Published in final edited form as:

Transplantation. 2013 August 27; 96(4): 372–378. doi:10.1097/TP.0b013e31829f7515.

Assessing the effect of immunosuppression on engraftment of pancreatic islets

Prashanth Vallabhajosyula^{1,2}, Atsushi Hirakata¹, Akira Shimizu¹, Masayoshi Okumi¹, Vaja Tchipashvili³, Hanzhou Hong¹, Kazuhiko Yamada¹, and David H. Sachs¹

¹Transplantation Biology Research Center, Massachusetts General Hospital, Boston, Massachusetts

²Division of Cardiovascular Surgery, Hospital of the University of Pennsylvania, Philadelphia, Pennsylvania

³Department of Islet Transplantation and Cell Biology, Joslin Diabetes Center, Boston, Massachusetts

Abstract

Objective—In addition to ischemia and immunologic factors, immunosuppressive drugs have been suggested as a possible contributing factor to the loss of functional islets following allogeneic islet cell transplantation. Using our previously described islet-kidney transplantation model in miniature swine, we studied whether an islet toxic triple-drug immunosuppressive regimen (cyclosporine + azathioprine + prednisone) affects the islet engraftment process and thus long-term islet function.

Design and Methods—Donor animals underwent partial pancreatectomy, autologous islet preparation and injection of these islets under the autologous kidney capsule to prepare an islet-kidney (IK). Experimental animals received daily triple drug immunosuppression during the islet engraftment period. Control animals did not receive any immunosuppression during this period. Four to eight weeks later, these engrafted IK were transplanted across a minor histocompatibility mismatched barrier into pancreatectomized, nephrectomized recipient animals at an islet dose of ~4500 islet equivalents (IE)/kg recipient weight. Cyclosporine was administered for 12 days to the recipients to induce tolerance of the IK grafts and the animals were followed long-term.

Results—Diabetes was corrected by IK transplantation in all pancreatectomized recipients on both the control (n=3) and the experimental (n=4) arms of the study and all animals showed normal glucose regulation over the follow-up period. Intravenous glucose tolerance tests

Author for correspondence: David H. Sachs, Professor of Surgery, Harvard Medical School, Director, Transplantation Biology Research Center, Massachusetts General Hospital, Building 149, 13th Street, Boston, MA 02129, Tel: 617-726-4065 Fax: 617-726-4067.

Author contributions/participation

Prashanth Vallabhajosyula – research design, writing of the paper, research performance, data analysis, analytical tools

Atsushi Hirakata – research performance

Akira Shimizu – research performance

Masayoshi Okumi – research performance

Vaja Tchipashvili – research performance

Hanzhou Hong – research performance

Kazuhiko Yamada – research design, analytical tools

David H. Sachs – research design, writing of the paper, data analysis, analytical tools

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

performed at 1, 2, > 3 months post-IK transplant showed essentially equivalent glycemic control in both control and experimental animals.

Conclusion—In this pre-clinical, in vivo large animal model of islet transplantation, the effect of triple drug immunosuppression on islet function does not negatively affect islet engraftment, as assessed by the long-term function of engrafted islets.

Keywords

Islet; Transplant; Pig; Immunosuppression; Islet-kidney

Introduction

Allogeneic islet cell transplantation is a treatment modality of type I diabetes with a goal of avoiding its serious complications (1-3). In the autologous setting, successful islet transplantation has been reported in several series of patients undergoing complete or near-total pancreatectomy for chronic pancreatitis (4-9). But even with a higher islet load, allogeneic islet transplantation is less successful than autologous islet transplantation. In addition to allogenicity, one of the proposed factors for increased islet toxicity is the immunosuppressive regimen in the allogeneic transplant recipient, something that is not administered in the autologous setting (10-18). In this study, we sought to test whether a potentially islet-toxic regimen of immunosuppression adversely affects the islet engraftment process, and thus decreases the effective functional islet mass. Initial clinical studies with allogeneic islet transplantation used a triple drug immunosuppressive regimen with azathioprine, prednisone, and cyclosporine. Outcomes with this regimen were poor, with studies suggesting that this drug regimen contributed to islet toxicity. The improvement in outcomes with the utilization of the Edmonton protocol revived efforts in allogeneic islet transplantation, with part of the success attributed to the use a less islet-toxic regimen. Since the Edmonton protocol is a less toxic regimen, for our study, we intentionally chose an immunosuppression cocktail used prior to the Edmonton era that was considered islet toxic-triple drug therapy with cyclosporine, prednisone, and azathioprine (15, 19-22).

To test this idea in an in vivo, preclinical, large animal model, we utilized an established model of vascularized islet transplantation in the miniature swine (23-25). Our previous work in the miniature swine showed that autologous transplantation of islets underneath the renal capsule leads to stable, long-term islet engraftment, and production of a composite organ that we have called an islet-kidney (IK). The IK, when transplanted into a diabetic, anephric recipient animal across a minor mismatch barrier immediately corrects both hyperglycemia and renal dysfunction. Utilizing this model, we tested whether immunosuppression adversely affects the islet engraftment process. To our knowledge, this is the first report assessing if immunosuppression independently affects the islet engraftment process in an in vivo, preclinical large animal model.

Results

Donor animals

Islet dose for IK transplantation and immunosuppressive regimen—Note that in order to study the effect of immunosuppression on islet engraftment, the triple drug regimen was given in the autologous setting in the experimental arm donors (Figure 1). This regimen was chosen because initial studies with islet transplantation suggested that it was islet toxic (15, 19-22). The IK from the donor was then transplanted into a diabetic, anephric recipient animal as an in vivo, physiologically and clinically relevant functional readout of the

transplanted islet mass that successfully engrafted. 12 days of intravenous cyclosporine was administered for tolerance induction in all the recipients in both arms of the study (26). In

IK transplants were performed at an islet dose of ~4500 IE/kg of recipient. Previous work showed that when the transplant is performed at islet dose of >5000 IE/kg recipient weight, the animals maintained long-term normoglycemia (fasting blood glucose (FBG) < 120 mg/dl) without any insulin requirements (23, 24). If the transplant was performed at ~3500 IE/kg, then the recipients maintained mildly elevated FBG around 160 mg/dl (unpublished data). If the islet toxic regimen adversely affected islet engraftment/function by 20%, the IK recipients in the experimental arm would display poorer fasting glucose regulation.

Clinical course—Three donor animals on the control arm were followed for at least 30 days post autologous islet transplant. The animals maintained normal FBG values throughout follow-up (Figure 2A). Four experimental arm donor animals also displayed essentially normal glucose regulation, even though they received an islet-toxic immunosuppressive regimen (Figure 2B). Intravenous glucose tolerance tests (IVGTT) were performed and both study arms showed near-normal response to glucose challenge (Figure 2C, 2D). As seen in this figure, blood glucose level was restored to baseline within 45 minutes post-challenge, although animals on the experimental arm showed a slightly higher early peak of blood glucose (average 350 versus 310 mg/dL). Thus, the islet engraftment process in both groups appeared to occur in similar, normal glycemic environments.

Since immunosuppression can have many adverse side effects in addition to islet stress that may indirectly affect islet engraftment and thus confound the results of the study, all the donors were followed for differences in other clinical parameters. All IK donors were clinically stable, and showed no signs of infection or surgical complications. The white blood count, hematocrit, and platelets remained in the normal range in the donors on both arms of the study (data not shown). Donors from both study arms maintained their pre-operative weight, showed normal electrolytes, and normal renal and liver function (data not shown). Thus, clinical parameters were essentially equivalent in donors from both arms of the study.

IK recipients

Fasting glycemic control—Diabetes was successfully induced in all IK recipients upon total pancreatectomy. Baseline FBG in these animals was <70 mg/dl, and on day 1 after total pancreatectomy it increased to >200 mg/dl. The hyperglycemic state was maintained until the day of IK transplantation.

Three IK recipients on the control arm showed immediate correction of the FBG one day after transplantation. The FBG increased again during the 12 day cyclosporine tolerance induction course, but resolved after and the animals maintained normal FBG long-term (Figure 3 A-C). To confirm that the glucose regulation was strictly dependent on the islet allograft, IK graftectomy was performed in the second animal (Figure 3B). The FBG dramatically increased on day 1 after graftectomy to >600 mg/dl. The third animal died acutely on postoperative day (POD) 50 from intestinal volvulus, a process unrelated to the function of the IK (Figure 3C).

Three IK recipients on the experimental arm also showed immediate correction of the diabetic state upon IK transplantation (Figure 3 D-F). These animals also showed transient hyperglycemia during the 12 day tolerance induction period with cyclosporine. Thus, just like the controls, these animals were also prone to diabetogenic stress. Glucose regulation in these animals was strictly dependent on the IK, as evidenced by the profound hyperglycemia ensuing post-graftectomy in one animal (Figure 3E). A fourth IK recipient on the

experimental arm died acutely on POD 11 from fungal sepsis, but maintained normoglycemia during the high dose cyclosporine tolerance induction period (data not shown). Therefore, overall there were only small differences in the long-term glucose regulation between the experimental and control arms.

Intravenous glucose tolerance tests—Both the control and experimental arms showed similar glucose tolerance curves (Figure 4 A-F). After the dextrose challenge, IK recipients typically required between 60 and 90 minutes for the blood glucose levels to return to baseline. For each animal on the control and the experimental arms of the study, there was no deterioration or improvement in the glucose tolerance curves over the long term follow-up

Renal function—IK recipients from both arms of the study showed normal renal function during the follow-up (data not shown). One animal developed renal failure around POD 100 that eventually led to its death. An earlier biopsy of the allograft on POD 42 showed normal renal histology, but repeat biopsy on POD 110 showed severe glomerular atrophy, without signs of cellular rejection. Interestingly, the FBG remained normal during this period of progressive renal failure (Figure 3D).

IK histology—IK histology was performed on all the recipient animals. Hematoxylin and eosin analysis of the islets in the IK from both arms of the study showed viable islet clusters underneath the renal capsule without any signs of rejection or injury. Insulin staining confirmed that the islets in the IK were functioning and producing insulin. Analysis of the renal parenchyma and the ureter failed to show any cellular rejection. Therefore, with the exception of unexplained renal failure in one animal, histological analysis confirmed long-term functionality in the IK recipients. Representative islet histology from the experimental arm recipient with renal failure with intact glucose regulation is shown (Figure 5).

Discussion

The design of the islet-kidney model in MGH miniature swine permits the investigation of controversial questions in the field of islet transplantation. One such question is the effect of immunosuppression on islet survival, especially during the engraftment period post-transplantation when the ischemic islets are most prone to injury. In this study, using a near-marginal islet transplant model, we addressed the potential negative role of immunosuppression on islet survival and engraftment. Since the Edmonton protocol is considered to be less islet-toxic, we hypothesized that the administration of a known islet-toxic immunosuppressive regimen would affect islet transplantation by either inhibiting engraftment/survival of islets, or affect their long-term function. To make this preclinical, large animal model more physiologically relevant, we studied the effects of immunosuppression on islet engraftment by transplanting the vascularized IK into diabetic recipients as an in vivo functional read-out. To our knowledge, this is the first report assessing if immunosuppression, as an independent factor, affects post-transplant islet engraftment/survival in an in vivo large animal model.

Previous work in our laboratory showed that when the transplant is performed at an islet dose of > 5000 IE/kg recipient weight, the animals maintained long-term normoglycemia (23, 24). If the transplant was performed at ~3500 IE/kg, then the recipients maintained mildly elevated FBG around 160 to 170 mg/dl. We therefore performed IK transplants at an islet dose of 4500 IE/kg, from which we could expect to reveal changes in islet engraftment/survival if there is an extra 20% functional loss of islets with immunosuppression compared to the control setting. This difference should have been detected in FBG and IVGTT of the experimental IK recipients compared to the controls. The results of the study do not support

this hypothesis. Thus., even with triple drug immunosuppression of azathioprine, prednisone, and cyclosporine, IK recipients on both arms of the study showed similar fasting glucose homeostasis and glucose tolerance over long-term follow-up.

IVGTTs were also performed in these animals. All of the animals showed mild glucose intolerance, attesting to the fact that islet function was equally marginal in both arms of the study. For each animal, IVGTT showed similar, small differences in responses followed over a >3 month period, with the greatest loss of function in one of the control IK recipients (Fig. 4A). This result suggests that there was no loss of functional beta cell mass during the follow-up, meaning there was no islet atrophy or exhaustion.

The marginality of the transplanted IKs in this study was supported by the finding that the IK recipients were susceptible to diabetogenic stress during the cyclosporine tolerance induction period. Also, the IK recipients showed a higher risk for catheter-associated systemic infections compared to the donor animals (data not shown). This was true on both arms of the study. These events often manifested as transient periods of mild hyperglycemia during the follow-up of the IK recipients (Figure 3). This finding validates the clinical significance of tight glycemic control in maintaining healthy homeostasis.

The clinical implications of this study include the likelihood that the immunosuppressive regimen, although possibly stressful to islets, is independently not an important cause of increased islet death in the peri-transplant period following islet cell transplantation. The study also suggests that transient immunosuppression (up to 60 days), including glucocorticoids, does not affect long-term islet function. The findings of this study may be especially relevant if the field of transplantation heads towards clinical experimentation with tolerance-inducing regimen. To achieve tolerance, more rigorous and diverse immunosuppressive regimens would likely be administered to patients, with the strongest suppression maintained in the peri-transplant period, during islet engraftment in a type I diabetic recipient. This study suggests that intense but transient immunosuppression may not necessarily be harmful to the islet survival and function long-term. Future studies using tolerance-inducing immunosuppressive regimens in this and other large animal islet transplant models would be important to test this hypothesis.

Although we realize that the number of animals used in this study is small compared to that feasible with smaller animal models, the MGH miniature swine are inbred and controlled across histocompatibility barriers. Therefore, there is high genetic and immunologic consistency between the experimental and control group animals. Also, the design of the model utilized in this study enables an in vivo, long-term readout of the islet engraftment process in a clinically more relevant model than in rodent/other small animal models. We believe that studying islet engraftment in this manner is physiologically more relevant, and strengthens the clinical implications of the study's findings.

In using the IK model of islet transplantation to address this question, we note that the transplantation site is different than that in clinical practice. It is possible that this difference may be responsible for providing resistance to an islet-toxic regimen during the engraftment process. Immunosuppression on islets in the liver may be more toxic than on islets transplanted under the renal capsule. In addition to exposure to drugs that occurs through the interface between the islet and the capillary, islets transplanted into the liver may also be directly exposed to drugs present in the portal venous blood. Therefore, the renal subcapsular site may provide relative protection to the islets. Future studies in our laboratory will investigate if the effects of immunosuppression on islet engraftment are affected by the transplantation site.

Although the goal of this study was to assess the effects of immunosuppression on islet cell engraftment/survival post-transplantation, in the clinical setting the patient is diabetic and is receiving allogeneic islets. Therefore, immunosuppressive regimen, in combination with allogenicity and the diabetic state may negatively affect islet cell survival/engraftment. To this effect, our laboratory is studying the effects of diabetic state on islet engraftment using the IK model. Future studies will focus on ascertaining the combined effects of these factors in affecting the islet engraftment process.

Research design and methods

Animals

All animals were housed at the Transplantation Biology Research Center, Massachusetts General Hospital, Boston, Massachusetts, in compliance with institutional guidelines, and studies were approved by the subcommittee on research animal care. Minor antigen mismatched donor and recipient pairs were selected from partially inbred miniature swine (27, 28). Typically, the IK transplant donors were >16 months of age, and the recipient animals were 2-3 months old.

Islet-kidney transplantation

An overview of the IK transplantation model is shown in Figure 1 (26).

Partial pancreatectomy and islet isolation

Distal pancreatectomy with splenectomy was performed in the donor animal through a midline laparotomy as previously described (8, 9). The pancreas was removed, distended on the back-table, and transported to Joslin Diabetes Center, Boston, Massachusetts, for islet isolation. The donor animal on the experimental arm of the protocol then underwent a gastric tube placement for reliable delivery of triple drug immunosuppression.

Islet transplantation (IK preparation)

Isolated islets were cultured overnight at 25 °C, 5% CO₂, and prepared the next day for transplantation (29). The donor animal was placed in a right lateral decubitus position, and the islet suspension was injected underneath the left renal capsule to prepare an IK on the day after the partial pancreatectomy procedure.

Immunosuppressive regimen in the experimental arm donors

Starting the day of autologous islet transplantation, donor animals received daily triple drug immunosuppression that was continued until the day of IK donation. The regimen was administered via the gastric tube, and consisted of: (a) prednisone taper once daily at 2 mg/kg × 3 days, 1.5 mg/kg × 3 days, 1 mg/kg × 3 days, 0.5 mg/kg × 3 days, and then 0.3 mg/kg per day; (b) cyclosporine at 4 to 6 mg/kg/day divided twice daily to maintain a blood trough of ~200 ng/ml, and; (c) azathioprine once daily at 1.5 mg/kg/day.

Total pancreatectomy

3 to 7 days before the IK transplantation, a total pancreatectomy was performed through a left paramedian incision as previously described (8, 9).

Islet-kidney transplantation

The composite IK graft was removed from the donor with the renal vessels and the ureter, and the organ was perfused with cold Euro-Collins solution on the back-table. The donor animal was sacrificed after IK procurement.

A right paramedian incision was made in the recipient animal, and bilateral native nephrectomy was performed. The IK was placed in the right abdomen, and the following anastomoses were performed using a running 6-0 prolene suture: renal vein to the IVC, renal artery to the aorta, and then an ureterocystostomy.

Donor and recipient animal monitoring

In early post-operative period, animals underwent daily blood sampling to check complete blood count (CBC), fasting blood glucose (FBG), blood urea nitrogen (BUN) and creatinine, amylase and lipase values. If normal, blood chemistry was checked only when warranted by clinical exam. Blood cyclosporine trough levels were checked at least three times a week in the experimental donor animals, with levels maintained between 200 to 250 ng/ml. Morning FBG was checked daily in all the animals.

In the IK recipients, 12 days of intravenous cyclosporine was administered for tolerance induction, maintaining blood trough level between 300 and 450 ng/ml.

Morning FBG was checked daily. All recipient animals received a high protein diet enriched with exocrine pancreatic enzymes.

Intravenous glucose tolerance tests (IVGTT)

Animals were fasted overnight and were sedated with ketamine given intramuscularly at 5 mg/kg. Dextrose was infused intravenously at a dose of 0.5 g/kg. Blood glucose was checked at 0, 2, 5, 10, 15, 30, 45, 60, 90, 120, 150, and 180 minutes post-infusion.

Kidney biopsy

The animal was placed in a lateral decubitus position, and 1 cm² open biopsy of the kidney was performed.

Graftectomy

Animal was placed in a lateral decubitus position and a flank incision was made. The renal vessels and the ureter were ligated and the organ procured. *Histology.* Tangential sections included islet and renal tissue. Formaldehyde-processed specimens were stained with hematoxylin and eosin (H&E) and insulin-specific antibodies. Insulin staining was performed on 4.0-µm sections. Sections were incubated at room temperature with 10% normal goat serum in PBS (pH 7.4) and then with guinea pig anti-porcine insulin antibody (Dako, Carpinteria, CA) diluted 1:10. They were next incubated with a 1:200 dilution of biotinylated goat anti-guinea pig secondary antibody (Vector Laboratories, Burlingame, CA) for 60 minutes. The tissue-bound antibodies were detected by avidin-biotin-peroxidase complex 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.

Acknowledgments

First author's work was supported by the following grants: American College of Surgeons Resident Research Scholarship, American Society of Transplant Surgeons – Roche Laboratories Scientist Scholarship, and Ruth L. Kirschstein National Research Service Award. The authors would also like to thank Aseda Tena, Jessica Sayre and Meghan Cochrane for help with animal care.

Support for author's work

Prashanth Vallabhajosyula: work supported by the following grants-American College of Surgeons Fellowship American Society of Transplant Surgeons - Roche Scientific Research Fellowship Ruth L. Kirschstein National Research Scholarship Award (5 F32 AI066699-02).

Abbreviations

BUN	Blood urea nitrogen
CBC	complete blood count
FBG	Fasting blood glucose
IE	Islet equivalents
IK	Islet-kidney
IVC	inferior vena cava
IVGTT	Intravenous glucose tolerance tests
PBS	phosphate-buffered saline

References

1. Shapiro AM, Lakey JR, Ryan EA, et al. Islet transplantation in seven patients with type 1 diabetes mellitus using a glucocorticoid-free immunosuppressive regimen. *N Engl J Med*. 2000; 343(4):230. [PubMed: 10911004]
2. Ryan EA, Lakey JR, Rajotte RV, et al. Clinical outcomes and insulin secretion after islet transplantation with the Edmonton protocol. *Diabetes*. 2001; 50(4):710. [PubMed: 11289033]
3. Matsumoto S, Okitsu T, Iwanaga Y, et al. Follow-up study of the first successful living donor islet transplantation. *Transplantation*. 2006; 82(12):1629. [PubMed: 17198249]
4. Farney AC, Najarian JS, Nakhleh RE, et al. Autotransplantation of dispersed pancreatic islet tissue combined with total or near-total pancreatectomy for treatment of chronic pancreatitis. *Surgery*. 1991; 110(2):427. [PubMed: 1858051]
5. Farney AC, Najarian JS, Nakhleh R, et al. Long-term function of islet autotransplants. *Transplant Proc*. 1992; 24(3):969. [PubMed: 1604690]
6. Gruessner RW, Sutherland DE, Dunn DL, et al. Transplant options for patients undergoing total pancreatectomy for chronic pancreatitis. *J Am Coll Surg*. 2004; 198(4):559. [PubMed: 15051008]
7. Robertson RP, Lanz KJ, Sutherland DE, Kendall DM. Prevention of diabetes for up to 13 years by autoislet transplantation after pancreatectomy for chronic pancreatitis. *Diabetes*. 2001; 50(1):47. [PubMed: 11147793]
8. Farney AC, Hering BJ, Nelson L, et al. No late failures of intraportal human islet autografts beyond 2 years. *Transplant Proc*. 1998; 30(2):420. [PubMed: 9532109]
9. Teuscher AU, Kendall DM, Smets YF, Leone JP, Sutherland DE, Robertson RP. Successful islet autotransplantation in humans: functional insulin secretory reserve as an estimate of surviving islet cell mass. *Diabetes*. 1998; 47(3):324. [PubMed: 9519735]
10. Basadonna G, Kakizaki K, Merrell RC. Effect of cyclosporine on established islet autografts. *J Surg Res*. 1986; 40(5):450. [PubMed: 3090372]
11. Fabian MC, Lakey JR, Rajotte RV, Kneteman NM. The efficacy and toxicity of rapamycin in murine islet transplantation. In vitro and in vivo studies. *Transplantation*. 1993; 56(5):1137. [PubMed: 8249114]
12. Ishizuka J, Gugliuzza KK, Wassmuth Z, et al. Effects of FK506 and cyclosporine on dynamic insulin secretion from isolated dog pancreatic islets. *Transplantation*. 1993; 56(6):1486. [PubMed: 7506454]
13. Desai NM, Goss JA, Deng S, et al. Elevated portal vein drug levels of sirolimus and tacrolimus in islet transplant recipients: local immunosuppression or islet toxicity? *Transplantation*. 2003; 76(11):1623. [PubMed: 14702535]
14. Hirshberg B, Rother KI, Digon BJ 3rd, et al. Benefits and risks of solitary islet transplantation for type 1 diabetes using steroid-sparing immunosuppression: the National Institutes of Health experience. *Diabetes Care*. 2003; 26(12):3288. [PubMed: 14633816]

15. Nanji SA, Shapiro AM. Islet transplantation in patients with diabetes mellitus: choice of immunosuppression. *BioDrugs*. 2004; 18(5):315. [PubMed: 15377174]
16. Hafiz MM, Faradji RN, Froud T, et al. Immunosuppression and procedure-related complications in 26 patients with type 1 diabetes mellitus receiving allogeneic islet cell transplantation. *Transplantation*. 2005; 80(12):1718. [PubMed: 16378067]
17. Shapiro AM, Hao E, Lakey JR, Finegood D, Rajotte RV, Kneteman NM. Diabetogenic synergism in canine islet autografts from cyclosporine and steroids in combination. *Transplant Proc*. 1998; 30(2):527. [PubMed: 9532162]
18. Rilo HL, Carroll PB, Shapiro R, et al. Human islet transplantation: results of the first 37 patients. *Transplant Proc*. 1995; 27(6):3162. [PubMed: 8539890]
19. Ricordi C, Tzakis AG, Carroll PB, et al. Human islet isolation and allotransplantation in 22 consecutive cases. *Transplantation*. 1992; 53(2):407. [PubMed: 1738936]
20. Menger MD, Wolf B, Jager S, Walter P, Messmer K. The influence of prednisolone on revascularization of pancreatic islet grafts. *Transplant Proc*. 1990; 22(4):2042. [PubMed: 1697120]
21. Scharp DW, Lacy PE, Santiago JV, et al. Results of our first nine intraportal islet allografts in type 1, insulin-dependent diabetic patients. *Transplantation*. 1991; 51(1):76. [PubMed: 1987709]
22. Socci C, Falqui L, Davalli AM, et al. Fresh human islet transplantation to replace pancreatic endocrine function in type 1 diabetic patients. Report of six cases. *Acta Diabetol*. 1991; 28(2):151. [PubMed: 1777651]
23. Kumagai N, O'Neil JJ, Barth RN, et al. Vascularized islet-cell transplantation in miniature swine. I. Preparation of vascularized islet kidneys. *Transplantation*. 2002; 74(9):1223. [PubMed: 12451257]
24. Kumagai N, LaMattina JC, Kamano C, et al. Vascularized islet cell transplantation in miniature Swine: islet-kidney allografts correct the diabetic hyperglycemia induced by total pancreatectomy. *Diabetes*. 2002; 51(11):3220. [PubMed: 12401713]
25. Vallabhajosyula P, Griesemer A, Yamada K, Sachs DH. Vascularized composite islet-kidney transplantation in a miniature swine model. *Cell Biochem Biophys*. 2007; 48(2-3):201. [PubMed: 17709890]
26. Rosengard BR, Ojikutu CA, Guzzetta PC, et al. Induction of specific tolerance to class I-disparate renal allografts in miniature swine with cyclosporine. *Transplantation*. 1992; 54(3):490. [PubMed: 1412729]
27. Sachs DH, Leight G, Cone J, Schwarz S, Stuart L, Rosenberg S. Transplantation in miniature swine. I. Fixation of the major histocompatibility complex. *Transplantation*. 1976; 22(6):559. [PubMed: 137560]
28. Mezrich JD, Haller GW, Arn JS, Houser SL, Madsen JC, Sachs DH. Histocompatible miniature swine: an inbred large-animal model. *Transplantation*. 2003; 75(6):904. [PubMed: 12660524]
29. O'Neil JJ, Stegemann JP, Nicholson DT, Gagnon KA, Solomon BA, Mullon CJ. The isolation and function of porcine islets from market weight pigs. *Cell Transplant*. 2001; 10(3):235. [PubMed: 11437069]

Figure 1.

The porcine islet-kidney (IK) model is shown. Donor animal underwent partial pancreatectomy + autologous IK preparation. The autologous islets were allowed to engraft over a period of 4 to 8 weeks. In the experimental arm, during the islet engraftment period, the donor animal received triple drug immunosuppression to test if these drugs adversely affect islet engraftment and function. As an in vivo functional readout, in both the control and experimental arms, the IK was then transplanted across an immunologic minor mismatch barrier into a pancreatectomized, nephrectomized recipient at an islet load of ~4500 IE/kg. All IK recipients received a 12 day course of cyclosporine for tolerance induction.

Figure 2.

IK donors on the control and experimental arms showed similar and normal glucose homeostasis, even with the administration of an islet-toxic immunosuppressive regimen in the latter group. Fasting blood glucose and IVGTTs are shown. (A) Fasting blood glucose regulation in three control donors (D1-3). (B) Fasting blood glucose in four experimental donors (D4-7). (C) IVGTT in control donors showed return to baseline glucose within 45 minutes post-infusion of dextrose bolus (D1-3). (D) Experimental donors showed similar IVGTT response as the control donors (D4-7).

Figure 3.

Daily fasting blood glucose levels in the IK recipients is shown. (A-C) Control arm animals showed immediate correction of the diabetic state upon IK transplantation, followed by transient hyperglycemia during the cyclosporine period. Overall, the animals showed long-term normal fasting glycemic control. One animal died on POD 50 from intestinal volvulus (C). (D-F) Experimental arm animals also showed similar fasting glycemic regulation as the control animals. One animal died late into the follow-up from renal failure of an unknown cause, but still maintained normoglycemia (D). IK graftectomy was performed to confirm the role of the IK in maintaining glucose homeostasis (B, E). Bars represent insulin dosing, line graph represent FBG level. TPx: total pancreatectomy; IK Tx: IK transplant; Gx: IK graftectomy; IV: intestinal volvulus; cyclosporine period marked

Figure 4.

Intravenous glucose tolerance tests were performed in the IK recipients around POD 30, 60, 90, and 120. (A-C): Three IK recipients on the control arm showed a mild delay in glucose clearance upon an intravenous challenge as compared to the donor animals (R1-3). (D-F): IVGTT responses in 3 experimental arm animals were similar to the control IK recipients (R4-6).

Figure 5.

Representative histology is shown in the IK recipient on the experimental arm with progressive renal failure of unknown cause (Animal R4). Islet clusters under the renal capsule stained positive for insulin, without any histological evidence of rejection. The renal parenchyma in this animal showed severe glomerular atrophy late in the follow-up. The animal maintained normoglycemia in the face of renal failure.