

## Vascularized composite islet-kidney transplantation in a miniature swine model

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**Abstract** Previous work from this laboratory has demonstrated that transplantation of allogeneic thymic tissue as part of a composite vascularized graft is far more successful in terms of both engraftment and long-term survival than transplantation of thymic tissue or cells alone. We have subsequently extended this concept to transplantation of allogeneic islets, comparing survival of islet cell suspensions to that of vascularized composite islet-kidneys (IK), prepared by injection of autologous islets underneath the renal capsule 2–3 months prior to allogeneic transplantation of the composite organ.

We have utilized partially inbred miniature swine with defined MHC loci as the experimental large animals for this study, permitting reproducible transplantation across specific MHC barriers. Composite IK have been transplanted successfully across minor and full MHC mismatch barriers, using treatment regimens previously demonstrated to induce long-term tolerance of kidney allografts across these barriers. IK allografts containing  $\geq 5000$  islet equivalents (IE)/kg recipient body weight were found capable of reversing surgically induced diabetes, while injection of comparable numbers of purified islets via the portal vein or under the renal capsule did not. Studies are also being directed toward preparation of autologous “thymo-islet-kidneys” (TIK), for potential use as xenografts, in which the thymic component is intended to induce tolerance and the islets to reverse diabetic hyperglycemia. The use of both types of composite organ transplants may eventually

be applicable to the treatment of type I diabetic patients suffering from end-stage diabetic nephropathy.

**Keywords** Islet · Transplantation · Islet-kidney · Swine · Thymo-islet-kidney · Kidney

### Introduction

Until recently, the results of whole organ pancreas transplantation in correcting the hyperglycemia of diabetes were far superior to those of islet transplantation [1–3]. Then, in 2000, the “Edmonton Protocol” [4] was demonstrated to achieve much improved survival of islets following transplantation, with initial normalization of blood sugar in 11 of 12 patients, and complete correction of hyperglycemia for over one year in four of these patients. However, this protocol required  $>9000$  IE/kg, necessitating the use of two or sometimes three pancreases for a single recipient. In addition, by one year post-transplant, three of the patients were again diabetic, two requiring insulin [5]. By 5 years, in a much larger series of patients at several centers, only 10% of patients have remained insulin independent [6, 7]. These data, while improved over previous islet transplantation data, therefore remain inferior to the results of whole organ pancreas transplantation. However, the significant morbidity of whole organ pancreas transplantation [8], suggests that further improvements in islet transplantation are still worth pursuing.

One of the main reasons for the disparity between the capacity of free islets and of the islets in a whole pancreas to survive after transplantation is likely to be the fact that newly transplanted islets must revascularize in the recipient before they can function. During the period of

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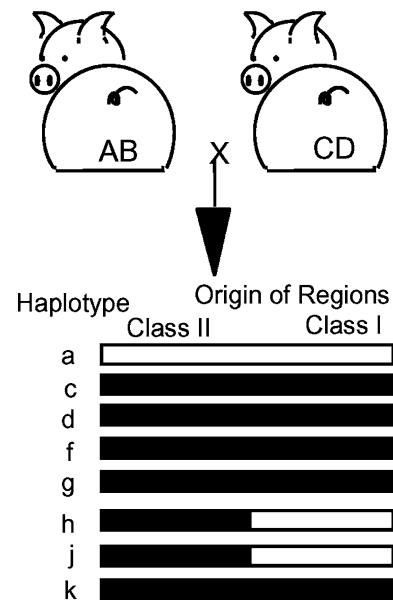
revascularization, one might anticipate an increased susceptibility to ischemic injury. In addition, while vascularized grafts are relatively tolerogenic [9–11], skin and tissue grafts are relatively immunogenic, probably because they release cells or antigens from cells into the lymphatic system, where they sensitize the host [12]. Thus, the non-vascularized islet graft may be damaged via both non-immunologic and immunologic mechanisms.

We have previously reported a marked difference in survival between vascularized and nonvascularized transplants of thymic tissue [13]. Transplantation of MHC-mismatched free thymic tissue led to loss of most of the thymic grafts, even with immunosuppression [14]. However, implantation of autologous thymic tissue under the kidney capsule of miniature swine allowed the grafts to vascularize and survive, presumably because even after ischemic injury, autologous tissue did not sensitize immunologically [15]. When the resulting composite “thymo-kidneys” were transplanted into thymectomized allogeneic recipients several months later, they survived long-term, and the thymic tissue showed evidence of function, including the induction of transplantation tolerance [13, 16].

We have reasoned that a similar survival advantage might be extended to islets by transplanting them as part of a composite vascularized graft. On this basis, we have developed the techniques required for preparing composite “islet-kidneys” (IK) by transplantation of autologous islets under the kidney capsule of the prospective donor 2–3 months prior to transplantation of the composite organ into pancreatectomized miniature swine. We present here a summary of our data so far using this modality for the treatment of surgically induced diabetes.

### The miniature swine transplantation model

Many successful rodent models of organ transplantation have failed when applied to large animals or to patients [17–19]. For this reason, there is need for well-defined, preclinical, large animal models in which new findings from rodent models can be tested before clinical applications are attempted. MGH miniature swine have been developed in this laboratory over the past 30 years as a preclinical, large animal model for studies of transplantation biology [20]. They represent the only large animal model in which MHC genetics can be reproducibly controlled. As such, these animals have been particularly useful in assessing the effects of MHC matching on rejection and/or tolerance induction. At present, we maintain swine of three homozygous SLA haplotypes, SLA<sup>aa</sup>, SLA<sup>cc</sup>, SLA<sup>dd</sup>, and five lines bearing intra-SLA recombinant haplotypes as illustrated in Fig. 1. All of these



**Fig. 1** Origin of haplotypes of MGH inbred miniature swine

lines differ by minor histocompatibility loci, thus providing a model in which most of the transplantation combinations relevant to human transplantation can be mimicked. Thus, for example, transplants within an MHC homozygous herd simulate transplants between HLA identical siblings, while transplants between herds resemble cadaveric or non-matched sibling transplants. Likewise, transplants between pairs of heterozygotes can be chosen to resemble parent into offspring or one-haplotype mismatched sibling transplants. In addition, we have chosen one subline of our SLA<sup>dd</sup> animals for further inbreeding, in order to produce a fully inbred line of miniature swine. This subline, which has reached a coefficient of inbreeding of 94%, is internally histocompatible, as reciprocal skin grafts within the subline are accepted long-term without immunosuppression [21]. We anticipate that the availability of these animals will permit easier production of islet-kidneys for our experimental work than the current method of utilizing autologous islets.

### Induction of tolerance to renal allografts in miniature swine

We have previously reported that a twelve-day course of high-dose (10–15 mg/kg/day) cyclosporine (CyA) induces long-term, specific tolerance to renal allografts across a minor antigen mismatch or across class I plus minor mismatches in miniature swine [9]. In order to cross a full-haplotype MHC mismatch, a twelve-day course of high-dose (.15–.2 mg/kg/day) tacrolimus (FK) was required

[22]. Tolerance induced by a short course of calcineurin inhibitors was found to be thymus-dependent [23, 24], requiring the transplants to be performed in juvenile recipients. In the initial studies of IK transplantation we utilized these methods of tolerance induction to attain survival of composite renal transplants. However, we expect that any means for prolonging the renal allograft would also be sufficient to prolong the survival of vascularized islets in an IK, so that tolerance induction will not necessarily be required for success of this treatment modality.

### Extension of the concept of composite organ transplantation to islets-kidneys

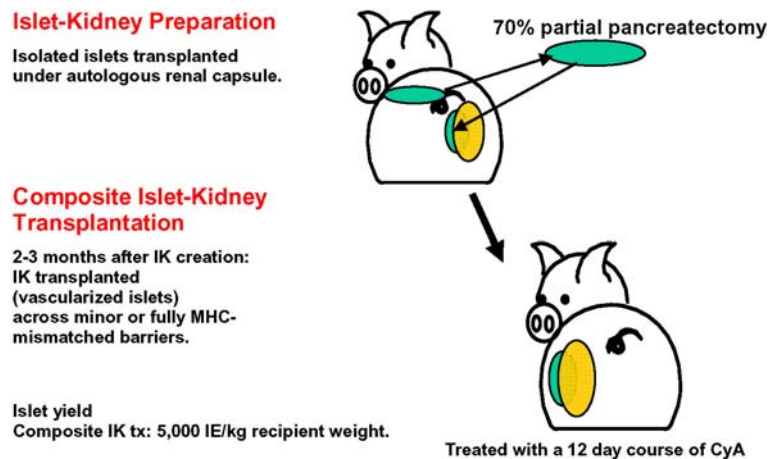
A schematic of the preparation and testing of vascularized islet-kidneys (IK) in miniature swine [25] is shown in Fig. 2. Donors were subjected to partial pancreatectomy, leaving approximately 30% of the pancreas (the head and part of the body) intact. The excised pancreas was placed in crushed ice immediately, and modified UW solution was infused through the pancreatic duct. Pancreatic tissue was then processed using methods adapted from those of Ricordi, as described [26], and the viability and purity of the preparation were evaluated by dual fluorescence staining with acridine orange and propidium iodide staining and by dithizone (DTZ) staining. The islets were cultured for 3 days to reduce antigen presenting cells and exocrine products.

Islet-kidneys (IK) were prepared by use of a paramedian or flank abdominal incision to expose the kidney, and injecting islets beneath the renal capsule using a 20G

angiocatheter [25]. As for the previous thymic grafts, islets were allowed to heal for about 2 months prior to the intended date of allogeneic transplantation of the composite organ. To test the function of these IK, diabetic hyperglycemia was induced in recipient animals by total surgical pancreatectomy 3–7 days prior to islet transplantation. After pancreatectomy, animals received pancreatase (Pancrezyme, Daniels Pharmaceuticals) in their diet to replace exocrine pancreatic enzymes. On the day of transplantation, these recipients were subjected to bilateral nephrectomy and orthotopic transplantation of the IK with urinary drainage through a ureterovesical anastomosis. Immunosuppression was either with 12-day course of either CyA or FK506, depending on whether the mismatch was for minor or major histocompatibility antigens, respectively [9, 22]. Islet function was assessed by fasting blood sugar (FBS) and glucose tolerance tests. Rejection was monitored through measurement of plasma creatinine as well as FBS, and was confirmed by histological assessment of biopsy tissue.

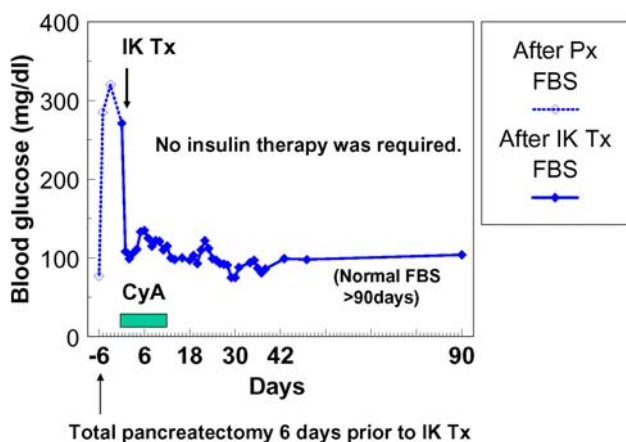
### Results of IK transplantation across a minor antigen mismatch

As described by Kumagai et al. [27], four animals were completely pancreatectomized 1 week prior to receiving a minor-mismatched composite IK. FBS levels reached >300 mg/dl without insulin administration. IKs containing approximately 5,000 IE/kg recipient body weight were transplanted, with a 12-day course of CyA at 10 mg/kg/day. As shown for one representative animal (Fig. 3), FBS levels fell immediately to normal levels (<120 mg/dl)



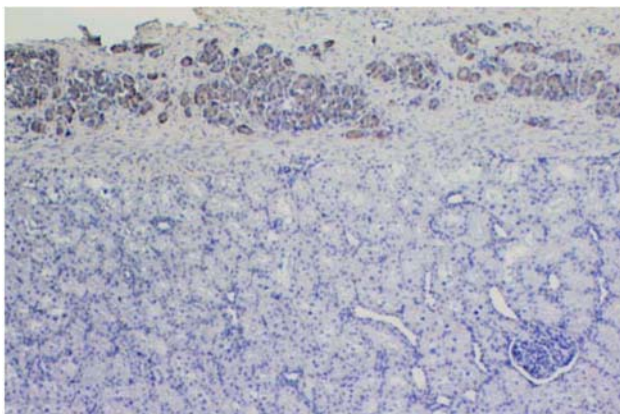
**Fig. 2** Schematic representation of autologous IK preparation and transplantation. Top: Donor animal undergoes partial pancreatectomy, followed by isolation of islets and injection of purified, cultured autologous islets back under the renal capsule, where they are allowed

to revascularize for 2–3 months. Bottom: The islet-kidney is then transplanted into allogeneic recipient animal that underwent prior total pancreatectomy to induce diabetes



**Fig. 3** Transplantation of a vascularized IK across a minor mismatch reverses surgically induced diabetes. Recipient animal underwent pancreatectomy (Px) 6 days before IK transplantation. Immunosuppression consisted of a 12-day course of cyclosporine (CyA) to induce tolerance. The IK restored fasting blood glucose (FBS) in the recipient rapidly and the animal had no further requirement for exogenous insulin. ([27] “Copyright © 2002 American Diabetes Association From Diabetes, Vol. 51, 2002; 3220–3228. Reprinted with permission from The American Diabetes Association”)

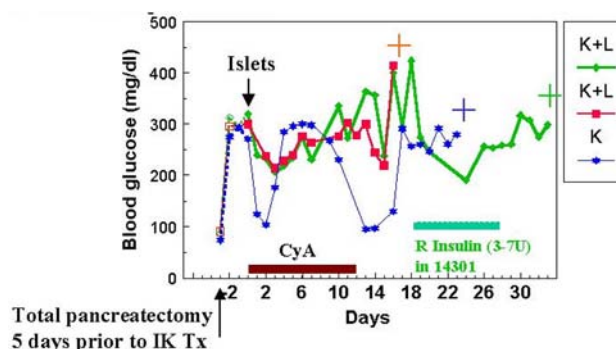
following IK transplantation. The IK grafts were accepted long-term, and the recipients maintained normoglycemia and normal creatinine thereafter. A glucose tolerance test was normal 3 months post transplantation. As seen in Fig. 4, a biopsy on POD 128 revealed numerous insulin-producing beta cells with minimal cellular infiltrate. Similar results were observed in all four experimental animals [27]. The IKs were explanted on PODs 67, 90, 128 and 150, following which the FBS increased immediately in each animal, confirming that recipient pancreatectomies had been complete.



**Fig. 4** Insulin staining shows islets under the kidney capsule of an IK at 128 days post-transplantation. ([27], “Copyright © 2002 American Diabetes Association From Diabetes, Vol. 51, 2002; 3220–3228. Reprinted with permission from The American Diabetes Association”)

### Comparison to results of non-vascularized minor-mismatched islet transplantation

Three pancreatectomized animals received 7000 IE/kg recipient body weight of similarly minor antigen-mismatched islets transplanted directly beneath the recipient’s native renal capsule ( $n = 1$ ) or injected via the portal vein (90% of islets) and beneath the renal capsule (10% of islets) ( $n = 2$ ), again followed by a 12-day course of CyA (10 mg/kg/day). The clinical courses of these animals are shown in Fig. 5. Despite administration of a larger number of islets, and administration of insulin for up to 2 weeks, none of these animals demonstrated reversal of hyperglycemia, and autopsy results revealed mononuclear cell infiltrates and fibrosis beneath the renal capsule and no evidence of functioning islets. As a further control, two pancreatectomized animals received 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. Immediately following revascularization of the donor renal allograft, cultured donor islets (7000 IE/kg recipient body weight) were injected beneath the renal capsule. In both animals, FBS levels decreased transiently, but then returned to elevated values, requiring insulin. Histologic analyses of autopsy specimens revealed few remaining islets and a diffuse mononuclear cell infiltrate in the site of islet-transplantation. Furthermore, an interstitial mononuclear cellular infiltrate was seen in the renal parenchyma, a finding not previously observed for minor-mismatched renal transplantation alone, suggesting sensitization of the recipient associated with the islet tissue rejection.



**Fig. 5** Islet cell transplantation across minor mismatch barriers fails to induce long term glucose control in diabetic recipient animals: Recipients were made diabetic through total pancreatectomy, and islets were injected into 3 animals, one receiving islets underneath the renal capsule (K), and two receiving renal subcapsular and portal vein injections (K + L). All three animals failed to regulate blood glucose. ([27], “Copyright © 2002 American Diabetes Association From Diabetes, Vol. 51, 2002; 322–228. Reprinted with permission from The American Diabetes Association”)

## Results of IK transplantation across a full MHC-mismatch

Two pancreatectomized animals received fully mismatched IKS containing approximately 5,000 IE/kg recipient body weight, with a 12-day course of FK506. Blood levels of FK were maintained at 25–35 ng/ml, since this was shown previously to induce tolerance to kidney transplants across full MHC barriers in miniature swine [22]. The composite IKS were accepted and FBS again fell from >300 mg/dl to normal levels immediately after revascularization. In this case, both animals exhibited a transient period of hyperglycemia from days 5 to 15, presumably due to FK506 associated islet-toxicity. Biopsies revealed numerous insulin-producing beta cells with minimal cellular infiltrate, similar to those observed for matched islets. In neither case was additional immunosuppression or insulin therapy required, and creatinine levels were maintained below 1.5 mg% throughout the experiment (>4 months).

## Vascularized thymo-islet-kidneys (TIK) as a future direction of this concept

Two of the major problems facing the field of islet transplantation are: (1) the limited availability of allogeneic islets; and (2) the requirement for chronic immunosuppressive medications to avoid rejection. Both of these problems might be solved if we could extend our work on IK transplantation to pig-to-primate xenotransplantation, using composite organs including thymic tissue to induce tolerance (see above) and islets to cure diabetes. We have named such a composite organ a “thymo-islet-kidney” (TIK), and have already reduced the concept to practice [25]. Indeed, we have demonstrated a marked salutary effect on the survival of minor-mismatched porcine islets when they were co-transplanted with autologous thymic tissue to make TIK grafts [25].

However, until recently, xenotransplantation of pig organs into primates has been stymied by the presence of high levels of natural antibodies. Using absorption techniques, complement inhibition, complement transgenic donors and tolerance-inducing protocols effective for allotransplants, we have had considerable success in tolerizing the T cell response across this xenogeneic barrier [28, 29]. However, these xenografts have been lost after several weeks due to the return of these natural antibodies. In humans and Old World non-human primates, the vast majority of natural antibodies to pig are directed against the single antigen alpha-1,3-Gal (Gal), on the surface of porcine cells. We have recently participated in the production of a Gal knock-out (GalT-KO) pig, derived by nuclear transfer of a genetically modified nucleus from our

most inbred line of miniature swine [30]. Utilizing these pigs as donors of kidneys and thymic tissue, we have recently achieved survivals of up to 83 days with excellent renal function [31].

Currently, studies are on going in our laboratory to determine the ability of TIK transplantation across defined MHC mismatch barriers to restore normoglycemia to recipient animals made diabetic by total surgical pancreatectomy. Our intent is to then extend the model to pig-to-baboon TIK transplantation, which could have potential clinical applicability for overcoming the shortage of allogeneic islets through xenotransplantation.

## Conclusion

The improved survival seen with composite IK grafts seems to be dependent on the prevascularization of the islets, and not on antigen loading or potential help from the co-transplantation of the donor kidney, since simultaneous transplantation of islets as cellular transplant, along with donor matched kidney transplantation, failed to restore normoglycemia to diabetic recipients. IK transplantation was not only effective in treating diabetes, but it also rendered long term tolerance using the same induction protocols that we have used for induction of tolerance to kidneys in our laboratory [9, 22]. Although not mentioned in this review, IK transplantation appears also to be a safe procedure for the donor, since as little as 30% residual pancreas was found to leave the donors with a normal glucose tolerance test for well over one year (Yamada et al., unpublished data). On the basis of these results, we have proposed the potential use of IK as a treatment modality in patients suffering from end-stage diabetic nephropathy [27]. In addition, the use of composite thymo-islet-kidneys could potentially extend this modality to xenotransplantation, thereby enormously widening its applicability for treating end-stage diabetic nephropathy.

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