Successful Islet Transplantation

Continued Insulin Reserve Provides Long-Term Glycemic Control

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Clinical islet transplantation is gaining acceptance as a potential therapy, particularly for subjects who have labile diabetes or problems with hypoglycemic awareness. The risks of the procedure and long-term outcomes are still not fully known. We have performed 54 islet transplantation procedures on 30 subjects and have detailed follow-up in 17 consecutive Edmonton protocol–treated subjects who attained insulin independence after transplantation of adequate numbers of islets. Subjects were assessed pretransplant and followed prospectively posttransplant for immediate and long-term complications related to the procedure or immunosuppressive therapy. The 17 patients all became insulin independent after a minimum of 9,000 islets/kg were transplanted. Of 15 consecutive patients with at least 1 year of follow-up after the initial transplant, 12 (80%) were insulin independent at 1 year. In 14 subjects who have maintained demonstrable C-peptide secretion, glucose control has been stable and glycemic lability and problems with hypoglycemic reactions have been corrected. After 2 of the 54 procedures, some thrombosis was detected in the portal vein circulation. Five subjects had bleeding related to the percutaneous portal vein access procedures: three required transfusion alone, and in one subject, who had a partial thrombosis of the portal vein, an expanding intrahepatic and subscapular hemorrhage occurred while on anticoagulation, requiring transfusion and surgery. Elevated liver function test results were found in 46% of subjects but resolved in all. Complications related to the therapy have been hypercholesterolemia requiring statin therapy in 65%; a rise in creatinine in two patients, both of whom had preexisting renal disease; a rise in protein in four, all of whom had preexisting proteinuria; and antihypertensive therapy increased or started in 53%. Three of the 17 patients have required retinal laser photocoagulation. There have been no cases of posttransplant lymphoproliferative disorder or cytomegalovirus infection, and no deaths. The acute insulin response to arginine correlated better with transplanted islet mass than acute insulin response to glucose (AIR_g) and area under the curve for insulin (AUC_i), but the AIR_g and AUC_i were more closely related to glycemic control. The AUC_i directly posttransplant was lower in those who eventually became C-peptide deficient. Our results, with a maximum follow-up of 34 months, indicate that prolonged insulin independence can be achieved after islet transplantation. There are some risks associated acutely with the procedure, and hypercholesterolemia and hypertension are treatable concerns on longer-term follow-up. All patients with persisting C-peptide secretion have had a resolution of both glycemic lability and problems with hypoglycemic reactions. Apart from the rise in serum creatinine in two subjects, no serious consequences of immunosuppressive therapy have been encountered. Islet transplantation is a reasonable option in those with severe problems with glycemic lability or hypoglycemia. *Diabetes* 51:2148–2157, 2002

Interest in islet transplantation has increased enormously, because of the improved success rates with newer immunosuppressive regimens and islet preparation techniques (1,2). After our early results, many other centers are now initiating islet transplant programs (3,4). A favorable outcome depends primarily on having excellence in harvesting human islets, appropriate immunosuppression regimens, and medical expertise in selecting patients and in follow-up. Although successful islet transplant programs are relatively new, the concept of transplanting islets rather than the whole pancreas is attractive because of its technical ease and the ability to transplant early in the course of the disease. The early promise of islet transplantation is now a reality, but the process still carries potential risks.

In deciding whether it is worthwhile to undergo the risk of immunosuppression for labile diabetes or concerns with hypoglycemia, it is important to be aware of the problems and outcomes in the long term. The simplest measure of success is insulin independence, which must

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AIR_{arg}, acute insulin response to arginine; AIR_g, acute insulin response to glucose; AUC, area under the curve; $AUC_{C\text{-}p}$, area under the curve for C-peptide; AUC_i, area under the curve for insulin; CBC, complete blood count; CMV, cytomegalovirus; GAD, glutamic acid decarboxylase; HOMA, homeostasis model assessment; ICA, islet cell antigen; IE, islet equivalents; IVGTT, intravenous glucose tolerance test; K_G, glucose disposal; LFT, liver function test; MAGE, mean amplitude of glycemic excursion; OGTT, oral glucose tolerance test; PTLD, posttransplant lymphoproliferative disorder; WBC, white blood cell count.

be judged in the context of glycemic control. However, other parameters have to be assessed, including the risks associated with both the procedure and immunosuppression, the improvement in glycemic lability, the eradication of problems with hypoglycemia, the effect of transplantation on long-term diabetes complications, and finally, the economic costs involved. In addition, early measures of success of the transplant other than the absolute measure of insulin independence would be helpful to assess function and outcomes. Measures of insulin release after the secretagogues glucose or arginine have been suggested but not formally studied in the setting of successful allotransplantation.

As of 1 January 2002, we performed 54 procedures as part of our islet transplant program, and these serve to delineate issues related to the procedure. Seventeen subjects have completed the Edmonton protocol and are the basis of this report of longer-term outcomes. Our results demonstrate the ability to stabilize labile diabetes and greatly improve problems with hypoglycemia. The better measure of insulin reserve appears to be the insulin response after intravenous glucose.

RESEARCH DESIGN AND METHODS

Thirty consecutive patients had islet transplantation, for a total of 54 procedures. Seventeen of these had islet transplantation using the Edmonton protocol, with provision of adequate numbers of islets for insulin independence. These 17 subjects have had a total of 38 procedures as part of their original transplant protocol. Three others have had their first transplant procedure and are in the process of completing the Edmonton protocol. Eight further subjects have had an islet transplant with another protocol using infliximab (nine procedures). Two more subjects had a transplantation under the Immune Tolerance Network protocol (two procedures), and 2 of the original 17 Edmonton protocol subjects had a supplemental infusion of islets.

The major indications were labile diabetes or decreased awareness of hypoglycemia giving rise to frequent or severe hypoglycemic reactions or progressive complications of diabetes. Further to our previous report (4), the latter is now a rare indication for an islet transplantation in our current subjects. Problems with hypoglycemia were characterized with a month's monitoring of glucose values and documentation of all occurrences of capillary glucose values <3.0 mmol/l with minimal or no symptoms of hypoglycemia or primarily neuroglycopenic symptoms. Typically, evidence of severe hypoglycemia (requiring outside help to treat the hypoglycemia) was required. Labile diabetes was characterized using mean amplitude of glycemic excursion (MAGE), (5) and evidence for disruption of daily lifestyle was also sought. Approval for either indication required confirmation that the problems had not been rectified by a period of intensive insulin therapy and intensive glucose monitoring. Of the 30 patients, 90% had problems with hypoglycemia awareness, 50% had labile diabetes, and 2 patients had progressive complications. All patients were fully briefed on the risks and complications associated with the procedure and immunosuppressive therapy, and all gave written informed consent. The protocols were approved by the Research Ethics Board of the University of Alberta.

The 30 patients, 14 men and 16 women with type 1 diabetes (absent C-peptide), had a mean age of 41.3 ± 1.6 years and a duration of diabetes of 25.5 ± 2.1 years. In the 17 subjects who completed the Edmonton protocol, 10 men and 7 women (mean age 39.7 ± 2.0 years and duration of diabetes 27.2 ± 2.8 years), 8 had evidence of treated proliferative retinopathy (1 was blind), and 3 others had nonproliferative retinopathy. Three subjects had elevated serum creatinine, five had both microalbuminuria and macroproteinuria, and two each had microalbuminuria or macroproteinuria. One subject had known coronary artery disease that had been previously treated by angioplasty and was stable. Two subjects had severe painful peripheral neuropathy, four gave a history suggesting possible autonomic neuropathy (impotence), and one patient had Charcot arthropathy. Pretransplant, all patients underwent a complete blood count (CBC), liver function tests (LFTs), and tests for electrolytes, calcium, magnesium, thyroid function, lipid profile, and renal function. Both Addison's disease and celiac disease as causes of labile diabetes or recurrent hypoglycemia were excluded; prostate-specific antigen levels were determined in men >40 years old, and women >40 underwent mammograms. An ultrasound of the abdomen was performed, as were chest X-ray, dental exam, and electrocardiogram. Further cardiac tests were performed if it was believed they were required. Screening was done for cytomegalovirus (CMV) IgG, Epstein-Barr nuclear antigen IgG, varicella-zoster IgG, hepatitis, syphilis, toxoplasmosis, and HIV. Lymphocytotoxic antibody screens were performed. All patients were screened for C-peptide status with determination of glucose and C-peptide levels before and 90 min after ingestion of a standard mixed meal.

Transplant procedures. Islets were prepared as previously described (3,6–8). Briefly, human cadaveric pancreata were removed from brain-dead multiorgan donors following in situ vascular flushing with cold University of Wisconsin solution and transported to Edmonton. On arrival at the laboratory, the pancreatic duct was cannulated and liberase enzyme (Boehringer Mannheim, Indianapolis, IN) (8) was perfused. The pancreas was enzymatically and mechanically dissociated before the islets were separated on a refrigerated Cobe 2991 centrifuge (Cobe BCT, Lakewood, CO). A group of pancreata (n = 14) were preserved for a period of 2–3 h with a two-layer (University of Wisconsin/perfluorochemical) cold storage method, as the retrieval results for situations of longer cold ischemic time appear to improve islet recovery with this modification (T. Tsujimura, Y. Kuroda, T. Kin, J.G. Avila, R.V.R., G.S.K., E.A.R., A.M.J.S., J.R.T.L., unpublished observations). In addition, in some cases, isolated islets were cultured at 22°C for up to a maximum of 12 h before transplantation (n = 14) to facilitate timing of islet infusion. This did not appear to adversely affect viability, as measured in vitro after transplantation (3). Islet numbers were quantified in duplicate using an islet standard diameter of 150 μm (9). Once the islets were obtained, the patient was admitted and (for the Edmonton protocol) had the following tests: CBC, chest X-ray, LFTs, and coagulation screen. The patient was then brought to the Radiology Department, and portal vein cannulation was performed. Typically, a size 4F catheter is now used to minimize the risk of bleeding, and with this a Gelfoam plug is not required as previously reported (4). Once the portal vein was cannulated, the islets were slowly injected. To facilitate the slow pace, for the four most recent procedures, a gravity feed system has been used: the islets in culture medium were placed in an intravenous fluid bag and were allowed to infuse under gravity pressure, which may decrease the shear forces on the islets. Portal pressure was monitored after each infusion, and once the transplant was completed, the patient returned to the ward. The glucose was monitored hourly initially, and insulin therapy was withheld until glucose was >8.0 mmol/l premeal or ≥10.0 mmol/l 2-h postmeal.

Patients were usually discharged the following day once an ultrasound had confirmed the absence of any portal vein thrombosis and the CBC and LFT results were acceptable. Immunosuppressive therapy consisted of dacluzimab every 14 days for 10 weeks (1 mg/kg), 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 a day with a target trough level of 3–5 ng/ml. Inhaled pentamidine was used for pneumocystis carinii prophylaxis originally, but sulfamethoxazole/trimethoprim has been used in its place for the most recent 13 patients because of its more broad coverage. Ganciclovir 1,000 mg three times a day for 3 months was given for CMV prophylaxis. CBC, drug levels, and basic parameters (LFTs, electrolytes, calcium, and magnesium) were measured three times a week for the first 2 weeks, twice a week for the next 2 weeks, and then weekly.

Metabolic monitoring. Before transplantation, all patients had C-peptide and glucose measurements before and after mixed-meal stimulation tests (Ensure HP; Abbott Laboratories). After transplant, fasting C-peptide and glucose levels were measured daily for the first week and then weekly. If insulin was used, the dose was recorded as the amount used on the day before the clinic visit. The Edmonton protocol subjects had Ensure tests performed 2 weeks after the first transplant and then at 2 weeks off insulin therapy, 3 and 6 months off insulin, and every 6 months afterward. These subjects also had intravenous glucose tolerance tests (IVGTTs) and intravenous arginine stimulation tests between the first and second transplants, 1, 3, and 6 months after becoming insulin independent after the second transplant, and every 6 months thereafter. An oral glucose tolerance test (OGTT) was performed at 6 and 12 months and then at yearly intervals posttransplant in insulin-independent subjects. HbA_{1c} and lipid profiles were checked monthly. In seven subjects, arginine and IVGTTs were performed pretransplant.

The 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 360 ml Ensure (391 kcal with 8.5 g fat, 44 g carbohydrate, and 17 g protein). The morning of the test, subjects did not take insulin until the test was completed, and typically the test was postponed if the glucose was >15 mmol/l before starting. The OGTT was performed using 75 g of oral glucose, with blood samples drawn at baseline and 30, 60, 90, and 120 min.

IVGTTs were performed in the fasting state using 50% dextrose, 300 mg/kg body wt, given over 1 min after two baseline samples (–10 and 0 min) for glucose, insulin, and C-peptide were drawn. Sampling was then at 3, 4, 5, 7, 10,

15, 20, 25, and 30 min, with time 0 being the start of the infusion. On a separate day, 5 g arginine HCl was infused intravenously over 0.5 min into the patient in the fasting state, and insulin levels were checked at the following time periods: −10, 0, 2, 3, 4, 5, 7, and 10 min.

The IVGTT allowed calculation of the acute insulin response to glucose (AIR_g) based on the mean of the insulin level at 3, 4, and 5 min after the infusion less the mean basal insulin level. Glucose disposal (K_G) was calculated as the slope of the natural log of the glucose values from 10 to 30 min. The areas under the curve for insulin and C-peptide (AUC_i and AUC_{C-p}) were calculated as the area under the curve above baseline over 30 min postinfusion. Acute insulin response to arginine (AIR_{arg}) was calculated as the mean of the three highest values for 2, 3, 4, and 5 min postinfusion less the mean basal value. Homeostasis model assessment (HOMA) was calculated based on the formula of Levy et al. (10) for the assessment of insulin sensitivity.

Glucose was measured by the hexokinase method using the Hitachi 917 system (Roche Diagnostics, Indianapolis, IN). As of December 2000, serum insulin was measured by Elecsys radioimmunoassay using a commercial kit (Roche Diagnostics) with local laboratory intra-assay and interassay coefficients of variation <8.2% and a lower limit of detectability of 0.2 μU/ml; all samples were measured in duplicate. Serum C-peptide was measured using a commercial assay (Diagnostic Systems Laboratories, Webster, TX). The lower limit of sensitivity of this assay was 0.1 nmol/l and the intra-assay and interassay coefficients of variation were <12.3%. An ischemic index was calculated by dividing the number of islets transplanted × 10^{-3} by the total cold ischemia time (time from cross clamp until time of islet infusion into the patient) as previously described (4). The ischemic index for each infusate was summed for a total for each patient.

Statistics. All statistical and regression analyses were performed using Sigma-Stat from Jandel Scientific (San Rafael, CA), and descriptive statistics are given as means ± SE or medians (25–75% CI). Significance was taken at a P value of <0.05, and groups were compared with Student's t test and Mann-Whitney U rank sum test or Wilcoxon signed rank test when normality tests failed. ANOVA was used for multiple comparisons, with the Tukey test for assessing significance.

Control subjects. Ten volunteers (six women, four men; age 32 ± 4 years, BMI 23.7 ± 0.8 kg/m^2) were studied as control subjects for metabolic tests. All had confirmed normal OGTTs, with a fasting glucose of 4.2 ± 0.2 mmol/l; 2-h postload glucose was 4.4 ± 0.4 mmol/l. The subjects underwent mixed-meal tolerance testing for determination of glucose and C-peptide and both arginine and IVGTTs.

RESULTS

Procedures. Of 54 islet transplant procedures, all were successful in achieving portal vein access and being able to use the islets for transplantation. The median hospital stay was 1 day (CI, 1–2). Two patients had transient bradycardia during the procedure, believed to be vasovagal attacks. Five patients had a bleed after the transplant, four of whom required blood transfusions. In one of these subjects, a thrombus occurred in the right portal vein (the main and left portal veins were patent), and anticoagulation was commenced. Three days later, an expanding intrahepatic and subcapsular bleed occurred, requiring transfusion and surgery to evacuate the hematoma with segmental hepatic resection. One other thrombus occurred as reported previously (4). In 12 of 54 procedures, moderate abdominal pain occurred, which was transient. In two patients, puncture of the gallbladder occurred, but in both cases it settled spontaneously and was noted at the time of the portal vein cannulation. LFT results rose to more than twice normal levels in 46% of cases; this peak typically occurred at 6 days posttransplant (CI, 4–9). In all these patients, LFTs returned to normal within a median time of 22 days (CI, 17–41). In only eight instances did alkaline phosphatase rise, and in all patients the peak aspartate aminotransferase was 189 ± 23 U/l (range, 62–563) (normal, <40 U/l) and the peak alanine aminotransferase was 208 ± 26 U/l (range, 59–614) (normal, <50 U/l).

Glycemic outcomes with the Edmonton protocol. Seventeen patients have completed the islet transplant procedure using the Edmonton protocol. The median follow-up time for these 17 patients was 20.4 months (CI, 15–29), with a range of 3.2–34.2 months from the first transplant. HbA_{1c} pretransplant was 8.21 ± 0.36, and the most recent value was 6.08 ± 0.77 ($P < 0.001$) (normal <6.1%). At 1 year after the initial transplant, 12 of 15 subjects were off insulin. As of 1 January 2002, of the 17 subjects, 11 remained off insulin and in these 11, HbA_{1c} pretransplant was 8.48 ± 0.49% and the most recent value was 5.8 ± 0.13 ($P < 0.001$) at a median follow-up of 20.4 months (CI, 14.2–30.5) (Fig. 1*A*). Of these 11 subjects, 2 are on oral hypoglycemic agents but have good glycemic control. The oral hypoglycemic agents were started because the HbA_{1c} rose above 6.5% in one subject and the postprandial glucose levels were consistently >10.0 mmol/l in the other subject. Glucose and C-peptide levels before and after the mixed-meal stimulation are shown in Fig. 1*B* and *C* for the pretransplant and most recent posttransplant tests. Although the C-peptide response was clearly improved and the stimulated C-peptide values were equivalent to those of control subjects, the glucose levels post-Ensure are higher in the posttransplant group than the control group but much better than pretransplant. All the subjects off insulin have stable glucose values and do not have hypoglycemic reactions.

Of the 6 patients who are now >2 years posttransplant, 4 remain off insulin, and of the 15 patients who are >1 year posttransplant, 9 are still off insulin. The more stringent criteria of the OGTT revealed that only two subjects currently meet the criteria for normal glucose tolerance. Of the 17 patients, 11 have diabetes by American Diabetes Association criteria, and in all but 3 of these there is detectable C-peptide secretion. The MAGE value pretransplant for the subjects judged to have increased lability was 10.6 (CI, 9.0–12.5), and the most recent value was 3.3 (CI, 2.6–5.7 mmol/l), indicative of more stable glucose control ($P = 0.003$). Fasting C-peptide was maintained over prolonged follow-up (Fig. 2). Of the six patients who are back on insulin, three lost C-peptide secretion at 7.5, 16, and 17 months posttransplant. Two of the three have become positive for glutamic acid decarboxylase (GAD) and islet cell antigen (ICA) antibodies and are believed to have had a recurrence of their autoimmune disease. The other subject who lost C-peptide remained negative for ICA and GAD antibodies, and one of the subjects on insulin with continuing C-peptide secretion (to 31 months) also had a rise in ICA and GAD antibodies. In the six patients requiring insulin, the median interval from the first transplant to restarting insulin was 10.1 months (CI, 2.5–14.1; range, 2–22.8 months). The current daily insulin dose in the three C-peptide–positive subjects on insulin is 0.33 ± 0.1 units/kg, which is significantly less than their pretransplant use of insulin (0.58 ± 0.08 units/kg; $P = 0.015$).

Immunosuppression regimen. As previously reported, two of the three patients with preexisting elevation of serum creatinine had a further rise of serum creatinine. In both of these subjects, the creatinine level has remained stable over the last year after replacing tacrolimus with mycophenolate mofetil: baseline serum creatinine of 160, peak of 371, and most recent 234 μmol/l for the first

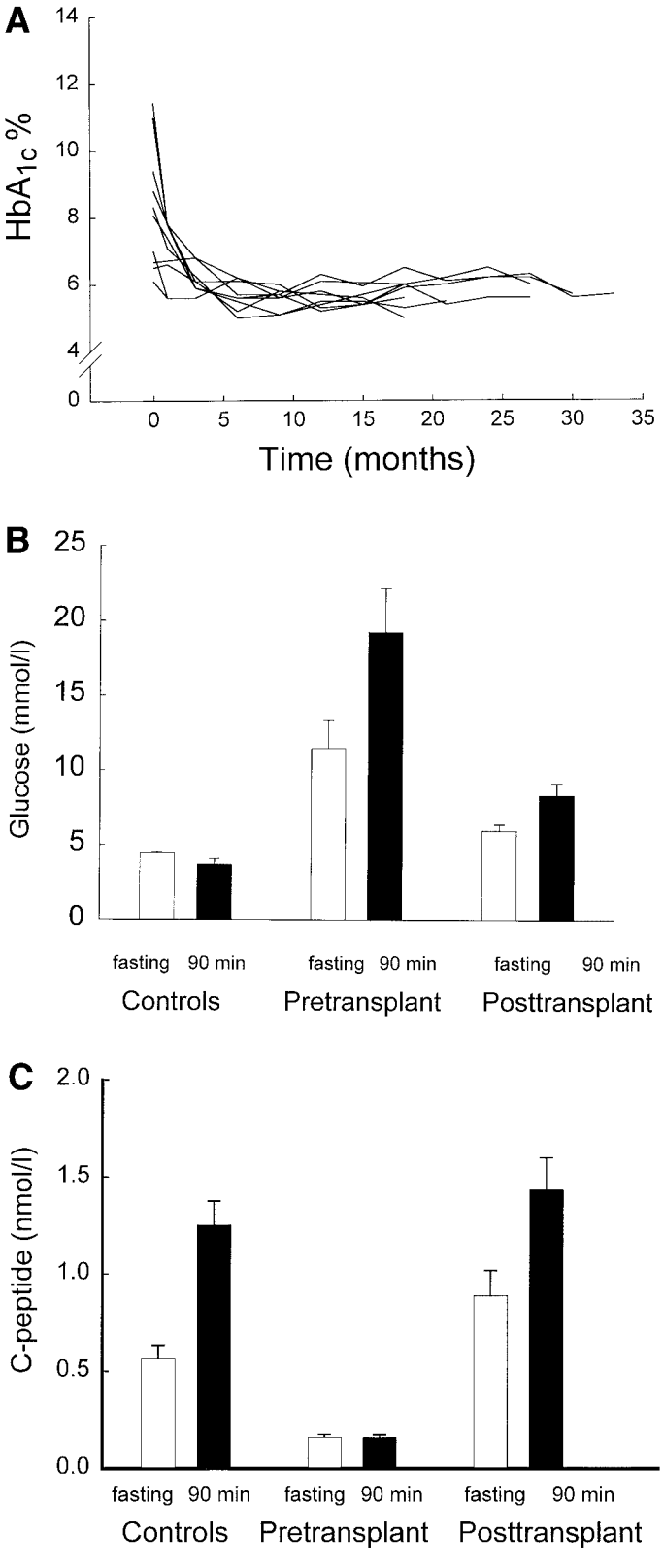

FIG. 1. *A*: HbA_{1c} at 3-month intervals after islet transplantation in subjects who remained insulin independent ($n = 11$). Each line represents an individual subject. *B*: Plasma glucose levels derived from standard meal tolerance tests in control subjects ($n = 10$) and patients before and after islet transplantation who have remained insulin independent ($n = 11$). Values are means ± SE. *C*: Plasma C-peptide levels derived from standard meal tolerance tests in control subjects ($n = 10$) and patients before and after islet transplantation who have remained insulin independent ($n = 11$). Values are means ± SE. Levels pretransplant were below detectability of the assay.

patient; and baseline of 158, peak of 269, and most recent of 251 μmol/l for the second patient. Four others had an increase in urine protein and, again in the three subjects with follow-up for more than a year, the urine protein has remained stable. Only 2 of 17 subjects did not develop mouth ulcers, most of which were small and superficial and resolved within a few weeks. Two patients have had episodes of recurrent nausea and vomiting requiring intravenous hydration. Acne occurred in two subjects and arthralgias in one, and one patient has developed rheumatoid arthritis. Ten patients have had problems with diarrhea posttransplant, but the bowel problems have generally subsided with time. Eight patients developed anemia to a hemoglobin level of <100 g/l. Two of these patients had a rise in serum creatinine, two were subjects who had bleeding posttransplant, one had an ileal ulcer, one had transient colitis, and in two the finding was unexplained. The mean hemoglobin level for the group of 17 fell from 140 ± 4 g/l pretransplant to the most recent level of 123 ± 4 g/l posttransplant ($P < 0.001$). The white blood cell (WBC) count also fell from a pretransplant level of $6.2 \pm 0.3 \times 10^9$/l to the most recent posttransplant level of $4.9 \pm 0.3 \times 10^9$/l ($P = 0.002$). In four subjects, the WBC fell below 2.5×10^9/l (for at least two consecutive readings), and in two subjects the WBC fell below 2.0×10^9/l; granulocyte colony-stimulating factor was used in two patients briefly. This problem may have been related to high levels of sirolimus. The platelet count did not change significantly after transplantation. All patients currently have negative panel-reactive antibodies. Most patients lost weight: pretransplant 70.3 ± 2.3 kg versus posttransplant 65.4 ± 2.3 kg ($P < 0.001$).

Diabetes complications. Three patients have had progression of their retinopathy so as to require laser photocoagulation. The mean serum creatinine for the 17 subjects rose slightly from 89 ± 8 pretransplant to 104 ± 14 μmol/l posttransplant ($P = 0.047$) and, excluding the two patients who had serious deterioration in renal function, there was no difference pre- and posttransplant (79 ± 5 vs. 85 ± 16 μmol/l, respectively; $P = 0.117$). Urine protein was unchanged in 15 of 17 subjects (excluding the two with a significant rise in serum creatinine), 0.2 (CI, 0.1–0.3) pretransplant versus the most recent posttransplant value 0.2 g/day (CI, 0.1–0.4) ($P = 0.492$), and creatinine clearance was unchanged pre- and posttransplant (1.68 ± 0.08 vs. 1.62 ± 0.13 ml · s^{-1} · 1.73 M^2, respectively; $P = 0.195$). Four patients were hypertensive pretransplant, and 10 had a rise of blood pressure posttransplant, such that a total of 9 either started or increased antihypertensive therapy. No dramatic changes have been seen in neuropathy. Anecdotally, two patients with severe neuropathic pain, both off insulin with a duration of insulin independence of 30 and 20 months, have had no change in their pain. Cholesterol rose in 15 of 17 subjects, and in 4 it has dropped again on diet therapy. In the 11 subjects who are on statin therapy, the median cholesterol is 4.8 mmol/l (CI, 4.4–5.2), not significantly different from baseline in these subjects, 4.7 mmol/l (CI, 4.5–5.1), but the maximum cholesterol in these subjects was 6.1 ± 0.2 mmol/l. Triglyceride levels did not change, and the total-to-HDL cholesterol ratio for the entire group pretransplant was 3.2 ± 0.2 and reached a maximum level of 4.6 ± 0.3 ($P < 0.001$), but

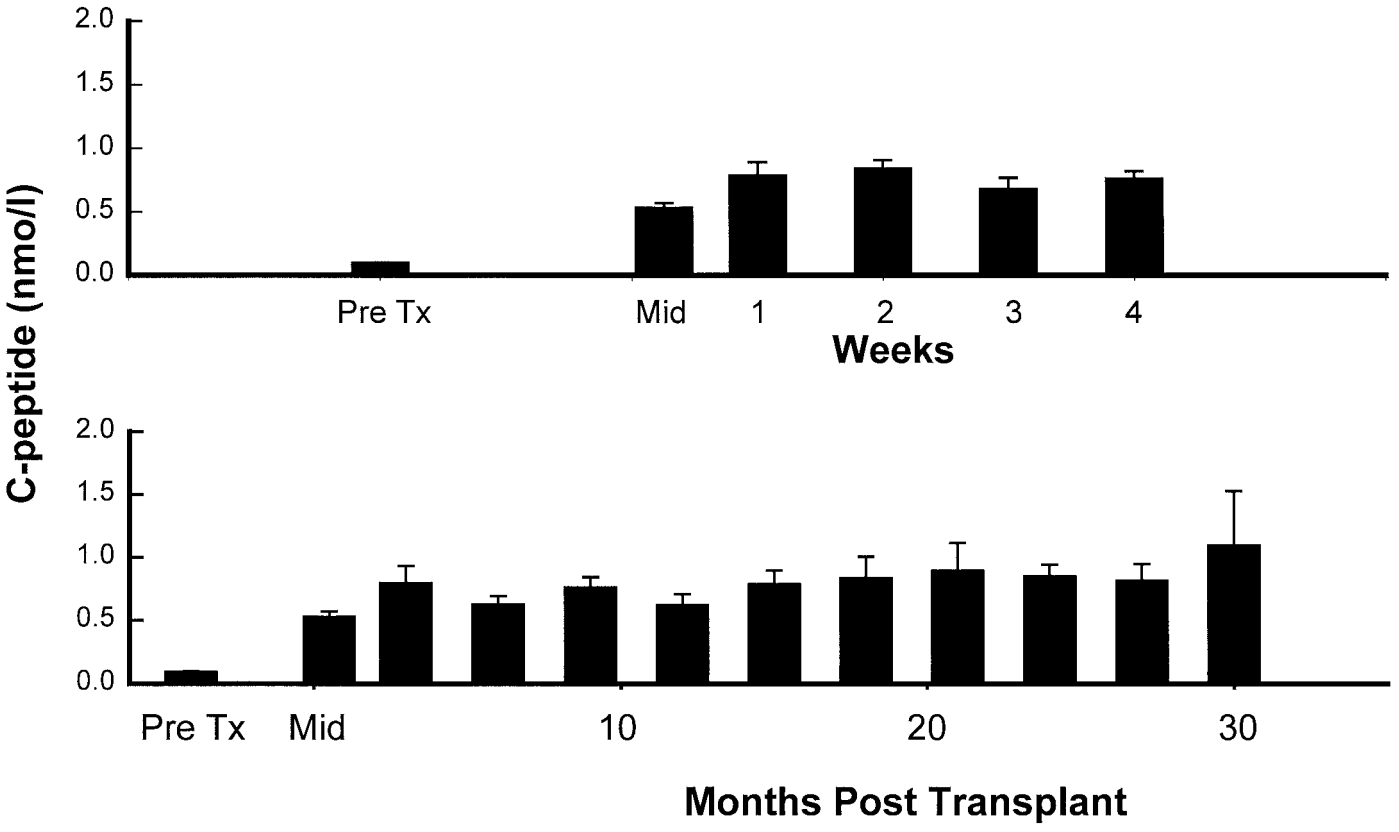

FIG. 2. Fasting plasma C-peptide levels during the first 4 weeks (top panel) and at 3-month intervals for the full duration of follow-up (lower panel) in the 11 patients who remained insulin independent. Shown also are the values pretransplant (pre-Tx) and after the first transplant (mid). Values are means ± SE.

once treated the group ratio was 3.3 ± 0.1. One patient with preexisting coronary artery disease has been stable but has required further angioplasty. No cases of post-transplant lymphoproliferative disease have occurred, and in none of the 12 CMV mismatches (i.e., donor positive, recipient negative for CMV) has there been any occurrence of CMV. There have been no deaths.

Transplant details and metabolic outcomes. The median interval between the first and second transplant was 29 days (CI, 21–54). All of the 17 subjects required at least two transplant procedures, and 4 required three procedures. In two instances, two pancreata were used for a single infusion (both pancreases became available simultaneously). The mean number of islet equivalents (IE) for the first and second transplants were 374,283 ± 20,247 and 391,647 ± 32,921, respectively; the total delivered islet mass was 850,035 ± 37,911 IE per patient (including the four patients who had three islet infusions). The mean numbers of IE/kg for the first and second transplants were 5,442 ± 327 and 5,678 ± 487, respectively, for a total of 12,330 ± 581 IE/kg provided. For the 17 patients who became insulin independent with the Edmonton protocol, the minimum number of islets required for insulin independence appears to be 9,000 IE/kg (Fig. 3). The subject with >12,000 IE/kg who was not insulin independent likely lost islets from a thrombosis of a peripheral branch of the portal vein. Two patients who resumed insulin therapy have had a supplemental infusion of islets but remain on exogenous insulin (one of these supplemental infusions resulted in the partial portal thrombus and hepatic bleed on subsequent anticoagulation).

As can be seen in Fig. 4*A* and *B*, measures of insulin secretion after intravenous glucose were reduced but clearly maintained in the subjects who remained insulin independent to 24 months. In a subanalysis of the four followed for 24 months, there were no significant changes in these parameters. Only one subject has been studied at 30 months, and his islet function is stable (insulin independent; HbA_{1c} 5.7%). In addition, $AUC_{C\text{-}p}$, K_G, and insulin

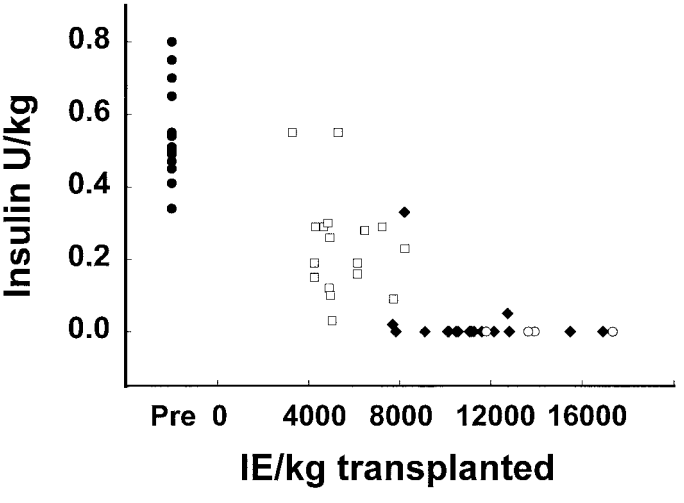

FIG. 3. Exogenous insulin use (units/kg) in relation to the number of islets transplanted (IE/kg). Values for pretransplant (●), after first transplant (□), after second transplant (◆), and after the third procedure (○) are shown. All patients became insulin independent once adequate islet numbers were provided.

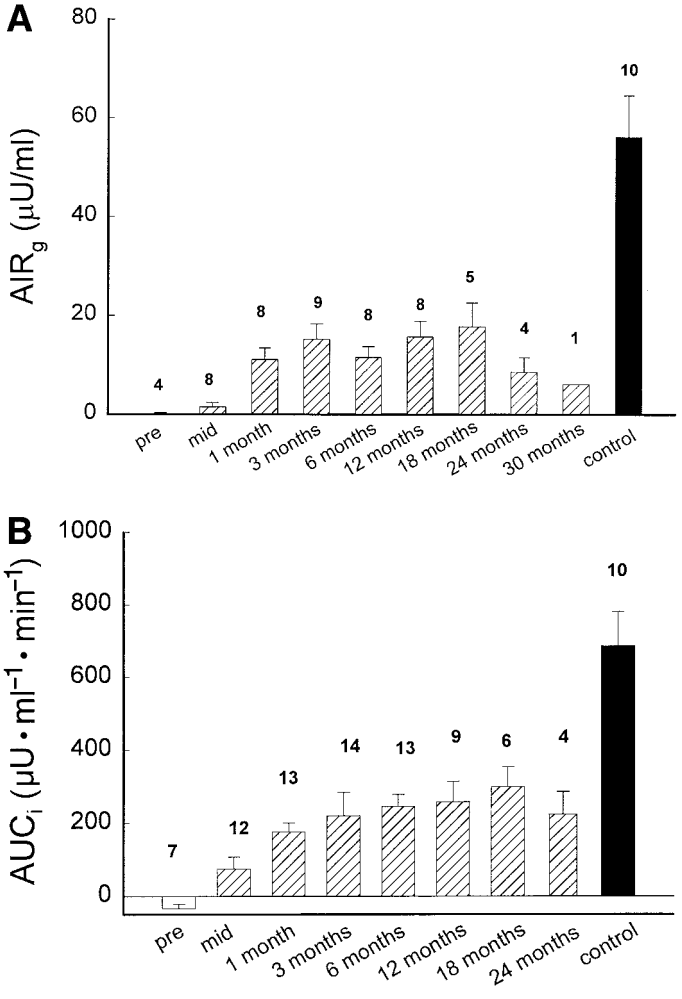

FIG. 4. AIR$_g$ (*A*) and AUC$_i$ (*B*) over time in insulin-independent subjects (n = 11) before and after islet transplantation as derived from the IVGTT. The number of subjects studied is shown across the top of the figure. Mean values ± SE are provided for each time point. Values for all time periods up to 24 months are significantly different from those of control subjects.

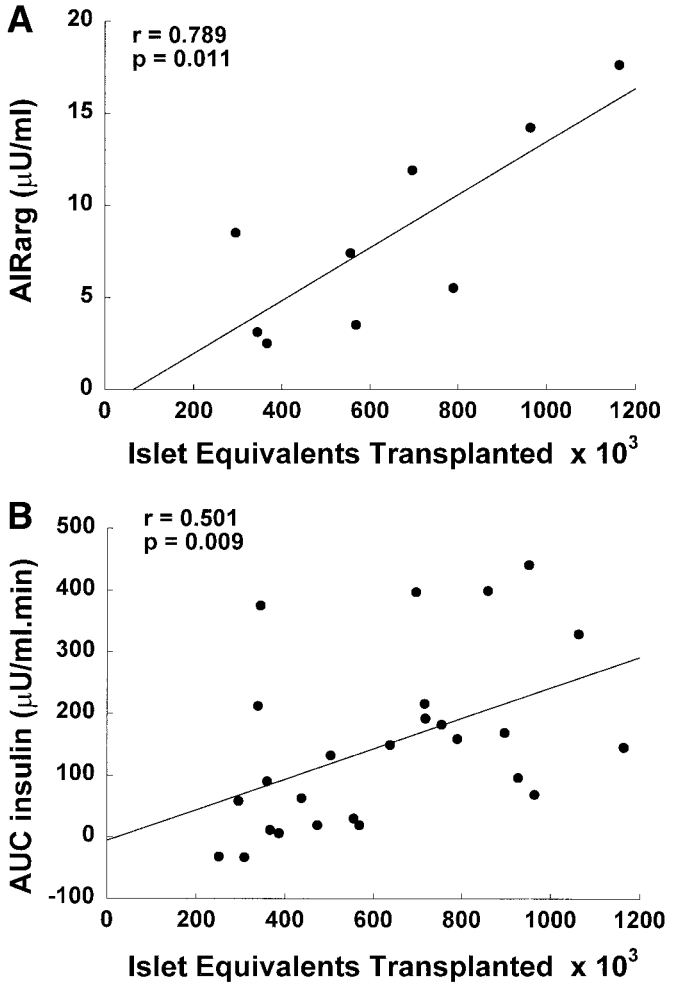

FIG. 5. *A*: Relationship of the number of islets transplanted and AIR$_{arg}$ as derived from intravenous arginine infusion test at midtransplant (n = 6) and 3 months (n = 3) after transplant. *B*: Relationship of AUC$_i$ and the number of islets transplanted as derived from IVGTT at midtransplant (n = 13) and 3 months (n = 13) after transplant.

sensitivity HOMA did not change significantly over the follow-up period. In particular, the five patients with persisting C-peptide secretion who have had to resume either oral hypoglycemic agents or insulin therapy did not have a lower HOMA value than the remaining nine with persistent C-peptide secretion. The AIR$_g$ appears proportionately better after the second transplant than after the first transplant (Table 1), but this may reflect the limits of the test. The AIR$_{arg}$ was also significantly less than that of control subjects even after the final transplant (Table 1).

TABLE 1
Measures of insulin secretion after the first and final transplant

	After first transplant	After final transplant
IE infused	374,283 ± 20,247	850,035 ± 37,911
AIR$_g$ (% of control)	2 ± 1	21 ± 5
AIR$_{arg}$ (% of control)	17 ± 3	56 ± 11
AUC$_i$ (% of control)	10 ± 4	34 ± 5

Data are means ± SE based on the 17 subjects (for IE infused) who completed the Edmonton protocol; n = 13 for the glucose studies (both after first and final transplant) and n = 6 after first transplant and n = 8 after final transplant for the arginine studies. Nondiabetic subjects are reported as having 1.0–1.7 × 10^6 islets (23,24).

In control subjects, the AIR$_g$ and AIR$_{arg}$ did not correlate significantly with each other (r = 0.613, P = 0.059), whereas the AUC$_i$ did correlate significantly with AIR$_g$ (r = 0.9, P < 0.001). In the transplanted patients, this association of AIR$_g$ and AUC$_i$ held (r = 0.901, P < 0.001), but the correlation between AIR$_g$ and AIR$_{arg}$ was not nearly as strong (r = 0.294, P = 0.288). All the measures of insulin reserve correlated with the islet mass transplanted (Fig. 5 *A* and *B*; Table 2). The AIR$_{arg}$ showed a stronger correlation, but we have smaller numbers in this group and the level of significance is similar with all measures of function. Interestingly, the correlation with islet mass was stronger for the studies at 3 months than those at 1 month (data not shown), and the correlation was stronger when cold ischemia time was considered (Table 2). The relationship of these measures of insulin secretion and glycemic control were also determined. Insulin responses to intravenous glucose were more strongly correlated than insulin response after arginine, with both fasting plasma glucose and 2-h glucose after oral glucose load (Fig. 6*A* and *B*; Table 3). The 90-min glucose post-Ensure was related to

TABLE 2
Relationship of both IE transplanted and the cold ischemic index with measures of insulin secretion and glucose disposal

	n	*r*	*P*
Islet equivalents versus			
AIR_g	26	0.463	0.017
AIR_{arg}	9	0.789	0.011
AUC_i	26	0.501	0.009
$AUC_{C\text{-}p}$	26	0.522	0.006
K_G	26	0.490	0.011
Cold ischemia index versus			
AIR_g	26	0.589	0.002
AIR_{arg}	9	0.827	0.006
AUC_i	26	0.684	<0.001
$AUC_{C\text{-}p}$	26	0.728	<0.001
K_G	26	0.684	<0.001

The cold ischemia index is based on the cold ischemia time and the number of islets transplanted (4). The measures of insulin secretion are AIR_g, μU/ml; AIR_{arg}, μU/ml; AUC_i, $\mu U \cdot ml^{-1} \cdot min^{-1}$; and $AUC_{C\text{-}p}$, $nmol \cdot l^{-1} \cdot min^{-1}$.

the AIR_g ($n = 60$, $r = -0.593$, $P < 0.001$) and AIR_{arg} ($n = 21$, $r = -0.697$, $P < 0.001$), as were AUC_i, $AUC_{C\text{-}p}$, and K_G. The 90-min glucose post-Ensure was significantly related to the 2-h glucose post-OGTT ($r = 0.718$, $P < 0.001$) in the posttransplant subjects. Finally, on long-term follow-up, the AUC_i within 3 months of transplantation in the three subjects who lost C-peptide production was significantly lower than those who remained off insulin or oral hypoglycemic agents (94 ± 17 vs. 299 ± 38 $\mu U \cdot ml^{-1} \cdot min^{-1}$; $P < 0.05$).

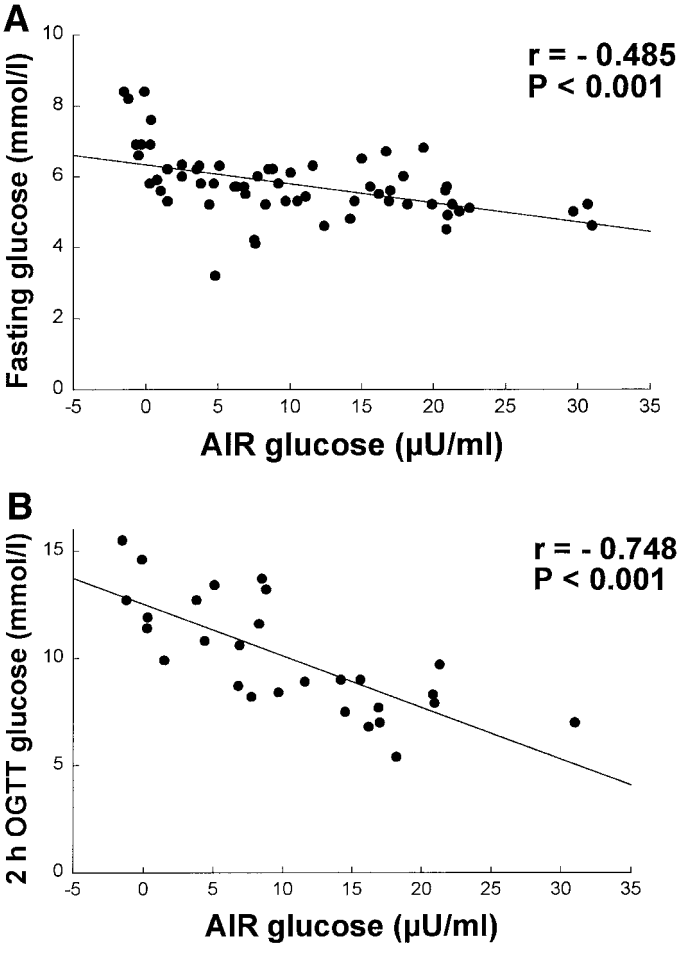

FIG. 6. *A*: Relationship of AIR_g as derived from IVGTT and fasting plasma glucose in subjects after islet transplant who were not taking exogenous insulin. *B*: Relationship of AIR_g as derived from IVGTT and 2-h plasma glucose during the OGTT in subjects after islet transplant who were not taking exogenous insulin.

TABLE 3
Relationship of glycemia (fasting plasma glucose and the 2-h glucose during the OGTT) with measures of insulin secretion and glucose disposal

	n	*r*	*P*
Fasting plasma glucose versus			
AIR_g	61	−0.485	<0.001
AIR_{arg}	14	−0.075	0.8
AUC_i	61	−0.422	<0.001
$AUC_{C\text{-}p}$	61	−0.434	<0.001
K_G	61	−0.35	0.006
2-h Glucose post-OGTT versus			
AIR_g	28	−0.748	<0.001
AIR_{arg}	4	−0.8	0.2
AUC_i	28	−0.74	<0.001
$AUC_{C\text{-}p}$	28	−0.69	<0.001
K_G	28	−0.474	0.011

Measure of insulin secretion and glucose disposal are as in Table 2.

DISCUSSION

Our results show that as a therapy for subjects with unstable, labile diabetes or severe problems with hypoglycemia, islet transplantation can achieve consistent glucose values and alleviate these problems. Even in the six subjects who resumed insulin therapy, the three who have clearly present C-peptide have stable glucose levels and are using about one-half the pretransplant dose of insulin. Thus, for the majority of patients with major problems of hypoglycemia or lability, the islet transplant procedure can provide an excellent solution.

The risks involved primarily relate to the procedure itself and the drugs used for immunosuppression. The procedure was associated with a 10% chance of bleeding and a 4% chance of some thrombus within the portal venous circulation. In only one case was bleeding a major concern, the patient who had hemorrhage while on anticoagulation for a partial portal vein thrombosis. Moderate abdominal pain occurred in ~20% of the subjects and settled readily. With a median hospital stay of 1 day and only one serious complication after 54 procedures, the overall safety profile of the percutaneous approach is acceptable. Other approaches such as using computed tomography guidance for percutaneous access (11), approaches by the transjugular route, or laparotomy could be considered as viable alternatives but would likely take longer, and the latter may be associated with further delay in recovery or potential long-term risk of adhesion formation. The rise in LFT results has become more frequent compared with our original findings without any obvious cause. Reassuringly, all the elevations in liver enzymes have resolved with time.

The only serious immunosuppression-related complication in the 17 patients to date has been a rise in the creatinine in 2 subjects with known preexisting renal impairment. This level of renal dysfunction is stable with

the discontinuation of the potentially nephrotoxic tacrolimus (12). The previous rise in urine protein (4) has now stabilized on longer-term follow up. These findings prompted changes in the protocol so that tacrolimus levels in the lower range of the target level of 3–5 ng/ml are accepted and hypertension is more aggressively treated. If blood pressure is consistently >135/85 mmHg, antihypertensive therapy is initiated and a target level of <125/75 mmHg on therapy is sought. With these targets, we have had to use or increase antihypertensive therapy in 53% of subjects. Of the 17 patients, 3 have required laser photocoagulation. This is of concern, and it is likely that the acute stabilization of glucose control is contributing (13,14). These results demonstrate the need for confirmation of stable eye disease pretransplant and monitoring for retinopathy posttransplant. It is too early to comment on neuropathy, but no dramatic changes have been noted. A concern is the degree to which hypercholesterolemia is being encountered. The rise in cholesterol is associated with a rise in the HDL so that the total to HDL cholesterol ratio is not grossly elevated, but we believe that this hypercholesterolemia warrants aggressive therapy given the history of unstable diabetes in the subjects. All have responded well to statin therapy when required, but the implications of this for the vasculature are unknown and merit close observation. Interestingly, hypertriglyceridemia has not been a problem, although it is common in other transplant immunosuppressive regimens (15); the absence of glucocorticoids with the regimen used in our patients may be the difference. Significant anemia was seen in eight patients and although explainable in most cases (renal or gastrointestinal disease), in other cases it most likely reflects a side effect of the medications. The WBC has also fallen, and in two subjects granulocyte colony-stimulating factor was used temporarily, but no serious infections have occurred. No posttransplant lymphoproliferative disorder has been encountered, and no CMV infection occurred despite a significant number of mismatches. It may be that in the islet preparation there are few contaminating leukocytes, making CMV infection less likely.

Most patients lose weight over time after the islet transplant procedure. No specific diet plan is prescribed other than eating healthily; in particular, the timing of meals is not specified. Although the frequent occurrence of mouth ulcers may be contributing to weight loss, these are usually transient and resolve as sirolimus levels stabilize. The occurrence of diarrhea may also be contributing, but even this is not as much of a problem as previously. Finally, once hypoglycemia is no longer a problem, the subjects are likely not eating as much to treat low glucose values.

For the patient with labile diabetes or recurrent hypoglyemia, the results continue to be excellent. Even for those who are now using insulin again, glucose values are more stable than pretransplant, and in only three subjects has complete C-peptide secretion been lost. The more stringent criteria of the OGTT confirmed problems with islet mass and/or function. Although only two patients meet the criteria for normal glucose tolerance with OGTT, the remainder with continuing C-peptide secretion have stable glucose control and do not have problems with hypoglycemia. In general, patients still feel that they are much better off with stable posttransplant diabetes than with the labile type 1 diabetes they had pretransplant. Two of the three who have lost C-peptide secretion appear to have had a recurrence of autoimmunity, as others have found (16). The other three subjects on insulin have persisting fasting C-peptide levels not different from those when they were insulin independent, yet their HbA_{1c} has risen. Why these individuals required insulin therapy is not clear. The acute insulin response to glucose was low from the time of transplant in these subjects. Whether the need for insulin simply reflects borderline islet mass that is failing over time or some insulin resistance induced by the tacrolimus or is a direct effect of the immunosuppressive drugs on islet function is unknown (17–19). We have considered supplemental islet infusions and will proceed cautiously with them in the absence of a recurrence of autoimmunity. In the presence of a raised titer of GAD or ICA antibodies, it would appear prudent to accept that the current immunosuppressive regimen may be inadequate and alternative regimens would be required for these subjects. Finally, in terms of glucose control, it is of interest that the C-peptide response to the mixed meal challenge was normal, albeit with a higher glucose level, yet the response to intravenous glucose was blunted. Perhaps the incretin response is more marked in this setting to account for the better C-peptide response to the oral challenge. The higher glucose level than the control subjects with equivalent C-peptides may be indicative of insulin resistance, other subtle metabolic derangements (20), or the venous drainage of the islets (whether portal or systemic), an issue yet to be resolved.

It continues to appear that an absolute minimum of 9,000 IE/kg is required for insulin independence in the absence of steroids (Fig. 3). The lack of previous long-term success with islet transplantation (21,22) was likely related to the presence of glucocorticoids combined with inadequate prophylaxis of rejection and autoimmune events. However, in the current study, each subject had an average of 850,000 islets transplanted; the nondiabetic pancreas is believed to have $1.0–1.7 \times 10^6$ islets (23,24). Thus the poor acute insulin response to the IVGTT we documented might suggest either loss of islets or poor β-cell function. Assessing β-cell mass has been difficult in this setting and yet is of importance. Many measures of insulin reserve are available. Although insulin independence is the most important end point, it has to be tempered with coexisting good glycemic control. Islet function can be severely impaired with the subject still insulin independent; thus the need for more detailed measures. Suggested tests of insulin secretion have included AIR_g, AIR_{arg}, AUC_i, insulin response to glucagon, and glucose-potentiated response to arginine (25). We believe that any tests should be considered in the light of four issues: are they equivalent, do they correlate to islet mass, do they relate to glycemic control, and can they predict future function of the graft.

Both glucose and arginine have been used as acute stimuli of insulin secretion. A bolus of glucose stimulates insulin release by increasing intracellular ATP, leading to closure of islet potassium channels, influx of calcium, and insulin secretion. Arginine, a cationic amino acid, can

directly lead to depolarization of the β-cell, calcium influx, and insulin release. The response to arginine is increased when hyperglycemia is present (26). Our results show that whereas AIR_g and AUC_i were closely related, there was no significant relationship of AIR_g and AIR_{arg} in control subjects or study subjects. Thus whereas both stimulate insulin release, they are not measuring the same mechanisms. The differential response to these secretagogues is also evident in early type 1 and 2 diabetes, where AIR_{arg} is typically preserved after AIR_g is lost (27–29). All the parameters used gave a measure of islet mass. The correlation coefficient was higher for AIR_{arg} and islet mass than for AIR_g or AUC_i. Detailed assessments in streptozotocin-treated baboons, which had in vivo measures of insulin reserve that were then correlated with in vitro anatomical determinations of islet mass or direct measures of pancreatic insulin content, have indicated that AIR_g correlates more closely with pancreatic insulin content than AIR_{arg} (30). In that study, AIR_g was linearly correlated ($r = 0.92$) with pancreatic insulin content, and the regression line passed through zero. Interestingly, islet mass was still detectable when insulin reserve in vivo was completely depleted. The correlation of AIR_g with β-cell mass, although linear, had a y intercept of 0.2 g/pancreas, indicating that at a negligible AIR_g there was no insulin reserve but there was persisting islet mass (30). AIR_{arg} and glucose-potentiated arginine-stimulated insulin release did not correlate as well as AIR_g with either insulin reserve or islet mass. Tobin et al. (31), using a rodent islet transplant model of known islet mass, likewise found that AIR_g was the better correlate with islet mass. Our results appear to be following a slightly different pattern. With the first transplant procedure, we have provided a reasonable islet mass, adequate to reduce exogenous insulin requirements and stabilize glycemic control but with a poor AIR_g (Table 1). When the second transplant is provided, AIR_g appears to improve disproportionately, although it still remains reduced compared with that of control subjects. AIR_{arg} appears independent of this and thus may provide the better measure of islet mass posttransplant. In addition, if nondiabetic subjects have closer to 1.7×10^6 rather than 1.0×10^6 islets (23,24), then the results for AIR_{arg} as a percentage of control (Table 1) are consistent with this proposition.

Our results indicate that the insulin response to glucose correlates better with the measures of glycemia we used (fasting plasma glucose and glucose level at 2 h during the OGTT) than AIR_{arg} and provides a better composite measure of islet mass and function. Finally, when we examined the dynamic tests in light of the final outcome of C-peptide production, the responses to intravenous glucose were lower in those whose islets eventually failed, but there were too few AIR_{arg} studies available to assess this parameter. It is clear that an improved measure of islet mass and/or function is needed for evaluating these patients. It needs to be determined whether proinsulin determinations will permit detection of islets that are beginning to fail (32). The glucose-potentiated arginine stimulation test may help, but the possibility of imaging islets is more exciting as a measure of mass (33). In the meantime, 90-min post-Ensure glucose is a simple and helpful test for following these patients in the long term. Many of our patients have problems with the palatability of the Ensure, and some other standard meal test would be of benefit. Of the more invasive tests, AIR_g and AUC_i derived from an IVGTT appear to give the most information and correlate better with function and long-term outcome; AIR_{arg} may be a better measure of islet mass.

In conclusion, our results show that continued insulin independence for up to 2 3/4 years is possible after islet transplantation. The glucose values are stable in 14 of 17 transplanted subjects, and at 1 year, 80% of patients were insulin independent, as were 4 of 6 subjects >2 years after their initial transplant, a major improvement over previous results (21,22). There are acute risks of the procedure and risks associated with the immunosuppressive drugs, demonstrating the need for further improvement. The insulin reserve is not normal but adequate to correct the problems with glycemia. Thus for many subjects who have very labile diabetes and severe problems with hypoglycemia, the risks are worth the outcome; careful patient selection remains essential to maximize the risk-benefit ratio for any individual patient as islet transplantation becomes more widespread.

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