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Prolonged Insulin Independence after Islet Allografts in Recipients with Type 1 Diabetes

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Abstract

We sought to determine the long-term outcomes in type 1 diabetic recipients of intraportal islet allografts on a modified immunosuppressive protocol. Six recipients with hypoglycemia unawareness received 1 to 2 islet infusions. Induction therapy was with antithymocyte globulin (ATG) plus etanercept for tumor necrosis factor- α blockade. Recipients received cyclosporine and everolimus for maintenance immunosuppression for the first year posttransplant, with mycophenolic acid or mycophenolate mofetil subsequently substituted for everolimus. Recipients have been followed for 1173 ± 270 days since their last infusion for islet graft function (insulin independence, hemoglobin A_{1c} levels, and C-peptide production) and for adverse events associated with the study protocol. Of the 6 recipients, 5 were insulin-independent at 1 year, and 4 continue to be insulin-independent at a mean of 3.4 ± 0.4 years posttransplant. None of the 6 recipients experienced recurrence of severe hypoglycemia. Measured glomerular filtration rate decreased from 110.5 ± 21.2 mL/min/1.73m² pretransplant to 82.6 ± 19.1 mL/min/1.73m² at 1 year posttransplant. In conclusion, islet transplants restored insulin independence for a mean of >3 years in 4 of 6 recipients treated with ATG and etanercept induction therapy and with cyclosporine and, initially, everolimus for maintenance. Our results suggest this immunosuppressive protocol may allow long-term graft survival.

Keywords

allograft survival; islet graft; islet transplantation; islets; T-cell depletion; thymoglobulin; cyclosporine; everolimus; mycophenolic acid; mycophenolate mofetil; diabetes mellitus; hypoglycemia

Introduction

Reliable restoration of insulin independence in type 1 diabetic recipients of islet allografts has been reported by several programs (1–4). Short-term results have been

promising, with 82% of patients maintaining insulin independence at 1 year posttransplant (5). This success rate in selected recipients had previously been attainable only with vascularized pancreas transplants.

However, the proportion of recipients maintaining insulin independence declines after the first year posttransplant (3,6). The reason for this decline remains unclear, but suggested causes include alloimmune rejection, autoimmune recurrence, toxicity of immunosuppressive medications, and inhospitability of the liver as a site (7,8). However, the liver's inhospitability is argued against by the long-term function of islet autografts (9). Rejection, autoimmune recurrence, and toxicity may be ameliorated by refined immunosuppressive protocols.

Most recent experience with immunosuppression for islet transplant recipients has been with daclizumab for induction therapy and sirolimus plus low-dose tacrolimus for maintenance therapy (1,3). Sirolimus and tacrolimus have been shown to inhibit beta-cell regeneration, presenting a potential disadvantage posttransplant (8). Use of T-cell-directed antibodies for induction therapy is limited to a small number of islet transplant recipients reported in the medical literature (2,10,11). The addition of tumor necrosis factor- α (TNF- α) blockade during induction therapy has been attempted infrequently (2,4).

We report herein our long-term results (2.4 to 4.4 years posttransplant) in 6 islet recipients on a modified immunosuppressive protocol consisting of antithymocyte globulin (ATG) and the soluble TNF- α receptor blocker etanercept at induction with cyclosporine and everolimus for maintenance immunosuppression.

Methods

Study Design

From August 2003 through March 2005, we enrolled 6 alloislet transplant recipients in a 1-year single center study. The immunosuppression protocol consisted of ATG and etanercept induction therapy and cyclosporine and everolimus maintenance.

The primary objective of our study was to examine the safety of alloislet transplants with a modified immunosuppressive protocol. The secondary objective was to assess the proportion of recipients who obtained insulin independence in the first year posttransplant, as well as the proportion with full or partial graft function at 1 year after the last islet infusion. Insulin independence was defined by fasting blood glucose levels ≤ 126 mg/dL and 2-hour postprandial levels ≤ 180 mg/dL without exogenous insulin. Full graft function was defined by insulin independence with hemoglobin A_{1c} (HbA_{1c}) $\leq 7\%$; partial function was defined by insulin dependence but C-peptide ≥ 0.5 ng/mL and HbA_{1c} $\leq 7\%$.

Beyond 1 year after the last islet infusion, recipients were enrolled into a long-term follow-up protocol for continued monitoring. Due to the risk of nephrotoxicity with calcineurin inhibitor and mTOR inhibitor combination therapy, we substituted an inosine monophosphate dehydrogenase inhibitor (mycophenolic acid or mycophenolate mofetil) for everolimus in 2 recipients due to toxicity before 1-year after final infusion (#2 at day 153

and #4 at day 280 relative to last infusion), and in the remaining 4 at 1–1.4 years after final infusion. During long-term follow-up, 5 recipients continued on cyclosporine with either mycophenelic acid or mycophenolate mofetil (choice based on insurance coverage, recipients # 1, 3, 4, 5, 6); in 1 recipient tacrolimus was substituted for cyclosporine because of gingival hypertrophy (# 2); and 1 recipient received additional treatment with sirolimus for 5 months for concern of new positive PRA results and increasing insulin needs (#4).

Study protocols were reviewed and approved by the University of Minnesota (UM) Institutional Review Board; all participants gave informed consent.

Recipients

Recipients were age 18 years or older and had a history of type 1 diabetes with severe hypoglycemia and reduced awareness of hypoglycemia (Clark hypoglycemia unawareness score 4 of 7) despite intensive diabetes management. Severe hypoglycemia was defined as an event with hypoglycemic symptoms in which the patient required the assistance of another person to treat and which was associated with a blood glucose <50 mg/dL or prompt recovery after oral carbohydrate, intravenous glucose, or glucagon administration.

Exclusion criteria included the following: body mass index (BMI) >26 kg/m², insulin requirement of > 50 IU per day, positive C-peptide levels, untreated proliferative retinopathy, creatinine clearance < 60 ml/min/1.73 m² for women and <70 ml/min/1.73 m² for men, and panel-reactive anti-HLA antibody levels >10%.

Islet Preparation

Islets were prepared as previously described (2). Briefly, pancreases were procured from brain-dead, ABO-compatible donors; preserved using the 2-layer technique; and dissociated using Liberase HI (Roche Diagnostics Corp, Indianapolis, IN) and the Ricordi method. Free islets were separated from non-islet tissue on continuous iodixanol density gradients, and cultured free-floating in supplemented CMRL 1066 at 37°C for 1 day and 22°C for 1 day pretransplant.

Transplant Procedure

Portal vein access was obtained via percutaneous transhepatic portal vein catheterization (7 infusions in 4 recipients) or minilaparotomy (3 infusions in 3 recipients), and the islet preparation was infused intraportally over 15 to 60 minutes. Portal vein pressure was monitored periodically during the infusion, with injection halted if portal pressures exceeded 30 cmH₂O and resumed if portal pressures dropped below 25 cmH₂O. Prophylactic anticoagulation was with intraportal heparin initially (70 units/ kilogram), followed by continuous intravenous heparin infusion for 48 hours (target, partial thromboplastin time of 60 seconds). Recipients were on subcutaneous enoxaparin prophylaxis from day 2 to day 7 posttransplant.

They received a continuous intravenous insulin infusion from day –2 to day 2 (target, blood glucose levels of 80 to 120 mg/dL).

Subsequent Islet Transplants

Recipients who received 5,000 IE/kg with the initial transplant but did not obtain insulin independence were eligible for a second transplant, performed within 18 months after the initial transplant. A minimum of 3,000 IE/kg was administered during the second islet infusion.

Immunosuppression

Induction immunosuppression for the first islet infusion was with ATG (Thymoglobulin, Genzyme) given on day -2 (0.5 mg/kg), day -1 (1.0 mg/kg), and days 0 through 2 (1.5 mg/kg/day) relative to transplant. Premedications included acetaminophen, diphenhydramine, and pentoxifylline, and with the first ATG infusion, methylprednisolone (2 mg/kg). For subsequent islet transplants, induction therapy was with basiliximab (Simulect, Novartis; 20 mg intravenously 2 hours pretransplant and 4 days posttransplant). Etanercept (Enbrel; Amgen) was given for peritransplant antiinflammatory therapy (loading dose, 50 mg intravenously 1 hour pretransplant, then 24 mg subcutaneously on days 3, 7, and 10 posttransplant).

Cyclosporine was initiated on day 1 after initial islet transplant (initial dose 3 mg/kg/day divided twice daily, then adjusted to these target blood levels: 2-hour post-cyclosporine level 350 to 500 ng/mL for the first 3 months posttransplant, 200 to 350 ng/mL thereafter). Everolimus was initiated on day-2 (3 mg orally once on day -2, then 1.5 mg orally twice daily and adjusted as needed to these target trough levels: 3 to 15 ng/mL for the first 3 months posttransplant, 3 to 12 ng/mL thereafter; mean levels maintained at 7.2 ± 1.8 ng/mL at 3 months, 6.3 ± 3.4 ng/mL at 6 months, 7.3 ± 2.3 ng/mL at 12 months). At any time, if deemed clinically appropriate, mycophenolate mofetil (320 to 720 mg orally twice daily) replaced everolimus.

Concomitant Therapy

All recipients received antimicrobial prophylaxis. Filgrastim was used as clinically indicated for absolute neutrophil count $< 1000/\mu\text{L}$. Intravenous insulin was administered in the peritransplant period and subsequently transitioned to subcutaneous therapy. Subcutaneous insulin therapy was withdrawn if the recipient's blood glucose levels were maintained at < 126 mg/dL fasting and < 180 mg/dL 2-hours postprandial.

Safety and Efficacy

Patients were admitted to the General Clinical Research Center at UM for days -2 through 2 and were seen in outpatient follow-up at regular intervals for 12 months posttransplant. Following enrollment into the long-term islet survival study (1 year after the last islet infusion), participants underwent laboratory testing locally and submitted blood glucose levels and insulin logs regularly. Recipients and their primary care physicians were asked to complete questionnaires annually regarding current medical health, insulin use, hypoglycemia episodes, and diabetes complications.

Laboratory safety assessments included complete blood counts, coagulation studies, hepatic panel, creatinine, 24-hour urinary albumin excretion, glomerular filtration rate (GFR), and immunosuppressive medication levels measured at regular intervals.

Metabolic follow-up testing was defined by time since last islet infusion. Self-monitored blood glucose levels, documentation of insulin requirements, and hypoglycemia history were reviewed at each follow-up visit. HbA_{1c} levels, measured by high-performance liquid chromatography (BioRad, Hercules, CA), were obtained at regular intervals. Participants underwent a 2-hour 75-gram oral glucose tolerance test (OGTT) on days 90 and 365 and a 5-gram arginine stimulation test (AST) on days 90, 180, and 365 posttransplant. The acute insulin response (AIR_a) and acute C-peptide response (ACR_a) to arginine stimulation testing were defined by the mean of the highest 3 post-arginine values between 2 and 5 minutes with the basal value subtracted. Insulin levels were measured by chemiluminescent immunoassay, and C-peptide levels were measured by double-antibody radioimmunoassay.

Alloimmunity and Autoimmunity

Titers of anti-glutamic acid decarboxylase (GAD65) antibodies, insulin autoantibodies (IAA), and anti-islet cell antibodies (ICA) were measured by radioassay (12,13). Panel-reactive antibody (PRA) levels were measured using ELISA pre-transplant and flow luminex posttransplant.

Statistical Analysis

Data are expressed as mean $\pm$ standard deviation. Because of the low number of study participants, we did not perform formal statistical analysis.

Results

Recipient and Donor Characteristics

Our 6 recipients (table 1) all had a history of severe hypoglycemia in the year preceding their first islet infusion and a Clark score = 4. Pretransplant, they required a mean of 23.7 ± 4.4 units per day of insulin and had a mean HbA_{1c} of $7.3\% \pm 0.7\%$.

The 6 recipients received a total of 11 islet grafts (Table 2). Recipients who failed to reach insulin independence with a single infusion underwent a second infusion 54 to 476 days after the first. One recipient required only 1 islet graft to obtain insulin independence, 1 received 2 islet grafts simultaneously, and 4 underwent 2 consecutive islet infusions. Recipients received a mean of $11,872 \pm 2541$ islet equivalents/ kilogram body weight (IE/kg).

Islet graft function

Recipients have been followed for 1173 ± 270 days since their last islet infusion (range, 969 to 1685 days). Insulin use and results of metabolic testing for all recipients are displayed in Table 3. Insulin independence was attained in 5 recipients; 1 recipient (#6) did not attain insulin independence after 2 islet infusions. This insulin-dependent recipient (#6) had a 60% reduction in insulin needs and met the definition of partial graft function at 1 year

posttransplant. Of 6 recipients, 5 were insulin-independent with full graft function at 1 year posttransplant, and 4 were insulin-independent with full graft function at 2 years posttransplant. These 4 recipients continue to be insulin-independent at a mean of 3.4 ± 0.8 years posttransplant.

All 6 recipients, including those on insulin, showed evidence of C-peptide production at their most recent follow-up (> 2.6 years). All 6, including the 1 on insulin, had an $\text{HbA}_{1c} < 6\%$ at 1 year posttransplant. None experienced recurrence of severe hypoglycemic episodes posttransplant.

At 2 years posttransplant, mean HbA_{1c} levels were $6.0\% \pm 0.7\%$ for all recipients and $5.6\% \pm 0.2\%$ for insulin-independent recipients, indicating good glycemic control. At 2 years posttransplant, insulin-independent participants had a mean fasting blood glucose level of 102 ± 11 mg/dL; mean C-peptide, 1.9 ± 1.3 ng/mL.

At their most recent follow-up (mean 3.4 ± 0.8 years), of the 4 insulin-independent recipients, mean HbA_{1c} levels were $5.7\% \pm 0.3\%$; mean fasting glucose level, 106 ± 17 mg/dL. Of the 2 insulin-dependent recipients, 1 (#4) demonstrated euglycemia on insulin with a HbA_{1c} level of 5.8% and a fasting glucose level of 85 mg/dL; the other had poor metabolic control with a HbA_{1c} level of 9.3% and no recent fasting laboratory glucose level.

Only 2 recipients had a 2-hour blood sugar level consistent with diabetes on an OGTT performed at 1 year posttransplant—namely, 1 on insulin and 1 who resumed insulin shortly after the 1 year follow-up. Of the 4 currently insulin-independent recipients, 2 had a normal response to an OGTT and 2 had impaired glucose tolerance.

The lowest ACR_a and AIR_a at 6 months and 12 months were in the recipient on insulin and the recipient who later resumed insulin.

Auto- and alloimmunity

Results of pretransplant and posttransplant PRA and autoantibody testing are displayed in Table 4. No recipient had evidence of allosensitization pretransplant. All had evidence of autoimmunity pretransplant: 2 of 3 antibodies were positive in 5 recipients and all antibodies positive in 1 recipient.

On most recent posttransplant PRA (mean 2.4 ± 0.8 years posttransplant), 4 recipients had positive results—class II only in 2 and both class I and class II positive in the other 2, although 1 of the recipients with both positive had a low class I PRA at 4%. Of these 4 recipients, 3 had donor-specific antibodies. In the 2 recipients on insulin, 1 was positive for class II donor-specific antibodies (#4); 1 was positive for both class I and class II donor-specific antibodies (#6).

Adverse Events

We noted no episodes of opportunistic infections, posttransplant lymphoproliferative disease, or other malignancies. All recipients experienced aphthous ulcers, leukopenia, and transient elevations of liver enzymes in the early posttransplant period. Two recipients

transplanted by percutaneous approach experienced symptomatic cholelithiasis or acute cholecystitis (days 8 and 72 posttransplant) and required cholecystectomy; this was possibly related to the transplant procedure, although no known injury to the gallbladder occurred during portal vein catheterization. We noted no other procedure-related adverse events.

Serum creatinine levels increased in all recipients: from a mean of 0.8 ± 0.1 mg/dL at baseline to 1.1 ± 0.2 mg/dL at 1 year posttransplant. At the most recent follow-up, levels were relatively stable at 1.1 ± 0.3 mg/dL.

Measured GFR decreased from pretransplant to 1 year posttransplant in 5 of 6 recipients: from a mean of 110 ± 21 mL/min/1.73m² at baseline to 83 ± 19 mL/min/1.73m² at 1 year posttransplant. Two recipients had >25% decrease in GFR, with an overall mean decrease of $28 \pm 19\%$ among all participants (table 5).

Mean urinary microalbumin increased from 12 ± 12 mg/g Cr pretransplant to 23 ± 19 mg/g Cr at 1 year posttransplant.

Pretransplant, 1 recipient required antihypertensive medications; posttransplant, 4 did, with 2 of these requiring 2 medications for adequate control of hypertension. One recipient required lipid-lowering medication pretransplant; posttransplant, 3 did.

Discussion

In this study, 4 of 6 recipients attained prolonged insulin independence after 1 to 2 islet infusions. Induction immunosuppression included ATG and etanercept; initial maintenance therapy was with cyclosporine and everolimus. Those 4 recipients have remained insulin independent for a mean of 3.4 years posttransplant, with normal HbA_{1c} levels in all 4. No insulin-independent recipient experienced recurrence of severe hypoglycemia, the main indication for transplant. This prolonged insulin independence in 4 of 6 recipients is promising, especially in view of other reports of a 15-month median duration of insulin independence (3,6).

Of the 2 recipients who did not attain prolonged insulin independence, 1 (#4) was initially insulin-independent but resumed insulin therapy at 1.5 years posttransplant; the other (#6) never became insulin-independent. Recipient #6 was the only 1 of our 6 recipients with a BMI above the normal range, which may have placed increased metabolic stress on the graft. Both #4 and #6 showed evidence of donor-specific alloimmunity posttransplant: class II donor-specific antibodies in #4 and both class I and class II donor-specific antibodies in #6. Positive PRA levels and donor-specific antibodies have been associated with increased rates of graft failure in other series (14). In our study, islet autoantibodies were common among all 6 recipients in our study, including the 2 insulin-dependent subjects. Some evidence suggests that recurrence of autoimmunity may be an important factor in graft decline (15). However, the relative contributions of allosensitization and autoimmunity to posttransplant graft decline are not well understood in islet transplantation and warrant further investigation.

All 6 of our recipients had a general decline in kidney function, most pronounced in the first year, with an increase in serum creatinine levels in all 6 and a decrease in measured GFR in 5. Variable rates of decline in kidney function have been previously reported in islet transplant recipients on tacrolimus and sirolimus; in contrast, those on mycophenolate mofetil and sirolimus have experienced a change in kidney function no greater than that seen in medically treated diabetics (16,17). Preservation of kidney function is a major concern after islet transplants, so minimizing immunosuppression-related kidney toxicity is of critical importance in future research.

In our study, duration of islet graft survival was promising in light of the gradual but invariable loss of insulin independence seen in recipients on a protocol of daclizumab induction and maintenance with tacrolimus and sirolimus (3,6). We have previously transplanted 4 patients at our institution using daclizumab, tacrolimus, and sirolimus; these patients participated in the international trial of the Edmonton protocol (3). These 4 recipients achieved insulin independence posttransplant, but in contrast to outcomes in our current study, only 1 of the 4 recipients was insulin-independent at 2 years after last infusion and all resumed insulin therapy by 26 months after final infusion, at a mean of 1.7 ± 0.3 years posttransplant.

Our protocol differs from that widely used protocol, both in induction therapy and maintenance. Recent evidence suggests that immunosuppression with daclizumab, tacrolimus, and sirolimus increases autoreactive CD8+ T cells and induces autoimmune recurrence after islet transplants (7). The presence of autoreactive CD8+ T cells and recurrent autoimmunity are associated with islet graft failure (15). Conversely, induction therapy with a T-cell antibody, such as ATG, may be protective against this autoimmune response. In 1 islet-after-kidney transplant study, only ATG used at the time of the kidney transplant was associated with long-term islet graft survival and lack of autoimmune recurrence (18). In mouse models, antilymphocyte globulin has induced long-term reversal of autoimmune diabetes, further suggesting that immunosuppression with T cell directed antibodies has profound and prolonged effects on the autoimmune repertoire (19). Combination therapy with sirolimus and tacrolimus has also been shown to interfere with β -cell regeneration, another potential disadvantage of such therapy (8). Additionally, tacrolimus frequently induces hyperglycemia even in nondiabetic patients, while this effect is significantly less frequent with the related calcineurin-inhibitor cyclosporine (20).

Most patients in this series required more than one infusion to obtain insulin independence, in contrast to our prior experience (2). Less suitable enzyme batches may have precluded the preparation of high islet yields needed to permit insulin independence with a single infusion. Also of note, this study is unusual in the high proportion of young islet donors. Because these young donors were obese (BMI >28.0), donor pancreases were preferentially allocated for islet transplantation; one pancreas from a non-obese donor (for recipient #1, BMI 27) was procured locally

Our study was limited by its nonrandomized design involving a small number of recipients. Duration of graft function may be different in a larger population. Moreover, we have no control group on daclizumab, tacrolimus, and sirolimus, so we could not make a direct

comparison. Several other factors beyond immunosuppression may have promoted graft survival in our recipients.

In conclusion, our pilot study provides proof-of-concept evidence that insulin independence can be maintained for >3 years in type 1 diabetic islet allograft recipients on ATG and etanercept (induction) and cyclosporine and everolimus (initial maintenance). It will be important to conduct carefully designed, prospective studies of the effects of immunosuppressive protocols on autoimmune and alloimmune responses to, and posttransplant regeneration of, transplanted islets.

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Abbreviations

ATG	antithymocyte globulin
TNF-α	tumor necrosis factor- α
HbA_{1c}	hemoglobin A _{1c}
BMI	body mass index
GFR	glomerular filtration rate
OGTT	oral glucose tolerance test
AST	arginine stimulation test
AIR_a	acute insulin response
ACR_a	acute C-peptide response
GAD65	anti-glutamic acid decarboxylase antibodies
IAA	insulin autoantibodies
ICA	anti-islet cell antibodies
PRA	panel-reactive antibody
IE/kg	.islet equivalents/ kilogram body weight

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Recipient Characteristics Pretransplant

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PR= proliferative retinopathy; NPR= nonproliferative retinopathy; AN= autonomic neuropathy (including gastroparesis); PN= peripheral neuropathy

Table 2

Donor and Graft Characteristics

	Recipient No. (Transplant No.)											
Characteristics	#1 (T1)	#2 (T1)	#2 (T2)	#3 (T1)	#3 (T2)	#4 (T1)	#4 (T2)	#5 (T1)	#5 (T2)	#6 (T1)	#6 (T2)	
Donor												
Age (years)	22	45	24	33	24	24	24	24	24	41	44	
Body weight (kg)	95.2	85.3	108.9	77.6	82.0	94.4	114.0	108.0	114.0	142.0	108.4	
Body mass index (kg/m²)	27.0	32.1	43.5	34.5	32.0	29.7	37.1	29.0	37.1	42.4	37.3	
Cause of death	Tr	Tr	Non-Tr	Tr	Non-Tr	Tr	Tr	Tr	Tr	Tr	CV	
Blood glucose during hospitalization												
lowest (mg/dL)	121	121	99	100	122	101	119	116	119	124	138	
highest (mg/dL)	300	279	269	206	234	214	235	314	235	281	305	
Elevated vasopressors *	Yes	Yes	No	Yes	No	No	Yes	Yes	Yes	No	Yes	
Cardiac or respiratory arrest	No	Yes	Yes	No	Yes	No	No	No	No	No	Yes	
HLA-A/B, DR matches	1/6	1/6	2/6	1/6	2/6	0/6	0/6	3/6	3/6	2/6	3/6	
Graft												
Cold storage time (hours)	4.0	12.0	6.1	5.0	5.0	4.2	6.5	2.8	6.5	2.5	4.1	
Tissue volume (mL), gravity sedimentation	5.5	1.0	3.5	3.0	3.5	1.8	3.0	9.5	3.0	9.5	3.0	
Islet equivalents (IE) per kg body wt	11,454	5,482	4,841	4,914	4,275	5,086	7,635	8,837	7,635	6,199	4,874	
Total IE/kg	11,454	10,323		9,189		12,721		16,472		11,073		
Beta cells/kg * 10 ^{6**}	9.8	1.9	4.9	4.4	7.1	7.0	6.8	12.0	6.8	5.6	5.2	
Islet purity (%)	57.5	62.5	40.0	52.5	57.5	66.1	72.0	47.1	72.0	56.2	80.2	
Islet viability (%)	92.5	92.7	96.0	99.0	99.3	94.8	94.5	94.1	94.5	95.7	94.1	
GSIR Index †	2.84	1.91	1.84	1.47	2.28	2.83	3.35	1.72	3.35	1.75	1.30	
Endotoxin (EU/kg)	0.61	0.63	0.64	0.67	0.67	0.32	0.03	1.24	0.03	0.99	1.04	

* Elevated vasopressors defined as dopamine 20µg/kg/min or norepinephrine at any dose

** Calculated from total DNA content and the percentage of beta-cells in the graft

† Glucose Stimulated Insulin Release (GSIR) - Amount of insulin released in vitro at high glucose level (16.7 mM) divided by amount of insulin released at low glucose level (1.67 mM) (each corrected for DNA content)

Table 3

Long-term Metabolic Outcomes

	Recipient No.					
	#1	#2	#3	#4	#5	#6
Last day of follow-up	1685	1116	1234	972	969	1060
Days between first and second infusion		476		178	148	54
Insulin use (U/day)						
Pretransplant	17.3	27.3	19.7	22.9	26.9	27.9
1 year	0	0	0	0	0	11.0
2 years	0	0	0	6	0	11.0
3 years (or last day of follow-up*)	0	0	0	9.3*	0*	15.0*
Hemoglobin A1c (%)						
Pretransplant	6.7	7.1	7.0	6.5	8.4	7.9
1 year	4.7	5.7	5.0	5.9	5.9	5.8
2 years	5.5	5.9	5.5	6.6	5.5	7.1
3 years (or last day of follow-up*)	5.4	5.9	5.3	5.8*	5.8*	9.3*
Posttransplant C-peptide (ng/mL) basal/stimulated						
1 year	2.43/4.48	2.27/3.67	1.17/2.31	1.07/1.30	1.35/3.08	0.72/1.05
2 years	1.2	3.9	1.8	<0.5/2.3	1.0	2.2
3 years (or last day of follow-up*)	2.2	1.4	1.0/2.0	0.4/2.1*	1.1*	0.9*
OGTT 2-hour glucose, 1 year post-transplant (mg/dL)	197	154	114	232	104	228
ACR _{arg} day 180 (ng/mL)	0.77	1.08	1.21	0.57	0.87	0.34
ACR _{arg} day 365 (ng/mL)	1.94	1.39	1.11	0.27	1.68	0.21
AIR _{arg} day 180 (mIU/mL)	20.30	19.33	16.33	12.00	18.66	4.00
AIR _{arg} day 365 (mIU/mL)	ND	22.30	16.67	1.40	28.60	4.67

OGTT= oral glucose tolerance test

ACR_{arg} = acute C-peptide response to arginine

AIR_{arg} = acute insulin response to arginine

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Table 4

PRA, Donor-Specific Antibodies, and Autoantibodies

	Recipient No.					
	#1	#2	#3	#4	#5	#6
Insulin-Independent at 2 years after last transplant	Y	Y	Y	N	Y	N
Pretransplant PRA *						
Class I	0%	0%	0%	0%	0%	0%
Class II	0%	0%	0%	0%	0%	0%
Posttransplant PRA **						
Class I	0%	4%	0%	0%	0%	78%
Class II	0%	97%	0%	14%	31%	54%
Donor-Specific Antibodies, T1						
Class I	-	B60	-	-	-	A1, B7
Class II	-	DR11, DR12	-	DQ6	-	DR1, DR7
Donor-Specific Antibodies, T2						
Class I	-	A31, B60	-	-	-	A32, B44, B61
Class II	-	DR57	-	DQ6	-	DR9
Autoantibodies $\neq$						
Pretransplant						
GAD65 (0.032)	0.132	0.039	0.075	-	-	1.387
ICA-512 (0.049)	0.074	-	-	0.053	0.124	-
IAA (0.01)	4.561	0.049	0.215	0.066	0.281	2.076
Posttransplant, 1 year						
GAD65 (0.032)	0.036	0.041	0.088	0.045	-	0.983
ICA-512 (0.049)	0.055	-	-	-	0.141	-
IAA (0.01)	1.161	-	0.012	-	0.121	1.282

T= transplant number; PRA= panel reactive antibodies

* ELISA method

** Flow luminex method; 2 to 4 years after transplant 1

† Cutoff for positive result in parentheses; GAD65= glutamic acid decarboxylase antibodies; ICA-512 = islet cell antibodies; IAA = insulin autoantibodies

Table 5

Creatinine, GFR, and LDL

	Recipient No.						Mean	SD
	#1	#2	#3	#4	#5	#6		
Creatinine (mg/dL)								
pretransplant baseline	1.00	0.80	0.77	0.77	0.66	0.80	0.80	0.11
1 year posttransplant	1.25	1.35	0.97	0.91	0.82	1.13	1.07	0.21
most recent follow-up	1.10	1.34	0.88	0.91	0.80	1.40	1.07	0.25
GFR (mL/min/1.73m²)								
pretransplant baseline	86	138	85	120	111	123	111	21.2
1 year posttransplant	65	55	88	107	92	89	83	19.1
most recent follow-up	65*	55*	88*	90	92*	75	83	10.6
LDL (mg/dL)								
pretransplant baseline	84	46	61	72	62	64	65	13
1 year posttransplant	128	73	88	87	111	89	96	20
most recent follow-up	75	99	112	90	103	100	97	13
on lipid-lowering medication	N	Y	N	Y	N	Y*		

GFR= glomerular filtration rate; LDL= low-density lipoprotein; SD= standard deviation

* Last measured GFR at 1 year posttransplant

** Required lipid-lowering medication both pre- and posttransplant.