1366

CHAPTER 40

ISLET TRANSPLANTATION IN DIABETES: THE PITTSBURGH EXPERIENCE

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INTRODUCTION

Nineteen-ninety was a significant year for clinical islet transplantation, since after many attempts reports of short term[1] and prolonged[2-6] 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. The development of improved procedures for islet isolation and purification from large animals[7-11] and human[12-16] pancreata have resulted in significant progress in both the number and purity of islets that can be obtained from each pancreas. In addition, the use of more powerful immunosuppressive agents such as cyclosporine A[1,3,16] or FK506[2,6] resulted in prolonged human islet allograft survival in some cases. This report summarized our initial experience on islet isolation and intrahepatic allotransplantation.

MATERIALS AND METHODS

PATIENTS

Twenty-five intrahepatic islet allografts were performed in 24 patients between January 10, 1990 and May 4, 1991.[6]

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.[17,18] More detailed results on nine of these patients have been reported previously.[2,6] Liver, pancreas, spleen, stomach, duodenum, proximal jejunum, terminal ileum, ascending and transverse colon (three cases), and part of the right atrium (one case) was removed. A cadaveric orthotopic liver allograft was done[18] and the graft portal vein was anastomosed to the recipient superior mesenteric vein. Arterialization was from the recipient aorta or celiac axis. A 14 g catheter with a heparin lock was placed in a superior mesenteric vein.[2] Bowel continuity was reestab-

lished and biliary drainage was via a choledocojejunostomy.

Group 2. Two patients had near total pancreatectomy for pain relief due to chronic pancreatitis and received an autograft of islets obtained from the excised pancreas.

Group 3. Three type I diabetic patients aged 22-56 years received a combined liver-islet allograft. The indications for liver transplantation were cirrhosis secondary to hepatitis C, alcoholic cirrhosis and cryptogenic cirrhosis.

Group 4. Nine patients aged 28-42 years received 10 combined cadaveric kidney-islet grafts (one retransplant) for end-stage renal disease secondary to type I diabetes mellitus. Immediately after renal transplantation, an upper midline incision was performed and a 16-18 g 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 zero to three. There were two positive cytotoxic crossmatches in Group 1 (Cluster-Islet) and two in Group 3 (Liver-Islet). The livers, kidneys and pancreata were obtained from multi-organ donors.[17-20] In situ perfusion of the abdominal aorta was with 1500-2000 ml of University of Wisconsin solution (UWS). An additional 500-1000 ml of UWS were 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 4 who received islets from a third party pancreas donor. Four patients in Group 1 and three patients in Group 4 were given islets from 1-2 additional donors 1-7 days after the principal operation. One patient in Group 4 was retransplanted (Kidney-Islet) seven months after the first combined graft because of irreversible kidney rejection.

Islet preparation and administration

Cold ischemia time of the 28 pancreata averaged 7.5 hours (range 4-12) with no statistically significant difference between groups. The human islets were obtained by a modification[10] of the automated method for human islet isolation[13] that is described in details in a previous chapter of this book. 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 minutes. The main modifications of the isolation procedure compared to the previously described automated method[13] were the volume of the isolation chamber that is now of 500 ml with an outlet port diameter of 6mm, and the pore size of the screen that was increased from 280 to 400u. The cooling system as well as the heating circuit bypass were eliminated, resulting in a simpler isolation apparatus.[10] 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 two minutes to monitor digestion. After approximately 20-30 minutes of recirculation the digestion was stopped by dilution (4°C Hanks, 400 ml/min flow rate) and cooling. 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, 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.[21,22] Determination of number, volume and purity of the human islets obtained after islet separation and purification was performed according to recently proposed criteria.[23] The final 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 six hours with 2 ml saline containing heparin (100 U/ml). The catheter was removed after completion of the last islet infusion. Two patients in Group 4 received islets through transcutaneous catheterization of the portal vein.

Immunosuppressive Management

In Group 1, immunosuppression with FK506 began with intravenous doses of 0.075 mg/kg every 12 h followed by 0.15 mg/kg orally every 12 h. The dose was adjusted on clinical grounds and by monitoring plasma FK506 levels. In Group 2, the patients with the autografts did not receive any immunosuppression. In Group 3, FK506 was administered at a dose of 0.1 mg/kg IV over 24 h, beginning immediately after transplantation. In addition, the patients received a 1000 mg IV bolus of methylprednisolone during the operation, followed by a maintenance dose of 20 mg prednisolone IV daily, until conversion to the oral route. The oral dose of FK506 was 0.15 mg/kg every 12 h (0.3 mg/kg per day), and 20 mg of prednisone per day were given. This dose was reduced and discontinued according to clinical criteria. In Group 4, following the intraoperative IV bolus of 1000 mg methylprednisolone, a decreasing prednisone dose (from 200 to 20 mg/day) was administered over 6 days. FK506 was given as in Group 3. 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 IV (Group 1 and 2) and a Sustacal (6 Kcal/kg)[24] or glucagon (Group 3 and 4) challenges. All patients had absent C-peptide responses preoperatively except for one of the patients with the autograft.

Posttransplant assessment of donor islet function: After islet transplantation, plasma glucose and C-peptide levels were monitored. An intravenous glucose tolerance test (IVGTT), 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 Group 3 and 4, a Sustacal tolerance test (STT) was selected as provocative test of C-peptide secretion. Glycosylated hemoglobin (HbA1c) was measured before and every six weeks after transplantation or when the patients were evaluated in follow-up clinics.

RESULTS

Islet isolation and purification: Pancreas cold ischemia time (CIT) before the islet isolation and purification procedure was comparable in the three groups, ranging 4-12 hours. 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 µl. Purity in islets was 61% (range 25-80%). In Group 3, islet preparation yielded an average of over 800,000 islets, representing 625,300 IEq. Average endocrine volume and purity in islets were 1,105 µl and 67% respectively. In Group 4, islet isolations resulted in an average of 644,600 islets (597,000 IEq) with an endocrine volume of 1,055 µl. The average purity in islets was 72%. Patients in Group 3 and 4 received a number of islets that was significantly higher ($p < 0.05$) compared to the cluster-islet patients of Group 1. No significant difference was observed in the degree of purity in islets infused in the three groups or in the number of islets transplanted in Group 3 and 4.

Patient survival: Following our preliminary report on cluster-islet allotransplantation,[2] additional patients died from cancer recurrence 9-20 months following transplantation, leaving 3 of 10 patients in Group 1 with follow-up of 24, 21, and 8 month. In Group 3, one patient died 36 hours following combined liver-islet transplantation. The patient had a positive crossmatch

(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 five postoperative months, died of hepatitis B and sepsis six months after transplantation. In Group 4, one patient died of aspiration pneumonia five days following combined kidney-islet transplantation.

Posttransplant islet function: In Group 1, six patients did not require insulin for 5 to over 24 months. The first patient, who received the islet allograft on January 10, 1990, is still insulin independent over 24 months postoperatively. nine months after transplantation the average value of pre- and postprandial blood glucose determinations progressively increased until the 14th postoperative month, but has spontaneously improved during the last eight months. It is of interest that this patient required over 3,000 and 2,000 units of intravenous insulin on her fourth and fifth postoperative day respectively. This is the most insulin we have used in any patient in the three groups. Five patients who recently died of tumor recurrence did not require insulin at the time of recurrence and expired with functioning islet grafts. In one patient (No. 6) who was insulin dependent,[2] the islet function progressively improved and insulin treatment was discontinued during the third postoperative month. She did not require insulin for five months. Insulin treatment was resumed eight months after islet allotransplantation (2.5 - 4.1 units/day, SQ) 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 once again does not require insulin. One patient (No. 8), did not require daytime insulin treatment, but was unable to discontinue night parenteral nutrition (10 units of insulin/night, IV). 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, both patients became insulin-independent one and four weeks after islet autotransplantation and still do not require any exogenous insulin 1 and 10 months following islet graft.

In Group 3, one patient is alive 16 months after transplantation. She had a 100% positive cytotoxic crossmatch and a rejection episode during the first postoperative week. Approximately 80% decrease in her insulin requirement was observed over the first six postoperative months (from 70 to 15 units of insulin per day[11] It was evident that glycemic control was extremely stable compared to preoperative values and HbA1c has been within the normal range (< 5.9%). In addition, Sustacal challenge tests two, three and six months after transplantation have shown progressive improvement of plasma C-peptide. A delay in C-peptide secretion and prolonged elevation during the challenge was evident in this patient, as previously reported in recipients of cluster islet grafts.[2] The second patient, who died six months after transplantation from hepatitis B and sepsis, also demonstrated significant islet function. His insulin requirement rapidly decreased during the first three postoperative weeks. A rejection episode on week four imposed a significant increment in the daily insulin dose, that never returned to prerejection levels. 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, two months posttransplant).

In Group 4, no 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 six month after the first combined transplant but never became insulin independent although receiving the highest number of islets (>2,000,000 IEq) in the study. Basal and stimulated C-peptide was measurable in all cases.[6] Two patients had 48% 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 compared to kidney-islet recipients.[6] Diabetes was stabilized in all patients, despite they all had at least one episode of rejection confirmed on biopsy.

DISCUSSION

Several cases of intrahepatic human islet allografts have been recently reported[1-4] with transient[1] or prolonged[2-4,6] insulin independence. Two patients with type I, insulin-dependent diabetes mellitus[3,4] received islets from multiple donors (four and five pancreata). One of these patients[1] was still insulin-independent 20 months after islet allotransplantation. In the present report, prolonged (5 to >24 months) insulin independence was observed in six patients who underwent upper abdominal exenteration and liver-islet replacement.[2,6] Four of them received islets from two donors. The first patient of this series is still insulin independent over 24 months after the islet allograft and received islets from a single donor. In contrast, in our experience none of the type I diabetic patients who received either a liver-islet or a kidney-islet allograft are insulin independent. Although our best result in type I diabetic patients was obtained in a case of positive crossmatch (100%), we currently consider a positive crossmatch an absolute contraindication to human islet allotransplantation. Differences in islet isolation and/or purification techniques do 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 longstanding diabetes mellitus;[25,26] 2) steroid treatment that may have a detrimental effect on islet engraftment and/or function[27] 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;[28] 4) inadequacy of the liver as a transplant site when islets are allogeneic to the liver, possibly for the intrinsic ability of the hepatic microenvironment (hepatocytes, Kupffer cells, endothelium) to generate high levels of nitric oxide that is toxic to the islets (unpublished observations).

Based on our data we favor the hypothesis of the protective effect of a liver that is syngeneic to the transplanted islets, since it could provide a better microenvironment at the islet transplant site, e.g., inferior nitric oxide generation for the absence of a local allogeneic response. The detrimental effect of steroid treatment could have played a determinant role as well. Furthermore, weight loss was observed during the first 2-3 postoperative months in all patients receiving a cluster-islet graft. The nutritional problem associated to upper abdominal exenteration could also result in reduced insulin requirement in these patients. In addition, the native pancreas is removed in these patients who probably have less glucagon than type I diabetic patients.

In conclusion, our results indicate that pancreatectomy-induced diabetes represents a favorable setting for long term successful function of islet cell grafts. Rejection is still a major factor limiting the clinical application of islet transplantation in patients with type I diabetes mellitus although other factors such as the microenvironment at the transplant site and steroid treatment may contribute to deteriorate islet engraftment and/or function.

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