Vascularized Islet Cell Transplantation in miniature swine
Islet-Kidney Allografts Correct the Diabetic Hyperglycemia Induced by Total Pancreatectomy
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We have previously reported the preparation of vascularized islet-kidneys (IKs) by transplantation of islets under the autologous kidney capsule. Here, we compare the efficacy of transplanting vascularized versus nonvascularized islets into diabetic allogeneic swine recipients. In the vascularized islet transplantation (5,000 islet equivalents [IE/kg]), recipients received minor-mismatched (n = 4) or fully-mismatched (n = 2) IKs after pancreatectomy, with a 12-day course of cyclosporine A (CyA) or FK506, respectively. For the nonvascularized islet transplantation (7,000 IE/kg), three recipients received minor-mismatched islets alone and two recipients received minor-mismatched donor islets placed in a donor kidney on the day of transplantation. All recipients of nonvascularized islets were treated with a 12-day course of CyA. With vascularized islet transplantation, pancreatectomized recipients were markedly hyperglycemic pretransplant (fasting blood glucose >300 mg/dl). After composite IK transplantation, all recipients developed and maintained normoglycemia (<120 mg/dl) and stable renal function indefinitely (>3 months), and insulin therapy was not required. Major histocompatibility complex-mismatched recipients demonstrated in vitro donor-specific unresponsiveness. In contrast, recipients of nonvascularized islets remained hyperglycemic. In conclusion, IK allografts cured surgically induced diabetes across allogeneic barriers, whereas nonvascularized islet transplants did not. These data indicate that prevascularization of islet allografts is crucial for their subsequent engraftment and that composite IKs may provide a strategy for successful islet transplantation. Diabetes 51: 3220–3228, 2002

Replacement of the islets of Langerhans is currently the only treatment for type 1 diabetes that achieves an insulin-independent, normoglycemic state. Recent data using the Edmonton Protocol (1) have demonstrated a much improved survival of islets after transplantation, with initial normalization of blood glucose levels in patients; however, this protocol required >9,000 islet equivalents (IE)/kg, necessitating the use of two to four pancreas donors for a single recipient. In addition, by 1-year posttransplantation, 3 of the 12 patients were diabetic, and two required insulin (2). Thus, these data, while much improved over previous islet transplantation data, are limited in clinical applicability by the small number of donor organs currently available and by the requirement of islets from multiple donors for a single diabetic recipient. Therefore, methods for transplanting islets from single donors that approach the results of whole-organ pancreas transplantation, for which an ~80% 1-year graft survival and function has been reported (3–7), must continue to be sought. In addition to the problems of engraftment of transplanted islets, current clinical regimens for islet transplantation require indefinite daily administration of immunosuppressive drugs. Therefore, strategies aimed at the induction of tolerance to islet grafts, using a short-course immunosuppression, would not only improve the efficacy of islet transplantation, but would also obviate the need for chronic immunosuppression.

One of the most likely reasons for the poor success thus far in islet cell transplantation is that these tissue grafts must establish new vasculature from the host to survive. During the time required for such revascularization, there is a much increased susceptibility to loss from ischemic injury (e.g., lack of oxygen or nutrients), as well as possible immunologic impairment as a consequence of the allogeneic environment. We have hypothesized that greater success in islet transplantation may be achieved through the use of primarily vascularized islets, eliminating the period required for revascularization after allogeneic transplantation. Therefore, we recently described the preparation of composite islet-kidney (IK) grafts containing vascularized autologous islets (31). The transplanta-
tion of prevascularized islets enables a comparison between transplanting vascularized versus nonvascularized islets in our miniature swine.

Our initial study demonstrated that autologous islets engraft and function when implanted beneath the renal capsule, whereas minor-mismatched islets are rejected when similarly implanted (31). In the present study, we examine the ability of IKs to cure surgically induced diabetes with a short course of immunosuppression, as compared with that of nonvascularized direct islet transplants across the same allogeneic barriers. The transplantation of IKs could have significant relevance as a strategy for the induction of tolerance to allogeneic islets.

RESEARCH DESIGN AND METHODS

Animals. Minor and fully major histocompatibility complex (MHC)-mismatched transplant donors (>1.5 years of age) and recipients (3–5 months of age) were selected from our herd of partially inbred miniature swine (8,9).

Islet harvest and transplantation. Islet donors were adult miniature swine, subjected to either total or partial pancreatectomy, as described elsewhere (31). For partial pancreatectomy, ~30% of the pancreas (the head and the remainder of the body) was left intact. For total pancreatectomy, the pancreas was carefully dissected so as not to damage the duodenum or the pancreas. The excised pancreas was placed in crushed ice immediately, and modified University of Wisconsin solution was infused through the pancreatic duct. Pancreatic tissue was processed at the Joslin Diabetes Center (Boston, MA) (27). After islet isolation, the purity of the preparation was evaluated with fluorescence-activated cell sorting (FACS) analysis.

Two semipermanent indwelling silastic central venous catheters were placed orthotopically into the recipient, using end-to-side aortocaval anastomoses. Total pancreatectomy was performed surgically by total pancreatectomy 3–1.5 years after the intended date of allogeneic transplantation.

Induction of type 1 diabetes in recipients. Type 1 diabetes was induced surgically by total pancreatectomy 3–7 days after either composite IK or direct islet transplantation. Total pancreatectomy was performed using a similar procedure to that described above for islet donors. Blood supply to duodenum and bile ducts was well preserved, and no blood transfusions were required (Fig. 1). Thereafter, these animals received pancreatase (Pancrezyme; Daniels Pharmaceuticals, St. Petersburg, FL) supplementation in their diet to replace exocrine pancreatic enzymes.

IK preparation. IKs were prepared in donors as previously described (31). Briefly, islets were suspended in 1.0 ml saline and injected beneath the renal capsule using a 20G angiocatheter. IKs were prepared 2–3 months before the intended date of allogeneic transplantation.

IK transplantation. Allogeneic transplantation of composite IKs was performed by the same technique as described previously for renal transplantation (10,11). Briefly, recipients underwent bilateral nephrectomy. The IK was harvested from the donor, flushed immediately with cold perfusion solution, and then placed on ice until transplantation. The IK was transplanted orthotopically into the recipient, using end-to-side aortocaval anastomoses. Urinary drainage was accomplished through a vesicoureteral anastomosis. Two semipermeable indwelling silastic central venous catheters were placed into the external jugular veins of the recipient. One catheter was used for the administration of immunosuppression and the other to obtain blood samples. Total cold ischemic time was kept to <60 min. No blood transfusions were required.

Immunosuppression. Cyclosporine A (CyA) (Sandimmune) was generously provided by Novartis Pharmaceutical (East Hanover, NJ) and was mixed and administered as an intravenous suspension, according to specifications of the manufacturer. CyA was given daily as a single infusion (10 mg · kg⁻¹ · day⁻¹) for 12 consecutive days, beginning on postoperative day (POD) zero. Whole-blood trough levels were determined by a fluorescence polarization immunoassay (Abbott Laboratories, Dallas, TX), which measured the parent compound but not metabolites, and the results were expressed in nanograms per milliliter. FK506 was generously provided by Fujisawa Healthcare (Deerfield, IL) and was administered continuously through an infusion pump (Baxter Healthcare, Deerfield, IL) for 12 consecutive days, beginning on POD zero. FK506 doses were started at 0.15 mg · kg⁻¹ · day⁻¹ and adjusted to maintain whole-blood levels between 30 and 40 ng/ml. Whole-blood levels were determined by microparticle enzyme immunoassay (Tiacolumn II, IMX System; Abbott Laboratories).

Monitoring of blood glucose. Islet function was assessed through fasting blood glucose (FBG) levels, glucose tolerance tests, and histological examination of biopsies. Glucose tolerance testing was performed by intravenous infusion of a glucose load (0.5 g/kg body wt) after a 24-h fasting period. Blood samples were obtained at 0, 5, 15, 30, 60, and 120 min postinfusion and analyzed for plasma creatinine and FBG levels, as well as histological assessment of biopsy tissue. The clinical end points used in this study were either graft survival >100 days or a morbund animal with uremia or hyperglycemia. Biopsied tissues were examined by a pathologist, and rejection of kidneys was diagnosed according to a standardized grading system (12).

Histological features. Tangential sections were taken to include islet and renal tissue. Formaldehyde-processed specimens were examined using H&E staining and insulin-specific antibodies. Insulin staining was performed on 4.0-μm sections cut from a paraffin-embedded block. Sections were incubated at room temperature in a humidified chamber for 20 min with 10% normal goat serum in PBS (pH 7.4) and then for 60 min with guinea pig anti-porcine insulin antibody (Dako, Carpinteria, CA) diluted 1:10 in PBS. They were next incubated with a 1:200 dilution of biotinylated goat anti–guinea pig antibody (Vector Laboratories, Burlingame, CA) for 60 min. The tissue-bound primary antibodies were detected by an avidin-biotin-peroxidase complex (Dako) that was visualized by staining with 0.02% hydrogen peroxide containing 0.3 mg/ml 3,3′-diaminobenzidine in 0.05 mol/l Tris buffer. Sections were counterstained with Gill's single-strength hematoxylin.

Preparation of peripheral blood lymphocytes. Blood was drawn from the external jugular vein on PODs 30, 60, and >100. For separation of peripheral blood lymphocytes, freshly heparinized whole blood was diluted ~12 with Hank's balanced salt solution (HBSS; Gibco BRL, Grand Island, NY), and the mononuclear cells were obtained by gradient centrifugation using lymphocyte separation medium (Organon, Teikika, Durham, NC). The mononuclear cells were washed once with HBSS, and contaminating red cells were lysed with ammonium chloride potassium buffer (B&D Research Laboratory, Fiskeville, RI). Cells were then washed with HBSS and resuspended in tissue culture medium. All cell suspensions were kept at 4°C until used in flow cytometry assays.

Cell-mediated lymphocytoxicity assay. Cell-mediated lymphocytoxicity (CML) assays were performed as previously described using our standard
CML tissue culture medium (10). Briefly, lymphocyte cultures containing $4 \times 10^6$ responder and $4 \times 10^6$ stimulator peripheral blood mononuclear cells (PBMCs) (irradiated with 2,500 cGy) were incubated for 6 days at 37°C in 7.5% CO2 and 100% humidity. Bulk cultures were harvested, and effectors were tested for cytotoxic activity on 51Cr (Amersham, Arlington Heights, IL)-labeled lymphoblast targets. Effector cells were incubated for 5.5 h with target cells at E/T ratios of 100:1, 50:1, 25:1, and 12.5:1. Three target cells were tested in each assay: swine leukocyte antigen (SLA)-matched PBMCs to the effectors, donor-matched PBMCs, and third-party PBMCs. Supernatants were then harvested using the Skatron collection system (Skatron, Sterling, VA), and 51Cr release was determined on a gamma counter (Micromedics, Huntsville, AL). The results were expressed as PSL (percent specific lysis), calculated as:

$$PSL = \left( \frac{\text{experimental release (cpm) - spontaneous release (cpm)}}{\text{maximum release (cpm) - spontaneous release (cpm)}} \right) \times 100\%$$

Mixed lymphocyte response assays. Mixed lymphocyte response (MLR) assays were performed by plating $4 \times 10^6$ responder PBMCs in triplicate in 96-well flat-bottom plates (Costar, Cambridge, MA). Cells were stimulated with $4 \times 10^6$ stimulator PBMCs irradiated with 2,500 cGy. Cultures were incubated for 5 days at 37°C in 6% CO2 and 100% humidity in our standard MLR culture medium (11). 3H-thymidine was added for an additional 6 h of culture. Wells were then harvested onto Mash II glass fibers and counted for β emission.

RESULTS

IKs reverse the hyperglycemia of surgically induced diabetes across a minor-mismatched barrier. Four animals were completely pancreatectomized within 1 week before receiving a minor-mismatched composite IK. After total pancreatectomy, the FBG of the recipients markedly increased, reaching levels >280 mg/dl (Fig. 2A). No insulin therapy was given.

Donors were selected by weight to achieve the required number of IE per kilogram for the intended recipient. IKs containing ~5,000 IE/kg recipient body wt were transplanted, with a 12-day course of CyA at 10 mg · kg$^{-1}$ · day$^{-1}$. The recipients' native kidneys were removed on the day of IK transplantation. After IK transplantation, FBG levels fell immediately to normal levels (<120 mg/dl) by POD 1. IK grafts were permanently accepted, and recipients maintained normoglycemia for at least 90 days in three of four cases (Fig. 2A, C, and D), without the need for insulin therapy. The fourth animal had to be sacrificed because of complications from a catheter infection on POD 128. Representative biopsy obtained on POD 128 demonstrates minimal cellular infiltrate (F, H&E) and numerous insulin-producing β-cells (G, insulin staining).

FIG. 2. A–D: Totally pancreatectomized recipients of minor-mismatched IKs maintained stable FBG and plasma creatinine levels after IK transplantation. Both FBG and creatinine levels sharply increased after IK nephrectomy (IK Gx). B: Animal was killed secondary to complications arising from a catheter infection (#). E: Representative data demonstrating a 0.5-g i.v. glucose tolerance test in an IK recipient are compared with both a naïve animal and a totally pancreatectomized (total Px) animal. Glucose tolerance testing reveals normal responses to intravenous glucose 2 months after IK transplantation. Representative biopsy obtained on POD 128 demonstrates minimal cellular infiltrate (F, H&E) and numerous insulin-producing β-cells (G, insulin staining).
POD 67; however, this animal had also maintained normoglycemia after IK transplantation (Fig. 2B).

Glucose tolerance testing revealed normal responses to intravenous glucose loads of 0.5 g at 2 months after IK transplantation (Fig. 2E, representative data shown). In contrast, glucose levels in pancreatectomized control animals reached >450 mg/dl (Fig. 2E). Upon gross examination of the IK allografts, no edema or spot hemorrhages were visible. As seen in Fig. 2F and G, a biopsy on POD 128 in a representative animal revealed numerous insulin-producing \( \beta \)-cells with minimal cellular infiltrate (Fig. 2F, H&E staining; Fig. 2G, insulin staining). Renal structure and function were followed throughout the transplant period. Creatinine levels remained <1.5 mg/dl throughout the clinical course (Fig. 2A–D), and biopsies revealed intact kidney parenchyma with minimal cellular infiltrate (renal parenchyma in Fig. 2F). The recipient animals survived long term (>3 months) and demonstrated a significant increase in weight during the experiment (40, 63, and 8% weight gain on the day of IK graftectomy compared with day of IK transplant in each animal). The IKs were explanted on PODs 67, 90, 128, and 150, and follow-up blood glucose levels were monitored to assure the completeness of pancreatectomies. The blood glucose levels rose immediately after IK explantation in each animal, indicating that the total pancreatectomies were complete in all cases.

Nonvascularized minor-mismatched islets did not reverse the hyperglycemia of surgically induced diabetes. Three pancreatectomized animals received direct minor-mismatched islets transplanted beneath the renal capsule \( n = 1 \) or injected via the portal vein (90% of islets) and beneath the renal capsule (10% of islets) \( n = 2 \). A total of 7,000 IE/kg recipient body wt were injected into each animal, followed by a 12-day course of CyA (10 mg \( \cdot \) kg \( ^{-1} \) \( \cdot \) day \( ^{-1} \)). This increased number of islets was ~40% more than that used in the preparation of IKs to compensate for any \( \beta \)-cell proliferation that might have occurred during the 2- to 3-month period after IK preparation. Recipient FBG decreased on POD 1; however, the levels subsequently increased and the islets were rejected by histological criteria by POD 23 (Figs. 3 and 4A and B) in the two animals that underwent direct islet transplantation. The third animal was maintained on exogenous insulin during postoperative weeks 2–4 to determine whether prolonged survival of the animal by insulin therapy would provide direct islet allografts additional time to engraft. This animal’s FBG was maintained between 180 and 250 mg/dl with insulin therapy; however, blood glucose levels immediately increased after cessation of insulin at week 5 (Fig. 3). Autopsy results revealed mononuclear cell infiltrates and fibrosis beneath the renal capsule (the site of islet implantation) (Fig. 4C). No functioning islets were found on histological evaluation (Fig. 4D).

Simultaneous transplantation of nonvascularized minor-mismatched islets and a renal allograft did not reverse the hyperglycemia of surgically induced diabetes. Two pancreatectomized animals received direct minor-mismatched islets and renal allografts, isolated from the same donor and transplanted on the same day, with a standard 12-day course of CyA. Three days before transplantation, donor pancreata were partially removed through a 70% pancreatectomy, and islets were harvested for a 3-day culture. On the day of transplantation, renal harvest was performed on the islet donor and recipients underwent bilateral nephrectomies before renal transplantation. Immediately after revascularization of the donor renal allograft, cultured donor islets (7,000 IE/kg recipient body wt) were injected beneath the renal capsule. In both animals, FBG levels decreased on POD 1 (Fig. 5A). However, thereafter, both animals became markedly hyperglycemic. One moribund animal was killed on POD 14 (Fig. 5A), while the other animal was maintained with exogenous insulin from POD 5 until POD 29 (Fig. 5B). The target FBG level in this period was established between 180 and

**FIG. 2—Continued**

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250 mg/dl. Regular and/or lente insulin was titrated between 4 and 10 units/day to maintain target FBG levels. The animal required less insulin (from 10 units/day to 6 units/day) to maintain target FBG levels during week 4, and insulin therapy was discontinued on POD 29. However, FBG levels never fell below 180 mg/dl after the cessation of insulin and levels increased markedly to 600 mg/dl on POD 31 (Fig. 5B). Creatinine levels remained normal in the animal that died on POD 14 (3 days after cessation of CyA); however, creatinine levels increased slightly in the animal that demonstrated a prolonged survival on insulin therapy (Fig. 5D). Histological analysis of the autopsy specimen on POD 31 revealed few remaining islets and a diffuse mononuclear cell infiltrate in the site of islet transplantation (Fig. 5C). Furthermore, an interstitial mononuclear cellular infiltrate was seen in the renal parenchyma (Fig. 5D), a finding not noted in our previous experience with minor-mismatched renal transplantation alone in miniature swine.

**IKs cured surgically induced diabetes across a two-haplotype fully-mismatched barrier.** Two pancreatectomized animals received fully-mismatched IKs containing ~5,000 IE/kg recipient body wt, with a 12-day course of FK506, which has been shown to facilitate the induction of tolerance to fully mismatched renal allografts in our miniature swine (13). Native kidneys were removed on the day of IK transplantation. In each case, FBG levels rose to >280 mg/dl after pancreatectomy. The composite IKs were accepted, and normoglycemia was immediately restored. However, both animals exhibited a transient period of hyperglycemia that lasted, approximately, from POD 5 to POD 18. These episodes resolved spontaneously within a week after the cessation of FK506, suggesting that the transient rise in blood glucose may have been due to FK506-associated islet toxicity (14,15). The clinical, pathological, and histological outcome in these animals was otherwise similar to that seen in minor-mismatched IK transplantation with a 12-day course of CyA. All IKs were permanently accepted, and recipients maintained normoglycemia for at least 120 days without insulin therapy (Fig. 5B). Glucose tolerance testing of IK recipients was normal 2 months after IK transplantation (Fig. 6C). Gross examination of the grafts revealed no edema, spot hemorrhages, or vasculopathy. Biopsy results revealed numerous insulin-producing β-cells with a minimal cellular infiltrate (Fig. 6D). Additional immunosuppression or insulin therapy required was not required in either case, and creatinine levels were maintained below 1.5 mg/dl throughout the experiment (Fig. 6A). The blood glucose levels rose immediately after IK explantation in each animal.

**IKs induced donor-specific tolerance in vitro when transplanted across fully-mismatched barriers.** IK recipients developed in vitro evidence of donor-specific
tolerance within 30 days after IK transplantation. Figure 7A demonstrates the CML responses on POD 30 in both animals. Strong anti-third party reactivity, but no anti-donor reactivity, was observed. MLR analysis also revealed anti-donor specific unresponsiveness (Fig. 7B), which was maintained for the duration of the experiment, confirming that these animals were tolerant.

**DISCUSSION**

We report here that vascularized islet cells, when transplanted as part of a composite IK, cured surgically induced diabetes across minor- and fully MHC-mismatched barriers. The present study demonstrates that 1) nonvascularized islet transplants isolated from a single donor are not capable of curing surgically induced diabetes and that 2) a similar number of prevascularized islets, within composite IK grafts, can cure surgically induced diabetes, as well as induce tolerance, after a short course of immunosuppression. Recipient animals that were hyperglycemic after complete pancreatectomy maintained normoglycemia indefinitely after IK transplantation. Upon removal of the IK, these animals returned to their pretreatment, hyperglycemic state, indicating that the pancreatectomies were indeed complete and that the transplanted vascularized islets within the composite IK were responsible for the normal regulation of blood glucose levels. An additional important note is the fact that each IK contained 5,000 IE/recipient body wt on the day of the IK transplantation. These recipients gained body weight, which effectively diluted the ratio of IE per kilogram recipient body weight after IK transplantation. Therefore, by the conclusion of the experiment, the recipients maintained normoglycemia with ~3 × 10^3 IE/kg recipient body wt.

Our previous studies of allogeneic and xenogeneic thymic tissue transplantation have demonstrated the role of prevascularization on graft survival (18,19), a principle that has already been noted for transplants of kidney tissues in a rodent model (28). The present studies provide further evidence of the critical nature of a graft’s vascular supply. Nonvascularized islets neither engrafted nor controlled blood glucose levels in transplanted recipients. While nonvascularized islet transplants transiently decreased FBG levels during the first 2 days after transplantation, this decrease was most likely secondary to the release of insulin from damaged ischemic islet β-cells. Histological and physiological data indicated that such grafts were rapidly rejected within the first weeks after transplantation. Vascularized grafts appeared less susceptible to this nonspecific graft loss and did not sensitize the host to donor antigen—processes that are likely to have

**FIG. 5.** A and B: Animals that underwent simultaneous transplantation of nonvascularized minor-mismatched islets and a renal allograft across minor-mismatched barriers were unable to maintain stable fasting blood glucose levels after total pancreatectomy. The recipient (B), otherwise identical to A, was maintained with exogenous insulin from POD 5 until POD 29. Regular and/or lente insulin was titrated between 4 and 10 units/day to maintain target FBG levels between 180 and 250 mg/dl. Autopsy specimens obtained from the animal depicted in B on POD 31 demonstrate few remaining islets and a diffuse mononuclear cell infiltrate at the site of islet transplantation (C) and an interstitial mononuclear cellular infiltrate in the renal parenchyma (D).
limited the success of nonvascularized islet grafts. In fact, it has been shown that transplants of composite kidney-islet grafts were more successful in avoiding autoimmune destruction in BB rats than freely grafted islets (30).

While previous studies have reported increased islet cell survival when injected via the portal vein (2,20), our studies in miniature swine were unable to confirm this finding.

This study also demonstrated that the success of the IK graft is not due to antigen load or specific help by a donor kidney, as cotransplantation of a donor kidney did not facilitate engraftment of nonvascularized islets. The improved survival of composite IK grafts appears to be based almost entirely on the prevascularization of the islet tissue. Creation of autologous IKs allowed for ischemia, reperfusion, and revascularization to all occur in a non-allogeneic setting, whereas directly transplanted islets must encounter all of these barriers to engraftment in an allogeneic setting. Autologous IK creation permitted not only enhanced islet engraftment by avoidance of typical

![FIG. 6. A and B: Recipients of two-haplotype fully MHC-mismatched IKs maintained stable FBG and plasma creatinine levels after total pancreatectomy (Px). C: Both recipients demonstrated a normal response to a 0.5-g i.v. glucose tolerance test. D: Representative biopsy obtained on POD 143 demonstrates numerous insulin-producing β-cells.](image-url)
rejection responses observed during this period, but also facilitated the long-term induction of tolerance by minimizing the presentation of allogeneic antigens after transplantation. When renal transplantation was immediately followed by the subcapsular implantation of cultured islets isolated from the same donor, the composite grafts were unable to cure diabetes in pancreatectomized recipients. Furthermore, the renal parenchyma in these grafts demonstrated an increased infiltrate as compared with historical renal allograft controls performed across similar immunological barriers (21). This indicated that 1) the process of renal transplantation itself does not lead to enhanced islet survival, 2) nonvascularized islets may be more immunogenic than a kidney transplanted alone, and 3) the presence of nonvascularized islets may actually sensitize the host to donor antigen.

The present studies also indicated that immunosuppressive regimens that induced tolerance to renal allografts also induced tolerance to associated vascularized islets within the IK graft. However, such regimens are not capable of inducing tolerance to islets directly transplanted into recipients. Our previous studies have demonstrated that a 12-day course of CyA (10) or FK506 induces tolerance to partial MHC– and fully MHC–mismatched renal allografts, respectively (13). These same regimens were capable of inducing tolerance to vascularized islets across similar barriers in the present study. Although short-term high-dose FK506 has not been used for tolerance induction clinically, we have used it successfully for this purpose recently in baboons (P.A.V., R.N.B., S.Y., J.M.L., J.C.L., C.K., D.H.S., K.Y., manuscript in preparation), and similar doses have been used clinically for the treatment of ABO-incompatible renal transplantation (16) and GvHD (17).

We have suggested that the transient rise in blood glucose in our fully MHC–mismatched recipients may have been due to FK506-associated islet toxicity (14,15). This level has never induced diabetes in our miniature swine recipients of kidneys (13), and FK506 blood levels were controlled between 25 and 35 ng/ml in both cases. Thus, transplanted islets could be sensitive to this drug, especially in the induction period after transplantation. Even though the regimen of FK506 needed to induce tolerance might have a slightly diabetogenic effect, the benefit of using a short course of immunosuppression for the establishment of long-term tolerance is very attractive. In fact, the hyperglycemic period, which was insulin free, was only transient and easily reversed upon the discontinuation of FK506. In the future, strategies that are currently demonstrating success in preclinical and early clinical models of renal allograft transplantation, such as mixed chimerism (22,29), costimulatory blockade (23), and FN18 immunotoxin (24,25), may be used to induce tolerance to vascularized islet allografts and avoid any secondary islet toxicity.

Although the feasibility of islet transplantation has previously been demonstrated (1,2), to our knowledge, the successful transplantation of islets isolated from a single living donor has not achieved the success reported from the use of multiple islet donors. The IK strategy permitted the isolation of a sufficient number of islets, while leaving the donor normoglycemic and with intact exocrine function. Such a strategy may eventually permit successful living-related islet donation for diabetic patients with end-stage diabetic nephropathy. We can envision the laparoscopic preparation of IK grafts (26) several months in advance of kidney transplantation.

While we have chosen to initiate our studies in an allogeneic model to assess cellular responses without attendant complications from natural antibodies, this
model may be even more applicable for IK transplants across xenogeneic barriers. In xenotransplantation, the preparation of IKs could provide an unlimited source of organs for transplantation into diabetic recipients requiring kidneys.

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