

Reversal of Diabetes in Pancreatectomized Pigs After Transplantation of Neonatal Porcine Islets

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Neonatal porcine islets (NPIs) are able to grow and to reverse hyperglycemia after transplantation in immunoincompetent mice. The aim of this study was to demonstrate the feasibility of allogeneic NPI grafts to achieve normoglycemia in a pancreatectomized diabetic pig. NPIs were isolated from pancreases of 1- to 3-day-old pigs, cultured, and then transplanted via the portal vein into the liver of totally pancreatectomized pigs (mean body weight, 20.8 kg). Each pig received NPIs consisting of $3.1 \pm 0.3 \times 10^6$ β -cells/kg ($12,476 \pm 1,146$ islet equivalent/kg). The six pigs that were given cyclosporine and sirolimus achieved normoglycemia by day 14 without insulin therapy. Three pigs died of surgical complications shortly after transplantation, whereas the other three remained insulin independent up to day 69. Of seven nonimmunosuppressed recipients, four pigs became normoglycemic by day 14 without insulin treatment, with two of the animals remaining normoglycemic long term. Well-preserved insulin-positive cells were found in the graft at the end of follow-up with a significant increase in insulin content in long-term survivors of both groups. This study demonstrates for the first time that allogeneic NPIs can reverse hyperglycemia in totally pancreatectomized diabetic pigs. *Diabetes* 54:1032–1039, 2005

Clinical islet transplantation is now an alternative treatment for brittle diabetes (1–5); however, the challenge we now face is to develop an unlimited source of tissue so that this therapy can be offered to more diabetic patients. There are several alternative approaches that are being pursued, including islets from living donors, nonhuman islet xenografts, genetically engineered insulin-secreting cells, in vitro expansion of β -cells, and differentiation of pancreatic precursor cells into β -cells (6,7). With respect to xenografts, the pig is the most likely source because it shares many physio-

logical features with humans, breeds rapidly, and produces large litters. Porcine insulin has also been used successfully for a long time to treat diabetic patients. Adult porcine islets, however, have been difficult and expensive to obtain on a consistent basis with the additional problem that, once isolated, adult porcine islets are fragile and difficult to maintain in culture (8–10). A more attractive source of tissue that does not have the technical problems is neonatal pigs. Neonatal porcine islets (NPIs) constitute a potential source of cells for clinical transplantation because of their inherent ability for proliferation and differentiation. We have developed a simple, reliable, and reproducible method to obtain a large number of NPIs from pigs aged 1–3 days (11). NPIs were shown to consist of fully differentiated endocrine cells (35%) and endocrine precursor cells (57%) (11). Studies from our laboratory (11–13) and others (14–16) have demonstrated that NPI grafts grow and are able to reverse hyperglycemia after transplantation in immunoincompetent diabetic mice. The next logical step, before clinical transplantation, is to determine whether we can also cure diabetes by transplanting NPIs in a large-animal model. The aim of this study was to demonstrate the feasibility of NPI allografts to reverse hyperglycemia in a preclinical pancreatectomized porcine model.

RESEARCH DESIGN AND METHODS

Landrace-Yorkshire crossbred pigs (Swine Research and Technology Centre, University of Alberta) were used as donors and recipients. Donor pancreases were obtained from 1- to 3-day-old neonatal pigs (1.5–2.0 kg body wt) of either sex. Recipients were male, 2- to 3-month-old (16.0–26.5 kg body wt) pigs. The recipient pigs were housed individually in a large-animal vivarium and cared for in accordance with the recommendations of the Canadian Council on Animal Care. They were fed in the morning (at 0800) and in the afternoon (at 1400) with a standard commercial chow. The recipients were acclimatized for 2 weeks before surgery.

Preparation of NPIs. The method used to isolate NPIs has been previously described (11). Briefly, neonatal pigs were anesthetized under halothane and subjected to laparotomy and exsanguination. The pancreas was removed, placed in Hanks' balanced salt solution (HBSS) supplemented with 0.25% BSA (Sigma, St. Louis, MO), cut into 1- to 2-mm pieces, and digested with 1.0 mg/ml collagenase (Type V, Sigma). After filtration through a nylon screen (500 μ m), the tissue was cultured in Ham's F10 medium (Life Technologies, Burlington, ON, Canada) containing 10 mmol/l glucose, 50 μ mol/l isobutylmethylxanthine, 0.5% BSA, 2 mmol/l L-glutamine, 10 mmol/l nicotinamide, 100 U/ml penicillin, and 100 μ g/ml streptomycin at 37°C for 5–7 days before transplantation with subsequent media changes every 2 days.

Graft characterization. On the day of transplantation, NPIs were recovered from the culture dishes, washed in Ham's F10 medium, and assessed for total cellular insulin and DNA contents as well as cellular composition by immunocytochemical methods as previously described (11). All measurements were assessed from duplicate aliquots of the islet cell suspensions. For cellular composition, NPI aggregates were dissociated into single-cell suspensions by gentle agitation in calcium-free medium containing trypsin (15 μ g/ml)

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CsA, cyclosporine; HBSS, Hanks' balanced salt solution; IE, islet equivalent; IVGTT, intravenous glucose tolerance test; NPI, neonatal porcine islet; RIA, radioimmunoassay.

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and DNase (4 $\mu\text{g/ml}$). Cell preparations were fixed and stained for insulin. Total number of β -cells per graft was calculated on the basis of the DNA content of the graft and the percentage of insulin-positive cells in the graft (11). The total number of neonatal islets [islet equivalent (IE)] per graft was calculated on the basis of the DNA contents of the graft [$\text{IE} = \text{DNA}(\text{ng})/9.23$] (11).

Indwelling catheters. Catheters were placed into an external jugular vein in all pigs 1–2 weeks before total pancreatectomy. Pigs were fasted overnight but were allowed access to water. They were premedicated with atropine sulfate (0.05 mg/kg) and ketamine hydrochloride (20 mg/kg) intramuscularly. Anesthesia was induced with a mixture of isoflurane and oxygen. Pigs were intubated and maintained on 2% isoflurane and 100% oxygen. The right or left external jugular vein was exposed and cannulated by two catheters (Silastic laboratory tubing, internal diameter 1.02 mm; Dow Corning, Midland, MI). Catheters were tunneled beneath the skin, exteriorized, and placed in a pouch to protect the catheters.

Total pancreatectomy and transplantation. Total pancreatectomy and transplantation were performed during one surgery in recipient pigs under general anesthesia as described above. The abdomen was entered through an upper vertical midline incision. The head of the pancreas was visualized, and the pancreatic capsule was incised over this portion of the pancreas. The pancreatic duct was identified, ligated, and divided, and the head of the pancreas was then separated from the duodenal wall. Careful attention was paid to preserving the pancreaticoduodenal vascular arcade. The uncinate process was dissected free from the surrounding structures, and then the posterior loop of the pancreas was divided where it passes dorsal to the superior mesenteric vein. Next, the tail and body of the pancreas were dissected free from its posterior attachments. The dissection was continued to the head of the pancreas. The posterior loop of the pancreas was dissected free from the right part of the superior mesenteric vein. The complete pancreas was then removed after ligation and division of the pancreatic artery and vein. After total pancreatectomy, a catheter (Silastic laboratory tubing, internal diameter 1.02 mm; Dow Corning) was inserted into the portal vein through the short gastric vein. The islets suspended in 10 ml HBSS were infused over 5 min through this catheter. Portal venous pressure was measured before and after infusion using a water manometer.

Postoperative care. The animals received 1 week of postoperative antibiotic prophylaxis (cefazolin, 500 mg twice a day). Normal diet was given starting from the day after transplantation. Each pancreatectomized pig received pancreatic enzyme (Cotazyme, four capsules per day; Organon Canada, Toronto, Canada) mixed with food to prevent pancreatic exocrine deficiency. Animals were clinically assessed daily and were weighed once a week. Blood glucose levels were determined twice a day before feeding with a portable glucose meter (Fast Take; LifeScan, Burnaby, British Columbia, Canada). Blood samples were obtained at weekly intervals for measurement of serum levels of alanine aminotransferase and aspartate aminotransferase using the Multistat III analyzer (Instrumentation Laboratory, Lexington, MA). Subcutaneous insulin (Novolin geNPH; Novo Nordisk, Bagsvaerd, Denmark) was given once or twice a day during meals as needed to maintain blood glucose below 30 mmol/l.

Immunosuppression. Some animals were treated with an immunosuppressive regimen consisting of cyclosporine (CsA) and sirolimus (Rapamune, Wyeth-Ayerst, Markham, ON, Canada). Immunosuppression was initiated 1–3 weeks before total pancreatectomy. CsA dissolved in medium-chain triglyceride oil, and sirolimus were given mixed with food every morning, with levels adjusted to maintain a trough level of 400 ng/ml CsA and 10 ng/ml sirolimus. Blood samples were taken immediately before the preceding administration three times a week for measurement of CsA and sirolimus by a monoclonal radioimmunoassay (RIA) and reverse-phase high-performance liquid chromatography, respectively.

Intravenous glucose tolerance test. Intravenous glucose tolerance tests (IVGTTs) were conducted before pancreatectomy/transplant and at 1 month posttransplant. A dextrose solution (0.5 g/kg body wt) was injected through an indwelling catheter over 1 min. Blood samples were taken through another catheter at 0, 5, 15, 60, and 120 min. *K* values [percentage decline of natural logarithm (blood glucose)/min] were calculated with blood glucose measurements from 5 to 120 min during IVGTT. Serum samples were also collected to assay porcine insulin and C-peptide using RIA specific to porcine peptide (Linco Research, St. Louis, MO).

Characterization of harvested NPIs grafts. At the end of the study, the whole liver was removed for histological examination and to determine insulin content of the harvested grafts (11). For histology, several portions of the liver were immersed in buffered zinc formalin, embedded in paraffin, sectioned, stained with hematoxylin and eosin, and immunostained for insulin and glucagon. For measurement of insulin content, the whole liver was weighed, and then five to eight pieces of the liver were taken randomly,

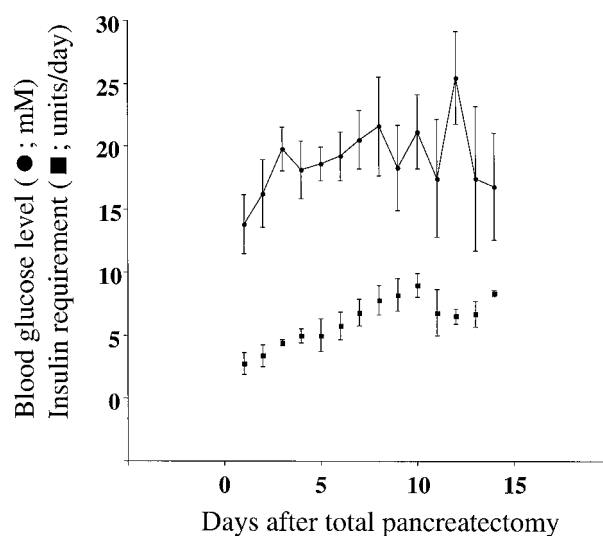


FIG. 1. Morning blood glucose levels and daily insulin requirements of totally pancreatectomized control pigs ($n = 5$). Bars represent means \pm SE.

weighed, immersed in liquid nitrogen, and stored at -80°C . At least one piece of the liver from the surface of both lobes and one piece from the central part of both lobes were taken in an attempt to get a representative sample. Liver tissues were homogenized and then sonicated at 4°C in 10 ml of 2 mmol/l acetic acid. After a 2-h incubation at 4°C , tissue homogenates were resonicated and centrifuged at $8,000g$ for 20 min; supernatants were then collected, and the pellets were further extracted by sonication in an additional 10 ml of acetic acid. The second supernatant was collected after centrifugation and combined with the first supernatant. The samples were assayed for insulin content by RIA using porcine insulin standards.

Statistical analysis. Mean values are presented with SE. Paired or unpaired Student's *t* test was used for statistical comparison. $P < 0.05$ was considered statistically significant.

RESULTS

Blood glucose levels in apancreatic pigs. Initially, five pigs (15.0 ± 0.3 kg) were pancreatectomized, given immunosuppression, and maintained on insulin for 15 days to serve as controls. Figure 1 illustrates the profile of morning blood glucose levels and insulin requirements of these five pigs after total pancreatectomy. All pigs became hyperglycemic within 2 days after total pancreatectomy, and the mean daily insulin requirements ranged from 2.8 ± 0.9 to 9.0 ± 0.9 units/day, resulting in mean \pm SE morning blood glucose levels ranging from 13.8 ± 2.4 to 25.4 ± 4.2 mmol/l.

Recipients and graft characterization. Thirteen pigs underwent total pancreatectomy and transplantation of NPIs obtained from multiple donors. The total number of β -cells, a cellular insulin content, and number of IEs per recipient's body weight were $3.1 \pm 0.3 \times 10^6$ cells/kg, 11.8 ± 1.2 $\mu\text{g/kg}$, and $12,476 \pm 1,146$ IE/kg, respectively (Table 1). A mean packed cell volume of 1.8 ± 0.1 ml was infused, resulting in an increase in the portal pressure from 95.5 ± 7.9 to 121.6 ± 8.8 mm H_2O ($P = 0.001$); however, no significant increases in liver enzymes were observed (aspartate aminotransferase/alanine aminotransferase, $33.4 \pm 9.8/48.2 \pm 4.0$ units/l at pretransplant, $26.7 \pm 6.5/51.8 \pm 4.8$ units/l at 1 week posttransplant, and $24.6 \pm 2.8/53.3 \pm 9.3$ units/l at 2 weeks posttransplant).

Islet transplantation with immunosuppression. Six pigs received immunosuppressive therapy (Table 1) with mean trough levels of CsA for each week at 292.0 ± 100.3 ,

TABLE 1
Islet graft characteristics

Pig	Immuno-suppression	Body weight (kg)	Graft insulin content ($\mu\text{g}/\text{kg}$ recipient's body weight)	β -Cell mass ($\times 10^6/\text{kg}$ recipient's body weight)	Mass of islets (IE/kg recipient's body weight)	Packed cell volume (ml)
1	Yes	16.0	7.2	2.1	8,352	1.50
2	Yes	23.0	8.8	1.9	6,498	2.00
3	Yes	25.5	6.3	2.5	11,400	2.00
4	Yes	21.5	11.5	4.9	12,416	2.00
5	Yes	22.5	12.7	3.3	19,001	2.00
6	Yes	23.5	9.6	3.3	10,522	1.75
7	No	26.5	11.3	1.4	16,064	2.25
8	No	20.0	20.6	5.7	17,439	2.00
9	No	22.5	9.9	2.5	7,409	1.50
10	No	15.0	13.1	2.1	14,217	1.75
11	No	18.0	18.9	2.7	18,912	1.75
12	No	14.0	15.7	3.9	10,405	1.50
13	No	21.5	7.7	3.7	9,557	1.75
Means \pm SE		20.8 \pm 1.0	11.8 \pm 1.2	3.1 \pm 0.3	12,476 \pm 1,146	1.8 \pm 0.1

313.4 \pm 44.9, 283.0 \pm 61.3, and 224.4 \pm 106.4 ng/ml, for -1, 1, 2, and 3 weeks after transplantation, respectively. Mean trough levels of sirolimus for each week were 14.4 \pm 2.5, 9.0 \pm 1.1, 10.7 \pm 1.3, and 9.2 \pm 1.4 ng/ml, for -1, 1, 2, and 3 weeks after transplantation, respectively. The blood glucose levels in these six recipients gradually decreased after transplantation and were normalized by 14 days. During this immediate posttransplant period, only a small amount of insulin (0–2.2 units/day) was required, which was considerably less than the pancreatic controls (2.8–9.0 units/day). Three pigs (4, 5, and 6) died of a small bowel volvulus shortly after transplantation; however, the remaining three pigs (1, 2, and 3) became normoglycemic within 14 days posttransplant, and insulin independence was maintained throughout the follow-up period for these three pigs (Fig. 2). One of these recipients (1) was killed on day 39 (Fig. 2A), and in the remaining two pigs, CsA and sirolimus were stopped on day 25 (Fig. 2B) and day 36 (Fig. 2C), respectively; however, these pigs remained normoglycemic without further insulin injection throughout the follow-up period. One pig (3) was killed 5 days after discontinuation of immunosuppressive therapy because of infection related to the indwelling catheter. Another pig (2) was followed until 69 days posttransplant. CsA and sirolimus levels in this pig were not detectable 5 days after discontinuation of the drugs. At the end of the study, the liver was removed for histological examination and for determination of cellular insulin content. Histological analysis of the liver from all six recipients revealed well-preserved insulin- and glucagon-positive cells in the portal tract with negligible lymphocytic infiltration (Fig. 3A and B). The three pigs (4, 5, and 6) that died shortly after transplantation had 3.9-, 1.2-, and 3.3-fold more insulin in the liver, respectively, than what was initially transplanted (Table 2). The three pigs (1, 2, and 3) with prolonged graft function had 6.4-, 31.0-, and 21.5-fold more insulin in the liver, respectively (Table 2). Body weight of these six pigs decreased to 96.0 \pm 1.3% of the original weight by 1 week posttransplant, whereas in the three long graft survivors, there was an increase to 106.5 \pm 2.2% of the original weight at 4 weeks posttransplant.

Islet transplantation without immunosuppression.

On the basis of the observation that some recipients showed graft function after discontinuation of the immunosuppressive drugs, we decided to transplant NPIs without immunosuppression for the next seven pigs (Table 1). In contrast to apantretic controls not receiving an NPI graft, all recipients except for one did not require insulin therapy after the transplantation of NPIs. Two pigs (9 and 11) died of a complication—a small bowel volvulus—on days 4 and 7, respectively. Hyperglycemia persisted after transplantation in one recipient (13), and this pig was killed on day 13. The remaining four pigs became normoglycemic within 6–14 days after transplantation (Fig. 4). Two pigs (8 and 10) returned to hyperglycemia by day 14, whereas the other two (7 and 12) remained normoglycemic throughout the experimental follow-up period (>49 days). In three pigs (8, 10, and 13) that were hyperglycemic at the end of the study, a few intact islets were observed, and a massive lymphocytic infiltration was within the portal space (Fig. 3C). Their liver had only 10–30% of initial amount of insulin (Table 2). The two pigs (9 and 11) that died of surgical complications had insulin-positive cells as well as glucagon-positive cells in the portal tract with negligible lymphocytic infiltration (data not shown). The insulin contents of these livers were 70–80% of pretransplanted values (Table 2). The two pigs (7 and 12) that had prolonged graft function had well-preserved insulin-positive cells (Fig. 3D) as well as glucagon-positive cells in the portal space without lymphocytic infiltration. Their livers contained considerably more insulin than what was initially transplanted (Table 2). The body weight of recipient pigs decreased to 94.2 \pm 2.2% of the original weight by 1 week posttransplant, whereas in the two long-term graft survivors, there was a progressive increase to 130.3 \pm 1.8% of the original weight at 6 weeks posttransplant.

IVGTT. Although IVGTTs were conducted in all recipient pigs before total pancreatectomy, only animals with long-term graft function (pigs 2, 3, 7, and 12) had an IVGTT performed at 1 month posttransplant (Fig. 5). Blood glucose levels declined rapidly to basal levels within 60 min after glucose injection in both prepancreatectomy and

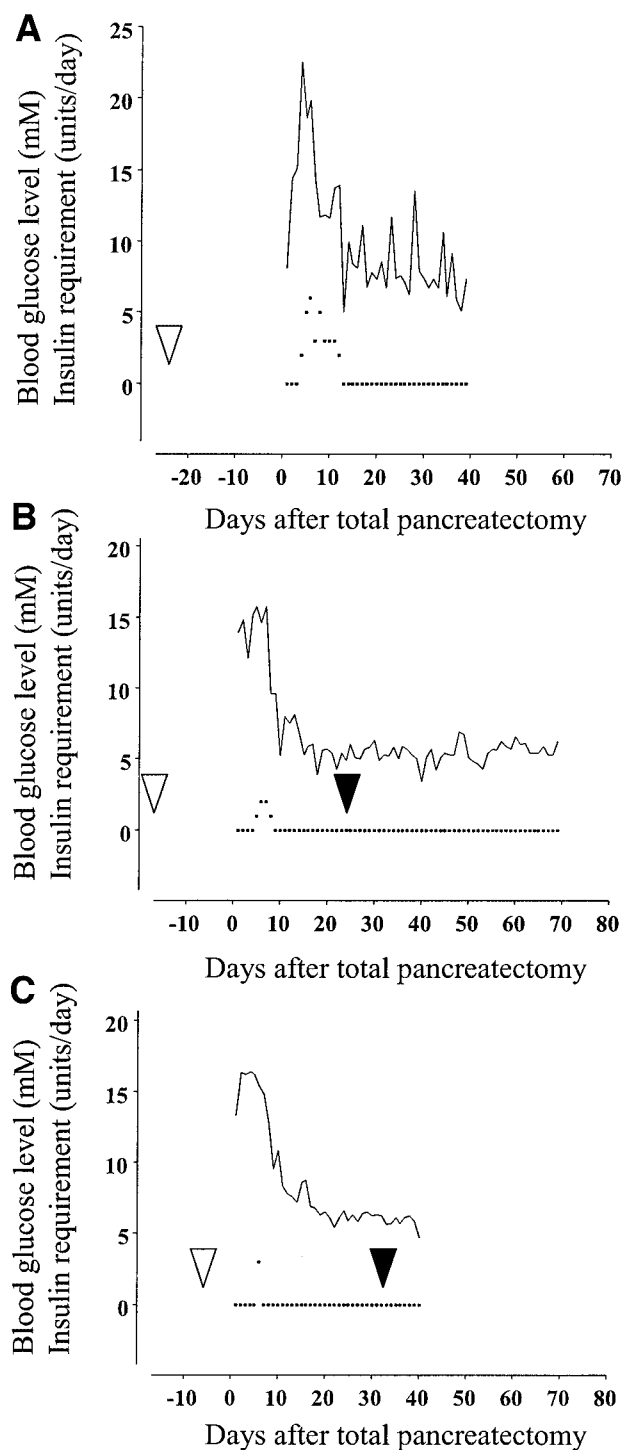


FIG. 2. Morning blood glucose levels (—) and daily insulin requirements (⋯) in three pigs after transplantation of NPIs with immunosuppressive drugs. In pig 1 (A), 8,352 IE/kg was injected into the liver. Insulin was discontinued on day 13, and normoglycemia (<8.4 mmol/l) was achieved on day 13. In pig 2 (B), 6,498 IE/kg was injected into the liver, and insulin was discontinued on day 9 with normoglycemia obtained on day 10. In pig 3 (C), 11,400 IE/kg was injected into the liver. Insulin was discontinued on day 6, and normoglycemia was reached on day 11. White arrow, begin immunosuppressive therapy. Black arrow, stop immunosuppressive therapy.

posttransplant animals. The blood glucose levels during IVGTT at posttransplant were similar to those in the prepancreatectomized nondiabetic state except for the value at 5 min. *K* values posttransplant (1.16 ± 0.12) were

not significantly different from those for their prepancreatectomized nondiabetic state (0.94 ± 0.07). Sharp increases in serum insulin and C-peptide were observed in response to glucose loading posttransplant; however, these responses were significantly blunted when compared with prepancreatectomized nondiabetic values (Fig. 5B and C).

DISCUSSION

Fetal porcine islets and NPIs have been used to cure experimental diabetes in rodents (11–17); however, reports describing reversal of diabetes in large animals have been scarce, and the results are discouraging. Fetal porcine islet-like cell clusters transplanted into the thymus of allograft recipients exhibited good glucose control in only one of four animals (18). Unfortunately, there was no evidence that the graft was responsible for normalization of blood glucose in this one pig. Fetal porcine islets have also been transplanted into diabetic patients in Sweden (19), and, even though functional and histological survival of the grafts was observed in some patients, no patients were able to reduce their insulin requirement.

Our study demonstrates for the first time that NPIs can reverse hyperglycemia in a totally pancreatectomized diabetic young adult pig. The most surprising result was the time needed to achieve normoglycemia, which was considerably shorter than the time needed to cure diabetes in immunoincompetent mice, which took 6–8 weeks to reverse hyperglycemia (11). Also, the β -cell mass needed to cure diabetes in this study was 10,000 IE/kg body wt, which was far less than 100,000 IE/kg body wt needed in our previous mouse study (11). One possible explanation for the more rapid correction of hyperglycemia is that the liver might provide an environment and growth factors that enhance the maturation of the NPI graft. Higher glucose levels in the portal vein after diet might play a role in enhancing growth of intraportally transplanted NPIs, because studies in rodents have shown that elevated glucose level enhances growth of transplanted neonatal rat islets (20) and that transient or mild hyperglycemia increases β -cell replication and mass in transplanted adult islets (21–23). Increased shear stress in the portal vein wall as a result of portal hypertension after transplantation, which is known to induce release of growth and transcription factors (24,25), might also facilitate the maturation of NPIs. Another possible explanation is the difference in recipients' own growing capacity; juvenile pig recipients that grow rapidly could provide a better environment for grafts having a capacity for growth. The more plausible explanation is the difference in the host species; the NPIs might function and grow faster in an allogeneic environment than in a xenogeneic host, because it is known that some hormones fail to function across species (26,27). Porcine insulin could be metabolically less active on rodent tissue because a large number of adult porcine islets are needed to cure diabetes in mice (28).

Because of their anatomical and physiological similarities to humans, pigs have been used as a model for allogeneic endocrine cell replacement (29–32). However, pigs have been shown to be relatively easy to induce into a tolerant state after a solid organ transplant (33–35). When our recipients were given immunosuppression, suc-

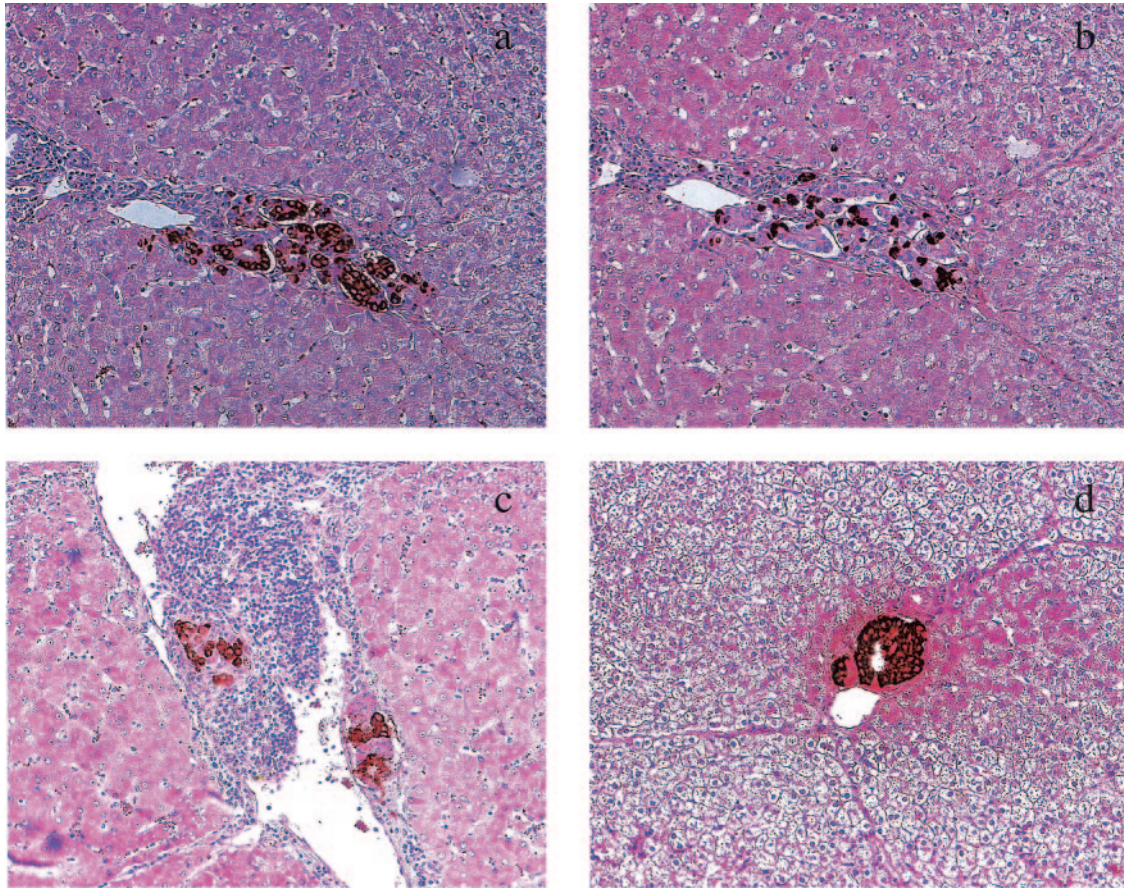


FIG. 3. Immunohistological analysis of transplanted intraportal NPI grafts. *A* and *B*: Insulin staining of a liver section from pig 3, which was treated with CsA and sirolimus for 5 weeks after transplantation. Grafts were harvested on day 41, and well-preserved insulin-positive (*A*) and glucagon-positive (*B*) cells were observed with a few lymphocytes at the portal space. *C*: A liver section from a nonimmunosuppressed pig 10 harvested on day 27 when hyperglycemia recurred. Note the presence of numerous lymphocytes in the portal space, invading the graft. *D*: A liver section from a nonimmunosuppressed pig 7 harvested on day 49 while normoglycemia was maintained. Original magnification $\times 200$.

cessful engraftment of NPIs grafts was achieved in all cases as judged by histological analysis and insulin reserve. In addition, episodes of chronic rejection were not observed in any of the recipients. In particular, two pigs did not show any episodes of rejection even after discontinuation of the drugs, suggesting that a state of immune unresponsiveness to NPIs might be induced; however, follow-up was only up to 41 days after withdrawal of the drugs. It should be noted that it took 7–50 days (36) or

31–445 days (37) for hyperglycemia to recur after cessation of CsA in allograft studies in dogs. In our study, trough levels of CsA were more variable than those of sirolimus, even though both drugs were administered exactly the same way. One possible explanation is that absorption of CsA might be more variable than sirolimus in a pancreatectomized pig that is on cotazyme. In some cases, we encountered unexpected high levels of CsA, which might have contributed to operational tolerance because it has

TABLE 2
Graft insulin content

Pig	Immuno-suppression	Pretransplant (μg)	Graft insulin content posttransplant (μg)	Fold increase	Day killed
1	Yes	115.4	733.8	6.4	39
2	Yes	203.0	6,299.7	31.0	69
3	Yes	160.0	3,438.3	21.5	41
4	Yes	246.2	958.7	3.9	14
5	Yes	285.7	336.2	1.2	5
6	Yes	225.4	751.7	3.3	9
7	No	298.3	6,007.3	20.1	49
8	No	411.6	26.8	0.1	14
9	No	223.4	153.2	0.7	4
10	No	196.2	60.3	0.3	27
11	No	340.2	282.8	0.8	7
12	No	219.7	38,427.5	174.9	79
13	No	166.3	31.6	0.2	13

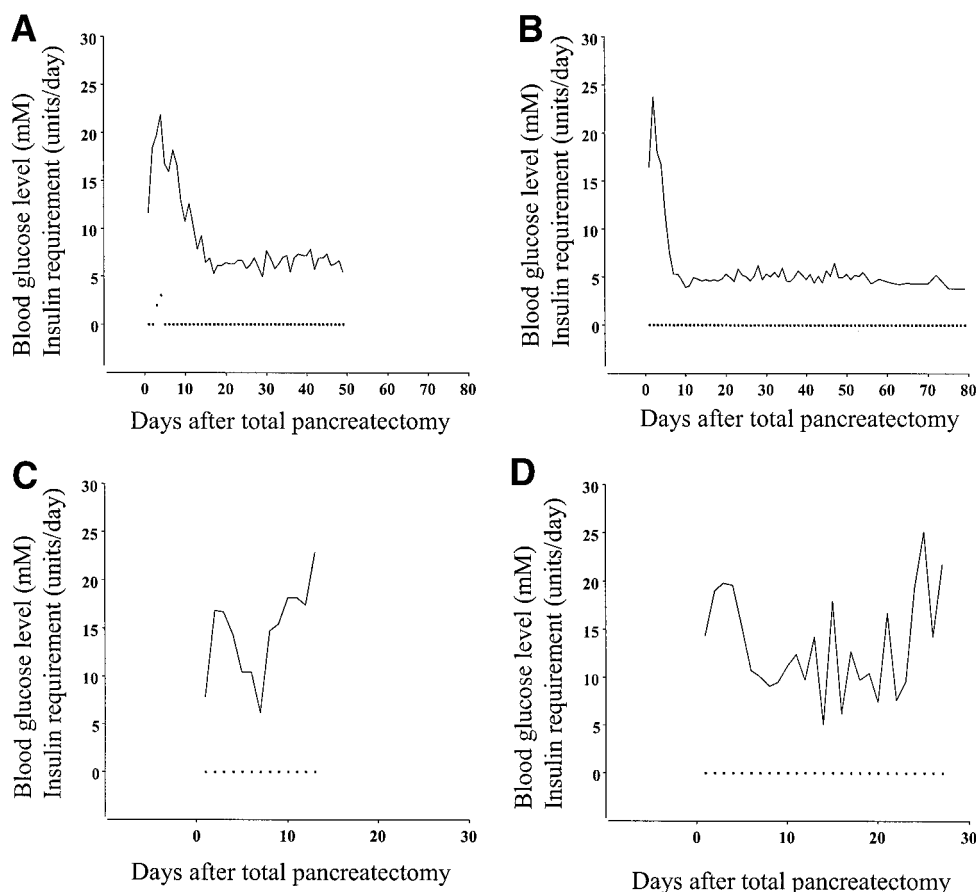


FIG. 4. Morning blood glucose levels (—) and daily insulin requirements (···) in four pigs that achieved normoglycemia after transplantation of NPIs without immunosuppressive drugs. In pig 7 (A), 16,064 IE/kg was injected into the liver. Insulin was discontinued on day 5, and normoglycemia (<8.4 mmol/l) was achieved on day 13. In pig 12 (B), 10,405 IE/kg was injected into the liver. Normoglycemia was achieved on day 6. Insulin was not given at any time throughout the study period. In pigs 8 (C) and 10 (D), 17,439 IE/kg and 14,217 IE/kg were injected into the liver, respectively. They achieved normoglycemia without any insulin injections within 14 days and then returned to hyperglycemia.

been shown that tolerance to renal and liver allografts can be achieved with high-dose calcineurin inhibitor induction in pigs (33–35).

Even though no immunosuppression was given to some of our recipients, histological examination showed remarkable preservation of the islets without infiltrating lymphocytes in four of the seven pigs. This observation raises a question regarding swine leukocyte antigen matching of pigs used in this study. Because we used the same strain of pigs (although they were not inbred animals) for recipients and donors, there is a possibility that some of the recipients were genetically related to the donors. We performed, however, a mixed lymphocyte reaction using donor's splenocyte and recipient's peripheral blood lymphocyte and observed that the recipient lymphocyte strongly reacted against the donor cells (data not included). We also performed skin transplantation using pigs obtained from the same vendor and found that young adult pigs rejected the skin graft of neonatal pigs 2 weeks after transplantation, whereas autografts were accepted long-term (data not included). It is conceivable, however, that multiple donors might provide an immunological advantage in our study, as previously described in rodents (38,39) and pigs (32). Also, it has been recently reported that embryonic derived cells can be successfully transplanted as allografts or xenografts in immunocompetent animals without immunosuppression (40–44). NPIs contain a high number of undifferentiated cells or precursor cells, which might share the same reduced immunogenicity as embryonic-derived cells. Bloch et al. (45) have shown that NPIs induce lower human T-cell proliferation

compared with adult pig islets. It was also shown that NPIs resist human natural killer or cytotoxic T-cell-mediated cytolytic damage probably because of insufficient expression of costimulatory and/or adhesion molecules on NPIs (46). Islets were recently shown to release tissue factor as well as chemokines such as monocyte chemoattractant-1, which trigger an injurious inflammatory reaction (47). Although it is not known whether NPIs release proinflammatory factors, NPIs could be resistant to inflammatory damage.

Pigs with prolonged graft function exhibited comparable glucose tolerance to the prepancreatectomized nondiabetic state; however, insulin response to glucose was reduced, which is a similar observation seen in clinical studies, even in the patients that achieve insulin independence after an islet transplant (4).

In summary, our data provide the first evidence that allogeneic NPIs can reverse hyperglycemia when transplanted into the liver of totally pancreatectomized diabetic pigs, and correction of diabetes is much faster compared with our previous mouse studies. Based on these findings, we hypothesize that NPIs are likely to provide us with an alternative practical source of insulin-producing tissue that we could use to treat more diabetic patients.

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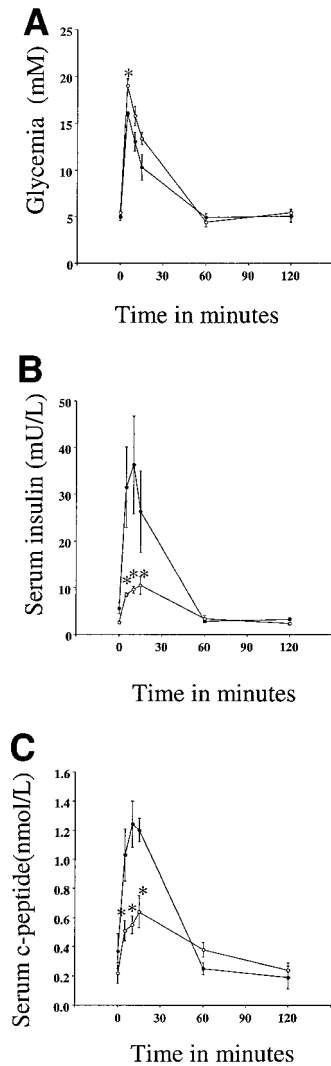


FIG. 5. IVGTT in totally pancreatectomized pigs transplanted with NPIs ($n = 4, 2, 3, 7,$ and 12). **A:** Blood glucose values in pigs 1 month after transplantation (○) and in pretransplanted nondiabetic pigs (●). **B:** Serum insulin levels in pigs 1 month after transplantation (○) and in pretransplanted nondiabetic pigs (●). **C:** Serum porcine C-peptide levels in pigs 1 month after transplantation (○) and in pretransplanted nondiabetic pigs (●). Bars represent means \pm SE. * $P < 0.05$.

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