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Classic and current opinion in embryonic organ transplantation

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Abstract

Purpose of Review—Here, we review the rationale for the use of organs from embryonic donors, antecedent investigations and recent work from our own laboratory exploring the utility for transplantation of embryonic kidney and pancreas as an organ replacement therapy.

Recent Findings—Ultra structurally precise kidneys differentiate in situ in rats following xenotransplantation in mesentery of embryonic pig renal primordia. The developing organ attracts its blood supply from the host. Engraftment of pig renal primordia requires host immune suppression. However, insulin-producing cells originating from embryonic pig pancreas obtained very early following initiation of organogenesis [embryonic day 28 (E28)] engraft long term in non-immune suppressed diabetic rats or rhesus macaques. Engraftment of morphologically similar cells originating from adult porcine islets of Langerhans occurs in animals previously transplanted with E28 pig pancreatic primordia.

Summary—Organ primordia engraft, attract a host vasculature and differentiate following transplantation to ectopic sites. Attempts have been made to exploit these characteristics to achieve clinically relevant endpoints for end-stage renal disease and diabetes mellitus using animal models. We and others have focused on use of the embryonic pig as a donor.

Keywords

chronic renal failure; diabetes mellitus; organogenesis; organ primordia; xenotransplantation

Introduction

It has been known for close to a hundred years that primordia of mammalian organs, once morphologically defined, can maintain themselves and undergo differentiation following transplantation to sites such as the mesentery, kidney capsule, or anterior eye chamber [1]. Classically, transplantation to mesentery was deemed to be particularly favorable in terms of its permitting undisturbed expansion of a growing organ primordium, hence morphogenesis that is not physically constrained over time, and resulting in vascularization by host blood vessels [2]. Under some circumstances engraftment was shown to occur following transplantation of embryonic organs to adult animals of a different species without the need

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to immune suppress hosts [3]. Within the past few decades efforts have been made by us and others to exploit the body of classic knowledge about embryonic organ transplantation to achieve a therapeutic end. Important studies antecedent to our own include those of Woolf et al who explored the possibility of adding new nephrons to the mammalian kidney via isotransplantation of embryonic metanephric tissue within renal parenchyma and reported that functioning nephrons can be added to mammalian kidneys by this technique in neonatal mice [4]; those originating in the Lazarow [5] and Brown [6] laboratories showing that experimental diabetes can be controlled in rats by isotransplantation of embryonic pancreas and that a novel organ consisting of islets of Langerhans in stroma without exocrine tissue differentiates in hosts post-procedure; and the work of Eloy et al who demonstrated that chick embryo pancreatic transplants reverse experimental diabetes in rats without a host immune-suppression requirement [7]. While the transplantation of human embryonic organs in human hosts has been contemplated by others, we have focused on the use of embryonic organs from the pig, a physiologically suitable donor for human pancreas or kidney replacement [reviewed in 8].

Transplantation of embryonic organ primordia to replace the function of failed organs

We have shown that it is possible to ‘grow’ new kidneys [9–11] or endocrine pancreatic tissue [11–17] in situ via xenotransplantation of organ primordia from pig embryos (organogenesis of the endocrine pancreas or kidney). The developing renal organ attracts its blood supply from the host [10]. In the case of pancreas, selective development of endocrine tissue takes place post-transplantation, developing beta cells enter lymphatic vessels and engraft in mesenteric lymph nodes from which they secrete insulin in response to elevated blood glucose [11–17]. Glucose intolerance can be corrected in formerly diabetic rats [11–14,16] and ameliorated in rhesus macaques [15,17] on the basis of porcine insulin secreted in a glucose-dependent manner by beta cells originating from transplants. In the case of kidney, an anatomically-correct functional organ differentiates in situ at the transplantation site [9–11]. Life can be prolonged in an otherwise anephric rat on the basis of renal function provided by a single transplanted rat renal primordium the ureter of which is anastomosed to a ureter of the host [18]. If obtained within a ‘window’ early during embryonic pancreas development, pig pancreatic primordia engraft in non-immune suppressed diabetic rats [11–14,16] or rhesus macaques [15,17]. In contrast, engraftment of pig renal primordia transplanted into rats requires host immune suppression [11].

Shown in Figure 1A is a photograph of an E28 pig renal primordium. The ureteric bud is labeled (ub). Figure 1B is a photograph of a kidney that has developed in situ. The ureter is labeled (u). Figure 1C is a photomicrograph that shows an E28 pig renal primordium. It consists of undifferentiated stroma, branched ureteric bud, and primitive developing nephrons (arrow) (11). The renal cortex of the kidney shown in Figure 1B consists of normal-appearing glomeruli (g), proximal tubules (pt) and distal tubules (dt) (Figure 1D). Its glomeruli are vascularized by host vessels that stain (brown) with anti-rat endothelial antigen 1 (RECA-1) that is specific for rat endothelium (Figure 1E).

An E28 pig pancreatic primordium with separate dorsal pancreas (dp) and ventral pancreas (vp) components is shown as an inset in Figure 2 (Figure 2A). Figure 2B shows the mesentery of a streptozotocin (STZ) -diabetic rhesus macaque at the time of transplantation. A primordium between sheets of mesentery is delineated (arrowhead). Figure 3 shows photomicrographs originating from a mesenteric lymph node of a rhesus macaque transplanted previously with E28 pig pancreatic primordia in mesentery. Sections in Figure 3 A and C are stained with an anti-insulin antibody. Sections in Figure 3 B and D are incubated with control serum. Individual cells that stain positive (red) are present in medullary sinus (arrow). The cells are polygonal, consistent with a beta cell identity (Figure 3 C arrow). No positive-staining cells are found in sections incubated with control serum (Figure 3 B and D). Engraftment of pig tissue in the mesenteric lymph nodes is documented using in-situ hybridization for porcine proinsulin mRNA. Cells expressing porcine mRNA stain with use of an antisense probe to porcine proinsulin mRNA (Figure 3 E), but not a sense probe (Figure 3 F).

As noted, glucose tolerance can be nearly normalized in nonimmune-suppressed diabetic rhesus macaques following transplantation of E28 pig pancreatic primordia [15, 17]. Exogenous insulin requirements are reduced in transplanted macaques, and animals have been weaned off insulin for short periods of time, but not permanently as is the case for rats transplanted with pig pancreatic primordia [11–14, 16]. The most likely explanation for the difference between rats and macaques is that macaques weigh 20 times as much as rats. An STZ-diabetic rat can be rendered normoglycemic lifelong with no exogenous insulin requirement by transplantation of 5–8 pig pancreatic primordia. Extrapolating, it would take 100–160 primordia to render a diabetic rhesus macaque independent of exogenous insulin. This would require the sacrifice of about 7–12 pregnant sows and multiple surgeries with the attendant complications.

In lieu of increasing the numbers of transplanted primordia or transplant surgeries in diabetic rhesus macaques, we embarked on a series of experiments to determine whether porcine islets, a more easily obtainable and possibly more robust source of insulin-producing cells, could be substituted for animals in which embryonic pig pancreas already had engrafted. To this end, we implanted adult porcine islets beneath the capsule of one kidney from rats [16] or macaques [17] that several weeks earlier had been transplanted with E28 pig pancreatic primordia in mesentery. We employed the renal subcapsular site for islet implantation so that we could differentiate engrafted porcine tissue originating from the islets from tissue originating from prior mesenteric E28 pig pancreatic transplants that never engraft in host kidney. In this setting, the contralateral (non-transplanted) kidney served as a control as did kidneys from rats or macaques implanted with islets without prior transplantation of E28 pig pancreatic primordia in mesentery. Figure 4 shows sections from a kidney of a STZ-diabetic rat (Figure 4 A and B) or rhesus macaque (Figure 4 C and D) implanted with porcine islets following transplantation of E28 pig pancreatic primordia in mesentery. Sections are stained using anti-insulin antibodies (Figure 4 A and C) or control serum (Figure 4 B and D). Cells that stain for insulin (Figure 4 A and C), but not with control serum (Figure 4 B and D) are present in an expanded renal subcapsular space. Nuclei of cells in the subcapsular space hybridize to antisense probes for porcine proinsulin mRNA, but not sense probes [16,17]. Neither cells that stain for insulin nor cells to which a the probe for porcine proinsulin

mRNA binds are present in contralateral (non-implanted) kidneys of STZ diabetic rats or macaques in which E28 pig pancreatic primordia were transplanted previously in mesentery or in kidneys from STZ-diabetic rats or macaques into which porcine islets are implanted without prior transplantation of E28 pig pancreatic primordia in mesentery [16,17].

To ascertain whether cells originating from kidney-implanted porcine islets function in rats or rhesus macaques, we determined whether the glucose tolerance of STZ-diabetic animals normalized partially by prior transplantation of E28 pig pancreatic primordia in mesentery was rendered normal by subsequent islet implantation, and measured glucose-stimulated insulin release from-islet implanted kidneys in vitro. Rats were rendered fully glucose tolerant by subsequent implantation of porcine islets in one kidney [16]. The glucose tolerance of macaques normalized partially by prior transplantation of E28 pig pancreatic primordia in mesentery was not improved by subsequent implantation of islets in kidney. However, a rapid release of insulin by macaque kidney slices was demonstrated in vitro in response to elevation of glucose levels across the threshold for insulin release [17].

Intact porcine islets do not engraft following renal subcapsular implantation [16,17]. Rather, a population of cells originating from donor islets with beta cell morphology that express insulin and porcine proinsulin mRNA engraft in kidneys of rats transplanted previously with E28 pig embryonic pancreas. Our observations are consistent with induction of tolerance to a cell component of adult porcine islets by previous transplantation of E28 pig pancreatic primordia in rats. We designate the phenomenon organogenetic tolerance [19]. Whatever its etiology might be, Induction of organogenetic tolerance to porcine islets in humans with diabetes mellitus would enable the use of pigs as islet donors with no host immune suppression requirement.

Ours is not the only group that has undertaken studies to ascertain whether transplantation embryonic pig renal or pancreatic primordia can be exploited to treat renal failure or diabetes mellitus in humans. Detailed comparisons of the findings described herein and the investigations of others are published elsewhere [8, 20–22] and will not be repeated here. Our body of work includes not only studies for which embryonic pigs serve as donors for organs transplanted in mesentery, but also investigations in which organs from embryonic rats were transplanted in mesentery and elsewhere. The findings are broadly confirmatory of preceding investigations. Thus, we recapitulated the findings of Woolf et al [4], that nephrons can be added to developed kidneys via transplantation of renal primordia beneath the capsule [23] prior to introducing use of the mesenteric site [9 – 11,18, 23, 24]. Interestingly, allotransplantation of renal primordia (rat to rat) can be performed in mesentery without host immune suppression [24]. We confirmed the observations of Hegre et al [5] and Brown et al [6], in that the novel organ they described (islets of Langerhans within connective tissue stroma) differentiates post- isotransplantation in rat [25]. We extended [11–17] the observation of Eloy et al [7], that engraftment following xenotransplantation of embryonic pancreas can occur in non- immune suppressed hosts (chick-to-rat), to different xenogeneic pairs (pig-to-rat and pig-to-rhesus macaque [11–17].

However broadly confirmatory our findings may have been of classic observations [1–7], there is an element of unpredictability to them. For example, the results of transplanting

embryonic kidney to mesentery (formation of a structurally correct renal organ) are different from results of transplanting embryonic pancreas (formation of the novel organ described above or dissemination of beta cells along a lymphatic distribution). Furthermore, outcomes following transplantation of embryonic pancreas differ not only depending on whether isotransplantation is carried out (novel organ), but also depending on what xenogeneic barrier is crossed (rat to mouse transplantation results in formation of the novel organ and requires host immune suppression; pig to rat or pig to rhesus macaque results in lymphatic dissemination of beta cells and no immune suppressions is required). In this regard, it has been impossible to predict what sort of structure will differentiate, or whether it will engraft at the implantation site or migrate elsewhere. Furthermore, it has been impossible to know whether or not host immune suppression if applicable (for xenotransplantation) will be required for engraftment.

Summary

A major advantage inherent in the use of embryonic kidney or pancreas for transplantation relative to more pluripotent undifferentiated cells is that the former differentiate spontaneously along defined organ-committed lines, albeit with a different outcome relative to what would occur if the primordia remained undisturbed within the embryo. In the case of pig renal primordia transplanted in mesentery, a kidney differentiates in situ with host vasculature [9–11]. In the case of embryonic pig pancreas all that remains in hosts post-transplantation are beta cells engrafted in mesenteric lymph nodes, for which glucose sensing and insulin-releasing mechanisms are functionally linked [11–17]. Transplantation of embryonic pancreas is one of many cellular strategies that can be employed to replace beta cell function. Others include islet implantation and transplantation of stem cells that differentiate into insulin producers [8, 21, 22]. In contrast, applications for cell transplantation to replace the function of a structurally complex organ such as the kidney are more limited. In order for glomerular filtration, reabsorption, and secretion of fluid and electrolytes to take place in a manner that will sustain life, individual nephrons must be integrated in three dimensions with one another and with a collecting system, the origin of which is yet another separate structure, the ureteric bud. Concomitantly, vascularization must occur in a unique organ-specific manner from endothelial precursors that may originate from both inside and outside of the developing renal primordium. While it is conceivable that endocrine functions of the kidney, such as erythropoietin production, could be replaced by transplanting one particular type of renal cell, recapitulation of glomerular filtration and reabsorption in kidneys and excretion of urine will be a much more formidable challenge for renal cell therapy.

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References

1. Rawles ME. Transplantation of normal embryonic tissues. *Ann New York Academy of Sciences*. 1952; 55:302–312.
2. Hamburger V. Morphogenetic and axial self-differentiation of transplanted limb primordia of 2-day chick embryos. *J Exp Zool*. 1938; 77:379–399.
3. Greene HSN. Attributes of embryonic tissues after growth and development in heterologous hosts. *Cancer Research*. 1955; 15:170–172. [PubMed: 14352209]
4. Woolf AS, Palmer SJ, Snow ML, Fine LG. Creation of a functioning chimeric mammalian kidney. *Kidney International*. 1990; 38:991–997. [PubMed: 2266685]
5. Hegre OD, Leonard RJ, Erlandsen SL, et al. Transplantation of the fetal rat pancreas: Quantitative morphological analysis of islet tissue growth. *Anat Rec*. 1976; 185:209–222. [PubMed: 776036]
6. Brown J, Molnar JG, Clark W, Mullen Y. Control of experimental diabetes mellitus in rats by transplantation of fetal pancreases. *Science*. 1974; 184:1377–1379. [PubMed: 4275321]
7. Eloy R, Haffen K, Keding M, Griener JF. Chick embryo pancreatic transplants reverse experimental diabetes of rats. *J Clin Invest*. 1979; 64:361–373. [PubMed: 156734]
8. Hammerman MR. Xenotransplantation of embryonic pig kidney or pancreas to replace the function of mature organs. *J Transplantation*. 2011:Article ID 501749.
9. Rogers SA, Talcott M, Hammerman MR. Transplantation of pig renal anlagen. *ASAIO Journal*. 2003; 49:48–52. [PubMed: 12558307]
10. Takeda S, Rogers SA, Hammerman MR. Differential origin for endothelial and mesangial cells after transplantation of pig fetal renal primordia into rat. *Transplant Immunology*. 2006; 15:211–215. [PubMed: 16431288]
11. Rogers SA, Liapis H, Hammerman MR. Normalization of glucose post-transplantation of pig pancreatic anlagen into non-immunosuppressed diabetic rats depends on obtaining anlagen prior to embryonic day 35. *Transplant Immunology*. 2005; 14:67–75. [PubMed: 15935296]
12. Rogers SA, Chen F, Talcott M, Hammerman MR. Islet cell engraftment and control of diabetes in rats following transplantation of pig pancreatic anlagen. *Am J Physiol*. 2004; 286:E502–E509.
13. Rogers SA, Chen F, Talcott M, et al. Glucose tolerance normalization following transplantation of pig pancreatic primordia into non-immunosuppressed diabetic ZDF rats. *Transplant Immunology*. 2006; 16:176–184. [PubMed: 17138051]
14. Rogers SA, Hammerman MR. Normalization of glucose post-transplantation into diabetic rats of pig pancreatic primordia preserved in vitro. *Organogenesis*. 2008; 4:48–51. [PubMed: 19279715]
15. Rogers SA, Chen F, Talcott MR, et al. Long-term engraftment following transplantation of pig pancreatic primordia into non-immunosuppressed diabetic rhesus macaques. *Xenotransplantation*. 2007; 14:591–602. [PubMed: 17991147]
16. Rogers SA, Mohanakumar T, Liapis H, Hammerman MR. Engraftment of cells from porcine islets of Langerhans and normalization of glucose tolerance following transplantation of pig pancreatic primordia in non-immune suppressed diabetic rats. *American Journal of Pathology*. 2010; 177:854–864. [PubMed: 20581052]
17. Rogers SA, Tripathi P, Mohanakumar T, et al. Engraftment of cells from porcine islets of Langerhans following transplantation of pig pancreatic primordia in non-immune suppressed diabetic rhesus macaques. *Organogenesis*. 2011; 7:154–162. [PubMed: 21654197]
18. Rogers SA, Hammerman MR. Prolongation of life in anephric rats following de novo renal organogenesis. *Organogenesis*. 2004; 1:22–25. [PubMed: 19521556]
19. Hammerman MR. Organogenetic Tolerance. *Organogenesis*. 2010; 6:270–275. [PubMed: 21220963]
20. Hammerman MR. Transplantation of renal primordia: Renal organogenesis. *Pediatric Nephrology*. 2007; 22:1991–1998. [PubMed: 17668242]
21. Hammerman MR. Xenotransplantation of pancreatic and kidney primordia: Where do we stand? *Transplant Immunology*. 2009; 21:93–100. [PubMed: 18992818]
- *22. Hammerman MR. Xenotransplantation of embryonic pancreas for treatment of diabetes mellitus in non-human primates. *J Biomedical Science and Engineering*. 2013; 6:6–11. Review of a body

of work directed toward development of a novel xenotransplantation therapy for diabetes mellitus.

23. Rogers SA, Lowell JA, Hammerman NA, Hammerman MR. Transplantation of developing metanephroi into adult rats. *Kidney Intl.* 1998; 54:27–37.
24. Rogers SA, Liapis H, Hammerman MR. Transplantation of metanephroi across the major histocompatibility complex in rats. *Am J Physiol.* 2001; 280:R132–R136.
25. Rogers SA, Liapis H, Hammerman MR. Intraperitoneal transplantation of pancreatic anlagen. *ASAIO Journal.* 2003; 49:527–532. [PubMed: 14524558]

Key Points

- Organ primordia engraft, attract a host vasculature and differentiate following transplantation to ectopic sites.
- Attempts have been made to exploit these characteristics to achieve clinically relevant endpoints for end-stage renal disease and diabetes mellitus using animal models.
- We and others have focused on use of the embryonic pig as a donor.
- What happens after transplantation of embryonic kidneys or pancreas as defined by what sort of structure differentiates, whether it engrafts at the implantation site or migrates elsewhere and by whether or not host immune suppression is required for engraftment differs from experimental case to experimental case.
- It is impossible to know what will happen after an embryonic organ obtained at a given developmental stage is isografted, allografted or transplanted across a narrow or wide xenogeneic barrier until one does the experiment.

Figure 1.

Photographs (A,B) and photomicrographs (C–E) of: A) a renal primordium freshly dissected from an E28 pig embryo; B) a developed pig renal primordium 7 weeks following transplantation after removal from mesentery.; C) a renal primordium freshly dissected from an E28 pig embryo. Branched ureteric bud and developing nephron (arrow); D) cortex of a developed pig renal primordium in rat mesentery 7 weeks following transplantation. Glomerular capillary loop (arrow); E) a glomerulus within the cortex of a developed pig renal primordium in rat mesentery 7 weeks following transplantation stained with rat endothelial cell antigen 1 (RECA-1). Abbreviations: ub, ureteric bud; u, ureter; pt, proximal

tubule; dt, distal tubule; g, glomerulus. Scale bar 80 μ m (A, C); 6 mm (B); 10 μ m (D); 5 μ m (E). Reproduced with permission [10,11].

Figure 2.

A) Photograph of a pancreatic primordium freshly dissected from an E28 pig embryo and B) A pancreatic primordium implanted between sheets of mesentery in a rhesus macaque. dp, dorsal pancreas; vp, ventral pancreas. Scale bar 10 μ m (A). Reproduced with permission [15].

Figure 3.

Photomicrographs of mesenteric lymph nodes from a STZ-diabetic rhesus macaque post-transplantation of E28 pig pancreatic primordia. Sections A and C are stained with an anti-insulin antibody. Sections B and D are stained using a control serum. Arrow delineates medullary sinus (A). Arrow delineates polygonal cell (C). In situ hybridization was performed using antisense (E) or sense probes (F). Arrows delineate a cells in consecutive sections to which the antisense probe binds (E). Scale bars 80 μ m (A & B); 10 μ m (C – E). Reproduced with permission [15].

Figure 4.

Sections of the islet-implanted kidney from a STZ-diabetic Lewis rat (A,B) or rhesus macaque (C,D) transplanted with E28 pig pancreatic primordia in mesentery followed by porcine islets in the renal subcapsular space stained using anti-insulin antibodies (A,C) or control antiserum (B,D). PT, proximal tubule. RC, renal capsule. Arrows, positively staining cells (A,C); negatively staining cells (B,D). Scale bar 10 μ m. Reproduced with permission [16,17].