# Islet Transplantation in Large Animals

## Long-Term Metabolic Follow-up With Different Preparations of Islet Autografts

S.M. GRIFFIN, D. ALDERSON, AND J.R. FARNDON

Large-animal islet autografts restore diabetic metabolism to normal in the short term. Complications of diabetes appear only after a significant duration of disease process. An assessment of long-term function of grafts needs to be made before allograft experiments are entertained. Twelve dogs were made diabetic by total pancreatectomy. Seven dogs received 50% of a graft prepared by an established technique (group 1). Five dogs received 33% of a graft prepared by a method previously shown to be initially effective (group 2) (1). Tissue was grafted into the splenic pulp in all animals. At 1, 3, 6, 12, 18, 24, and 30 mo, euglycemic animals were assessed by intravenous glucose tolerance test so glucose clearance, insulin, lactate, pyruvate, alanine, free fatty acids, glycerol, 3-hydroxybutyrate, and glucagon responses could be determined. Splenectomy was performed on one animal in each group at 2 mo to confirm graft dependence. All animals in group 1 became diabetic by 8 mo, with fasting hyperglycemia occurring at 2, 4, 4, 7, 8, and 8 mo, respectively. No animals in group 2 became spontaneously diabetic. Abnormalities in fat metabolism were noted up to 3 mo but had normalized by 6 mo, and metabolic profiles were normal up to 30 mo. Histology of the spleens confirmed the presence of the three islet cell types. Delayed graft failure occurs with the established technique of graft preparation (group 1), but long-term function can be achieved with the group 2 method. In addition, metabolic control remains normal for up to 30 mo.

From the Department of Surgery, The University of Newcastle upon Tyne, Newcastle upon Tyne, United Kingdom.

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## Canine Pancreatic Islet Isolation and Intrahepatic Transplantation

J.A. VAN DER VLIET, D.B. KAUFMAN, R.M. MELOCHE, AND D.E.R. SUTHERLAND

Human trials of islet transplantation have been impeded by the inability to isolate an adequate mass of functional tissue that ameliorates diabetes. Methods must be refined in the large-animal model that are successful in reversing hyperglycemia and that are undemanding of time and resources before clinical application will be feasible. A method for isolation of canine islets was developed utilizing total pancreatectomy, intraductal collagenase (type XI) perfusion, and stationary digestion at 37°C. Digested tissue filtered through a steel mesh (400 μm) was washed and suspended in 25% dextran (density 1.088 g/ml) overlaid with 11% (density 1.041 g/ml) and centrifuged at 500 × *g*. Tissue enhanced for islets was recovered from the dextran interface (1.5–4.0 ml) and autotransplanted intraportally. Fasting plasma glucose levels were measured daily, and intravenous glucose tolerance tests (IVGTTs) were performed 2 and 10 wk posttransplant. Eight dogs underwent the procedure, with six (75%) reverting to immediate and sustained normoglycemia (glucose <200 mg/dl). These dogs had a mean glucose level of 103.3 ± 8.4 mg/dl and mean IVGTT *K* values of −1.21 ± 0.13 ($n$ = 6) and −2.59 ± 0.89 ($n$ = 3) at 2 and 18 wk, respectively. Based on insulin content the islet yield was 33 ± 3% of the total pancreas, and the islet mass was enhanced 20 ± 6-fold based on insulin-to-amylase ratios. The success rate in this canine model compares favorably with previously published rates. The procedure, performed within 4 h, allows for intrahepatic engraftment by utilizing a small tissue volume. The method is inexpensive and can be applied in the allotransplant model, furthering efforts toward human clinical trials.

From the Department of Surgery, University of Minnesota, Minneapolis, Minnesota; and University of Limburg, Maastricht, The Netherlands.

# Outcome of Intrahepatic Canine Islet Autotransplantation Predicted by Weight-Corrected Islet Count

S.R. MUNN, D.B. KAUFMAN, R.M. MELOCHE, M.J. FIELD, L.G. VAN DER HEM, AND D.E.R. SUTHERLAND

To facilitate the testing of hypotheses regarding preservation and allotransplantation of canine islet-enriched tissue with the intraportal route, it became apparent that an accurate predictor of outcome in an autograft model was necessary to exclude failures due to an inadequate transfer of islets. We pancreatectomized 12 dogs with a method that obviated duodenal ischemia, distended each fresh pancreas with collagenase (1600 U/ml, Sigma type XI), and allowed the gland to digest at 37°C for 20 min. Mechanical dispersion of the digested tissue resulted in a mean islet count, with diphenylthiocarbazone (dithizone) staining, of 197,000 ± 22,000 per pancreas in a volume of 31 ± 1.3 ml. This tissue was then purified on discontinuous dextran (71,500 $M_r$) gradients, resulting in a greatly reduced tissue volume of 2.0 ± 0.3 ml; this was at the expense of a 55% reduction in yield, the mean islet count being 87,000 ± 7000. This material averaged 20.5% of the total pancreatic insulin as estimated by sampling all portions of the islet isolation procedure for insulin content. We then transplanted the islets back into the dogs via a mesenteric venous tributary and monitored the subsequent graft function with daily nonfasting blood glucose measurements as well as intravenous glucose tolerance tests in those with functioning transplants. Two of the animals suffered intraoperative technical problems related to islet infusion and were excluded. Of the remainder, 7 of 10 achieved durable euglycemia within 24 h, whereas 3 of 10 rapidly became hyperglycemic and were killed. The mean $K$ value of the dogs with successful grafts was −1.41 ± 0.20 2 wk posttransplant. Univariate analysis of parameters predictive of outcome is summarized in Table 1. Both weight-corrected islet counts and percentage of total insulin in the transplant were 100% specific and sensitive in predicting outcome; all grafts with >4350 islets/kg or with >19% of total insulin were successful, and the converse was also true. Of these two parameters, islet counting is the most convenient and is truly prospective in that it can be done before transplantation.

TABLE 1
Univariate analysis of parameters

| Parameter | Total islets | Islets/kg | Percent total insulin |
|---|---|---|---|
| Unsuccessful | 62,900 ± 4900 | 3500 ± 400 | 12.7 ± 1.8 |
| Successful | 94,400 ± 8900 | 5400 ± 500 | 24.4 ± 1.7 |
| $P$ | .060 | .004 | .004 |

Values are means ± SE.

From the Department of Surgery, University of Minnesota, Minneapolis, Minnesota.

# Pancreatic Islet Autotransplantation Protects Against Diabetic Retinopathy at 18 and 27 mo in Dogs

T. WALSH, D. MOONEY, AND S. TRAVERS

The development of diabetic retinopathy is related to persisting metabolic abnormalities during conventional insulin therapy. Dispersed islet-graft transplantation corrects fasting and postprandial insulin, glucose, and intermediary metabolic profiles and may prevent the secondary complications of diabetes. This study examines the effects of islet autotransplantation on the retina in successful recipients at 18 mo ($n$ = 5) and 27 mo ($n$ = 2). Six mongrel dogs weighing between 12 and 24 kg underwent total pancreatectomy, and a dispersed islet autograft was prepared and transplanted as described elsewhere. Blood glucose was assayed weekly, and glycosylated hemoglobin was assayed monthly. All animals were followed up for 18–27 mo or until graft failure. Fluorescein angiographic studies of the retina were performed at 18 mo (5 dogs) and repeated at 27 mo (2 dogs). Three grafts failed between 18 and 27 mo. Angiography was performed under general anesthesia with 1% tropicamide and 10% phenylephrine to dilate the pupils, with a Zeiss 30° fundus camera fitted with spectrotech exciter/barrier filter combination on Kodak Tri X Pan film. Films were compared with age-matched controls. Fluorescein angiograms at 18 and 27 mo were normal, showing no evidence of fluorescein leakage or microaneurysm formation. In the three animals with grafts that failed between 18 and 27 mo, the absence of diabetic changes was confirmed histologically. In conclusion, failure to detect retinal changes at 18 or 27 mo by fluorescein angiography suggests that successful islet transplantation may prevent or delay diabetic retinopathy.

From the Department of Surgery, Mater Misericordiae Hospital and Royal Victoria Eye and Ear Hospital, Dublin, Ireland.

# Short-Term Administration of Cyclosporin A in Normal Dogs Results in Irreversible Islet Secretory Defects

E.C. FELDMAN, R. ALEJANDRO, AND D.H. MINTZ

Although cyclosporin A (CsA) has been shown to inhibit insulin secretion in vitro and in vivo in several species, it is still unclear whether these effects are reversible. To examine this question, we assessed plasma glucose and insulin responses to glucose (0.5 g/kg i.v.), glucagon (1 mg i.v.), and oral glucose (1 g/kg) in six normal beagle dogs before, during, and 1 and 4 mo after administration of CsA in doses previously shown to be required for uniform prevention of canine islet-allograft rejection (20 mg/kg, mean trough radioimmunoassay serum levels not <500 ng/ml; 1). The results are given in Table 1. Insulin secretion in response to intravenous glucose and glucagon is significantly inhibited during administration of CsA. These secretory defects persist 4 mo after CsA is discontinued. Plasma glucose disposal in response to these secretagogues, however, returns to normal 1 mo after discontinuation of CsA. Insulin secretion and glucose disappearance in response to oral glucose were not statistically different during CsA administration ($P$ = .08 and .84, respectively; not shown). We conclude that although glucose disappearance rates are normal after discontinuation of CsA therapy, CsA causes an irreversible impairment in islet secretory responses detectable with intravenous glucose and glucagon but not with oral glucose. These results suggest that short-term CsA in doses required to prevent islet-allograft rejection in dogs can result in permanent loss of functionally competent β-cells.

From the University of Miami, Miami, Florida.

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TABLE 1
Results of IVGTT and glucagon TT

| | IVGTT (areas under curves) | | | | Glucagon TT (areas under curves) | | | |
|---|---|---|---|---|---|---|---|---|
| | Glucose (mg · min · dl$^{-1}$) | $P$ | Insulin (μU · min · ml$^{-1}$) | $P$ | Glucose (mg · min · dl$^{-1}$) | $P$ | Insulin (μU · min · ml$^{-1}$) | $P$ |
| Before CsA | 7325 ± 281 | | 1550 ± 179 | | 9857 ± 365 | | 2593 ± 391 | |
| During CsA | 10,066 ± 630 | .004 | 829 ± 160 | .004 | 14,568 ± 862 | .003 | 613 ± 68 | .005 |
| 1 mo later | 7858 ± 452 | .41 | 773 ± 112 | .001 | 10,016 ± 397 | .80 | 1494 ± 459 | .04 |
| 4 mo later | 6746 ± 131 | .16 | 607 ± 92 | .01 | 9485 ± 798 | .56 | 1472 ± 343 | .002 |

IVGTT, intravenous glucose tolerance test; glucagon TT, glucagon tolerance test.

# Canine Islet Auto- and Allotransplants Maintain Metabolic Control With Cyclosporin A Immunosuppression

A.J. GUY, S.M. GRIFFIN, D. ALDERSON, AND J.R. FARNDON

If islet transplants restore metabolic normality in diabetes, secondary complications could be prevented. The metabolic control maintained by canine islet auto- and allotransplants, immunosuppressed with cyclosporin A (CsA), has been investigated with pancreatectomized dogs. Beagles were studied in four groups: controls (group 1, $n$ = 65), islet

TABLE 1
Median fasting values

| | Glu (mM) | Ins (mU/L) | L/P | Ala (μM) | Gly (μM) | 3-HB (μM) | FFA (mM) | Chol (mM) | CsA (ng/ml) |
|---|---|---|---|---|---|---|---|---|---|
| Group 1 | 5.5 | 18.9 | 16.9 | 425 | 95 | 11.5 | 0.43 | 5.16 | |
| Group 2 | 5.5 | 15.3 | 13.1* | 444 | 96 | 14.5 | 0.53 | 6.40* | |
| Group 3 | 7.0* | 22.4 | 16.9 | 446 | 84 | 9.8 | 0.26 | 5.35 | 649 |
| Group 4 | 6.0 | 12.5 | 16.4 | 387 | 83 | 17.0 | 0.47 | 6.21 | 689 |

*See text; all comparisons by Mann-Whitney $U$ test.

autotransplants (group 2, $n = 9$) islet autotransplants + CsA (group 3, $n = 6$), and islet allotransplants + CsA (group 4, $n = 5$). Fasting and intravenous glucose tolerance test (IVGTT) profiles of glucose (Glu), insulin (Ins), lactate (L), pyruvate (P), alanine (Ala), glycerol (Gly), β-hydroxybutyrate (3-HB), free fatty acid (FFA), and cholesterol (Chol) were established before operation and repeated 1 mo after islet transplantation. Islet grafts were prepared by collagenase perfusion and were implanted in the splenic pulp. CsA was given by intramuscular injection at an initial dose of 20 $mg \cdot kg^{-1} \cdot day^{-1}$, adjusted to maintain the plasma CsA level >400 ng/ml. Results are given in Table 1. Compared with control animals, group 2 dogs had a low lactate-to-pyruvate ratio ($P < .02$) and raised cholesterol ($P < .001$) 1 mo post-transplantation, and group 3 dogs were mildly hyperglycemic ($P < .002$). Insulin response during IVGTT and glucose clearance were reduced in all transplanted animals. In all transplanted groups, appropriate changes in intermediary metabolites and lipids occurred during IVGTT. Despite mild hyperglycemia, CsA does not adversely affect intermediary or lipid metabolism of islet-autotransplanted dogs and allows islet allotransplants to maintain metabolic normalcy. If, in clinical practice, islet-graft survival could be maintained, metabolic normalcy would ensue, and diabetic complications would be prevented.

From the Department of Surgery, University of Newcastle, Newcastle upon Tyne, United Kingdom.

# Detrimental Effects of Triple Immunosuppressive Therapy on Pancreatic Islet Allografts in Canine Model

P. STOCK, D.E.R. SUTHERLAND, M. DUNNING, M.J. FIELD, D. CASANOVA, M. PRIETO, AND J.S. NAJARIAN

In spite of limited success with pancreatic islet autotransplantation in humans, transplantation of islet allografts has been totally unsuccessful. One contributing factor to allograft failure could be the toxic effect of the immunosuppressive drugs on islets. We investigated this possibility in canine pancreatic segmental and islet autografts. Fourteen dogs were divided into three groups: group 1 ($n = 4$), untreated controls; group 2 ($n = 5$), total pancreatectomy with intrasplenic islet autotransplantation; group 3 ($n = 5$), total pancreatectomy with segmental autotransplantation with bladder drainage. Intravenous glucose tolerance tests (IVGTTs) were performed on day 0. Dogs were subsequently given triple drug immunosuppression for 2 wk: 2.0 $mg \cdot kg^{-1} \cdot day^{-1}$ Imuran, 1.0 $mg \cdot kg^{-1} \cdot day^{-1}$ prednisolone, and 25 $mg \cdot kg^{-1} \cdot day^{-1}$ cyclosporin. On day 15, the IVGTT was repeated. The mean difference in $K$ values (negative slope of glucose disappearance 1–30 min) and mean fasting serum glucose levels are given in Table 1. Triple immunosuppression in dosages currently used were toxic in recipients of islet autotransplantation. All 5 dogs in this group became diabetic within 2 wk of treatment. None of the control group or recipients of segmental autotransplants did so, presumably because they had adequate β-cell reserve. These data suggest that different immunosuppressive regimens will be necessary for clinical islet allografts.

From the Department of Surgery, University of Minnesota Hospitals, Minneapolis, Minnesota.

TABLE 1
IVGTT $K$ values and fasting serum glucose

| | IVGTT $K$ values | | | Fasting serum glucose (ng/dl) | | |
|---|---|---|---|---|---|---|
| Group | Day 0 | Day 15 | Day 15 − day 0 | Day 0 | Day 15 | Day 15 − day 0 |
| 1 | −2.7 ± 0.5 | −3.2 ± 1.4 | −0.55 ± 0.9 | 82 ± 5.6 | 106 ± 14 | 25 ± 6.4 |
| 2 | −2.7 ± 0.5 | −2.3 ± 0.4 | +0.29 ± 0.1 | 95 ± 6.7 | 106 ± 16 | 11 ± 9.3 |
| 3 | −1.2 ± 0.3 | −0.1 ± 0.5 | +1.1 ± 0.8* | 96 ± 8.2 | 473 ± 95 | 376 ± 44* |

Values are means ± SD.
*$P < .05$ by paired Student's $t$ test.

# Maintenance of Functional Porcine Pancreatic Pseudoislets Embedded in Collagen-Gel Matrix

H. OHGAWARA, R. YUI, S. NISHIJIMA, N. IWASAKI, M. CHOSA, AND Y. HIRATA

Tissue culture techniques in which pancreatic islets are maintained in culture for varying periods are being used for transplantation. Cultured islets for transplantation should have a high clinical potential to supply insulin and glucagon in response to normal physiologic demand. We describe a method in which neonatal porcine pseudoislets are embed-

ded in collagen-gel matrix for maintenance of long-term survival. Neonatal porcine pancreases were dissociated by sequential treatment with EDTA-dispase. The cells were cultured in culture flasks for 24 h and incubated on a shaker that rotated with a figure-eight motion for 4–5 days. During this time, the endocrine cells formed cell clumps (pseudoislets) on sheets formed by the fibroblasts. These pseudoislets were recultured in collagen-gel matrix for 30 days. For the first 2 days, they were treated with cystine-free medium to eliminate the fibroblasts and endothelial and acinar cells. The cells were then cultured with RPMI-1640 containing 11 mM D-glucose, 10% fetal bovine serum, with or without 10 mM nicotinamide or 0.1 mM 3-isobutyl-1-methylxanthine (IBMX), and were refed every 4 days. Provocative stimulation with nicotinamide or IBMX confirmed a prolonged survival of the pseudoislets, which were maintained in an embedded culture in a collagen-gel matrix. Morphologically, excellent preservation of the pancreatic endocrine cells was confirmed by light microscopy with enzymatic staining method. The preliminary results of this study will form the pancreatic endocrine cells in collagen-gel matrix for maintenance of cell function. Also, the possible use of porcine endocrine cells as a source of transplantation will be investigated.

From the Diabetes Center, Tokyo Women's Medical College, Tokyo, Japan.

# Immunoisolation of Rat and Porcine Islets of Langerhans by Polyurethane-Silicone Macrocapsules

G. SOLDANI, P. MARCHETTI, R. GIANNARELLI, A. DI CARLO, A. MASONI, P. MASIELLO, M. PALLA, P. GIUSTI, AND R. NAVALESI

The immunological problems related to islet transplantation may be overcome by the immunoisolation of the graft with micro- or macrocapsules. In both cases, it is mandatory for the capsules to be biocompatible, impermeable to the immunocompetent cells, and freely permeable to nutrients and hormones. We prepared macrocapsules with a blend of polyurethane and silicone as biomaterial. The macrocapsules were obtained by plugging segments of tubes prepared by a spraying and phase-inversion technique that produced a microporous structure, with a thin skin on the inside of the tube. The macrocapsules were evaluated for permeability to glucose, insulin, albumin, immunoglobulins, and white blood cells. In addition, the response to glucose of macroencapsulated islets of Langerhans purified from rat and adult pig pancreases was studied. Glucose and insulin diffusion from the macrocapsules to the incubation medium reached the plateau after 10 and 30 min, respectively. Albumin, immunoglobulins, and white blood cells did not cross the macrocapsule walls. Macroencapsulated rat islets secreted 1.0 ± 0.05 and 1.8 ± 0.05 μU insulin · islet$^{-1}$ · min$^{-1}$ ($P < .05$) in the presence of 50 and 400 mg/dl glucose, respectively, in Krebs-Ringer bicarbonate solution, during 45 min incubation at 37°C. The same figures for macrocapsulated porcine islets were 3.7 ± 1.6 and 5.3 ± 1.4 μU insulin · islet$^{-1}$ · min$^{-1}$ ($P < .05$). Over a 2-h incubation period, insulin release from encapsulated and unencapsulated pig islets attained a similar plateau value, although the plateau was reached more slowly by encapsulated islets. These results suggest the possibility of using polyurethane-silicone macrocapsules for allo- and xenotransplantation of islets of Langerhans.

From the Centro Studio Tecnopolimeri e Biomateriali, CNR; the Cattedra Malattie del Ricambio, Istituto Clinica Medica II; the Istituto Fisiologia Clinica CNR; and the Dipartimento Ingegneria Chimica, Pisa, Italy.

# Long-Term Results After Islet Transplantation in Pigs

U.J. HESSE, J. WEYER, G. MEYER, N. KIPPING, H. PICHLMAIER, AND M. VIERBUCHEN

Because an affinity to the human pancreas and insulin is attributed to the porcine pancreas and insulin, the pig could be an ideal donor source for islet xenotransplantation (1). Long-term results after successful islet transplantation in pigs representing the isolation of a sufficient quantity of viable islets, however, are not reported. A newly refined intraductal peripancreatic collagenase perfusion technique was evaluated for islet isolation and transplantation in pigs. Landrace pigs of either sex weighing between 25 and 30 kg were totally pancreatectomized. The pancreatic duct was cannulated and perfused with collagenase solution (800 U/ml) for distension at 37°C for 10 min at a rate of 20 ml/min. Thereafter, the gland was immersed for 20 min in collagenase at 37°C while the perfusion with collagenase was continued. The pancreatic capsule and duct were removed after the pancreatic tissue was teased apart. Subsequently, the tissue was agitated in a shaking water bath, filtered through a screen (600-μm pore size), and washed with Hanks' solution in a centrifuge. The resulting suspension was transplanted into the spleen (IS) in four animals and as a pilot study into the portal vein (IP) in one pig. Daily fasting glucose levels were considered normoglycemic when <150 mg/dl. Intravenous glucose tolerance tests (IVGTTs) were performed 2 wk and 3 mo posttransplant. Four animals with IS

transplants and the IP-transplanted pig survived >3 mo. Two of four IS-transplanted animals and the IP-transplanted pig were normoglycemic. IVGTTs were nearly normal in these normoglycemic animals, whereas they were impaired in the hyperglycemic animals. Because pancreatectomy-induced diabetes is uniformly lethal in pigs within 10–12 days, these results represent a new model for the preparation of potential islet xenografts and the study of successful islet transplantation in pigs.

From the Chirurgische Universitätsklinik Köln, Cologne, Federal Republic of Germany.

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