Published in final edited form as:

Transplantation. 1992 February ; 53(2): 407–414.

HUMAN ISLET ISOLATION AND ALLOTRANSPLANTATION IN 22 CONSECUTIVE CASES^{1,2}

Camillo Ricordi³, Andreas G. Tzakis, Patricia B. Carroll, Yijun Zeng, Horacio L. Rodriguez Rilo, Rodolfo Alejandro, Ron Shapiro, John J. Fung, Anthony J. Demetris, Daniel H. Mintz, and Thomas E. Starzl

University of Pittsburgh, Transplant Institute, Pittsburgh, Pennsylvania 15213; and The Diabetes Research Institute, University of Miami, Miami, Florida 33101

Abstract

This report provides our initial experience in islet isolation and intrahepatic allotransplantation in 21 patients. In group 1, 10 patients underwent combined liver-islet allotransplantation following upper-abdominal exenteration for cancer. In group 2, 4 patients received a combined liver-islet allograft for cirrhosis and diabetes. One patient had plasma C-peptide >3 pM and was therefore excluded from analysis. In group 3, 7 patients received 8 combined cadaveric kidney-islet grafts (one retransplant) for end-stage renal disease secondary to type 1 diabetes mellitus. The islets were separated by a modification of the automated method for human islet isolation and the preparations were infused into the portal vein. Immunosuppression was with FK506 (group 1) plus steroids (groups 2 and 3).

Six patients in group 1 did not require insulin treatment for 5 to >16 months. In groups 2 and 3 none of the patients became insulin-independent, although decreased insulin requirement and stabilization of diabetes were observed.

Our results indicate that rejection is still a major factor limiting the clinical application of islet transplantation in patients with type 1 diabetes mellitus, although other factors such as steroid treatment may contribute to deteriorate islet engraftment and/or function.

Diabetes mellitus is the most common endocrine disease and is a worldwide public health problem, being the fourth leading cause of death by disease in Western countries (1). Estimates for insulin-dependent diabetes (type 1) indicate a prevalence of 0.26 percent by age 20 in the United States (2). There is evidence that the incidence of this disease is increasing in several world populations (3). Prolongation of life is achieved by current maintenance therapy with insulin, but an increased number of diabetic patients are treated for complications (4) including end-stage renal failure, now representing 10–40% of new patients on dialysis (5). Diabetes is also the leading cause of new cases of blindness in patients over the age of 20 (1).

In patients with type 1 diabetes mellitus, insulin production by the pancreatic islets progressively declines and finally disappears, as the beta cells within the islets are destroyed by an autoimmune process resulting from a complex interplay between genetic and unknown environmental factors (6). Replacement therapy with exogenous insulin is imperfect and has been ineffective in preventing the chronic complication of the disease. Thus, alternative

¹Presented at the 17th Annual Meeting of the American Society of Transplant Surgeons, May 29–31, 1991, Chicago, IL.

²This work was supported by Research Grant No. 1911421 from the Juvenile Diabetes Foundation International.

³Address correspondence to: Camillo Ricordi, M.D., The Transplant Institute, University of Pittsburgh School of Medicine, 5C Falk Clinic, 3601 Fifth Avenue, Pittsburgh, PA 15213.

methods for total endocrine replacement have been explored, including transplantation of isolated islets as free grafts (7).

1990 was a significant year for clinical islet transplantation. In fact, almost a century after the first attempt to treat a diabetic child by transplantation of pancreatic tissue (8), reports of short-term (9) and prolonged (10–13) insulin independence following human islet allotransplantation indicated that it is possible to replace the endocrine function of the pancreas by an islet transplant in man.

These encouraging results have been the product of recent improvements in isolation technology and immunosuppressive therapy. In fact, the procedures developed for the isolation (14) of rodent islets were ineffective to separate islets from the pancreas of larger mammals, including man.

It is estimated that the human pancreas contains approximately 1 million islets, which are mainly composed of insulin-producing cells (15). The development of more effective procedures for islet isolation and purification from large animals (16–20) and human (21–26) pancreases have resulted in significant progress in both number and purity of the islets that can be obtained from each pancreas.

In addition, the use of more powerful immunosuppressive agents such as cyclosporine A (9, 11,25) or FK506 (10) resulted in prolonged human islet allograft survival in some cases.

This report provides our initial experience in islet isolation and intrahepatic allotransplantation in 21 patients.

MATERIALS AND METHODS

Patients

Twenty-two intrahepatic islet allografts were performed in 21 patients between January 10, 1990, and May 4, 1991. One patient had significant C-peptide production before islet transplantation and was therefore excluded from data analysis. Data on patients with a follow-up of at least 2 months are summarized in Table 1.

Group 1—Ten patients aged 8–58 years underwent combined liver-islet allotransplantation following upper-abdominal exenteration for tumors too extensive to be removed with less drastic procedures (27,28). Preliminary results on nine of these patients have been reported previously (10). Liver, pancreas, spleen, stomach, duodenum, proximal jejunum, terminal ileum, ascending and transverse colon (three cases), and part of the right atrium (one case) were removed. A cadaveric orthotopic liver allograft was done (28) and the graft portal vein was anastomosed to the recipient superior mesenteric vein. Arterialization was from the recipient aorta or celiac axis. A 14G catheter with a heparin lock was placed in a superior mesenteric vein (10). Bowel continuity was reestablished and biliary drainage was via a choledocojejunostomy.

Group 2—Four patients aged 22–56 years received a combined liver-islet allograft. The indications for liver transplantation were cirrhosis secondary to cystic fibrosis, cirrhosis secondary to hepatitis C, alcoholic cirrhosis, and cryptogenic cirrhosis. All patients except one had type 1 diabetes as evidenced by an absent C-peptide response to glucagon or Sustacal challenge test. One patient (cystic fibrosis), who was tested in the operating room before the islet transplant, had basal and stimulated plasma C-peptide >3 pM and was therefore excluded from analysis.

Group 3—Seven patients aged 28–42 years received 8 combined cadaveric kidney-islet grafts (one retransplant) for end-stage renal disease secondary to type 1 diabetes mellitus. Immediately after renal transplantation, an upper midline incision was performed and a 16–18G catheter was placed in a jejunal vein for islet infusion. All patients had negative C-peptide in response to a Sustacal challenge test performed before islet transplantation.

Organ procurement

The cadaveric donor ABO types were the same as, or compatible with, the recipient ABO types. HLA matching was random and the antigen match was 0 to 3. There were two positive cytotoxic crossmatches in group 1 (cluster-islet) and two in group 2 (liver-islet).

The livers, kidneys, and pancreases were obtained from multiorgan donors (27–29). In situ perfusion of the abdominal aorta was with 1500–2000 ml of University of Wisconsin solution. An additional 500–1000 ml of UWS was infused directly into the liver via the portal vein, which was encircled below the catheter tip to prevent retrograde leakage. Venous hypertension of the pancreas was avoided by venting the portal and/or splenic vein. The specimens were immersed in UWS and packed on ice.

The pancreas of the liver or kidney donor was the source of the primary islet graft for all patients except one patient in group 1 and one patient in group 3 who received islets from a third-party pancreas donor.

Four patients in group 1 and two patients in group 3 were given islets from 1–2 additional donors 1–5 days after the principal operation. One patient in group 3 was retransplanted (kidney-islet) 7 months after the first combined graft because of irreversible kidney rejection.

Islet preparation and administration

Cold ischemia time of the 28 pancreases averaged 7.5 hours (range 4–12), with no statistically significant difference between groups.

The human islets were obtained by a modification (19) of the automated method for human islet isolation (22).

Briefly, after cannulation of the pancreatic duct 350 ml of Hanks solution containing 2 mg/ml collagenase solution (Boehringer-Mannheim, Type P) was injected through the duct. The pancreas was loaded into a stainless steel digestion chamber and islets were separated during a continuous digestion process that lasted 30–45 min.

The main modification of the isolation procedure compared with the automated method previously described (22) was the isolation chamber, whose volume is now 475 ml with an outlet port diameter of 6 mm, which is significantly wider than that of the previously used chamber. In addition, the pore size of the screen was increased from 280 to 400 μ , and the cooling system was eliminated, as well as the heating circuit bypass, resulting in a simpler isolation apparatus (Fig. 1).

During the recirculation phase (flow rate 85 ml/min) intrachamber temperature was increased at a rate of 2°C/min by passage of the solution through a stainless steel coil immersed in a water bath (50°C). The chamber containing the distended pancreas was gently agitated and samples were taken every 2 min to monitor digestion. After approximately 20–30 min of recirculation the digestion was stopped by dilution (4°C Hanks, 400 ml/min flow rate) and cooling. In this phase the digested tissue was rapidly collected in 1-liter sterile bottles containing 400 ml Hanks solution (4°C) with 10% fetal calf serum. The dilution phase lasted

15–20 min. Upon initiation of the dilution phase the chamber was connected to a shaker with oscillation amplitude of 10 cm and a variable rate of 0–320 oscillation/min.

Eurocollins solution was used as vehicle for the Ficoll powder (Ficoll DL-400, Sigma, St. Louis, MO). Eurocollins-Ficoll at densities of 1.108, 1.096, 1.037 was used in a three-layer discontinuous gradient (10), in which the digested pancreatic tissue was bottom-loaded with the 1.108 layer. A cell separator (COBE 2991, Lakewood, CO) was used for centrifugation of the gradients (30,31).

Determination of number, volume, and purity of the human islets obtained after islet separation and purification was performed according to recently proposed criteria (32). Briefly, the final islet preparation was suspended in 250 ml Hank's solution; 100- μ l samples were stained with dithizone to assess total islet yield, which was converted to total number of islets of an average diameter of 150 μ (IEq) (32). The contribution of the different size groups to the total islet volume was then expressed in μ l.

The preparation was pelleted and suspended in 100 ml Hank's solution containing 10% human albumin and infused into the portal vein catheter over 20–30 min. Portal venous pressure was measured and in some cases the portal flow was assessed by color doppler ultrasonography. In patients who received more than one islet preparation, the portal vein catheter was flushed every 6 hr with 2 ml saline containing heparin (100 U/ml). The catheter was removed after completion of the last islet infusion.

Immunosuppressive management

In group 1, immunosuppression with FK506 began with intravenous doses of 0.075 mg/kg every 12 hr followed by 0.15 mg/kg orally every 12 hr. The dose was adjusted on clinical grounds and by monitoring plasma FK506 levels.

In group 2, FK506 was administered at a dose of 0.1 mg/kg i.v. over 24 hr, beginning immediately after transplantation. In addition, the patients received a 1000-mg i.v. bolus of methylprednisolone during the operation, followed by a maintenance dose of 20 mg prednisolone i.v. daily, until conversion to the oral route. The oral dose of FK506 was 0.15 mg/kg every 12 hr (0.3 mg/kg per day), and 20 mg of prednisone per day was given. This dose was reduced and discontinued according to clinical criteria.

In group 3, FK506 was given as in group 2. Following the intraoperative i.v. bolus of 1000 mg methylprednisolone, a decreasing prednisone dose (from 200 to 20 mg/day) was administered over 6 days. When possible, the steroid dose was tapered over the first several weeks and stopped.

Supplementary steroids or OKT3 was given if rejection was suspected clinically or diagnosed by biopsy.

Pretransplant assessment of recipient islet function

Basal and stimulated plasma C-peptide levels were measured in all recipients before the infusion of the islets. The provocative tests were 1 mg glucagon i.v. (group 1) and a Sustacal (6 Kcal/kg) (33) or glucagon (groups 2 and 3) challenges. There were no C-peptide responses except in one patient in group 2 who had high pretransplant basal and stimulated C-peptide levels (>3 pM) during a glucagon test performed in the operating room.

Posttransplant assessment of donor islet function

After islet transplantation, plasma glucose and C-peptide levels were monitored. An intravenous glucose tolerance test was used as provocative test of C-peptide secretion in patients in group 1. IVGTT was chosen to avoid interpretative problems in the evaluation of the results, since the patients of this group underwent significant gastrointestinal resections. In groups 2 and 3, a Sustacal tolerance test was selected as provocative test of C-peptide secretion. Glycosylated hemoglobin (HbA1c) was measured before and every 6 weeks after transplantation, or when the patients were evaluated in follow-up clinics.

RESULTS

Islet isolation and purification

Islet isolation and purification results are summarized in Table 2.

Pancreas cold ischemia time before the islet isolation and purification procedure was comparable in the three groups, ranging 4 to 12 hr.

In group 1, the 14 human islet preparations that were transplanted comprised an average of 392,100 islets, representing an average of 279,800 IEq with an endocrine volume of approximately 495 μ l. Purity in islets was 61 % (range 25–80%).

In group 2, 3 islet preparation yielded an average of over 800,000 islets, representing 625,300 IEq. Average endocrine volume and purity in islets were 1105 μ l and 67%, respectively.

In group 3, 11 islet isolations resulted in an average of 644,600 islets (597,000 IEq) with an endocrine volume of 1055 μ l. The average purity in islets was 72%.

Patients in groups 2 and 3 received a number of islets that was significantly higher ($P<0.05$) compared with the cluster-islet patients of group 1. No significant difference was observed in the degree of purity in islets infused in the three groups, and in the number of islets transplanted in groups 2 and 3.

Patient survival

Following our preliminary report on cluster-islet allotransplantation (10), two additional patients died from cancer recurrence 9 and 14 months following transplantation, leaving 5 of 10 patients in group 1 with follow-up of 16, 14, 13, 13, and 1 month.

In group 2 (n=3), one patient died 36 hr following combined liver-islet transplantation. The patient had a positive cross-match (100%) with her liver-islet donor and had primary hepatic nonfunction because of humoral (hyperacute) rejection. A second patient, who demonstrated significant islet function for the first 5 postoperative months, died of hepatitis B and sepsis 6 months after transplantation.

In group 3 (n=7), one patient died 5 days following combined kidney-islet transplantation as a result of aspiration pneumonia on postoperative day 3.

Posttransplant islet function

The metabolic outcome of intrahepatic human islet allotransplantation is summarized in Table 1.

In group 1, six patients did not require insulin for 5 to over 16 months.

The first patient, who received the islet allograft on January 10, 1990, is still insulin-independent over 16 months postoperatively. Nevertheless, 9 months after transplantation the average value of pre- and postprandial blood glucose determinations progressively increased until the 14th postoperative month, but spontaneously improved during the last 60 days (Fig. 2). It is of interest that this patient required over 3000 and 2000 units of intravenous insulin on her fourth and fifth postoperative days, respectively (Fig. 3).

Two patients who recently died did not require insulin at the time of tumor recurrence and expired with functioning islet grafts 9 and 14 months after transplantation.

In one patient (No.6) who was insulin-dependent (10), the islet function progressively improved and insulin treatment was discontinued during the third postoperative month. She did not require insulin for 5 months. Insulin treatment was resumed 8 months after islet allotransplantation (2.5–4.1 units/day, s.c.) for increased fasting plasma glucose levels (>120 mg/dl). The patient was converted to oral hypoglycemic agents (glibenclamide 5 mg/day) 14 months after transplantation, since her insulin requirement was minimal. She now requires no insulin.

One patient (No.8) did not require daytime insulin treatment, but was unable to discontinue night parenteral nutrition (10 units of insulin/night, i.v.).

One patient (No.9) did not require insulin until the 10th postoperative month, when sudden development of symptomatic hyperglycemia in the absence of any evidence of liver rejection imposed reinstitution of exogenous insulin treatment.

In group 2, one patient is alive 7 months after transplantation. She had a 100% positive cytotoxic crossmatch and a rejection episode during the first postoperative week. An approximately 80% decrease in her insulin requirement was observed over the first 6 postoperative months (from 70 to 15 units of insulin per day; Fig. 4). It was evident that glycemic control was extremely stable compared with preoperative values and HbA1c has been within the normal range (<5.9%). In addition, Sustacal challenge tests 2, 3, and 6 months after transplantation have shown progressive improvement of plasma C-peptide (Fig. 5). A delay in C-peptide secretion and prolonged elevation during the challenge was evident in this patient, as previously reported in islet allograft recipients (10).

The second patient, who died 6 months after transplantation from hepatitis B and sepsis, also demonstrated significant islet function. His insulin requirement rapidly decreased during the first 3 postoperative weeks (Fig. 6). A rejection episode in week 4 imposed a significant increment in the daily insulin dose, which never returned to prerejection levels (Fig. 6). The islets were not completely rejected, as documented by persistence of significant basal and stimulated C-peptide levels of 0.76 and 1.59 pM, respectively (Sustacal challenge, 2 months posttransplant).

In group 3, none of the patients became insulin-independent. All patients had at least one rejection episode in the first postoperative month. One patient lost the transplanted kidney due to rejection. Of interest in this patient was documentation of islet function with basal and stimulated C-peptide of 0.30 and 0.75 pM, respectively, after the kidney was completely rejected. The patient received a second kidney-islet graft 6 months after the first combined transplant, but never became insulin-independent despite receiving the highest number of islets (>2,000,000 IEq) in the study. C-peptide was measurable in all cases, although only three of six patients with a follow-up of more than 1 month had significant basal and stimulated plasma C-peptide (basal = 1.62/0.36/0.38 and peak = 1.95/.57/.93 pM) following a Sustacal challenge test 4–8 weeks postoperatively. Two patients had 48% (Fig. 7) and 70% reduction in insulin requirements following transplantation. It is of interest that basal and stimulated C-peptide

levels in both cluster-islet and liver-islet groups were higher than in kidney-islet recipients (Fig. 8). Diabetes was stabilized in all patients, despite the fact that they all had at least one episode of rejection confirmed on biopsy.

DISCUSSION

Several cases of intrahepatic human islet allografts have been reported recently (9–12) with transient (9) or prolonged (10–12) insulin independence. Two patients with type 1, insulin-dependent diabetes mellitus (11,12) received islets from multiple donors (4 and 5 pancreases). One of these patients (12) was still insulin-independent 1 year after islet allotransplantation.

In the present report, prolonged (5 to >16 months) insulin independence was observed in six patients who underwent upper abdominal exenteration and liver-islet replacement (10). Four of them received islets from two donors. The first patient of this series is still insulin-independent over 16 months after the islet allograft and received islets from a single donor.

In contrast, in our experience none of the type 1 diabetic patients who received either a liver-islet or a kidney-islet allograft are insulin-independent. Although our best result in type 1 diabetic patients was obtained in a case of positive crossmatch (100%), we currently consider a positive crossmatch as an absolute contraindication to human islet allotransplantation, because of the increased risk of morbidity and mortality in this group.

Differences in islet isolation and/or purification techniques can not explain the inferior results obtained in the combined kidney-islet group, since the patients in the three groups represent consecutive cases in which the same separation and purification procedure was used for human islet isolation. Possible explanations for which there is experimental support include: (1) metabolic dysfunction and/or impaired vascular engraftment due to long-standing diabetes mellitus (34,35); (2) steroid treatment, which may have a detrimental effect on islet engraftment and/or function (36), was not used in the cluster-islet patients, and was higher in the kidney-islet group than in liver-islet recipients; (3) the immune barrier to islet acceptance might be lowered by the presence of a liver from the same donor (37). Based on our data we favor the hypothesis of the protective effect of the simultaneous liver graft and/or the detrimental effect of steroid treatment. In addition, weight loss was observed during the first 2–3 postoperative months in all patients receiving a cluster-islet graft. The nutritional problem associated with upper abdominal exenteration could also result in reduced insulin requirement in these patients.

In conclusion, our results indicate that rejection is still a major factor limiting the clinical application of islet transplantation in patients with type 1 diabetes mellitus, although other factors such as steroid treatment may contribute to deteriorate islet engraftment and/or function.

References

1. Harris, MI.; Hanaman, RF., editors. NIH publication No. 85–1468. Bethesda, MD: 1985. Diabetes in america.
2. La Porte RE, Fishbirt HA, Drash AL, et al. The Pittsburgh insulin dependent diabetes mellitus (IDDM) registry: the incidence of insulin dependent diabetes mellitus in Allegheny County, Pennsylvania (1965–1976). *Diabetes* 1981;30:279. [PubMed: 7202862]
3. Bennet, PH. Epidemiology of diabetes mellitus. In: Rifkin, H.; Porte, D., Jr, editors. *Diabetes mellitus: theory and practice*. New York: Elsevier; 1990. p. 357
4. Krolewski AS, Warram JH, Rand LI, et al. Epidemiologic approach to the etiology of type 1 diabetes mellitus and its complications. *N Engl J Med* 1987;317:1390. [PubMed: 3317040]
5. Goetz FC, Elick B, Fryd D, Sutherland DER. Renal transplantation in diabetes. *Clin Endocrinol Metab* 1986;15:807. [PubMed: 3536201]

6. Eisenbarth GS. Type 1 diabetes mellitus: a chronic autoimmune disease. *N Engl J Med* 1986;314:1360. [PubMed: 3517648]
7. Dubernard, JM.; Sutherland, DER. Introduction and history of pancreatic transplantation. In: Dubernard, JM.; Sutherland, DER., editors. *International handbook of pancreas transplantation*. Dordrecht: Kluwer Academic; 1989.
8. Williams PW. Notes on diabetes treated with extract and by grafts of sheep's pancreas. *Br Med J* 1894;2:1303.
9. Scharp DW, Lacy PE, Santiago JV, et al. Insulin independence after islet transplantation into type I diabetic patient. *Diabetes* 1990;39:515. [PubMed: 2108071]
10. Tzakis A, Ricordi C, Alejandro R, et al. Pancreatic islet transplantation after upper abdominal exenteration and liver replacement. *Lancet* 1990;336:402. [PubMed: 1974944]
11. Scharp DW, Lacy PE, Ricordi C, et al. Human islet transplantation in patients with type I diabetes. *Transplant Proc* 1989;21:2744. [PubMed: 2495688]
12. Warnock GL, Kneteman NM, Ryan E, Seelis REA, Rabinovitch A, Rajotte RV. Normoglycemia after transplantation of freshly isolated and cryopreserved pancreatic islets in type I (insulin-dependent) diabetes mellitus. *Diabetologia* 1991;34:55. [PubMed: 2055341]
13. Altman JJ, Cugnenc PH, Tessier C, et al. Epiploic flap: a new site for islet implantation in man. *Horm Metab Res (suppl)* 1990;25:136. [PubMed: 2088954]
14. Lacy PE, Kostianovsky M. Method for the isolation of intact islets of Langerhans from the rat pancreas. *Diabetes* 1967;16:35. [PubMed: 5333500]
15. Weir GC, Bonner-Weir S. Islets of Langerhans: the puzzle of intraislet interactions and their relevance to diabetes. *J Clin Invest* 1990;85:983. [PubMed: 1969425]
16. Gray DWR, Morris PJ. Developments in isolated pancreatic islet transplantation. *Transplantation* 1987;43:321. [PubMed: 3103272]
17. Gray DW, Warnock G, Sutton R, Peters M, McShane P, Morris PJ. Successful autotransplantation of isolated islets of Langerhans in the cynomolgus monkey. *Br J Surg* 1986;73:850. [PubMed: 3094618]
18. Warnock GL, Rajotte RV. Critical mass of purified islets that induce normoglycemia after implantation into dogs. *Diabetes* 1988;37:467. [PubMed: 3132412]
19. Ricordi C, Socci C, Davalli AM, et al. Isolation of the elusive pig islet. *Surgery* 1990;107:688. [PubMed: 2112787]
20. Alejandro R, Curfield RG, Scheinvald FL, et al. Natural history of intrahepatic canine islet cell autografts. *J Clin Invest* 1986;78:1339. [PubMed: 3095376]
21. Gray DWR, McShane P, Grant A, Morris PJ. A method for isolation of islets of Langerhans from the human pancreas. *Diabetes* 1984;33:1055. [PubMed: 6437895]
22. Ricordi C, Lacy PE, Finke EH, Olack B, Scharp DW. An automated method for the isolation of human pancreatic islets. *Diabetes* 1988;37:413. [PubMed: 3288530]
23. Scharp DW, Lacy PE, Finke E, OJack BJ. Low-temperature culture of human islets isolated by the distension method and purified with Ficoll or Percoll gradients. *Surgery* 1987;102:869. [PubMed: 3313779]
24. Rajotte RV, Warnock GL, Evans M, Dawidson I. Isolation of viable islets of Langerhans from collagenase-perfused canine and human pancreata. *Transplant Proc* 1987;19:916. [PubMed: 3152636]
25. Alejandro R, Noel J, Latif Z, et al. Islet cell transplantation in type I diabetes mellitus. *Transplant Proc* 1987;19:2359. [PubMed: 3152660]
26. Sutherland DER. Pancreas and islet transplantation: II. Clinical trials. *Diabetologia* 1981;20:435. [PubMed: 6786945]
27. Starzl TE, Todo S, Tzakis A, et al. Abdominal organ cluster transplantation for the treatment of upper abdominal malignancies. *Ann Surg* 1989;210:374. [PubMed: 2673085]
28. Tzakis A, Todo S, Starzl TE. Upper abdominal exenteration with liver replacement: a modification of the cluster procedure. *Transplant Proc* 1990;22:273. [PubMed: 2155493]
29. Starzl TE, Miller C, Broznick B, Makowka L. An improved technique for multiple organ harvesting. *Surg Gynecol Obstet* 1987;165:343. [PubMed: 3310285]

30. Lake SP, Basset PD, Larkins A, et al. Large-scale purification of human islets utilizing discontinuous albumin gradient on IBM 2991 cell separator. *Diabetes* 1989;38 (suppl):143. [PubMed: 2642839]
31. Alejandro R, Strasser S, Zucker PF, Mintz DH. Isolation of pancreatic islets from dogs: semiautomated purification on albumin gradients. *Transplantation* 1990;50:207. [PubMed: 1696406]
32. Ricordi C, Gray DWR, Hering BJ, et al. Islet isolation assessment in man and large animals. *Acta Diabetol Lat* 1990;27:185. [PubMed: 2075782]
33. Goetz, FC. Endocrine assessment of potential candidates for pancreas transplantation and post-transplant monitoring. In: Van Schilfgaarde, R.; Hardy, MA., editors. *Transplantation of the endocrine pancreas in diabetes mellitus*. New York: Elsevier; 1988. p. 333
34. Hayek A, Lopez AD, Beattie GM. Decrease in the number of neonatal islets required for successful transplantation by strict metabolic control of diabetic rats. *Transplantation* 1988;45:940. [PubMed: 3130698]
35. Hayek A, Lopez AD, Beattie GM. Factors influencing islet transplantation: number, location, and metabolic control. *Transplantation* 1990;49:224. [PubMed: 2105547]
36. Kaufman DB, Morel P, Condie R, et al. Beneficial and detrimental effects of RBC-adsorbed antilymphocyte globulin and prednisone on purified canine islet autograft and allograft function. *Transplantation* 1991;51:37. [PubMed: 1987703]
37. Morris PJ. Combined liver and pancreatic islet transplantation in the rat. *Transplantation* 1983;36:230. [PubMed: 6410561]

Figure 1.

Modified automated procedure for islet separation. (A) Lines that are occluded during the recirculation phase; (B) lines that are occluded during the dilution phase. In the first phase, collagenase solution recirculates in a closed system, in which the collagenase solution and the pancreas are progressively heated to 37°C. In a second phase, the islets that are progressively released from the digesting pancreas are saved in collecting flasks or bottles.

METABOLIC PROFILES OVER TIME **CLUSTER-ISLET PATIENT M.A.**

Figure 2.

Plasma glucose and daily insulin requirements of a cluster-islet patient (group 1, No.1, Tables 1 and 2), who is still insulin independent over 16 months following liver-islet allotransplantation.

INSULIN AND GLUCOSE PROFILES CLUSTER-ISLET PT M.A.

Figure 3.

Plasma glucose and insulin requirements in patient No. 1 (group 1, Tables 1 and 2) during the first postoperative week, demonstrating an episode of significant insulin resistance in which over 2000–3000 units i.v. per day were administered.

Figure 4. Plasma glucose and daily insulin requirements before and after human islet allotransplantation in one type 1 diabetic patient who received a combined liver-islet graft (group 2, No.1, Tables 1 and 2).

Figure 5.

Plasma glucose and C-peptide following Sustacal Tolerance Test (STT) before and 2, 3, and 6 months after human islet allotransplantation (group 2, No.1, Tables 1 and 2).

LIVER-ISLET PATIENT F.C. WEEKLY METABOLIC PROFILES

Figure 6.

Plasma glucose and daily insulin requirements in a type 1 diabetic recipient of combined liver-islet allograft (group 2, No.2, Tables 1 and 2). Insulin requirement rapidly decreased during the first 3 weeks following transplantation. A rejection episode in week 4 imposed a significant increment in the daily insulin dose, which never returned to prerejection levels.

KIDNEY-ISLET PT. G.S. WEEKLY METABOLIC PROFILES

Figure 7.

Plasma glucose and daily insulin requirements in a type 1 diabetic patient who received a combined kidney-islet allograft (group 3, No.1, Tables 1 and 2). Daily insulin requirement decreased by 48% in the first 40 weeks posttransplant, compared with pretransplant requirements.

Figure 8.

Basal and stimulated plasma C-peptide levels in patients following kidney-islet (n=7), liver-islet (n=2), and cluster-islet (n=9) allotransplantation. C-peptide appears higher in cluster-islet patients than in liver-islet and kidney-islet recipients.

Table 1

Description of recipients with a follow-up of at least 2 months and clinical outcome

Patient No. and diagnosis	Age/sex	Metabolic outcome	HbA1c ^a	C-peptide ^b (basal/stim)	Post-operative month	Outcome
Group 1 (cluster-islets)						
1 Hepatocellular Ca	17/F	NIR ^c	6.3%	1.14/2.82	16	Full activity
2 Hepatocellular Ca	8/M	IR, ^d 6 U/day	6.1%	0.30/1.68	5	Died: recurrence
3 Pancreatic AdCa	55/M	IR, 14 U/day	NA	0.07/0.53	2	Died: multiple-systemic failure
4 Neuroendocrine	36/F	IR, 6 U/day	5.4%	0.54/3.84	3	Died: multiple-systemic failure
5 Leiomyosarcoma	58/M	NIR	4.6%	1.50/3.90	14	Died: recurrence
6 Perianapillary AdCa	52/F	NIR- ^e	6.2%	1.56/3.60	14	Full activity
7 Metastatic AdCa colon	46/M	NIR	5.1%	0.90/6.60	9	Died: recurrence
8 Cholangiocarcinoma	44/F	NIR	6.6%	1.68/3.60	13	Full activity
9 Cholangiocarcinoma	33/F	IR, 10 months	5.6%	1.50/3.24	13	Full activity
Group 2 (liver-islets)						
1 Cirrhosis 2o hepatitis C	56/F	IR, 15 U/day	4.1%	0.74/2.38	7	Full activity
2 Cirrhosis 2o ETOH	42/M	IR	3.8%	0.76/1.59	6	Died: hepatitis B, sepsis
Group 3 (kidney-islets)						
1 ESRD 2o type 1 DM	38/M	IR, 20 U/day	6.6%	0.36/0.60	10	Full activity
2 ESRD 2o type 1 DM	28/M	IR, 60 U/day	8.4%	0.59/0.60	9	Full activity
3 ESRD 2o type 1 DM	35/M	IR, 12 U/day	6.5%	0.38/0.93	9	Full activity
4 ESRD 2o type 1 DM	36/F	IR, 30 U/day	7.0%	0.05/0.17	6	Full activity
5 ESRD 2o type 1 DM	36/M	IR, 40 U/day	8.1%	0.08/0.11	4	Full activity
6 ESRD 2o type 1 DM	32/M	IR, 50 U/day	7.0%	0.14/0.50	2	Full activity

^aHbA1c: glycosylated hemoglobin (nl 3.9–5.9), most recent values.

^bC-peptide plasma levels (pmol/ml), most recent values.

^cNon-insulin-requiring.

^dInsulin-requiring.

^eon oral hypoglycemic agent.

Table 2

Intrahepatic islet transplantation: donor data and description of isolation outcome

Patient No.	Donor age	Pancreas		Transplanted islets			
		Cold ischemia time (hr)	Weight (g)	No. (×1000)	Ieq. No. (×1000) ^a	Volume (μl)	Purity (% islets)
Group 1 (cluster-islets)							
1	18	6	42	505	474	838	50
2	7	10	24	659	562	993	50
3	36	6	63	428	205	363	25
4	19	8	47	536	289	511	70
5	26	6	58	692	369	652	70
	19	5	131	265	209	369	70
6	18	9	108	233	105	186	70
	40	6	110	220	153	270	50
7	18	6	100	283	285	504	60
8	17	10	35	427	459	811	70
	35	5	89	475	267	472	70
9	31	12	59	270	205	362	60
	50	7	114	261	127	224	60
10	17	4	70	235	208	368	80
Mean	23.4	7.1	69.9	406.8	297.0	525.0	59.5
SEM	3.2	0.6	8.7	46.5	43.2	76.3	4.9
Group 2 (liver-islets)							
1	43	8	57	1005	1114	1969	80
2	18	6	75	365	288	509	50
3	47	7	60	1110	474	838	70
Mean	36.0	7.0	64.0	826.7	625.3	1105.3	67.0
SEM	9.1	0.6	5.6	232.8	250.0	442.1	8.8
Group 3 (kidney-islets)							
1	16	6	90	555	448	792	70
2	23	8	85	1060	869	1536	60
	31	11	78	705	1065	1882	60
+	42	8	116	789	315	557	50

Patient No.	Donor age	Pancreas			Transplanted islets			
		Cold ischemia time (hr)	Weight (g)	No. ($\times 1000$)	Ieq. No. ($\times 1000$) ^a	Volume (μ l)	Purity (% islets)	
3	49	7	98	577	327	578	70	
	53	8	89	382	605	1069	50	
	17	9	50	155	180	318	65	
4	23	9	93	725	462	816	95	
5	21	8	84	737	438	774	85	
6	30	6	60	800	600	1060	75	
7	41	10	79	473	1110	1962	60	
Mean	29.0	8.0	82.6	644.6	597.0	1054.9	72.0	
SEM	3.6	0.6	4.4	68.0	98.0	173.2	5.3	
P	NS	NS	NS	1:2=<0.01	1:2=0.05	1:2=0.05	NS	
				1:3=<0.03	1:3=<0.03	1:3=<0.03		
				2:3=NS	2:3=NS	2:3=NS		

^a 150-micron equivalents.

+Preparation transplanted with 2nd kidney.

N.B.: Where more than one donor was used, mean values/patient were used in calculations.