: an integrated approach. *Endocrinol Metab Clin North Am* 33:135–148, 2004
- Ryan EA, Shandro T, Green K, Paty BW, Senior PA, Bigam D, Shapiro AMJ, Vantghem M-C: Assessment of the severity of hypoglycemia and glycemic lability in type 1 diabetic subjects undergoing islet transplantation. *Diabetes* 53:955–962, 2004
- Service FJ, Molnar GD, Rosevear JW, Ackerman E, Gatewood LC, Taylor WF: Mean amplitude of glycemic excursions, a measure of diabetic instability. *Diabetes* 19:644–655, 1970
- Linetsky E, Bottino R, Lehmann R, Alejandro R, Inverardi L, Ricordi C: Improved human islet isolation using a new enzyme blend, liberase. *Diabetes* 46:1120–1123, 1997
- Lahey JRT, Warnock GL, Shapiro AMJ, Korbitt GS, Ao Z, Kneteman NM, Rajotte RV: Intraductal collagenase delivery into the human pancreas using syringe loading or controlled perfusion. *Cell Transplant* 8:285–292, 1999
- Ricordi C, Lacy PE, Scharp DW: Automated islet isolation from human pancreas. *Diabetes* 38 (Suppl. 1):140–142, 1989
- Tsujimura T, Kuroda Y, Kin T, Avila JG, Rajotte RV, Korbitt GS, Ryan EA, Shapiro AMJ, Lakey JRT: Human islet transplantation from pancreases with prolonged cold ischemia using additional preservation by the two-layer (UW solution/perfluorochemical) cold storage method. *Transplantation* 74:1687–1691, 2002
- Ricordi C, Gray DW, Hering BJ, Kaufman DB, Warnock GL, Kneteman NM, Lake SP, London NJ, Alejandro R, Zeng Y, Scharp D, Viviani G, Falqui L, Tzakis A, Bretzel R, Federlin K, Pozza G, James RFL, Rajotte R, DiCarlo V, Morris PJ, Sutherland DER, Starzl T, Mintz DH, Lacy P: Islet isolation assessment in man and large animals. *Acta Diabetol Lat* 27:185–195, 1990
- Baidal DA, Froud T, Ferreira JV, Khan A, Alejandro R, Ricordi C: The bag method for islet cell infusion. *Cell Transplant* 12:809–813, 2003
- Sellers MT, McGuire BM, Hausteil SV, Bynon JS, Hunt SL, Eckhoff DE: Two-dose daclizumab induction therapy in 209 liver transplants single-center analysis. *Transplantation* 78:1212–1217, 2004
- Ryan EA, Paty BW, Senior PA, Lakey JRT, Bigam D, Shapiro AMJ: B-score: an assessment of B-cell function after islet transplantation. *Diabetes Care* 28:343–347, 2005
- Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC: Homeostasis model assessment: insulin resistance and β -cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* 28:412–419, 1985
- Vaccaro O, Masulli M, Cuomo V, Albarosa-Rivellese A, Uusitupa M, Vessby B, Hermansen K, Tapsell L, Riccardi G: Comparative evaluation of simple indices of insulin resistance. *Metabolism* 53:1522–1526, 2004
- Moberg L, Johansson H, Lukinius A, Berne C, Foss A, Källén R, Ørstraat Ø, Salmela K, Tibell A, Tufveson G, Elgue G, Nilsson Ekdahl K, Korsgren O, Nilsson B: Production of tissue factor by pancreatic islet cells as a trigger of detrimental thrombotic reactions in clinical islet transplantation. *Lancet* 360:2039–2045, 2003
- Bertuzzi F, Marzorati S, Maffi P, Piemonti L, Melzi R, de Taddeo F, Valtolina V, D'Angelo A, di Carlo V, Bonifacio E, Secchi A: Tissue factor and CCL2/monocyte chemoattractant protein-1 released by human islets affect islet engraftment in type 1 diabetic recipients. *J Clin Endocrinol Metab* 89:5724–5728, 2004
- Ryan EA, Lakey JRT, Paty BW, Imes S, Korbitt GS, Kneteman NM, Bigam D, Rajotte RV, Shapiro AMJ: Successful islet transplantation: continued insulin reserve provides long-term glycemic control. *Diabetes* 51:2148–2157, 2002
- Rickels MR, Schutta MH, Markmann JF, Barker CF, Najj A, Teff KL: B-cell function following human islet transplantation for type 1 diabetes. *Diabetes* 54:100–106, 2005
- Alejandro R, Lehmann R, Ricordi C, Kenyon NS, Angelico MC, Burke G, Esquenazi V, Nery J, Betancourt AE, Kong SS, Miller J: Long-term function (6 years) of islet allografts in type 1 diabetes. *Diabetes* 46:1983–1989, 1997
- Robertson RP, Lanz KJ, Sutherland DE, Kendall DM: Prevention of diabetes for up to 13 years by autoislet transplantation after pancreatectomy for chronic pancreatitis. *Diabetes* 50:47–50, 2001

27. Shapiro AM, Gallant HL, Hao EG, Lakey JR, McCready T, Rajotte RV, Yatscoff RW, Kneteman NM: The portal immunosuppressive storm: relevance to islet transplantation. *Ther Drug Monit* 27:35–37, 2005
28. Desai NM, Goss JA, Deng S, Wolf BA, Markmann E, Palanjian M, Shock AP, Feliciano S, Brunicaudi FC, Barker CF, Naji A, Markmann JF: Elevated portal vein drug levels of sirolimus and tacrolimus in islet transplant recipients: local immunosuppression or islet toxicity? *Transplantation* 76:1623–1637, 2003
29. Bell E, Cao X, Moibi JA, Greene SR, Young R, Trucco M, Gao Z, Matschinsky FM, Deng S, Markman JF, Naji A, Wolf BA: Rapamycin has a deleterious effect on MIN-6 cells and rat and human islets. *Diabetes* 52:2731–2739, 2003
30. Han D, Xu X, Baidal D, Leith J, Ricordi C, Alejandro R, Kenyon NS: Assessment of cytotoxic lymphocyte gene expression in the peripheral blood of human islet allograft recipients: elevation precedes clinical evidence of rejection. *Diabetes* 53:2281–2290, 2004
31. Henderson JR, Moss MC: A morphometric study of the endocrine and exocrine capillaries of the pancreas. *Q J Exp Physiol* 70:347–356, 1985
32. Like AA: The uptake of exogenous peroxidase by the beta cells of the islets of Langerhans. *Am J Pathol* 59:225–246, 1970
33. Hirshberg B, Mog S, Patterson N, Leconte J, Harlan DM: Histopathological study of intrahepatic islets transplanted in the nonhuman primate model using Edmonton Protocol immunosuppression. *J Clin Endocrinol Metab* 87:5424–5429, 2002
34. Robertson RP: Islet transplantation as a treatment for diabetes: a work in progress. *N Engl J Med* 350:694–705, 2004
35. Paty BW, Ryan EA, Shapiro AMJ, Lakey JRT, Robertson RP: Intrahepatic islet transplantation in type 1 diabetic patients does not restore hypoglycemic hormonal counterregulation or symptom recognition after insulin independence. *Diabetes* 51:3428–3434, 2002
36. Brennan DC, Shannon MB, Koch MJ, Polonsky KS, Desai N, Shapiro J: Portal vein thrombosis complicating islet transplantation in a recipient with the Factor V Leiden mutation. *Transplantation* 78:172–173, 2004
37. Hirshberg B, Rother KI, Digon BJ 3rd, Lee J, Gaglia JL, Hines K, Read EJ, Chang R, Wood BJ, Harlan DM: Benefits and risks of solitary islet transplantation for type 1 diabetes using steroid-sparing immunosuppression. *Diabetes Care* 26:3288–3295, 2003
38. Bertuzzi F, Grohovaz F, Maffi P, Caumo A, Aldrighetti L, Nano R, Hengster P, Calori G, Di Carlo V, Bonifacio E, Secchi A: Successful transplantation of human islets in recipients bearing a kidney graft. *Diabetologia* 45:77–84, 2002
39. Oberholzer J, Triponez F, Mage R, Anderegg E, Bühler L, Crétin N, Fournier B, Goumaz C, Lou J, Philippe J, Morel P: Human islet transplantation. *Transplantation* 6:1115–1123, 2000
40. Clayton HA, Davies JE, Pollard CA, White SA, Musto PP, Dennison AR: Pancreatectomy with islet autotransplantation for the treatment of severe chronic pancreatitis: the first 40 patients at the Leicester general hospital. *Transplantation* 76:92–98, 2003
41. Gupta V, Wahoff DC, Rooney DP, Poitout V, Sutherland DER, Kendall DM, Robertson RP: The defective glucagon response from transplanted intrahepatic pancreatic islets during hypoglycemia is transplantation site-determined. *Diabetes* 46:28–33, 1997
42. Cure P, Pileggi A, Froud T, Norris PM, Baidal DA, Cornejo A, Hafiz MM, Ponte G, Poggioli R, Yu J, Saab A, Selvaggi G, Ricordi C, Alejandro R: Alterations of the female reproductive system in recipients of islet grafts. *Transplantation* 78:1576–1581, 2004
43. Aboujaoude W, Milgrom ML, Govani MV: Lymphedema associated with sirolimus in renal transplant recipients. *Transplantation* 77:1094–1096, 2004
44. Senior PA, Shapiro AMJ, Ackerman TE, Ryan EA, Paty BW, Bhargava R: Magnetic resonance-defined perinephric edema after clinical islet transplantation: a benign finding associated with mild renal impairment. *Transplantation* 78:945–948, 2004
45. Markmann JF, Rosen M, Siegelman ES, Soulen MC, Deng S, Barker CF, Naji A: Magnetic resonance defined periportal steatosis following intraportal islet transplantation: a functional footprint of islet graft survival? *Diabetes* 52:1591–1594, 2003
46. Bhargava R, Senior PA, Ackerman TE, Ryan EA, Paty BW, Lakey JRT, Shapiro AMJ: Prevalence of hepatic steatosis after islet transplantation and its relationship to graft function. *Diabetes* 53:1311–1317, 2004
47. Ryan EA, Lakey JRT, Rajotte RV, Korbitt GS, Kin T, Imes S, Rabinovitch A, Elliott JF, Bigam D, Kneteman NM, Warnock GL, Larsen I, Shapiro AMJ: Clinical outcomes and insulin secretion after islet transplantation with the Edmonton Protocol. *Diabetes* 50:710–719, 2001
48. Trotter JF, Wachs ME, Trouillot TE, Bak T, Kugelmas M, Kam I, Everson G: Dyslipidemia during sirolimus therapy in liver transplant recipients occurs with concomitant cyclosporine but not with tacrolimus. *Liver Transpl* 7:401–408, 2001