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Normoglycaemia after transplantation of freshly isolated and cryopreserved pancreatic islets in Type 1 (insulin-dependent) diabetes mellitus

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Summary. Purified islets of Langerhans and a kidney were transplanted into a 36-year-old patient who suffered from renal failure secondary to a 25 year history of Type 1 (insulin-dependent) diabetes mellitus. The islet graft contained 243 000 fresh islets (mean islet diameter 150 μm) that were syngeneic with the kidney graft and 368 000 cryopreserved islets that had been collected from four other donors. The total of 10 000 islets/kg body weight was infused into the liver via the umbilical vein. Immunosuppression was induced with antilymphocyte globulin and maintained with prednisone, cyclosporine and azathioprine. Serum C-peptide levels (ng/ml) during fasting and after standard mixed meal feeding (Sustacal) were < 0.12 preoperatively. Postoperatively, insulin secretion was restored: fasting C-peptide rose during the first 4 weeks to levels of 4 to 5 and Sustacal elicited a further rise to 6 to 7. Transplant renal function was stable. Daily fasting glucose (mmol/l, mean ± SD) was 5.6 ± 1 and 5.3 ± 0.6 during the first and second months respectively and post-Sustacal glucose was 5.7 ± 0.8. Exogenous insulin therapy was progressively withdrawn and stopped during the ninth week. Thereafter, fasting glucose was 4.7 ± 0.5, 24 h mean glucose was 6.6 ± 0.5, and normoglycaemia was maintained after Sustacal. These data show that this mass of freshly isolated and cryopreserved islets from multiple donors provided sustained function (3 months) that reversed insulin-dependence in an immunosuppressed Type 1 diabetic patient treated with simultaneous islet-kidney transplantation.

Key words: Pancreatic islet transplantation, cryopreservation, Type 1 (insulin-dependent) diabetes, tissue bank.

Several advances have made it feasible to initiate a clinical trial of pancreatic islet transplantation for Type 1 (insulin-dependent) diabetes mellitus in our research centre. Mass isolation of purified human islets has improved significantly [1, 2] and dose response studies have defined the critical mass of autogenous or allogeneic islet tissue needed to reverse Type 1 diabetes in dogs treated with cyclosporine (CsA) [3]. Initial clinical transplants using a similar islet mass resulted in sustained insulin production, however, exogenous insulin therapy could not be withdrawn [4], suggesting that an increased Beta-cell mass is needed in human subjects with long-term diabetes. An approach to this problem is to collect and store islets in a tissue bank until sufficient donor tissue is available to treat an individual recipient, such as storage of islets by cryopreservation [5]. The present report details the results of transplantation of a combination of freshly-isolated and cryopreserved islets from multiple human donors into a Type 1 diabetic patient.

Subjects and methods

Patient

A 36-year-old woman had Type 1 diabetes resulting in proliferative retinopathy, neuropathy and end-stage nephropathy. Insulin therapy of 0.6 U/kg was required daily before onset of clinical nephropathy. Serum C-peptide was 0.12 ng/ml during fasting and did not rise after Sustacal or glucagon. Haemoglobin A_1c was 0.061 one month preoperatively. Procedures were performed with informed consent from the patient and in accordance with principles of the Declaration of Helsinki.

Islet isolation

Human pancreases were recovered from adult cadaver organ donors at teaching hospitals affiliated with the University of Alberta. The pancreases were removed before in situ flush and processed within 2 h by collagenase ductal perfusion, gentle dissociation and Ficoll

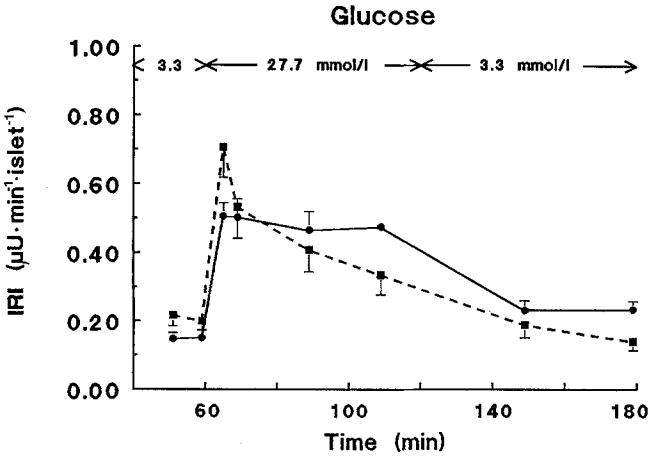

Fig. 1. Mean ± SEM insulin (IRI) release during perifusion of duplicate aliquots of freshly-isolated islets (●—●, $n = 2$ perifusion chambers) and cryopreserved islets from 4 donors (■---■, $n = 6$ chambers) before transplantation

gradient purification [1]. To assess islet quantity, aliquots of the purified suspension were stained with dithizone [6] and examined at microscopy immediately before transplantation. The number of islets was counted within each size range of 60–99 μm, 100–199 μm, 200–299 μm, 300–399 μm and >400 μm and converted to a number of islets equivalent to a diameter of 150 μm. Purity for insulin-containing islets was estimated by comparing the relative proportions of dithizone-stained and unstained tissue. Functional viability was assessed by perifusion in Krebs' ringer bicarbonate buffer supplemented with 3.3 mmol/l glucose for 1 h, then with 27.7 mmol/l glucose for 1 h, followed by an additional hour at 3.3 mmol/l. Perifusate was collected and assayed for insulin content by radioimmunoassay using kits (Pharmacia, Uppsala, Sweden).

Islet cryopreservation

Islets were cryopreserved according to the protocol of Rajotte [5]. Briefly, aliquots of 10000 islets were incubated in dimethylsulfoxide at increasing concentrations of 0.66, 1 and 2 mol/l, then supercooled to −7.6°C. Following ice nucleation and release of latent heat of fusion, controlled cooling of 0.25°C/min was used from −10°C to −40°C then the islets were plunged into liquid nitrogen (−196°C) for storage from 6 months to 1 year. Immediately before transplantation, they were thawed rapidly (150°C/min) and the cryoprotectant removed using a sucrose dilution (0.75 mol/l).

Transplant procedure

A silastic catheter was inserted via the umbilical vein when the kidney transplant was completed. The tip of the catheter was advanced to the left portal vein and secured. Twenty-four hours later, the freshly isolated and frozen-thawed islets were each suspended in medium 199. Each was infused via the catheter while portal venous pressure was monitored. Immunosuppression was induced with a 10 day course of Minnesota antilymphoblast globulin (MALG) and maintained with azathioprine, prednisone and CsA.

Patient monitoring and follow-up

During the first 14 postoperative days, insulin was infused intravenously to maintain capillary blood glucose as measured by test strips (Glucometer, Miles Canada Inc., Etobicoke, Ontario, Canada) between 4 and 7 mmol/l. During subsequent weeks, subcutaneous insulin was administered in decreasing doses to maintain similar glycaemic levels. Fasting serum glucose and C-peptide concentrations were measured daily postoperatively for 14 days, then 3 to 4 times weekly. At weekly intervals insulin therapy was withheld after 18.00 hours and C-peptide was measured after an 8 to 12 h overnight fast and 90 min after 360 ml of oral Sustacal (Mead-Johnson, Evansville, Ind., USA). At 69 days after islet transplantation, insulin therapy was stopped and glucose tolerance and C-peptide responses were measured for 4 h after Sustacal. C-peptide was measured by radioimmunoassay using kits (Incstar, Stillwater, Minn., USA).

Results

Fresh islets syngeneic with the donor kidney provided a dose of 4000 islets (volume 7.1 μl)/kg recipient body weight. Cryopreserved islets from four other donors provided 6072 islets (10.8 μl)/kg. Microscopic examination of the graft revealed a composition of 75% dithizone-positive tissue and the packed tissue volume was 2.5 ml. Portal venous pressure remained constant during islet infusion. HLA matching for the fresh islets showed that four antigens were matched with the recipient. For the cryopreserved islets, two donors had a one antigen match, one donor had a two antigen match, and one had no shared antigens. In no instance was an HLA-DR antigen shared. Donors and recipient were seropositive for cytomegalovirus.

The responses of donor islets to perifusate glucose are shown in Fig. 1. Fresh and cryopreserved islets showed a three-fold rise in first phase insulin release, from 0.15 to 0.51 $\mu U \cdot islet^{-1} \cdot min^{-1}$ and from 0.19 to 0.71 respectively. Second phase secretion was higher and more sustained in the fresh islets. Insulin levels returned to basal when the glucose concentration was reduced.

Postoperative immunosuppression and graft function during the first month are detailed in Figure 2a. MALG was initially given for 10 days, followed by CsA (whole blood trough levels 300–400 ng/ml). Azathioprine was maintained at 2 $mg \cdot kg^{-1} \cdot day^{-1}$ and prednisone at 0.5 to 0.6 $mg \cdot kg^{-1} \cdot day^{-1}$. Islet function (serum C-peptide level) was detected immediately after islet implantation. Fasting C-peptide levels (ng/ml) rose progressively to 4 to 5 and stabilized at this level. Simultaneous mean (± SD) fasting glucose was 5.6 ± 1 mmol/l. At weekly intervals, Sustacal elicited further increases in C-peptide, with peak values of 7.1 at 3 and 4 weeks (corresponding glucose 6.4). Insulin dosage ($IU \cdot kg^{-1} day^{-1}$) were progressively reduced from 2.2 before the transplant to 0.71. Mean daily glucose values obtained by blood glucose monitoring ranged from 5.5 to 8.1. Renal transplant function was stable with serum creatinine < 100 μmol/l after day 5, except for a slight increase when CsA level exceeded 400 ng/ml.

During the second month (Fig. 2b), the dosages of immunosuppressive drugs were tapered. Fasting C-peptide levels were 3 to 5 (simultaneous glucose 5.3 ± 0.6) and stimulated C-peptide levels were 4 to 6 (simultaneous glucose 5.7 ± 0.7). Insulin therapy was further reduced to 0.25 $IU \cdot kg^{-1} day^{-1}$. During this time, glucose monitoring showed mean 24 h levels ranging from 5.3–8.2. Renal transplant function remained stable.

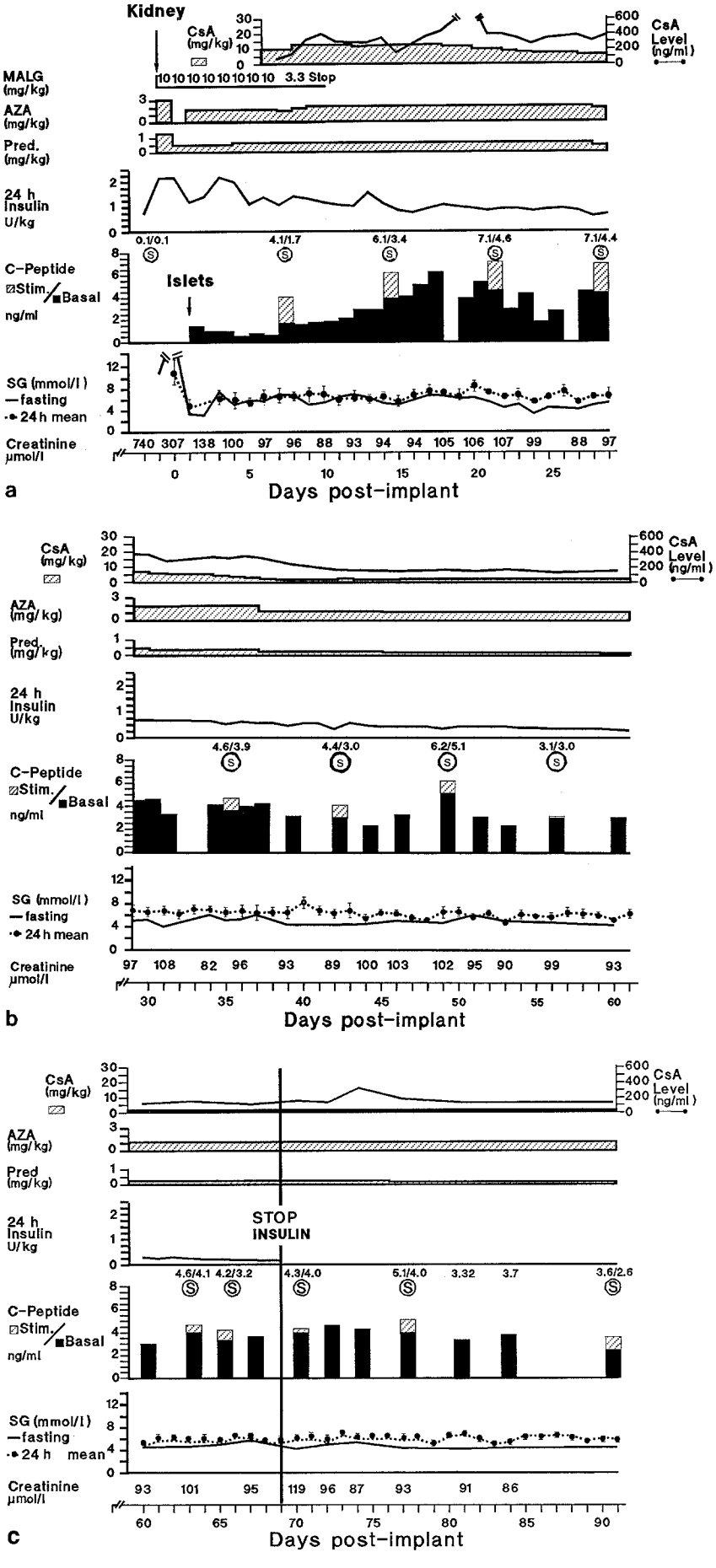

Fig. 2 a–c. Detailed profile of immunosuppression with cyclosporine (CsA), Minnesota antilymphoblast globulin (MALG), azathioprine (AZA) and prednisone (Pred) and corresponding 24 h insulin requirements, serum C-peptide, serum glucose (SG) and serum creatinine concentrations for the first (**a**), second (**b**) and third (**c**) months after transplantation of islets and kidney into a Type 1 diabetic recipient. (S) denotes Sustacal stimulation

During the third month (Fig. 2c), fasting serum glucose ranged from 4.2 to 5.6 and 24 h mean glucose was from 5.8 to 7.5. Serum HbA_1c was normal at 0.04. At 69 days after islet transplantation, insulin therapy was discontinued. Thereafter, mean fasting glucose was 4.7 ± 0.5 and 24 h glucose remained at 5.4 to 7.5 for 3 weeks (the time of this manuscript). Serum glucose values for 4 h after oral Sustacal at 65 days after transplantation ranged from 4.9 to 6.7 (C-peptide 3.2 to 4.9). Repeat assays at 77 days revealed glucose levels of 5.2 to 6.3 (corresponding C-peptide 3.6 to 5.3).

Discussion

Eleven cases of synchronous kidney-islet transplantation from human cadaver donors into immunosuppressed diabetic patients have been reported. A single adult recipient was reported by Largiader and associates to have stopped insulin therapy after implantation of 200,000 unpurified islets into the spleen [7]. That report did not document the preoperative insulin dependent status and postoperative islet function according to C-peptide levels. In further studies from our laboratory, a total of 250,000 purified islets provided prolonged Beta-cell secretion but this was insufficient to eliminate insulin therapy [4]. Compared with these earlier studies, a similar quantity of freshly isolated islets were also implanted in the present patient who was able to discontinue insulin therapy. These findings suggest that the additional Beta-cell function was at least partly attributable to the increased islet mass provided by the 368,000 cryopreserved islets, but further studies will be needed to verify this observation.

The present data compares favourably with that of Scharp et al. [8] who transplanted islets into a patient who did not receive a synchronous kidney transplant or prolonged steroid immunosuppression. Despite the larger islet mass used by Scharp et al., C-peptide levels were lower, and the glucose levels were higher than those observed in the present patient. The duration of insulin independence in their patient was 12 days.

Serum C-peptide levels in the present patient exceeded those observed in normal subjects and are similar to levels observed in non-diabetic recipients of renal allografts or diabetic recipients of vascularized pancreas allografts who are rendered insulin-independent [9]. These higher C-peptide levels, in the face of normal renal transplant function, are consistent with a degree of insulin resistance attributable to glucocorticoid therapy.

We administered exogenous insulin immediately after islet transplantation in order to minimize the potential for hyperglycaemia associated with the insulin resistant state created by immunosuppressive therapy, including high doses of glucocorticoids. However, insulin requirements were found to decrease progressively as immunosuppressive drugs were tapered, until at 69 days, insulin therapy could be completely withdrawn and normoglycaemia was maintained. Whereas we cannot state whether administration of insulin to avoid hyperglycaemia and uncontrolled diabetes served to protect the islet graft, others have reported that the diabetic state caused impaired blood perfusion of islet grafts [10]. Recent studies in our laboratory with purified islet allografts in dogs (unpublished observations) suggest that the function of frozen-thawed grafts is improved when hyperglycaemia is avoided.

In summary, this report documents sustained (3 month) function of a combined graft of freshly-isolated and cryopreserved human islets that induced normoglycaemia and insulin-independence after transplantation into a Type 1 diabetic patient. This represents an essential preliminary step to evaluate the efficacy of this therapy for treatment of serious complications of diabetes.

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Note added in proof: Since the time of submission of this manuscript, the patient has remained insulin-independent with normoglycaemia, now 5 months post-transplant.

References

1. Warnock GL, Ellis D, Rajotte RV, Dawidson I, Baekkeskov S, Egebjerg J (1988) Studies of the isolation and viability of human islets for Langerhans. Transplantation 45: 957–963
2. Gray DWR, McShane P, Grant A, Morris PJ (1984) A method for isolation of islets of Langerhans from the human pancreas. Diabetes 33: 1055–1061
3. Warnock GL, Cattral MS, Rajotte RV (1988) Normoglycemia after implantation of purified islets of Langerhans in dogs. Can J Surg 31: 421–426
4. Warnock GL, Kneteman NM, Ryan EA, Evans MG, Seelis REA, Halloran PF, Rabinovitch A, Rajotte RV (1989) Continued function of pancreatic islets after transplantation in type I diabetes. Lancet II: 570–572
5. Rajotte RV, Warnock GL, Coulombe MG (1988) Islet cryopreservation: methods and experimental results in rodents, large mammals and humans. In: van Schildgaarde R, Hardy MA (eds) Transplantation of the Endocrine Pancreas in Diabetes Mellitus. Elsevier science Publishers, Amsterdam, pp 125–135
6. McNary WF (1954) Zinc-dithizone reaction of pancreatic islets. J Histochem Cytochem 2: 185–194
7. Largiader F, Kolb E, Binswanger U (1980) A long-term functioning human pancreatic islet allotransplant. Transplantation 29: 76–77
8. Scharp DW, Lacy PE, Santiago JV et al. (1990) Insulin independence after islet transplantion into type I diabetic patient. Diabetes 39: 515–518
9. Diem P, Abid M, Radmon JB, Sutherland DER, Robertson RP (1990) Systemic venous drainage of pancreas allografts as independent cause of hyperinsulinemia in type I diabetic recipients. Diabetes 39: 534–539
10. Sandler S, Jansson L (1987) Blood flow measurements in autotransplanted pancreatic islets of the rat: impairment of the blood perfusion of the graft during hyperglycemia. J Clin Invest 80: 17–21

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