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Long-Term Immunosuppression After Solitary Islet Transplantation Is Associated With Preserved C-Peptide Secretion for More Than a Decade

J. E. Blau¹, M. R. Abegg¹, W. A. Flegel², X. Zhao¹, D. M. Harlan³, and K. I. Rother^{1,*}

¹Diabetes, Endocrinology, and Obesity Branch, NIDDK National Institutes of Health, Bethesda, MD

²Department of Transfusion Medicine, NIH Clinical Center National Institutes of Health, Bethesda, MD

³Division of Diabetes, Endocrinology, and Nutrition Diabetes Center of Excellence, Department of Internal Medicine, University of Massachusetts Medical School, North Worcester, MA

Abstract

We report on two patients with type 1 diabetes (T1D) after solitary islet transplantation in 2001. They received steroid-sparing immunosuppression (daclizumab, sirolimus, and tacrolimus according to the Edmonton protocol). Both patients became insulin independent for 2 years: Patient A, a 42-year-old female with a 12-year history of T1D, received two islet infusions; patient B, a 53-year-old female with a 40-year T1D history, received one islet infusion. Pretransplant, both had undetectable C-peptide concentrations and frequent and severe hypoglycemia. Pretransplant, hemoglobin A1c (HbA1c) was 7.8% and 8.8% and insulin requirements were 0.47 and 0.33 units/kg/day, respectively. Posttransplant, C-peptide levels remained detectable while immunosuppression was continued, but decreased over time. Insulin was re-started 2 years posttransplant in both patients. Since patient A's glycemia and insulin requirements trended toward pretransplant levels, immunosuppression was discontinued after 13 years. This resulted in a sudden cessation of C-peptide secretion. Patient B continues on immunosuppression, has better HbA1c, and half the insulin requirement compared to pretransplant. Both patients no longer experience severe hypoglycemia. Herein, we document blood glucose concentrations over time (>30 000 measurements per patient) and β cell function based on C-peptide secretion. Despite renewed insulin dependence, both patients express satisfaction with having undergone the procedure.

*Corresponding author: Kristina I. Rother, kristina.rother@nih.gov.

Disclosure

The authors of this manuscript have no conflicts of interest to disclose as described by the *American Journal of Transplantation*.

Disclaimer

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Introduction

Solitary allogeneic islet transplantation may provide clinical benefits for a select group of patients with type 1 diabetes (T1D), for example, transient insulin independence and long-term resolution of severe hypoglycemia. Even so, the treatment continues to face many challenges. Typical inclusion criteria of islet transplantation programs consist of undetectable C-peptide, severe glycemic lability with hypoglycemia unawareness, and recurrent, life-threatening episodes of hypoglycemia. Additionally, candidates must be willing to maintain long-term immunosuppressive therapy.

The Edmonton protocol raised the hope for sustained insulin independence, but 5 years after islet transplantation, only 10% of patients remained insulin free (1). Since that seminal report, technical improvements have increased the 3-year insulin-independence rate from 27% in 2002 to 44% in 2010 (2). More recently, a 5-year insulin-independence rate of 50% has been documented (3,4). However, the overall number of islet transplant recipients has remained small with just over 1000 patients worldwide (5). Long-term outcome data, as we present in this series, are sparse, and remain of critical importance to advance the field of islet transplantation.

We report on two patients 13 years after solitary islet transplantation. Detailed daily blood glucose records, baseline and stimulated C-peptide results and trend analyses of hemoglobin A1c (HbA1c), renal function, and immunological data are presented. We document acute changes after discontinuing immunosuppression therapy 13 years posttransplant. Our report is consistent with the experience of others and highlights the most important long-term outcome of islet transplantation in a subset of those treated: resolution of life-threatening hypoglycemia.

Methods

Patients A and B have had continuous medical follow-up at the National Institutes of Health Clinical Center (NIH-CC) in Bethesda, MD, since 2000. Both patients have been seen every 3 months since transplantation for baseline and arginine-stimulated C-peptide testing. However, patient B's venous access became problematic 4 years posttransplantation and, thus, only nonstimulated levels were obtained.

These two patients were among six patients who underwent isolated islet transplantation at the NIH between the years 2000 and 2001. Further details regarding the outcomes of the other four patients have been previously published (6). Only one of these four patients had achieved insulin independence (lasting 1 year), but had to discontinue immunosuppression due to side effects related to sirolimus (7). Written informed consent was obtained prior to study enrollment (NCT00006505). According to the NIH protocol, patients were to be followed until cessation of C-peptide secretion.

Laboratory evaluations were performed in the NIH-CC Department of Laboratory Medicine. The C-peptide assay at the NIH-CC from March 2000 through January 2006 utilized the Immulite 2000, which has a lower limit of detection at 0.1 ng/mL, with between-day coefficient of variation (CV) of 7.7% at 1.12 ng/mL, and a CV of 8.3% at 5.94 ng/mL. The

within run CV of this assay is 1.6% at 0.38 ng/mL, 2% at 1.73 ng/mL, and 1.9% at 3.88 ng/mL. From January 2006 through December 2011, NIH-CC utilized the Immulite 2500 with equivalent performance characteristics as the Immulite 2000. Since January 2012, NIH-CC utilizes a Roche Cobas 6000, which has a lower limit of detection at 0.01 ng/mL, with between-day CV of 2.6% at 1.83 ng/mL and 2.8% at 9.51 ng/mL, and within run CV of 0.9% at 1.97 ng/mL and 2.8% at 9.72 ng/mL.

The NIH-CC Department of Transfusion Medicine collected fresh serum samples in March 2015 which were analyzed for human leukocyte antigen (HLA) class I and II antibodies using a membrane independent solid phase assay and a flow analyzer (LABScreen and LABScan 100; OneLambda, Canoga Park, CA) (8). HLA antigens of recipients and donors had been determined at the time of transplantation (Organ Procurement Organizations) (9).

Case Reports

Patient A

This 54-year-old white woman (42 years old at transplantation) had been diagnosed with T1D at age 30, at which time she had positive anti-glutamate decarboxylase (GAD) antibodies. Prior to transplantation, she experienced frequent hypoglycemia (documented blood glucose of 40 mg/dL) and had about three life-threatening episodes of hypoglycemia per year (requiring third-party assistance). Her complications included mild neuropathy and nonproliferative retinopathy. She underwent her first allogeneic islet transplant in April 2001 and received a second infusion 1 month later with a total of 509 000 islet equivalents (IEQs) (approximately 10 000 IEQs/kg) after which she attained insulin independence immediately after the second infusion.

A regimen of tacrolimus (target trough plasma levels 3–6 ng/mL) and sirolimus (target trough plasma levels 6–10 ng/mL) was maintained following transplantation until recently. Target levels of the immunosuppressive drugs were achieved 76% and 71% of the time, respectively. Metformin and glitazones were introduced 19 months after transplantation but did not reverse the gradual rise of fasting and postprandial hyperglycemia. Thus, they were discontinued and insulin was re-started 2 years posttransplant with requirements that continuously increased to pretransplant levels (Table 1). Twelve years posttransplant, renal function remained normal, but serum creatinine increased continuously within the normal range. No significant changes in body mass index (BMI) occurred (Table 1). Daily mean fasting, prandial, and 2-h postprandial glucose values (obtained by home glucose monitoring) over the past 13 years demonstrate lower overall glycemic excursions compared to pretransplantation, but show a gradual increase of mean blood glucose concentrations (Figure 1). Severe hypoglycemia has not reoccurred. Immunosuppression was stopped after 13 years (August 2014). Two months after discontinuation, C-peptide secretion was undetectable after arginine stimulation for the first time since transplantation (Figure 2), which was confirmed during another stimulation test 3 months later. Insulin requirements have returned to pretransplant levels.

According to our original islet transplant protocol, donors and recipients were not required to be HLA matched. Patient A received islets from two different donors, which both

matched for 1 out of 10 HLA antigens (a “1/10 HLA match;” Table 2). After discontinuation of immunosuppression, the serum of patient A exhibited strong donor-specific antibodies against HLA-DQ6, -DQ5, and -DQ9, which represents a panel reactive antibody (PRA) of 43% for HLA class II antigens. In addition, donor-specific antibodies against HLA-B44 and -B62 were detected (2% PRA for HLA class I antigens). No HLA antibody test results were available prior to discontinuation of immunosuppression. High levels of GAD antibodies decreased to a nadir at 5 years posttransplant and have remained almost 10 times lower than at initial presentation (Table 1). No other significant antibody changes were evident.

Patient B

This 65-year-old white woman (53 years old at transplantation) was diagnosed with T1D at the age of 13 years. She progressively developed severe hypoglycemia unawareness with multiple weekly episodes (blood glucose < 40 mg/dL). She had hypoglycemia-related seizures as well as a motor vehicle accident due to loss of consciousness associated with hypoglycemia. Her pretransplant diabetes-related complications included cataracts and diabetic retinopathy for which she had laser photocoagulation. In June 2001, she received an islet preparation of 435 482 IEQs (approximately 10 000 IEQs/kg) and became insulin independent within 3 weeks. Patient B had been on 20 mg simvastatin daily pretransplant, which was changed to 40 mg atorvastatin daily in 2006 for elevated low density lipoprotein (LDL) concentrations. She has remained on this treatment until present.

Following transplantation, her immunosuppressive regimen included sirolimus and tacrolimus. The latter was discontinued 5 years posttransplant due to worsening kidney function (creatinine increased from 0.7 to 1.3 mg/dL) and substituted with mycophenolate mofetil, which she is still taking. Target levels of sirolimus were achieved 82% of the time. Renal function improved after discontinuation of tacrolimus. BMI remained stable and no other significant metabolic changes occurred (Table 1). Approximately 24 months after transplantation, metformin therapy was initiated because of gradually increasing fasting blood glucose concentrations (Figure 1). Similar to patient A, this trend was not reversed and a glitazone was started. Two years posttransplant both oral hypoglycemia agents were discontinued and insulin therapy was reinitiated due to increasing HbA1c (Figure 3). The current daily insulin dose remains at approximately half of the pretransplant dose. C-peptide secretion has remained detectable throughout (Figure 2).

Immunological data demonstrate that the single islet transplant of patient B was a 2/10 HLA match. In contrast to patient A, no antibodies against HLA class I or class II antigens were found in the current serum of patient B (0% PRA for HLA class I and II antigens). GAD antibody concentrations have remained low or undetectable while on immunosuppressant therapy (Table 1); no other antibody changes were apparent.

Complications

Neither patient experienced acute complications during the islet transplantation procedure, but both patients developed immunosuppression-related side effects, such as frequent mouth ulcers which occurred with higher frequency in the first 2 years after transplantation. Within the last 10 years, patient B sustained one serious adverse event resulting in hospitalization

and intravenous antibiotic treatment after developing a lower extremity cellulitis. As mentioned, patient B also required discontinuation of tacrolimus due to a decline in renal function, which returned to baseline. Neither patient's retinopathy progressed. Patient A had no worsening of neuropathy—but also no improvement.

Discussion

These two cases demonstrate long-term follow-up after solitary allogeneic islet transplantation. Both patients experienced a gradual decline in β cell function over the years, but maintained measurable C-peptide while receiving immunosuppressant therapy. In patient B, blood glucose concentrations remained stable and insulin requirements are 50% lower compared to pretransplant doses during the observation period of 13 years. In patient A, β cell function declined noticeably approximately 6 years after islet transplantation resulting in greater blood glucose excursions. Weighing the benefits of minimal C-peptide secretion with the continued potential long-term toxicities of immunosuppressant therapy, the decision was made to discontinue immunosuppressant therapy in patient A. This led to an immediate and complete cessation of C-peptide secretion and a step-up in insulin requirements to pretransplant levels.

The long-term benefits of islet transplantation include slowed progression of microvascular disease and some reports of sustained insulin independence (10–15). Another clinically important outcome is resolution of severe hypoglycemia (16,17). The mechanism is unknown but has been postulated to involve (1) lower insulin doses and (2) increased endogenous glucose production via lactate gluconeogenesis and decreased systemic glucose disposal (18). C-peptide secretion may play a protective role by increasing peripheral blood flow and preventing renal damage (19). However, restoration of hypoglycemia awareness and reduction of severe hypoglycemic episodes in these patients may reflect a training effect rather than a direct result of a functioning graft. While long-term islet survival and insulin independence are rare, improved glycemic control with insulin dependence remains an important and achievable long-term goal.

One of the major considerations for selecting patients suitable for islet transplantation includes the subject's willingness to remain on long-term immunosuppression. The balance between side effects of chronic treatment with immunosuppressive agents and the potential benefits of continued minimal C-peptide secretion remains debatable (13–15). This is in contrast to patients who received a life-saving organ transplantation (e.g. kidney or heart) and who would clearly experience harm if discontinuing immunosuppression.

It has been shown previously (20,21) that withdrawal of immunosuppression, including the potent T cell inhibitor tacrolimus, within 3 years of a failing islet transplant increases anti-donor HLA antibody formation (22). HLA matching may play a role in preservation of functional islets, demonstrated by two cases (13,15) with encouraging outcomes of 10- and 13-year insulin independence. In our cohort, Patient A had strong HLA antibodies reactive with both islet grafts at the end of this study. Her islet function was promptly lost once the immunosuppression was discontinued. Patient B, on the other hand, who has maintained low levels of C-peptide secretion for more than 13 years did not have any HLA antibodies.

Whether the different HLA alloimmunization of our patients explains their different clinical courses and outcomes remains speculative.

Furthermore, immunosuppression tapering has been inversely related to C-peptide secretion in functional grafts (23). However, complete cessation of C-peptide secretion after long-term immunosuppression withdrawal has not been previously reported. It should be noted, the C-peptide assay that was available at our center at the time of transplantation in 2001 had a lower sensitivity (lower limit of detection 0.1 ng/mL) than the current assay (lower limit of detection 0.01 ng/mL). Therefore, we cannot rule out that minimal pretransplant C-peptide secretion could have been detectable with the current assay. Using selective venous sampling performed many months after the recipients received their islet allograft(s), our group has previously shown (24) that two out of three islet transplantation patients studied secreted minimal but detectable insulin from their native pancreatic β cells, in addition to the insulin secreted from the allografted islet cells in liver.

In conclusion, we have demonstrated that improved long-term blood glucose control is sustainable over 13 years posttransplant, with subjective benefits for these two patients who no longer suffer from severe hypoglycemia. The benefit of continued minimal C-peptide secretion remains unclear, and, therefore, must be weighed against the risks of long-term immunosuppression.

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Abbreviations

CPK	creatine phosphokinase
CV	coefficient of variation
GAD	glutamic acid decarboxylase
HbA1c	hemoglobin A1c
HLA	human leukocyte antigen
IEQs	islet equivalents
NIH-CC	National Institutes of Health Clinical Center
PRA	panel reactive antibody
TPO	thyroid peroxidase
T1D	type 1 diabetes

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Figure 1. Daily fasting home glucose monitoring values since transplant (A) in patient A, demonstrating early improvement with gradual increase of mean blood glucose concentrations and (B) in patient B, demonstrating persistence of improved fasting glucose levels.

Figure 2.
HbA1c levels in (A) patient A and (B) patient B since transplantation. HbA1c, hemoglobin A1c.

Figure 3.

C-peptide levels in (A) patient A, demonstrating gradual decline of C-peptide concentrations, and in (B) patient B, demonstrating persistence of C-peptide secretion. C-peptide was measured in response to arginine except for years 5–13 posttransplantation in patient B when only fasting C-peptide could be obtained. HbA1c, hemoglobin A1c.

Table 1

Metabolic and immunologic data from pretransplant through 13 years posttransplant on patients A and B

Metabolic data	Patient A						Patient B						
	Pre	1 yr	3 yr	5 yr	10 yr	13 yr	2 mo after discontinuation of immunosuppression	Pre	1 yr	3 yr	5 yr	10 yr	13 yr
BMI (kg/m ²)	19.9	20	20.4	20.8	21.2	20.9	21.8	23.6	23.1	23	23.3	23.7	23
Creatinine (mg/dL)	0.5	0.6	0.7	0.7	0.8	0.9	0.9	0.8	0.7	0.9	1.3	0.8	0.74
HbA1c (avg)	7.8	5.4	6.6	6.6	6.9	7.5	7.5	8.8	6.2	6.6	6.4	6.6	7.3
Medications	Insulin	None	TZD + Insulin	TZD + Insulin	Insulin	Insulin	Insulin	Insulin	None	TZD + Metformin + Insulin	Insulin	Insulin	Insulin
Daily insulin requirement (u/kg/day)	0.47	0	0.11	0.14	0.33	0.37	0.47	0.33	0	0.06	0.12	0.14	0.15
C-peptide (ng/dL)	<0.2 (s)	2.5 (s)	0.5 (s)	0.4 (s)	0.3 (s)	0.4 (s)	<0.1 (s)	<0.2 (s)	2.8 (s)	0.9 (s)	0.6 (ns)	0.6 (ns)	0.3 (ns)
Microalbuminuria (mg/dL)	15.8	<2	7.8	6.6	2.2	4.5	3.0	13	75	15	15.3	5.3	9.3
Auto-immune markers													
GAD Ab (nmol/L; nL <0.02)	922	382–588	163–331	64–122	165–272	188	115	0.08	0.06–1.2	0–0.04	0.06	0.02	0.02
TPO Ab (IU/mL; nL 0–34.9)	<10						10.5	<10					<10

TZD, thiazolidinedione; (s), arginine stimulated, max value; (ns), nonstimulated, fasting; GAD, glutamate decarboxylase; TPO, thyroid peroxidase; HbA1c, hemoglobin A1c; BMI, body mass index.

Table 2

HLA antigen testing for patients A and B¹

Antigens	Patient A			Patient B	
	Patient	Donor 1	Donor 2	Patient	Donor
HLA-A	A1	A74	A24	A1	A2
	A2	A2	A2	A30	A26
HLA-B	B7	B44	B60	B8	B7
	B13	B70	B62	B18	B14
HLA-Cw	Cw6	Cw2	Cw1	Cw5	Cw8
	Cw7	Cw5	Cw3	Cw7	Cw7
HLA-DR	DR4	DR9	DR9	DR17	DR7
	DR7	DR13	DR13	–	DR12
HLA-DQ	DQ2	DQ6	DQ1 ²	DQ2	DQ2
	DQ8	DQ9	DQ9	–	DQ7

HLA, human leukocyte antigen.

Patient A was a 1/10 HLA match (two donors) and patient B was a 2/10 HLA match (one donor).

¹HLA antigens that are matching between patient and donor are boxed.

²DQ1 represents DQ5 or DQ6 in current HLA terminology.