Rapid Publications

Insulin Independence After Islet Transplantation Into Type I Diabetic Patient

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Effective clinical trials of islet transplantation have been limited by the inability to transplant enough viable human islets into patients with type I (insulin-dependent) diabetes mellitus to eliminate their exogenous insulin requirement. We report the first type I diabetic patient with an established kidney transplant on basal cyclosporin immunosuppression who was able to eliminate the insulin requirement after human islet transplantation into the portal vein. We successfully isolated ~800,000 islets that were 95% pure from 1.4 cadaver pancreases containing 121 U of insulin. Islets were proven viable by in vitro insulin response to glucose challenge. After 7 days of 24°C culture, the islets were transplanted into the portal vein under local anesthesia. Seven days of Minnesota antilymphoblast globulin (20 mg/kg) administration followed the islet transplantation, with maintenance of the cyclosporin. Blood glucose was kept under strict control via intravenous insulin for 10 days posttransplantation, when all insulin therapy was stopped. Off insulin, the average 24-h blood glucose level remained <150 mg/dl, with the fasting glucose level at 115 ± 6 mg/dl and the 2-h postprandial level at 141 ± 8 mg/dl for 22 days posttransplantation (the time of this study). The C-peptide values post-Sustacal testing, although initially rising slower, exceeded the normal range, with peak values of 1.0–1.8 pmol/ml. This preliminary result represents the first essential step required to determine the feasibility of islet transplantation by future clinical trials. *Diabetes* 39:515–18, 1990

Islet transplantation in diabetic rodents and canines successfully eliminates the requirement for exogenous insulin and protects recipients from the complications of diabetes (1). However, with the increasing success of human pancreas transplantation (2,3) and with the lack of success of islet transplantation to eliminate the exogenous insulin requirements in diabetic patients, questions have risen as to the potential of islet-transplant therapy for patients with type I (insulin-dependent) diabetes mellitus. This is a preliminary study of the first patient with type I diabetes who has been able to discontinue exogenous insulin after transplantation of islets isolated and purified from cadaver-donor pancreas. Although many important questions remain as to the degree and duration of islet function in this patient, this preliminary observation of insulin independence after islet transplantation represents the necessary first step to permit effective clinical trials of this procedure in type I diabetic patients.

RESEARCH DESIGN AND METHODS

Islet isolation. Cadaver-donor pancreases (human immune deficiency virus and hepatitis negative) were procured through regional Organ Procurement Organizations from multiple cadaver organ donors with coordination by the National Disease Research Institute. The pancreas was removed after flushing with University of Wisconsin solution, and then the organs were shipped to us. Islets were isolated in a class 100 laminar-flow room in class 10 hoods via the digestion-filtration procedure with collagenase (Boehringer Mannheim, Mannheim, FRG) as previously described (4). The islets were purified by centrifugation on Ficoll gradients (Sigma, St. Louis, MO) prepared with Eurocollins solution (Fresenius, Runcorn, UK) at densities of 1.091 and 1.108 (D.W.S., B.J. Oleck, E.H. Finke, L. Falqui, P.E.L., unpublished observations). Pooled islets were cultured overnight in uncoated 75-ml T flasks (Corning) containing 50 ml CMRL-1066 medium supplemented with 10% fetal calf serum, L-glutamine, HEPES at pH 7.4, and penicillin-streptomycin at 37°C followed by 24°C culture for 7 days. For this patient, one

Glucose 1 mM = 18 mg/dl

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complete pancreas and a part of a second pancreas were used for islet isolation.

Islet counts were made by islet size followed by calculation of islet equivalents (number of islets if all were 150 μm in diameter). Islet purity was also confirmed. Insulin extraction of the islet preparation was performed with acid-alcohol. Viability testing was performed at days 1 and 6 after isolation via perifusions with glucose concentrations of 3.3, 16.7, and 16.7 mM plus theophylline (10 mM) and 3.3 mM glucose after overnight culture at 37°C (4). The insulin assay was performed by the Diabetes Research and Training Center's (DRTC) Radioimmunoassay (RIA) Core Facility. Histology was performed with aldehyde fuchsin staining by the DRTC Histology Core Facility. Bacteriologic testing was performed on the pancreas transport fluid, at each step of the islet isolation process, and after islet culture.

Transplant recipients. Established diabetic kidney allograft recipients with stable kidney function were screened for islet-transplant selection. A Sustacal (Mead Johnson Nutritionals, Evansville, IN) challenge test was performed to confirm the total lack of stimulated C-peptide secretion (<0.01 pmol/ml).

The 36-yr-old patient described herein had diabetes for 27 yr with many documented episodes of diabetic ketoacidosis as a teenager. Before islet transplantation, she was on 30–35 U insulin/day with a glycosylated hemoglobin level of 13.6%. In 1983, she had a previous kidney-pancreas transplant that failed followed by a successful kidney transplant with a current creatinine clearance of 50 ml/min.

Transplant procedure. The islets were transplanted under local anesthesia (1% lidocaine) via a short midline abdominal incision to expose the umbilical vein, which was then cannulated. The catheter was passed into the portal vein and injected with contrast medium to confirm its location by X ray. Purified islets were suspended in 120 ml of Hanks' salt solution supplemented with 2% human albumin and were injected in 10-ml aliquots every 2 min to allow the monitoring of portal venous pressure after each injection. Heparin (3000 U i.v.) was given systemically before the islet injection. After the transplantation, the catheter was removed, and the fascia and skin were closed with sutures.

Immunosuppression. The patient had been on a stable cyclosporin dosage of 500 mg once daily for the existing kidney transplant with a pre–islet transplantation 12-h trough level of 151 ng/ml (whole-blood high-performance liquid chromatography). After negative skin testing, 7 days of Minnesota antilymphoblast globulin (MALG) was given for the islet transplant at 20 mg/kg starting 1 day after transplantation. The patient was premedicated with methylprednisolone (1 mg/kg), Benadryl, and acetaminophen for the first three doses of MALG. Imuran (1 mg/kg) was added. There were no other changes made in her immunosuppression.

Metabolic monitoring and testing. The patients for this protocol were observed in the General Clinical Research Center (GCRC), where hourly glucose determinations were made to keep the serum glucose between 80 and 150 mg/dl via intravenous insulin for the first 7 days after transplantation. Increases in insulin (5 times the basal rate) were given for the hour of each meal. The 2nd wk of insulin therapy was determined by the needs of the patient. This patient had an oral Sustacal test (5) before transplantation and 1 wk, 10 days, and 2 wk after transplantation that measured glucose (Beckman glucose analyzer, Fullerton, CA) and C-peptide performed by the DRTC RIA Core Facility. All Sustacal tests were performed without insulin on the day of the test except for the 1-wk posttransplantation test, when 0.2 U insulin/h was given to protect the islets from possible hyperglycemia.

RESULTS AND DISCUSSION

The first pancreas donor provided a partial pancreas weighing 41 g from which 206,400 islets were isolated with 240,980 islet equivalents, an islet volume of 0.43 ml, an insulin content of 37 U, and a purity of 95% (Table 1). This islet preparation was a three-antigen match with the recipient, two of which were HLA-DR (Table 1).

Because the quantity of purified islets was considered inadequate for a transplant, they were combined with those from a second donor of the same blood type (A) obtained 2 days later. This donor provided a whole pancreas weighing 110 g from which 440,400 islets were isolated with 541,570 islet equivalents, an islet volume of 0.96 ml, an insulin content of 84 U, and a purity of 95%. The second islet preparation was a four-antigen match, one of which was HLA-DR (Table 1). Cross matches from both donors' spleen cells were negative for recipient serum. The islets were cultured at 37°C overnight and then at 24°C for 7 days for the first donor and 5 days for the second donor until the islet transplantation. Perifusion tests (4) for each set of islets demonstrated a normal insulin response to glucose stimulation (16.7 mM) over basal glucose (3.3 mM), with an average of a 9.0 ± 2.3-fold insulin increase in the first 15 min after glucose stimulation. Histology of each set confirmed excellent purified islets. Bacteriologic cultures revealed sterile pancreas transport fluid and sterile aliquots from each stage of the islet processing and culture.

The islet transplantation was performed under local anesthesia with no rise in portal venous pressure during or after the islet injection. There was no change in liver function tests or serum amylase after transplantation.

Figure 1 presents the 24-h average serum glucose and insulin requirement for the first 22 days after transplantation. Basal insulin supplementation was stopped after 6 days, with only intravenous insulin given for the mealtime hour. All exogenous insulin therapy was stopped after 10 days, with acceptable glycemic control as shown.

Figure 2 presents the results of provocative testing with

TABLE 1
Donor and recipient data

	Islets (*n*)	Pancreas weight (g)	HLA typing		
			A	B	DR
Recipient			1/24	35/44	1/4
1983 donors					
Kidney-pancreas			1/24	44/57	6/0
Kidney only			2/0	1/8	*
Islet donors					
1	240,980	40	3/28	35/16	1/4
2	541,570	110	2/24	35/44	4/7
Total	782,550				

*Not available.

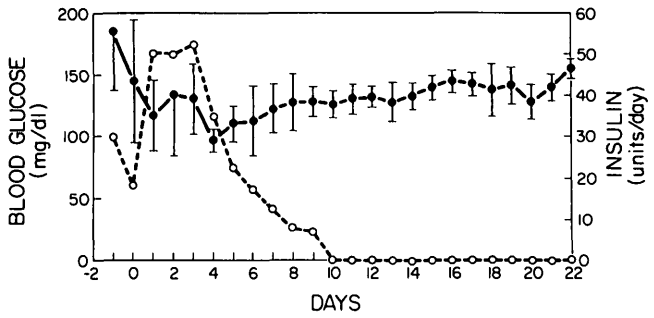

FIG. 1. Insulin glycemic profile before and after islet transplantation into patient with established kidney allograft. Average 24-h serum glucose level (●, means ± SD) is compared with 24-h insulin requirement (○).

an oral Sustacal challenge compared with those of 10 nondiabetic volunteers. Before islet transplantation, this patient had a maximal glucose response >300 mg/dl while on insulin, with no stimulated C-peptide secretion. After islet transplantation, the maximal glucose responses off insulin ranged from 183 to 206 mg/dl and returned to <160 mg/dl by 4 h. The fasting C-peptide values were all above control fasting levels after transplantation. In response to Sustacal challenge, the maximally stimulated C-peptide levels all markedly exceeded the normal values yet peaked later. Although the patient remained glucose intolerant off insulin, her level of glycemic control was in an acceptable range, with fasting glucose levels of 115 ± 6 mg/dl and 2-h postprandial values of 141 ± 8 mg/dl. Even though her basal immunosuppression did not include glucocorticoids, the elevated C-peptide levels seemed consistent with a degree of insulin resistance in the face of slightly impaired kidney function.

This is the first demonstration of the ability of purified human islets transplanted intraportally as an allograft to achieve exogenous insulin independence in a patient with type I diabetes. Note that during preparation of this manuscript and after significant personal stresses, including a family death, the recipient's fasting glucose level rose above 140 mg/dl, and her postprandial glucose level rose above 200 mg/dl, requiring reassessment in the GCRC 25 days after trans-

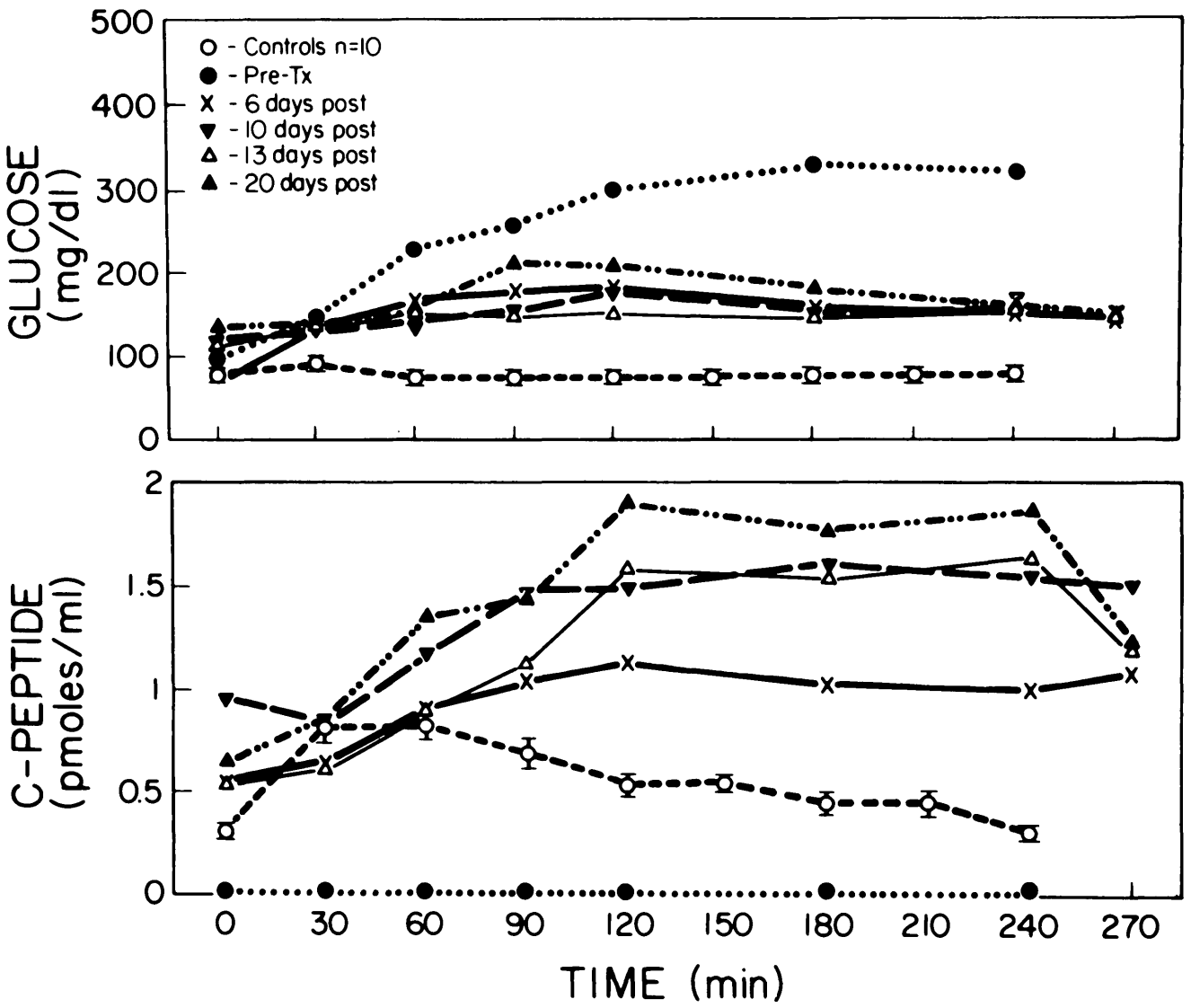

FIG. 2. Glycemic and C-peptide responses to oral Sustacal challenge test. Means ± SE of responses from 10 nondiabetic volunteers are compared with those of islet-transplant recipient at different times after transplantation.

plantation. Exogenous insulin therapy was reinstituted at 14–24 U/day. A repeat Sustacal challenge was performed showing a drop in her peak C-peptide response to 0.5 pmol/ml with a glucose level of 295 mg/dl. Whether this represents a marked stress response or a rejection of part of the transplanted islets is unclear. She is currently continuing at this level of insulin therapy.

Many questions raised can now be answered with additional clinical trials of islet transplantation. Initially, it is necessary to know how to optimally reproduce this result determining the correct islet dosage, duration of insulin therapy after transplantation, duration of islet culture, initial form of immunosuppression, potential of additional sites, and importance of HLA-DR matching. Expansion of these trials to patients with diabetes who are approaching kidney transplantation are in progress with the kidney and islets from the same donor.

The ultimate objective of islet transplantation is to be able to transplant islets sufficiently early in the course of diabetes to prevent or stabilize the complications of diabetes without the use of long-term immunosuppression. These clinical findings justify investigating optimal ways of transplanting islets without immunosuppression, e.g., by immunoalteration or immunoisolation techniques (1). This preliminary result is the first essential step toward determining whether islet transplants can function long term in patients with type I diabetes.

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