

The New England Journal of Medicine

© Copyright, 2000, by the Massachusetts Medical Society

VOLUME 343

JULY 27, 2000

NUMBER 4



ISLET TRANSPLANTATION IN SEVEN PATIENTS WITH TYPE 1 DIABETES MELLITUS USING A GLUCOCORTICOID-FREE IMMUNOSUPPRESSIVE REGIMEN

A.M. JAMES SHAPIRO, M.B., B.S., JONATHAN R.T. LAKEY, PH.D., EDMOND A. RYAN, M.D., GREGORY S. KORBUTT, PH.D., ELLEN TOTH, M.D., GARTH L. WARNOCK, M.D., NORMAN M. KNETEMAN, M.D., AND RAY V. RAJOTTE, PH.D.

ABSTRACT

Background Registry data on patients with type 1 diabetes mellitus who undergo pancreatic islet transplantation indicate that only 8 percent are free of the need for insulin therapy at one year.

Methods Seven consecutive patients with type 1 diabetes and a history of severe hypoglycemia and metabolic instability underwent islet transplantation in conjunction with a glucocorticoid-free immunosuppressive regimen consisting of sirolimus, tacrolimus, and daclizumab. Islets were isolated by ductal perfusion with cold, purified collagenase, digested and purified in xenoprotein-free medium, and transplanted immediately by means of a percutaneous transhepatic portal embolization.

Results All seven patients quickly attained sustained insulin independence after transplantation of a mean (\pm SD) islet mass of $11,547 \pm 1604$ islet equivalents per kilogram of body weight (median follow-up, 11.9 months; range, 4.4 to 14.9). All recipients required islets from two donor pancreases, and one required a third transplant from two donors to achieve sustained insulin independence. The mean glycosylated hemoglobin values were normal after transplantation in all recipients. The mean amplitude of glycemic excursions (a measure of fluctuations in blood glucose concentrations) was significantly decreased after the attainment of insulin independence (from 198 ± 32 mg per deciliter [11.1 ± 1.8 mmol per liter] before transplantation to 119 ± 37 mg per deciliter [6.7 ± 2.1 mmol per liter] after the first transplantation and 51 ± 30 mg per deciliter [2.8 ± 1.7 mmol per liter] after the attainment of insulin independence; $P < 0.001$). There were no further episodes of hypoglycemic coma. Complications were minor, and there were no significant increases in lipid concentrations during follow-up.

Conclusions Our observations in patients with type 1 diabetes indicate that islet transplantation can result in insulin independence with excellent metabolic control when glucocorticoid-free immunosuppression is combined with the infusion of an adequate islet mass. (N Engl J Med 2000;343:230-8.)

©2000, Massachusetts Medical Society.

ISLET transplantation has been investigated as a treatment for type 1 diabetes mellitus in selected patients with inadequate glucose control despite insulin therapy. However, the perennial hope that such an approach would result in long-term freedom from the need for exogenous insulin, with stabilization of the secondary complications of diabetes, has failed to materialize in practice. Of the 267 allografts transplanted since 1990, only 12.4 percent have resulted in insulin independence for periods of more than one week, and only 8.2 percent have done so for periods of more than one year.¹ In the majority of these procedures, the regimen of immunosuppression consisted of antibody induction with an antilymphocyte globulin combined with cyclosporine, azathioprine, and glucocorticoids.¹

In the past 10 years, techniques for isolating large numbers of human islets have advanced, permitting renewed attempts at islet transplantation.^{2,3} With the increase in the availability of new and more potent immunosuppressive agents, strategies can now be developed specifically for islet transplantation that will provide greater immunologic protection without diabetogenic side effects.

For any type of transplantation procedure, a balance is sought between efficacy and toxicity. With respect to islet transplantation a further difficulty is that many of the current agents damage beta cells or induce peripheral insulin resistance.⁴ To address this problem, we developed a glucocorticoid-free immunosuppressive protocol that includes sirolimus, low-dose tacrolimus, and a monoclonal antibody against the interleukin-2 receptor (daclizumab) for use in a trial of islet transplantation alone for patients with

From the Surgical-Medical Research Institute and the Department of Surgery (A.M.J.S., J.R.T.L., G.S.K., G.L.W., N.M.K., R.V.R.) and the Department of Medicine (E.A.R., E.T.), University of Alberta, Edmonton, Alta., Canada. Address reprint requests to Dr. Shapiro at 2D4.37 Department of Surgery, University of Alberta Hospitals, Mackenzie Health Sciences Center, 8440 112 St., Edmonton, AB T6G 2B7, Canada, or at amjs@powersurfr.com.

brittle type 1 diabetes. Most previous islet transplantations have been performed in combination with kidney transplantation in patients with end-stage diabetic nephropathy.¹ We limited our procedure to islet transplantation alone and in doing so selected patients who had severe hypoglycemia (defined as multiple hypoglycemic episodes) or uncontrolled diabetes despite compliance with an insulin regimen.

METHODS

Patients

Patients who were considered to have had type 1 diabetes for more than five years on the basis of a stimulated serum C-peptide concentration of less than 0.48 ng per milliliter (0.16 nmol per liter) were eligible to undergo islet transplantation if their serum glucose concentrations remained uncontrolled despite exogenous insulin therapy. Patients also had to have recurrent severe hypoglycemia with coma or metabolic instability to such an extent that the global risk of transplantation and immunosuppression was judged to be less than the risk of continued uncontrolled diabetes. All protocols were approved by the health research ethics board of the University of Alberta, and each patient gave written informed consent.

Glucocorticoid-free Immunosuppression

Immunosuppression was initiated immediately before transplantation. Sirolimus (Rapamune, Wyeth–Ayerst Canada) was given orally at a loading dose of 0.2 mg per kilogram of body weight, followed by a dose of 0.1 mg per kilogram per day, with monitoring of drug levels to maintain them in the range of 12 to 15 ng per milliliter for the first three months and in the range of 7 to 10 ng per milliliter thereafter. Low-dose tacrolimus (Prograf, Fujisawa Canada) was given orally at an initial dose of 1 mg twice daily, and the dose was subsequently adjusted to maintain a trough concentration at 12 hours of 3 to 6 ng per milliliter (IMX enzyme immunoassay, Abbott). Daclizumab (Zenapax, Roche Canada) was given intravenously at a dose of 1 mg per kilogram every 14 days for a total of five doses. If the second transplantation procedure occurred more than 10 weeks after the first, the course of daclizumab was repeated. No glucocorticoids were given at any time during the trial.

Conditioning Regimen and Post-Transplantation Therapy

As soon as there were sufficient numbers of islets for transplantation, the patient was given intravenous antibiotics prophylactically (500 mg of vancomycin and 500 mg of imipenem), and oral supplementation with vitamin E (800 IU per day), vitamin B₆ (100 mg per day), and vitamin A (25,000 IU per day) was initiated.⁵ Inhaled pentamidine (300 mg once a month) was given after transplantation to prevent infection with *Pneumocystis carinii*, and oral ganciclovir (1 g three times per day) was given for 14 weeks after transplantation irrespective of the patient's cytomegalovirus status to reduce the risk of graft loss^{6,7} and to protect against lymphoproliferative disorder.⁸

Islet Preparation

Pancreases were removed from brain-dead donors and stored in chilled University of Wisconsin solution after informed consent had been obtained from the donors' relatives. Donors were selected according to the results of a multivariate analysis of factors that influence the success of islet isolation.⁹

To isolate the islets, the ducts were perfused in a controlled fashion with a cold enzyme (Liberase human islet, Roche). The islets were then separated by gentle mechanical dissociation and purified with the use of continuous gradients of Ficoll–diatrizoic acid (Seromed-Biochrom) in an apheresis system (model 2991, Cobe Laboratories).^{2,3,10-13} The use of xenoprotein products (such as fetal-calf serum) was avoided during islet isolation and purifi-

cation, and 25 percent human albumin was used instead. To minimize the risk of islet injury as a result of cold ischemia, we transplanted freshly prepared islets immediately after harvesting them, thus eliminating the need for islet culture.

Samples were collected in duplicate for the quantification of the islets, expressed in terms of islet equivalents, the standard unit for reporting variations in the volume of islets, with the use of a standard islet diameter of 150 μm .¹⁴ Islet grafts were characterized with respect to cell composition, total cellular insulin, DNA, and the extent of insulin secretion in vitro during a glucose challenge.¹⁵ In brief, the islets were incubated for 24 hours at 37°C in CMRL 1066 medium with 10 percent fetal-calf serum and 25 mmol HEPES buffer. A known number of duplicate aliquots of islets were incubated in a low concentration of glucose (50 mg per deciliter [2.8 mmol per liter]) and a high concentration of glucose (360 mg per deciliter [20 mmol per liter]) for two hours, and the amount of insulin generated in response to the high-glucose challenge was divided by the amount generated by the low-glucose challenge to yield the mean insulin-release stimulation index.

Islet Transplantation

Islet preparations that had more than 4000 islet equivalents per kilogram of the recipient's body weight in a packed-tissue volume of less than 10 ml were judged safe for transplantation.¹⁶ Each islet preparation from a donor was matched to the recipient's blood type and cross-matched for lymphocytotoxic antibodies, but no attempt at HLA matching was made.

Patients were sedated, and a percutaneous transhepatic approach was used to gain access to the portal vein under fluoroscopic guidance. Once access was confirmed, we used the Seldinger technique to place a 5-French Kumpe catheter within the main portal vein. Portal venous pressure was measured at base line and after islet infusion. The final islet preparation was suspended in 120 ml of medium 199 that contained 500 U of heparin and 20 percent human albumin and was infused over a period of five minutes. In all but the first 2 of the 15 procedures, on completion of the islet infusion, as the catheter was partially removed, gelatin-sponge (Gelfoam) particles were embolized into the peripheral catheter tract in the liver. Doppler ultrasonography of the portal vein and liver-function tests were performed within 24 hours after transplantation.

Assessment of Glycemic Control after Transplantation

Insulin therapy was discontinued after each transplantation and was not resumed unless serum glucose concentrations rose above 200 mg per deciliter (11.1 mmol per liter), in which case another transplantation was performed. Serum glucose concentrations were monitored by memory capillary glucose meters, and the resulting data were analyzed by computer (with Medisense and Precision Link software). To determine the extent of fluctuations in glucose concentrations in each patient, we measured the mean amplitude of glycemic excursions, which was calculated as the mean of the differences in the major fluctuations in high and low glucose values during two 24-hour periods¹⁷; a minimum of seven measurements of capillary glucose were obtained (before a meal, two hours after a meal, at bedtime, and at 3 a.m.). The patients also underwent oral glucose-tolerance testing and mixed-meal testing. The homeostatic model assessment was used to calculate insulin sensitivity.¹⁸ We also measured glycosylated hemoglobin and serum C-peptide, creatinine, and lipid concentrations.

Statistical Analysis

Results are expressed as means \pm SD or, in the case of nonparametric variables, as medians and ranges. Analysis of variance was conducted with use of the Sigmastat program.

RESULTS

Characteristics of the Patients

Seven consecutive patients (median age, 44 years; range, 29 to 54) who had had type 1 diabetes mel-

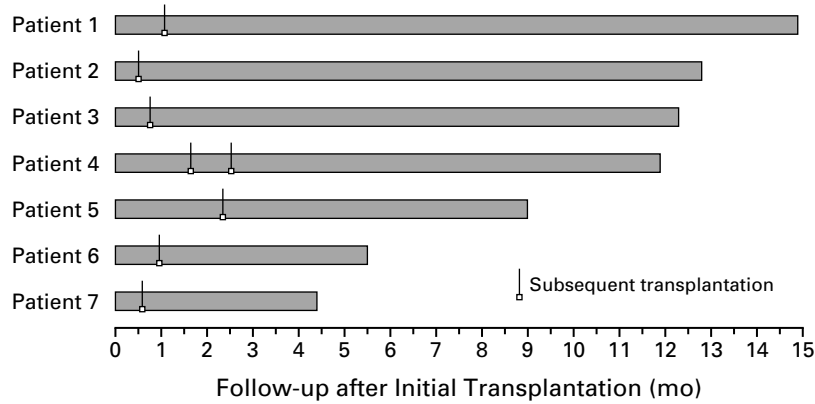


Figure 1. Length of Follow-up after the Initial Islet Transplantation and the Time at Which Subsequent Transplantations Were Performed.

TABLE 1. CHARACTERISTICS OF THE ISLET ALLOGRAFTS.

PATIENT AND PROCEDURE NO.	AGE OF DONOR	DURATION OF COLD ISCHEMIA		IMMUNOHISTOCHEMICAL ANALYSIS			TOTAL BETA-CELL MASS PER TRANSPLANT*	TOTAL NO. OF ISLETS PER ISOLATION	TOTAL NO. OF ISLETS TRANSFUSED	MEAN INSULIN-RELEASE STIMULATION INDEX‡
		FROM CROSS-CLAMPING TO ISLET ISOLATION	FROM CROSS-CLAMPING TO IMPLANTATION	BETA CELLS	ALPHA CELLS	AMYLASE				
	yr	hr		percent			×10 ⁻⁶	IE†	IE/kg of recipient's body weight†	
Patient 1										
1	35	4.0	7.5	25	16	26	102.2	376,838		11.9
2	41	9.5	14.5	13	8	42	192.4	361,577	9,407	5.4
Patient 2										
1	71	1.5	8.0	32	14	12	173.6	316,909		5.4
2	17	8.5	18.0	52	6	12	262.5	400,403	11,138	23.5
Patient 3										
1	48	3.0	11.4	14	5	49	113.8	502,636		8.4
2	22	5.0	14.2	22	7	42	42.9	251,185	13,225	3.5
Patient 4										
1	65	2.0	7.0	13	7	43	60.2	386,067		3.5
2	38	2.5	10.3	15	9	37	181.3	306,114		9.0
3§	42	5.0	43.0	45	19	5	139.1	125,317		3.5
3§	39	3.5	21.0	30	17	18	193.2	244,453	11,800	9.2
Patient 5										
1	54	6.5	13.3	27	10	35	100.6	359,198		3.8
2	57	1.5	7.0	17	8	53	166.2	591,278	13,978	3.1
Patient 6										
1	51	6.0	11.5	28	14	20	101.5	308,606		3.0
2	44	13.0	18.4	17	9	22	31.1	328,622	10,278	3.3
Patient 7										
1	55	5.0	10.5	21	12	46	50.1	472,861		3.7
2	41	1.0	6.5	12	2	21	197.8	385,305	11,002	3.8
Mean (±SD) values	45.0±14	4.8±3	13.9±9	24±12	10±5	30±15	132±67	357,336±109,042	11,547±1604	6.5±5

*The mean total beta-cell mass per transplant was based on the DNA content of the allografts (the DNA content of human islet cells is 6.0 pg per cell) and the percentage of insulin-positive cells in the allograft.¹⁵

†The islet equivalent (IE) is the standard unit used to report the volume of islets.

‡Values reflect the response of islet cells in vitro to a glucose challenge.

§Islets from two donors were used.

litus for a median of 35 years (range, 18 to 50) underwent islet transplantation between March 11, 1999, and January 23, 2000. As of June 2000, the median duration of follow-up was 11.9 months (range, 4.4 to 14.9). In all seven patients, exogenous insulin therapy quickly became unnecessary once sufficient numbers of islets were transplanted. At the time of the most recent follow-up, all patients remained free of the need for exogenous insulin. The patient who received the smallest number of islets (Patient 1) has briefly required 4 to 10 U of insulin per day on four occasions during times of stress from intercurrent illness. One patient required a total of 7 U of insulin on a single occasion during a two-day illness.

There have been no episodes of acute cellular rejection, as determined by measurements of glycemic control, serum insulin, and C peptide. None of the patients have died. Six of the seven required a second islet infusion from a second donor pancreas a median of 29 days (range, 14 to 70) after the first procedure (Fig. 1) to become insulin independent. One patient, the most obese (weight, 93 kg), required a third infusion to achieve insulin independence. The third infusion combined islets from two donors because of mechanical failure in one of the purification runs.

All patients had had repeated episodes of severe hypoglycemia before transplantation but have had no further episodes since transplantation. This change has dramatically improved their quality of life.

The mean (\pm SD) total number of islets required to induce insulin independence was $11,547 \pm 1604$ islet equivalents per kilogram of the recipient's body weight, with a mean total beta-cell mass per transplant of $132 \pm 67 \times 10^6$ (Table 1). A mean packed-cell volume of 3.5 ± 1.3 ml was infused, and this did not change the portal pressure significantly (mean increase, 0.8 mm Hg; $P=0.8$). The results of tests of liver function 24 hours after transplantation were within the normal range. Doppler ultrasonography demonstrated no evidence of thrombus within the portal vein in any of the patients. The patients were hospitalized for a median of 2.3 days (range, 0.5 to 14.7), and three patients who underwent transplantation most recently (40 percent) were discharged within 24 hours after the procedure.

Glycemic Control and Serum C-Peptide Concentrations after Islet Transplantation

Insulin requirements decreased in all patients after the first transplantation (Fig. 2). Computer analysis of data from capillary glucose meters showed a marked improvement in glycemic control in all patients. Overall mean serum glucose concentrations decreased and the mean amplitude of glycemic excursions decreased significantly with sequential islet transplantation (Fig. 2). The lability of glycemic control in a 24-hour period also decreased dramatically (Fig. 3). All patients had normal glycosylated hemoglobin values

after transplantation (Table 2). Serum C-peptide concentrations were undetectable in all patients before transplantation (less than 0.48 ng per milliliter after an overnight fast and in response to the mixed-meal test). Three months and six months after transplantation all patients had detectable serum C-peptide concentrations ($P<0.001$ by analysis of variance for the comparison with values before transplantation), and the concentrations did not decrease over time: at three months, the mean fasting value was 2.4 ± 0.3 ng per milliliter (0.8 ± 0.1 nmol per liter), and the mean value after a meal was 5.7 ± 0.9 ng per milliliter (1.9 ± 0.3 nmol per liter); at six months, the mean fasting value was 2.5 ± 0.2 ng per milliliter (0.8 ± 0.1 nmol per liter), and the mean value after a meal was 5.7 ± 0.6 ng per milliliter (1.9 ± 0.2 nmol per liter).

Autoantibody Analyses

Serum was analyzed for anti-insulin antibody, islet-cell antibody 512, and glutamic acid decarboxylase antibody before and after transplantation.¹⁹ Mean serum anti-insulin antibody concentrations fell from 0.26 ± 0.06 IU before transplantation to 0.07 ± 0.03 IU after transplantation ($P=0.04$ by t-test); this change may represent a beneficial effect of systemic immunosuppression. Serum glutamic acid decarboxylase antibody was undetectable before and after transplantation. One of four patients for whom data were available was positive for islet-cell antibody 512 before transplantation and remained so after transplantation.

Assessments of Oral Glucose Tolerance, Mixed-Meal Tolerance, and Homeostasis

The results of oral glucose-tolerance tests, completed after insulin independence had been achieved, indicated that none of the seven patients met current American Diabetes Association criteria for diabetes (Table 2).²⁰ However, in five patients, the response to the test at 120 minutes was impaired (glucose, 142 to 195 mg per deciliter [7.9 to 10.8 mmol per liter]), and two had fasting glucose concentrations that were at or above the upper limit of the normal range (110 mg per deciliter [6.1 mmol per liter]).

We used the homeostatic model assessment¹⁸ to estimate insulin sensitivity on the basis of paired fasting glucose and insulin data from the transplant recipients after they had achieved insulin independence and from normal subjects without diabetes. The values in the two groups did not differ significantly (103 ± 14 percent among transplant recipients and 118 ± 12 percent among control subjects, $P=0.43$).

Transplantation-Related Complications

None of the patients have had cytomegalovirus infection, despite the fact that four were seronegative for the virus before transplantation and received an allograft from a seropositive donor. In the first 2 of

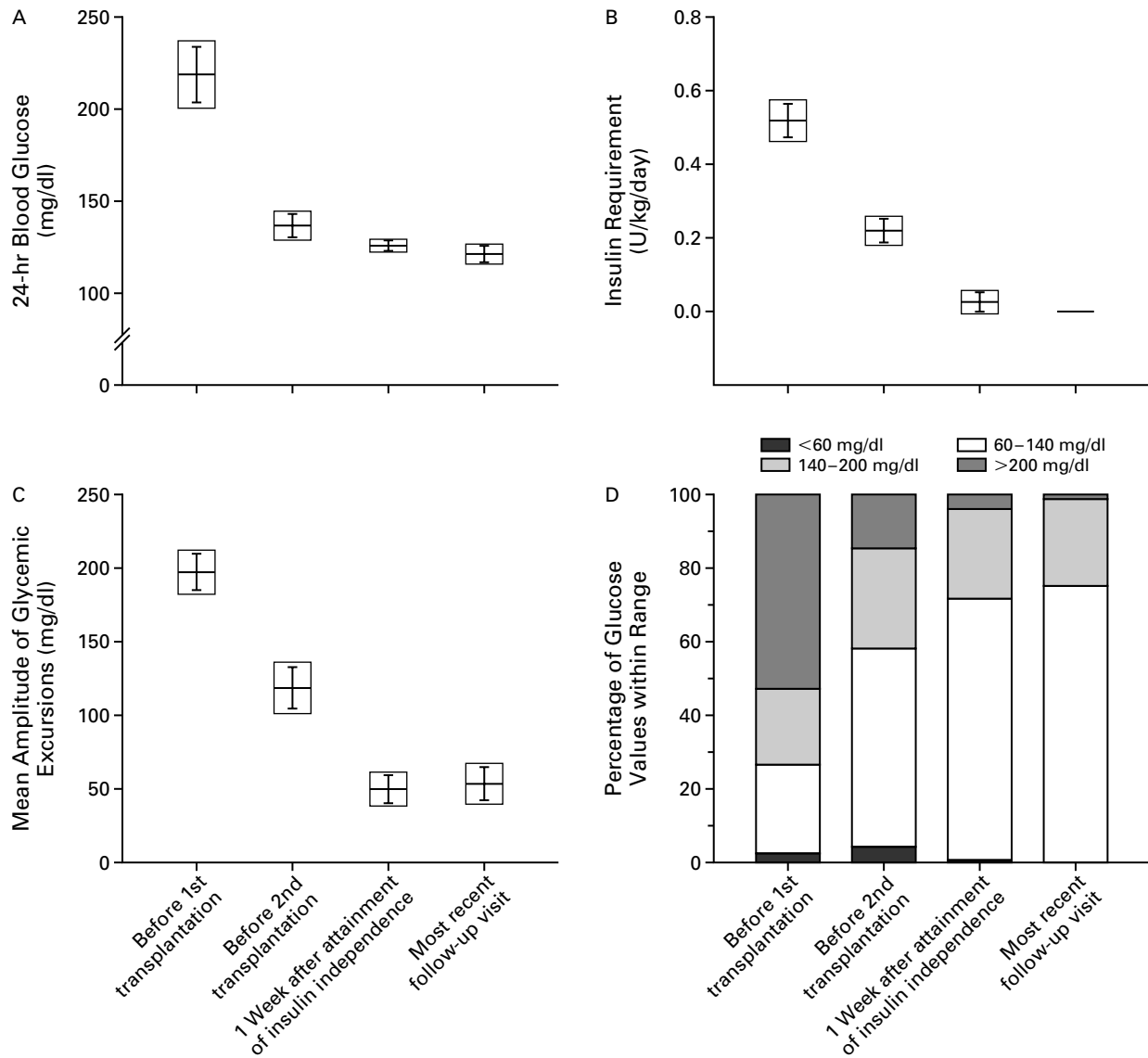


Figure 2. Mean (\pm SE) 24-Hour Blood Glucose Concentrations (Panel A), Mean (\pm SE) Daily Insulin Requirements (Panel B), Mean (\pm SE) Amplitude of Glycemic Excursions (Panel C), and Mean Percentage of Glucose Values That Fell within a Given Range (Panel D) Three Days before the First Islet Transplantation, Three Days before the Second Transplantation, One Week after the Attainment of Insulin Independence, and at the Most Recent Follow-up Visit.

Each box in Panels A, B, and C represents the 95 percent confidence interval. $P < 0.001$ (by analysis of variance) for each comparison of pretransplantation values with subsequent values. Blood glucose was measured seven times a day for the first four weeks, four times a day for the subsequent two months, and a minimum of four times a week thereafter. The mean amplitude of glycemic excursions is a measure of fluctuations in blood glucose concentrations. Values in Panel D were based on a computerized analysis of data from capillary glucose meters. For each value obtained after transplantation, there was a significant decrease in the percentage of glucose values that exceeded 200 mg per deciliter (11.1 mmol per liter) ($P < 0.001$ by analysis of variance) and a significant increase in the percentage of values that were within the range of 60 to 140 mg per deciliter (3.3 to 7.8 mmol per liter) ($P < 0.001$). To convert values for glucose to millimoles per liter, multiply by 0.0555.

the 15 procedures, moderate bleeding occurred at the site of the transhepatic puncture and required transfusion. This complication was subsequently avoided by injecting a Gelfoam plug through the catheter and by reducing the intraportal dose of heparin from 5000 to 500 U.

All patients had minor, superficial ulcerations of the buccal mucosa that resolved after the dose of sirolimus was reduced and the capsule formulation of sirolimus was substituted for the liquid form. None of the patients had sirolimus-related cytopenia. After transplantation, there were no significant increases in lipid con-

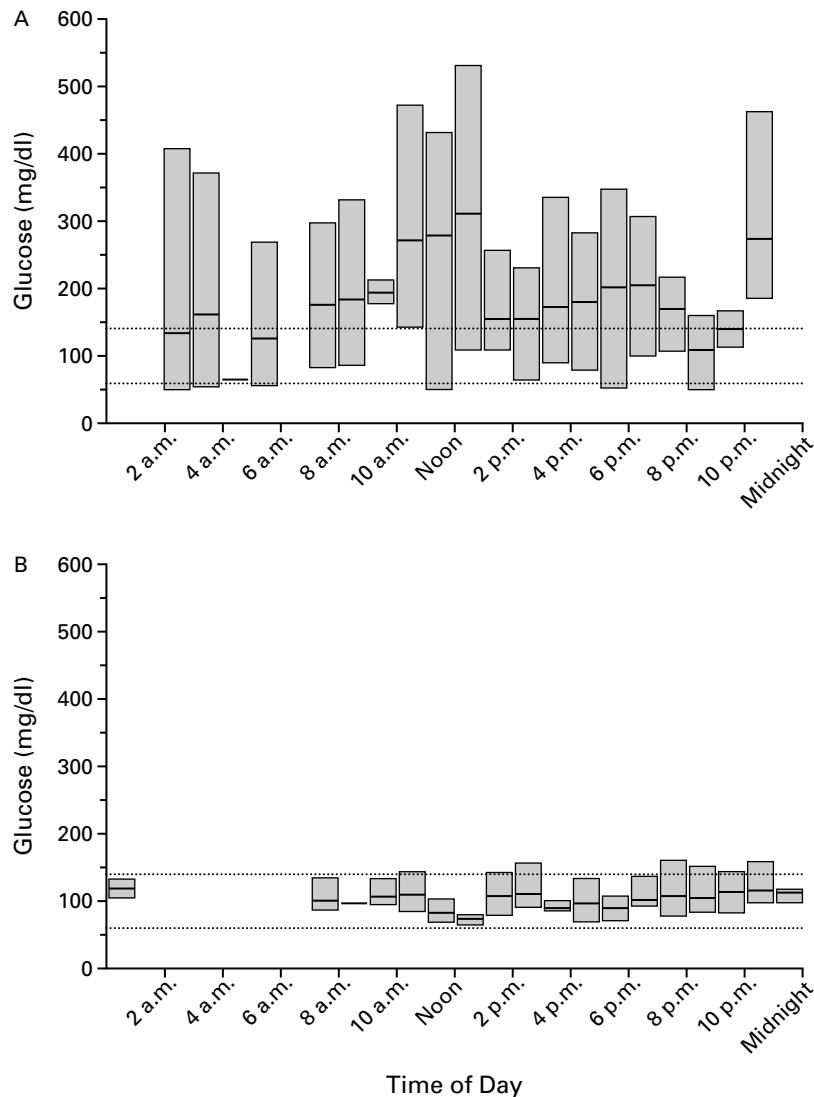


Figure 3. Fluctuations in Blood Glucose Concentrations over a 24-Hour Period One Month before Transplantation (Panel A) and after the Attainment of Insulin Independence (Panel B) in a Representative Patient.

Each bar represents the median and the range. The broken lines represent blood glucose concentrations of 60 and 140 mg per deciliter (3.3 and 7.8 mmol per liter). To convert values for glucose to millimoles per liter, multiply by 0.0555.

centrations and no patient required lipid-lowering therapy (Table 2). There were no significant changes in serum concentrations of creatinine ($P=0.92$), cholesterol ($P=0.90$), or triglycerides ($P=0.46$) during follow-up (Table 2). As of this writing, there has been insufficient follow-up for us to perform a prospective evaluation of secondary diabetic complications.

DISCUSSION

We found that in patients with type 1 diabetes the use of a glucocorticoid-free immunosuppressive protocol in conjunction with islet transplantation quick-

ly resulted in sustained freedom from the need for exogenous insulin. Our results represent an improvement in outcome as compared with previous reports.¹ Transplantation of an initial, suboptimal islet mass halted the episodes of severe hypoglycemia in our patients. Sirolimus, low-dose tacrolimus, and daclizumab provided effective immunosuppression, with no apparent diabetogenic or toxic effects. Indeed, there were no clinically evident episodes of graft rejection, and this combination appears to be effective in preventing autoimmune recurrence of diabetes.

A recent review of the potential barriers to insulin

TABLE 2. RESULTS OF ASSESSMENTS OF ORAL GLUCOSE TOLERANCE, MIXED-MEAL TOLERANCE, GLYCOSYLATED HEMOGLOBIN VALUES, AND SERUM CREATININE AND LIPID CONCENTRATIONS BEFORE AND AFTER TRANSPLANTATION.*

PATIENT NO.	DURATION OF FOLLOW-UP mo	GLUCOSE-TOLERANCE TEST†		MIXED-MEAL TEST‡		GLYCOSYLATED HEMOGLOBIN		CREATININE		CHOLESTEROL		TRIGLYCERIDE		
		GLUCOSE AFTER FASTING	GLUCOSE AT 120 MIN	C PEPTIDE AT 0 MIN	C PEPTIDE AT 90 MIN	GLUCOSE AT 90 MIN	BEFORE TRANSPLANTATION	AT 3 MO	AT 6 MO	BEFORE TRANSPLANTATION	AT FOLLOW-UP	BEFORE TRANSPLANTATION	AT FOLLOW-UP	
1	14.9	103	130	3.6	5.7	150	7.2	6.2	5.7	1.9§	149	155	78	77
2	12.8	97	195	1.4	3.0	157	8.1	5.6	5.9	0.8	210	208	134	117
3	12.3	108	142	1.6	3.6	182	9.0	5.6	5.8	1.1	210	149	86	124
4	11.9	88	139	2.3	5.1	83	8.6	5.7	5.5	1.5	166	189	80	137
5	9.0	110	150	2.8	4.8	106	9.0	5.5	5.8	0.9	247	168	163	93
6	5.5	113	157	2.0	4.8	153	8.5	5.5	—	1.8	180	260	132	167
7	4.4	92	175	—	—	—	8.6	5.8	—	1.0	188	240	66	120
Mean ±SD		101.7±10	155.2±23	2.3±0.8	4.5±1.0	138.4±37	8.4±0.6	5.7±0.2¶	5.7±0.2¶	1.3±0.4	193±33	196±43	105±37	119±29

*Values were determined after insulin independence had been achieved. To convert values for glucose to millimoles per liter, multiply by 0.0555. To convert values for creatinine to micromoles per liter, multiply by 88.4. To convert values for C-peptide to nanomoles per liter, multiply by 0.33. To convert values for cholesterol to millimoles per liter, multiply by 0.026. To convert values for triglyceride to millimoles per liter, multiply by 0.011.

†A total of 75 g of glucose in 300 ml of solution was given over a one-minute period after an overnight fast.

‡A total of 300 ml of a high-protein drink (Ensure) was given over a one-minute period.

§This patient had preexisting renal dysfunction, with a glomerular filtration rate of 46 ml per minute per square meter of body-surface area on radionuclide assay.

¶P<0.001 for the comparison with the value before transplantation by analysis of variance.

independence after islet transplantation identified several factors.²¹ The number of beta cells may be inadequate owing to insufficient engraftment of islets and immediate cellular loss through apoptosis and other nonimmune-mediated inflammatory pathways.^{22,23} The graft may be rejected as a result of ineffective immunosuppression of both alloimmune and autoimmune pathways.²⁴ This event is hard to identify initially, given the lack of tools available for the early diagnosis of rejection.²⁵ The high metabolic demand on the islets that results from preexisting insulin resistance in most patients who undergo combined islet and kidney transplantation is aggravated by the use of diabetogenic immunosuppressant agents.^{4,26} We addressed each of these key factors by transplanting an adequate number of viable, well-characterized islets, which had been prepared in xenoprotein-free medium, and minimizing the duration of cold ischemia. Nonspecific coating of islets by a xenoprotein could theoretically target such cells for immediate destruction. The immunosuppressive regimen that we used protected against alloimmune and autoimmune reactivity. The use of a glucocorticoid-free protocol that included low-dose tacrolimus and daclizumab further minimized the possibility of damaging beta cells and increasing insulin resistance.

Interest in the use of sirolimus increased when its molecular structure was found to be similar to that of tacrolimus.²⁷ Sirolimus-based trials of kidney transplantation reported a substantial reduction in the rate of acute rejection with minimal nephrotoxicity.^{28,29} Preclinical studies of the use of sirolimus with islet transplantation reported prolonged allograft survival and enhanced autograft function.^{30,31} In vitro studies suggested that sirolimus and tacrolimus could not be used in combination, since both drugs bind to the same cytosolic binding proteins (FKBP-12 and FKBP-25).³² This interaction does not occur when the two are used in vivo, and indeed, there is a strong synergistic potentiation of efficacy.^{33,34} The combination of sirolimus, low-dose tacrolimus, and glucocorticoids in liver, kidney, and pancreas transplantation has been associated with extremely low rates of rejection.³⁵

To avoid the diabetogenic effect of glucocorticoids in islet transplantation, we replaced them with daclizumab. This monoclonal antibody against the interleukin-2 receptor has been shown to be safe and effective in renal transplantation, and its use lowered the rates of rejection.³⁶ Daclizumab therapy is given over a 10-week period, thus allowing an extended period for a supplemental islet-transplant procedure. The combined glucocorticoid-free strategy of tacrolimus, sirolimus, and daclizumab therapy prevents activation of the immune cascade by inhibiting T-cell activation, the production of interleukin-2 and other cytokines, binding of the interleukin-2 receptor to its ligand, and the clonal expansion of lymphocytes.³⁷

Our findings show that an infusion of islets from a single donor (a mean of $389,016 \pm 73,769$ islet equivalents in the first transplant) did not result in insulin independence. Since glucocorticoids were not used and thus did not exert any adverse effects on islet function, other factors must be involved. The quantity of islets required to achieve insulin independence is approximately double that reported previously.¹ Recently, one center achieved insulin independence in 14.3 percent of patients after the transplantation of islets from a single donor, but insulin was not withdrawn until a mean of 10.6 months after transplantation.³⁸ In our study the need for more than one donor pancreas per recipient may be interpreted as a drawback, given the shortage of donors. At present, however, less than one third of available cadaveric pancreases are actually transplanted (United Network for Organ Sharing Registry: unpublished data).

In patients with type 1 diabetes, glycemic control can also be achieved with intensive insulin therapy and pancreatic transplantation. Intensive insulin therapy does not normalize glycosylated hemoglobin values and may cause severe hypoglycemia.³⁹ Pancreatic transplantation provides excellent glycemic control, and although the outcome of the procedure has improved dramatically over the past decade, it remains an invasive procedure with a substantial risk of morbidity.⁴⁰ Our findings indicate that islet transplantation alone is associated with a minimal risk and results in good metabolic control, with normalization of glycosylated hemoglobin values and sustained freedom from the need for exogenous insulin.

Supported by the Alberta Foundation for Diabetes Research, by a grant from the Medical Research Council–Juvenile Diabetes Foundation, by the Alberta Health Services Research Innovation Fund, by institutional support from the University of Alberta Hospitals Capital Health Authority, by the Muttart Diabetes Research and Training Centre, and by the Edmonton Civic Employees Charitable Assistance Fund, by the C.F. (“Curly”) MacLachlan and Gladys B. MacLachlan Fund, and by the University Hospital Foundation. Drs. Lakey and Korbitt are recipients of scholarships from the Canadian Diabetes Association and the Alberta Heritage Foundation for Medical Research. Drs. Warnock and Kneteman are Senior Scholars of the Alberta Heritage Foundation for Medical Research.

We are indebted to Roche Canada, Wyeth–Ayerst Canada, and Fujisawa Canada for their generous gifts of daclizumab, sirolimus, and tacrolimus, respectively; to all the technical staff members of the human islet transplant laboratory, including Dr. Tatsuya Kin, for their expertise; to Barbara Waters and Ingrid Larsen (islet transplantation coordinators) for excellent patient care; to Dr. Dalila Barama, Dawn Saik, Sharleen Innes, and the staff members of the clinical investigation unit and the University of Alberta Hospitals metabolic center for assistance with metabolic monitoring of patients; to our colleagues in interventional radiology for their assistance; to the organ-procurement programs in Alberta and across Canada for identifying cadaveric donors; to Dr. George Eisenbarth, Barbara Davis Diabetes Center, Denver, for assistance with the evaluation of autoimmune markers; and to Dr. Jonathan Levy, Radcliffe Infirmary, Oxford, United Kingdom, for providing the Homadisk program.

REFERENCES

1. Brendel M, Hering B, Schulz A, Bretzel R. International Islet Transplant Registry report. Giessen, Germany: University of Giessen, 1999:1-20.

2. Linetsky E, Bottino R, Lehmann R, Alejandro R, Inverardi L, Ricordi C. Improved human islet isolation using a new enzyme blend, liberase. *Diabetes* 1997;46:1120-3.
3. Lakey JR, Warnock GL, Shapiro AM, et al. Intraductal collagenase delivery into the human pancreas using syringe loading or controlled perfusion. *Cell Transplant* 1999;8:285-92.
4. Zeng YC, Ricordi C, Lendoire J, et al. The effect of prednisone on pancreatic islet autografts in dogs. *Surgery* 1993;113:98-102.
5. Weinand S, Jahr H, Hering BJ, Federlin K, Bretzel RG. Oxygen radical production in human mononuclear blood cells is not suppressed by drugs used in clinical islet transplantation. *J Mol Med* 1999;77:121-2.
6. Pak CY, Eun HM, McArthur RG, Yoon JW. Association of cytomegalovirus infection with autoimmune type 1 diabetes. *Lancet* 1988;2:1-4.
7. Numazaki K, Goldman H, Seemayer TA, Wong I, Wainberg MA. Infection by human cytomegalovirus and rubella virus of cultured human fetal islets of Langerhans. *In Vivo* 1990;4:49-54.
8. Darenkov IA, Marcarelli MA, Basadonna GP, et al. Reduced incidence of Epstein-Barr virus-associated posttransplant lymphoproliferative disorder using preemptive antiviral therapy. *Transplantation* 1997;64:848-52.
9. Lakey JR, Warnock GL, Rajotte RV, et al. Variables in organ donors that affect the recovery of human islets of Langerhans. *Transplantation* 1996;61:1047-53.
10. Ricordi C, Lacy PE, Scharp DW. Automated islet isolation from human pancreas. *Diabetes* 1989;38:Suppl 1:140-2.
11. Brandhorst H, Brandhorst D, Brendel MD, Hering BJ, Bretzel RG. Assessment of intracellular insulin content during all steps of human islet isolation procedure. *Cell Transplant* 1998;7:489-95.
12. Rosenberg L, Wang R, Paraskevas S, Maysinger D. Structural and functional changes resulting from islet isolation lead to islet cell death. *Surgery* 1999;126:393-8.
13. Vargas F, Vives-Pi M, Somoza N, et al. Endotoxin contamination may be responsible for the unexplained failure of human pancreatic islet transplantation. *Transplantation* 1998;65:722-7.
14. Ricordi C, Gray DW, Hering BJ, et al. Islet isolation assessment in man and large animals. *Acta Diabetol Lat* 1990;27:185-95.
15. Korbitt GS, Elliott JF, Ao Z, Smith DK, Warnock GL, Rajotte RV. Large scale isolation, growth, and function of porcine neonatal islet cells. *J Clin Invest* 1996;97:2119-29.
16. Shapiro AM, Lakey JR, Rajotte RV, et al. Portal vein thrombosis after transplantation or partially purified pancreatic islets in a combined human liver/islet allograft. *Transplantation* 1995;59:1060-3.
17. Service FJ, Molnar GD, Rosevear JW, Ackerman E, Gatewood LC, Taylor WF. Mean amplitude of glycemic excursions, a measure of diabetic instability. *Diabetes* 1970;19:644-55.
18. Levy JC, Matthews DR, Hermans MP. Correct homeostasis model assessment (HOMA) evaluation uses the computer program. *Diabetes Care* 1998;21:2191-2.
19. Verge CF, Stenger D, Bonifacio E, et al. Combined use of autoantibodies (IA-2 autoantibody, GAD autoantibody, insulin autoantibody, cytoplasmic islet cell antibodies) in type 1 diabetes: Combinatorial Islet Autoantibody Workshop. *Diabetes* 1998;47:1857-66.
20. Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. *Diabetes Care* 1997;20:1183-97.
21. Hering B, Ricordi C. Islet transplantation for patients with type 1 diabetes. *Graft* 1999;2:12-27.
22. Kaufman DB, Gores PF, Field MJ, et al. Effect of 15-deoxyspergualin on immediate function and long-term survival of transplanted islets in murine recipients of a marginal islet mass. *Diabetes* 1994;43:778-83.
23. Bennet W, Sundberg B, Groth CG, et al. Incompatibility between human blood and isolated islets of Langerhans: a finding with implications for clinical intraportal islet transplantation? *Diabetes* 1999;48:1907-14.
24. Kenyon NS, Ranunco A, Masetti M, Chatzipetrou M, Ricordi C. Islet transplantation: present and future perspectives. *Diabetes Metab Rev* 1998;14:303-13.
25. Shapiro AM, Hao E, Lakey JR, Elliott JF, Rajotte RV, Kneteman NM. Development of diagnostic markers for islet allograft rejection. *Transplant Proc* 1998;30:647.
26. Drachenberg CB, Klassen DK, Weir MR, et al. Islet cell damage associated with tacrolimus and cyclosporine: morphological features in pancreas allograft biopsies and clinical correlation. *Transplantation* 1999;68:396-402.
27. Sehgal SN, Baker H, Vezina C. Rapamycin (AY-22,989), a new antifungal antibiotic. II. Fermentation, isolation and characterization. *Antibiot (Tokyo)* 1975;28:727-32.
28. Kahan BD, Podbielski J, Napoli KL, Katz SM, Meier-Kriesche HU, Van Buren CT. Immunosuppressive effects and safety of a sirolimus/cyclosporine combination regimen for renal transplantation. *Transplantation* 1998;66:1040-6.
29. Groth CG, Backman L, Morales JM, et al. Sirolimus (rapamycin)-based therapy in human renal transplantation: similar efficacy and different toxicity compared with cyclosporine. *Transplantation* 1999;67:1036-42.
30. Yakimets WJ, Lakey JR, Yatscoff RW, et al. Prolongation of canine pancreatic islet allograft survival with combined rapamycin and cyclosporine therapy at low doses: rapamycin efficacy is blood level related. *Transplantation* 1993;56:1293-8.
31. Kneteman NM, Lakey JR, Wagner T, Finegood D. The metabolic impact of rapamycin (sirolimus) in chronic canine islet graft recipients. *Transplantation* 1996;61:1206-10.
32. Kahan BD. Cyclosporin A, FK506, rapamycin: the use of a quantitative analytic tool to discriminate immunosuppressive drug interactions. *J Am Soc Nephrol* 1992;2:Suppl:S222-S227.
33. Chen H, Qi S, Xu D, et al. Combined effect of rapamycin and FK 506 in prolongation of small bowel graft survival in the mouse. *Transplant Proc* 1998;30:2579-81.
34. Vu MD, Qi S, Xu D, et al. Tacrolimus (FK506) and sirolimus (rapamycin) in combination are not antagonistic but produce extended graft survival in cardiac transplantation in the rat. *Transplantation* 1997;64:1853-6.
35. McAlister VC, Gao Z, Peltekian K, Domingues J, Mahalati K, MacDonald AS. Sirolimus-tacrolimus combination immunosuppression. *Lancet* 2000;355:376-7.
36. Vincenti F, Kirkman R, Light S, et al. Interleukin-2-receptor blockade with daclizumab to prevent acute rejection in renal transplantation. *N Engl J Med* 1998;338:161-5.
37. Halloran PF. T-cell activation pathways: a transplantation perspective. *Transplant Proc* 1999;31:769-71.
38. Bretzel RG, Brandhorst D, Brandhorst H, et al. Improved survival of intraportal pancreatic islet cell allografts in patients with type-1 diabetes mellitus by refined peritransplant management. *J Mol Med* 1999;77:140-3.
39. The Diabetes Control and Complications Trial Research Group. Hypoglycemia in the Diabetes Control and Complications Trial. *Diabetes* 1997;46:271-86.
40. Bartlett ST, Schweitzer EJ, Johnson LB, et al. Equivalent success of simultaneous pancreas kidney and solitary pancreas transplantation: a prospective trial of tacrolimus immunosuppression with percutaneous biopsy. *Ann Surg* 1996;224:440-52.