2004

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[Organogenesis 1:1, 3-13; July/August 2004]; ©2004 Landes Bioscience

Review

Pancreas and Kidney Transplantation Using Embryonic Donor Organs

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Received 04/08/04; Accepted 06/01/04

Previously published online as an *Organogenesis* E-publication: http://www.landesbioscience.com/journals/organogenesis/abstract.php?id=1008

KEY WORDS

cell therapy, diabetes mellitus, end-stage renal disease, metanephros; organogenesis, xenotransplantation

ACKNOWLEDGEMENTS

M.R.H. was supported by grant DK63379 from the National Institutes of Health. M.R.H. and Washington University may receive income based on a license of related kidney technology by Washington University to Intercytex LTD and based on equity holdings in Intercytex LTD Manchester UK.

ABSTRACT

One novel solution to the shortage of human organs available for transplantation envisions 'growing' new organs in situ. This can be accomplished by transplantation of developing organ anlagen/primordia. We and others have shown that renal anlagen (metanephroi) transplanted into animal hosts undergo differentiation and growth, become vascularized by blood vessels of host origin and exhibit excretory function. Metanephroi can be stored for up to 3 days in vitro prior to transplantation with no impairment in growth or function post-implantation. Metanephroi can be transplanted across both concordant (rat to mouse) and highly disparate (pig to rodent) xenogeneic barriers. Similarly, pancreatic anlagen can be transplanted across concordant and highly disparate barriers, and undergo growth, differentiation and secrete insulin in a physiological manner following intra-peritoneal placement. Implantation of the embryonic pancreas, is followed by selective differentiation of islet components. Here we review studies exploring the potential therapeutic applicability for organogenesis of the kidney or endocrine pancreas.

INTRODUCTION

Transplantation of human kidneys is an established medical therapy, the application of which is limited by shortage of available donors. The use of animals in lieu of humans as donors (xenotransplantation) is a potential solution for the organ shortage.[1]

In that humans and pigs are of comparable size, and share a similar renal physiology it is likely that pigs could serve as a substitute animal donor for human kidney replacement.[1] Unfortunately, the transplantation of vascularized organs such as the kidney originating from pigs into humans is rendered problematic because of the processes of hyperacute and acute vascular rejection that occur across this xenogeneic barrier.[1-3]

Hyperacute rejection can be avoided, for example through the use of genetically altered kidneys originating from pigs transgenic for the human complement activators, human decay accelerating factor (hDAF) and CD59.[4] Unfortunately, kidneys even from transgenic pigs transplanted to susceptible primates (humans) are subject to acute vascular rejection and acute rejection. Acute rejection can be circumvented using immunosuppression or co-stimulatory blockade.[1] However, acute vascular rejection represents a major obstacle to the use of porcine organs in human hosts. Its etiology is multi-factorial and incompletely defined. Several processes implicated as causative of acute vascular rejection, reflect a fundamental incompatibility between host proteins/protein systems, and the vascular endothelium of the donor.[1,2]

Host immune responses directed against antigens located on the endothelium of a transplanted organ, or mediated by transplant endothelial cells are obviated in proportion to the extent that the organ can be vascularized by the host. Cellular transplantation, which employs the transplantation or placement of cells that have the potential for replacement of native cells damaged or destroyed by disease, represents a way to circumvent hyperacute and acute vascular rejection since many cellular transplants derive their vasculature from the host.[2,3] While cell transplantation may under some conditions provide an alternative to whole organ transplantation, replacing multiple functions in organs that are structurally complex such as the lung or kidney is probably beyond its therapeutic utility.[2] An alternative that could be more useful for complex organ replacement is organogenesis as applied through the growing of a new organ in situ from transplanted primodia/organ anlagen.[2] For the kidney this can be accomplished by metanephros (renal anlage) transplantation.[5]

Transplantation of isolated islets of Langerhans to diabetic humans, a form of cellular therapy, is a proven but still experimental means to treat diabetes mellitus.[6,7] As for the kidney, the shortage of human islet donors is such that it will not be possible to meet the demand from all the patients with Type 1 diabetes mellitus.[7] Limited availability of islets could be overcome through use of tissue derived from pigs since humans are sensitive to porcine insulin, and islets are not subject to hyperacute or acute vascular rejection post-transplantation from pigs to humans.[8] Unfortunately, the limited experience with pig to human islet transplantation suggests that even large quantities of transplanted porcine islets will not translate into insulin sufficiency for human hosts.[8]

It is likely that problems inherent in the isolation of islets result in diminished viability post-implantation.[9] Coupled with the limited ability of beta cells within mature islets to replicate,[10] the diminished viability results in a declining pool of functioning engrafted islets over time.[9]

One possible strategy to overcome the limited potential for growth or division of mature islet cells is to transplant developing fetal or neonatal pancreatic tissues (pancreatic anlagen) that have a greater capacity for beta cell expansion post implantation.[9,10] The potential for expansion of pancreatic anlagen sufficient to render a diabetic host euglycemic is reflected by the fact that every normal human pancreas originates from a single embryonic precursor organ.

This review will discuss progress relating to the potential for transplantation of renal or pancreatic anlagen as precursors for in situ organogenesis in the xeno-replacement of human kidney or endocrine pancreas.

DEVELOPMENT OF THE KIDNEY

Three paired renal organs develop from the intermediate mesoderm during mammalian embryogenesis, the pronephros, mesonephros and metanephros. Metanephroi are the anlagen of adult mammalian kidneys. They originate during the fifth week of gestation in humans, during day 12 of embryonic rat development (E12), during day 11 of embryonic mouse development (E11) and during day 21–28 of embryonic pig development (E21–28) when outgrowths of the mesonephric ducts, so-called ureteric buds, collect about their distal ends, intermediate mesoderm (metanephric blastema) located caudal to the mesonephros. Numerous outgrowths arise from the distal end of the ureteric bud which push radially into the surrounding mass of metanephric blastema and give rise to the collecting ducts of the kidneys. The proximal ends of the ureteric bud give rise to the ureter and renal pelvis. The metanephric blastema differentiates into all tubular structures of the adult nephron with the exception of the collecting system.[11]

The major vessels supplying the kidney originate from lateral branches of the abdominal aorta that terminate in a plexus of arteries in close proximity to the renal pelvis, the renal artery rete.[12] It is a matter of controversy whether the renal microvasculature arises exclusively via this angiogenic process, or also in part from endothelial cells resident in the developing metanephros. However, it is clear that during its development, the renal anlage is able to attract at least the major portion of its vasculature, from the developing aorta.[12] In that its blood supply originates from outside of the developing renal anlage, the kidney may be regarded as a chimeric organ. Its ability to attract its own vasculature in situ establishes the potential for a transplanted metanephros to attract a vasculature from an appropriate vascular bed.

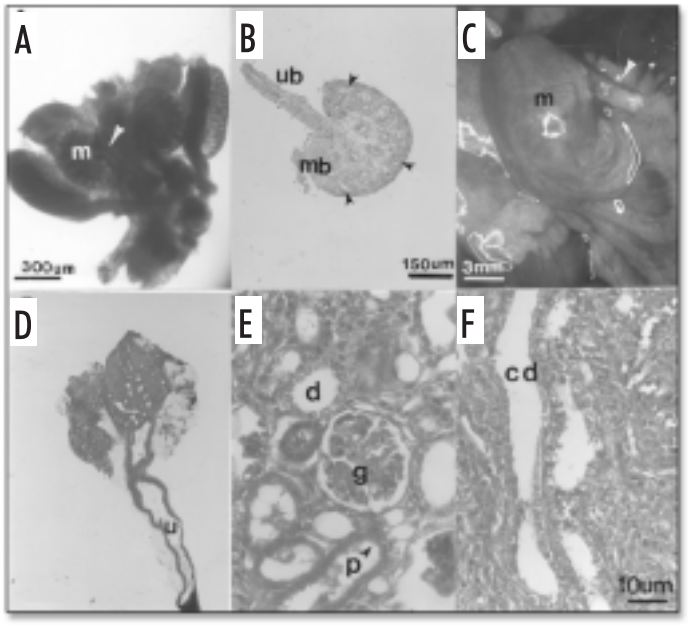

Figure 1. (A) Photograph of retroperitoneal dissection from an E15 rat embryo showing metanephros (m) and ureteric bud (arrowhead); (B) Photomicrograph of an E15 metanephros. A branched segment of ureteric bud (ub; arrowheads) and metanephric blastema (mb) are labeled; (C) Photograph of a developed metanephros (m) in the omentum of an adult host rat 3 weeks post transplantation. Arrowhead shows developed ureter. (D–F) Photomicrographs of H&E-stained sections: (D) A developed metanephros (u) ureter; (E) Cortex from a developed metanephros. Glomerulus (g), proximal tubule (p), brush border (arrowhead) and distal tubule (d) are shown; (F) Medulla from developed metanephros. Collecting duct (cd) is shown. Magnifications are shown in (A), (B) and (C) (for C and D), and (F) (for E and F). Reproduced with permission.[14,17]

RENAL ORGANOGENESIS

It was speculated that developing nephrons implanted beneath the renal capsule or in tunnels fashioned into the renal parenchyma might become incorporated into the collecting system of the host, and thereby increase host renal function.[13] However, such incorporation and a consequent enhancement of renal function have never been demonstrated for isotransplants,[13] allotransplants[14] or xenotransplants.[15] In addition, space limitation beneath the renal capsule has proven to be an impediment to the growth of transplants.[14]

Allotransplantation of whole metanephroi, at least across some barriers[14,16] can be carried out with no host immunosuppression. This is not possible if fully developed kidneys are transplanted across the same allogeneic barriers.[14]

Rat to Rat Allotransplantation. In contrast to the case for rat nephrons transplanted beneath the renal capsule, metanephroi transplanted into a host rat's fold of omentum, undergo differentiation and growth in hosts that is not confined by a tight organ capsule.[14] A metanephros in a retroperitoneal dissection from an E15 rat embryo is shown in Figure 1A. The ureteric bud is delineated by an arrowhead. Figure 1B is a photomicrograph of an E15 rat metanephros stained with hematoxylin and eosin (H&E) showing that it consists of branched ureteric bud (ub; arrowheads, Fig. 1B) and undifferentiated metanephric blastema (mb). If transplanted with its ureteric bud attached, the metanephros enlarges and becomes kidney-shaped within 3 weeks (Fig. 1C). Figures 1D–F show H&E-stained sections of: a developed renal anlagen six weeks post-transplantation into the omentum of a host rat with labeled ureter (u) (Fig. 1D) and developed

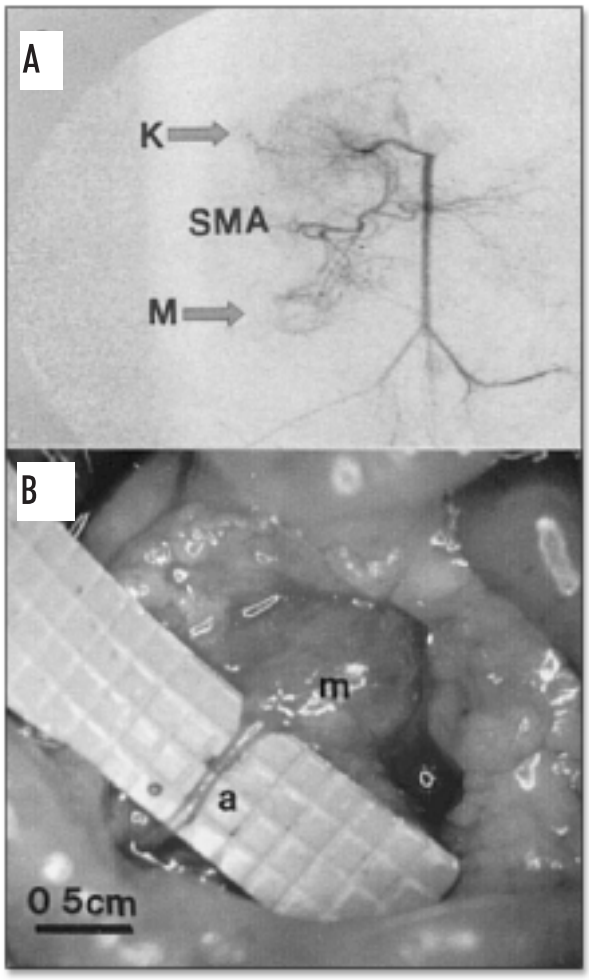

Figure 2. (A) Radiocontrast image of kidney (K) and developed metanephros (M), 6 weeks post-transplantation into the peritoneum of a host rat. SMA (superior mesenteric artery); (B) Artery (a) supplying the developed metanephros originating from the host's peritoneum. Magnification is shown for (B). Reproduced with permission.[14,17]

Table 1 **Urine Volumes and Inulin Clearances**

	Vehicle	Gr Factors
	(N = 7)	(N = 4)
Urine volume (UV)	31 ± 9.1	621 ± 62
(ul/hour)		
Inulin Clearance (GFR)		
(ul/min/100g)	0.24 ± 0.06	27 ± 8.2
(ul/hour)	38	5313
UV/GFR	0.8	0.12

Some data are expressed as mean ± SEM. UV/GFR is calculated using values expressed as ul/hour. Reproduced with permission.[17]

cortex with a labeled glomerulus (g), proximal tubule (p) with a brush border membrane (arrowhead), and distal tubule (d) (Fig. 1E). A developed medulla with a labeled collecting duct (cd) is shown in Figure 1F.

In contrast to transplanted developed kidneys,[14] metanephroi transplanted into non-immunosuppressed hosts have a normal kidney structure and ultrastructure post-development in situ and become vascularized via arteries that originate from the superior

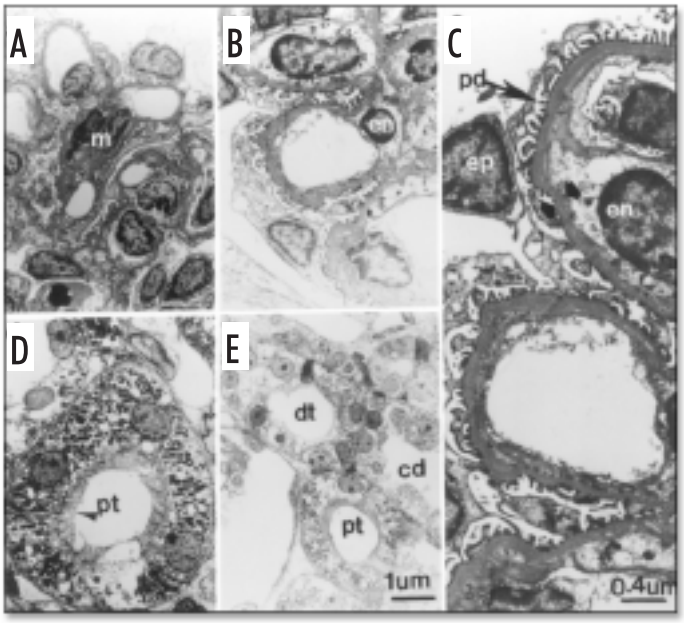

Figure 3. Electron micrographs of transplanted rat metanephroi. Glomerular capillary loops show labeled: (A) mesangial cell (m); (B) endothelial cell (en); and (C) epithelial cell (ep), endothial cell (en), podocytes (pd), and a basement membrane (arrows); (D) A proximal tubule (pt) with a brush border membrane (arrowhead); (E) proximal tubule (pt) distal tubule (dt), and collecting duct (cd). Magnifications shown for (C) and (E). Reproduced with permission.[18]

mesenteric artery (SMA) of hosts.[17] The SMA supplying a transplanted metanephros (M) is delineated in the radiocontrast study shown in Figure 2A. The artery (a) is labeled in Figure 2B. Electron microscopy of a developed renal anlage[18] reveals normal renal structures including the filtration barrier (Fig. 3).

Developed metanephroi transplanted onto the omentum produce urine that is excreted in the normal manner following ureteroureterostomy between transplant and host, a procedure that can be readily carried out at if metanephroi are implanted in close proximity to the host ureter.[14]

Levels of renal function in transplanted metanephroi [glomerular filtration rate (GFR)] were determined by measuring inulin clearance in otherwise anephric rats. In initial experiments GFRs were very low.[14] However as shown in Table 1 incubation of metanephroi with growth factors prior to implantation increased GFRs more than 100-fold compared to those in rats with non growth factor-incubated metanephroi implanted concurrently.[17] GFRs in growth factor treated metanephroi are about 6% of normal. This represents a 250-fold increase relative to the values obtained in our original studies employing non growth factor-treated renal anlagen (0.11 ± 0.02 µl/min/100 g rat weight).[14]

Hemodialysis provides renal failure patients with GFRs that are about 10% of normal. This level of renal function that sustains life in a human can also sustain life in a rat.[19] Therefore, 6% of normal approximates a level of renal function that would be expected to preserve life. Indeed, survival in an otherwise anephric rat (~2 days) can be prolonged to 7 days by a single transplanted developed metanephros.[20]

Renal plasma flow, another parameter of renal function, was measured in transplanted metanephroi by calculating P-aminohippurate (PAH) clearances. The ratio of GFR/PAH clearance (filtration fraction) was 0.6, higher than normal for the rat (0.3–0.4).[17] However, it is comparable to filtration fractions measured in rats

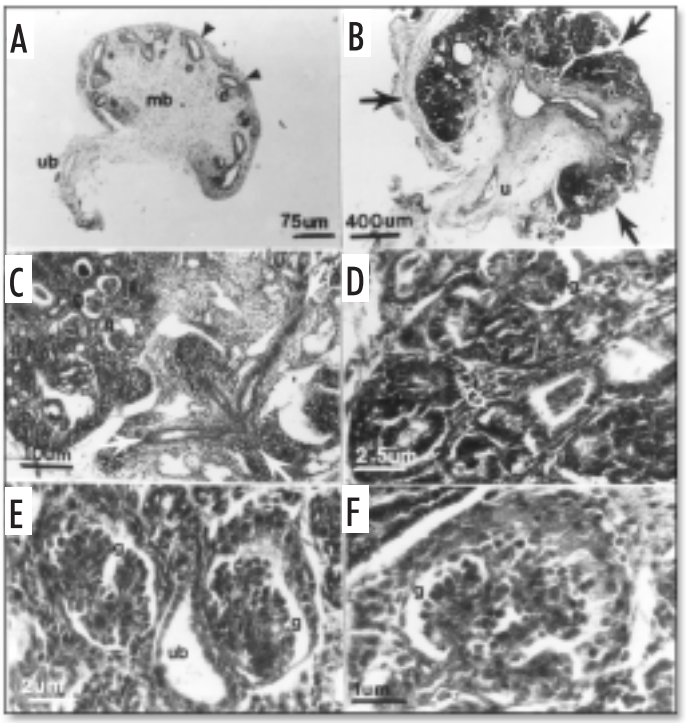

Figure 4. H&E-stained sections of paraffin-embedded pig metanephroi prior to transplantation (A) and 2 weeks post-transplantation into a pig (B–F): (A) An E28 pig metanephros. Branched ureteric bud (arrowheads), undifferentiated metanephric blastema (mb) and the ureteric bud (ub) are labeled; (B) Lobules are delineated (arrows). The ureter is labeled (u); (C) Glomeruli (g) are labeled as is a branched large artery (arrows); (D) A developing nephron (arrow) and a glomerulus (g) are delineated; (E); Glomeruli (g) and a section of the branched ureteric bud (ub) are labeled; (F); Higher power view of a glomerulus (g) The proximal tubule lumen is delineated (arrow). Magnifications are shown. Reproduced with permission.[21]

with reduced renal function (0.4–0.5), and demonstrates that tubules in transplanted metanephroi transport PAH.[17]

Urine flow rates in transplanted rats were calculated by measuring urine volumes (UV) as a function of time. UVs are about 12% of the inulin clearance (GFR) measured in growth factor-treated metanephroi (Table 1). This value is comparable to the upper range of normal for a mammal (humans). The UV/GFR of 0.12 demonstrates that transplanted metanephroi can concentrate urine.[17]

Pig to Pig Allotransplantation. Transplantation of metanephroi from E28 outbred Yucatan minipigs to adult outbred Yucatan minipigs can be carried out without host immunosuppression.[21] H&E-stained sections of paraffin-embedded pig metanephroi are shown in Figure 4. On E28 (pre-implantation), the Yucatan minipig metanephros consists of undifferentiated metanephric blastema (mb) and branched ureteric bud (arrowheads). The ureteric bud is labeled (ub) (Fig. 4A).

Two weeks post-transplantation into the omentum of an adult host (Fig. 4B), Yucatan minipig metanephroi are enlarged and have the characteristic lobular (arrows) pig kidney-like structure.[21] The ureter is labeled (u). Shown in Figure 4C are glomeruli (g) and a branched large artery (arrows). A glomerulus (g) and developing nephrons (arrow) are shown in Figure 4D. Higher power views of glomeruli (g) are shown in Figures 4E and F. A ureteric bud (ub) is delineated in Figure 4E. A proximal tubule lumen is shown in Figure 4F (arrow).

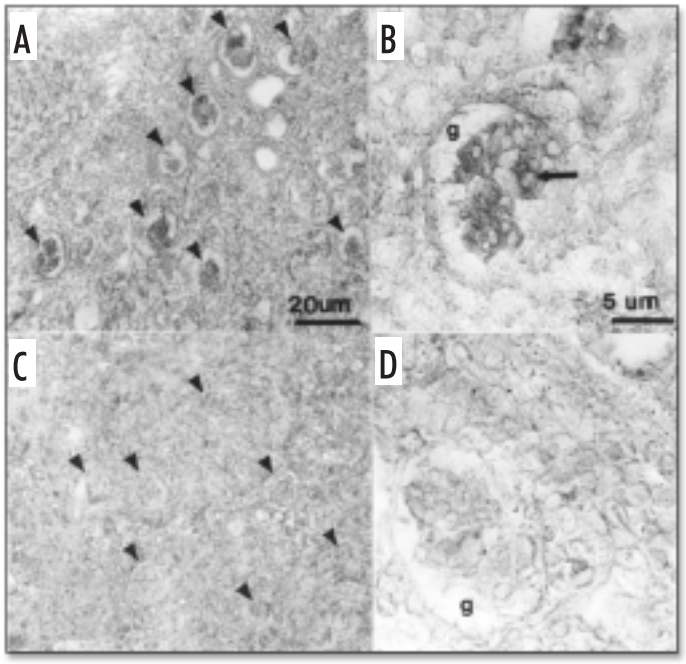

Figure 5. Photomicrographs of sections of rat metanephroi 2 weeks post-transplantation into a mouse omentum (A and B) or a rat omentum (C and D) stained using anti-mouse CD31. Arrowheads show (A) positively staining and (C) negatively staining glomeruli. Arrow shows positively staining glomerular capillary loops (B). Glomeruli (g) are labeled. Magnifications shown in A (A and C) and (B) (B and D) Reproduced with permission.[23]

AVAILABILITY OF SOURCE MATERIAL FOR METANEPHROS TRANSPLANTS

In the case of human renal allotransplantation, there is an unavoidable delay between the time of harvest from donors and the time of implantation into recipients. Prior to removal from the donor, human renal allografts are flushed with a preservation solution, and stored subsequently in ice-cold preservation solution.[22]

Theoretically, metanephroi could be harvested immediately prior to implantation into humans. However, practically it would be best if metanephroi could be stored in vitro for a period of time prior to transplantation. The ability to store metanephroi would permit their distribution to sites for transplantation, distant from the site of harvesting and would allow time to plan the transplant procedure.

To determine whether metanephroi can be stored in vitro prior to transplantation, we transplanted metanephroi from E15 rat embryos into the omentum of non-immunosuppressed uni-nephrectomized (host) rats either directly or suspended in ice-cold University of Wisconsin (UW) preservation solution for 3 days prior to implantation. The size and extent of tissue differentiation pre-implantation of E15 metanephroi implanted directly is not distinguishable from the size and differentiation of metanephroi preserved for 3 days.

By 4 weeks post transplantation, metanephroi that had been preserved for 3 days had grown and differentiated such that glomeruli, proximal and distal tubules, and collecting ducts with normal structure had developed. At 12 weeks post-transplantation, inulin clearances of preserved metanephroi are comparable to those of metanephroi that had been implanted directly, consistent with the viability of preserved metanephroi.[22]

Rat to Mouse Xenotransplantation. We transplanted metanephroi from an E15 Lewis rat embryo across a concordant xenogeneic

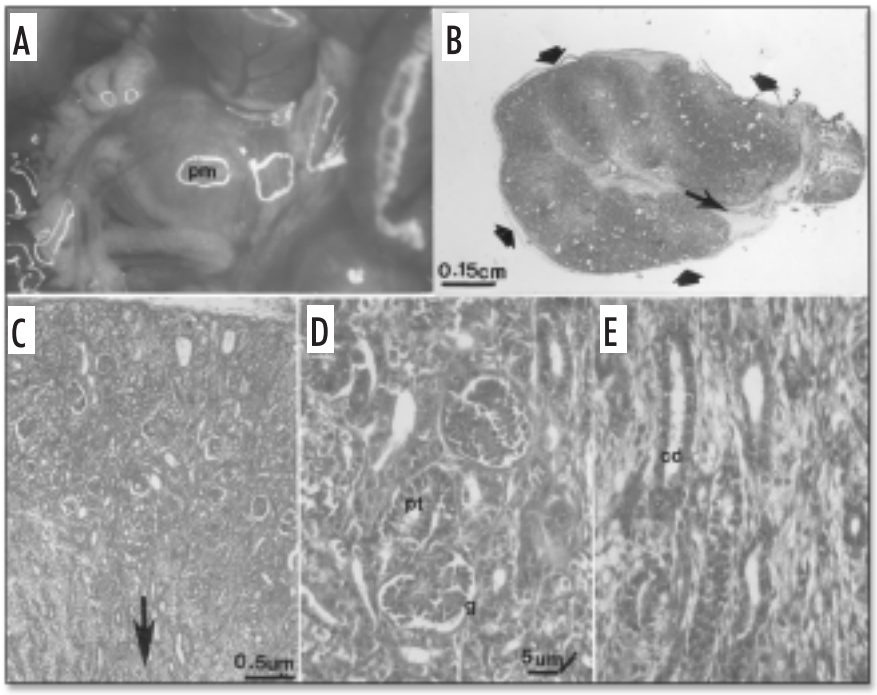

Figure 6. (A) A pig metanephros (pm) in the rat omentum 5 weeks post-transplantation; (B) An H&E-stained section of the pig metanephros shown in (A) with lobules (short arrows) and ureter direction (long arrow) delineated; (C) A H&E stained section of the cortex (arrow delineates the direction of the medulla); (D) A H &E stained section of the cortex with a glomerulus (g) and proximal tubule (pt) labeled; and (E) A H&E-stained section of the medulla with collecting ducts (cd) labeled. Magnifications are shown for (A and B) (B), (C) and (D and E) (D). Reproduced with permission.[24]

barrier into the peritoneum of 10 week-old C57Bl/6J mice. Two weeks later, either no trace of the metanephros could be found in mice, or a yellowish piece of tissue, too small to embed, was observed in the omentum. Failure to identify transplanted tissue post-implantation could reflect failure to engraft or tissue rejection. In contrast, in mice that receive co-stimulatory blocking agents, the transplanted rat metanephros undergoes differentiation and growth in situ.[23]

To gain insight into the origin of the vasculature (donor vs. host) of metanephroi transplanted in the omentum, using our rat to mouse model, we stained developing rat metanephroi using mouse specific antibodies directed against the endothelial antigen CD31.[23]

Shown in Figure 5 are photomicrographs of a paraffin-embedded section containing developed glomeruli (g) in a Lewis rat metanephros following 2 weeks of transplantation into a C57Bl/6J mouse (Fig. 5A and B) or a Lewis rat (Fig. 5C and D), stained using anti-mouse CD31. Positively staining glomeruli (rat to mouse) are shown in Figure 5A. Negatively-stained glomeruli (rat to rat) are shown in Figure 5C (arrowheads). The vasculature of the transplanted developed rat kidney transplanted into the mouse is largely of mouse origin including glomerular capillary loops (Fig. 5B). In contrast, glomerular capillary loops in rat metanephroi transplanted into rats to not stain for mouse CD31 (Fig. 5D).

Pig to Rodent Xenotransplantation. Using a highly disparate model (pig to rodent) we have transplanted E28 Yorkshire pig metanephroi, consisting only of branched ureteric bud and undifferentiated metanephric blastema[21] into the peritoneum of Lewis rats[24,25] or C57Bl/6J mice.[21]

Five weeks post-transplantation, no trace of the metanephroi transplanted as described[21,24,25] could be found in hosts that received no co-stimulatory blockers. Some rat hosts were treated to induce tolerance with a regimen consisting of anti-CD4; anti CD11a; antiTCR-2b; anti CD28; and CRH.[24] Figure 6A illustrates a pig metanephros (pm) in the rat omentum 5 weeks post-transplantation. A H&E-stained section of the pig metanephros is shown in Figure 6B with lobules (short arrows) and ureter direction (long arrow) delineated. Figure 6C illustrates a H&E stained section of the cortex, and Figure 6D a H&E stained section of the cortex with a glomerulus (g) and proximal tubule (pt) labeled. Figure 6E shows H&E-stained section of the medulla with collecting ducts (cd) delineated. The developed pig metanephros is slightly larger in volume (diameter and weight) than a normal rat kidney.

Dekel et al. have successfully transplanted metanephroi originating from pig embryos of ages ranging from E20–21 to E27–28 beneath the renal capsule of immunodeficient mice. They found that most transplants from the E20–21 donors fail to develop or evolve into growths containing few glomeruli and tubules, but other differentiated derivatives such as blood vessels, cartilage and bone. Furthermore, metanephroi from E24–25 donors also contained non-renal cell types and disorganized cell clusters. In contrast, the transplants originating from E27–28 pig embryos all exhibited significant growth and full differentiation into mature glomeruli and tubules.[15]

In addition, Dekel et al. transplanted adult pig kidney tissue or E27–28 pig metanephroi beneath the renal capsule or onto the testicular fat of immunocompetent Balb/c mice. Some hosts were treated with CTLA4-Ig. Evaluation of adult or E27–28 embryonic tissues 2 weeks post-implantation into non CTLA4-Ig-treated hosts showed rejection of tissues. In CTLA4-Ig-treated hosts, most E27–28 metanephroi underwent growth and differentiation. In contrast, all adult kidney grafts had a disturbed morphology, necrotic tissue and a high degree of lymphocyte infiltration. The authors interpreted these data as being consistent with an immune advantage of the developing precursor transplants over developed adult kidney transplants in fully immunocompetent hosts.[15]

DEVELOPMENT OF THE PANCREAS

The pancreas is derived from two separate primordia (the dorsal and ventral pancreas).[26] In the rat that has a 21 day gestation, the primordia arise from the duodenum during embryonic day 11 (E11) of development (dorsal pancreas) and from the endoderm of the hepatic diverticulum E12.1 (ventral pancreas), and later fuse (E13). The head of the pancreas develops from the ventral anlage and the tail from the dorsal anlage. The glandular (exocrine) tissue of the pancreas is formed by the budding and re-budding of cords of cells derived from this primordial mass. Islets are identifiable on ~E15. The terminal parts of the cords gradually take on the characteristics of pancreatic acini while their more proximal portion forms the duct system. Initially intermingled with other pancreatic cells in the ducts, islet cell precursors migrate from the duct system into the surrounding

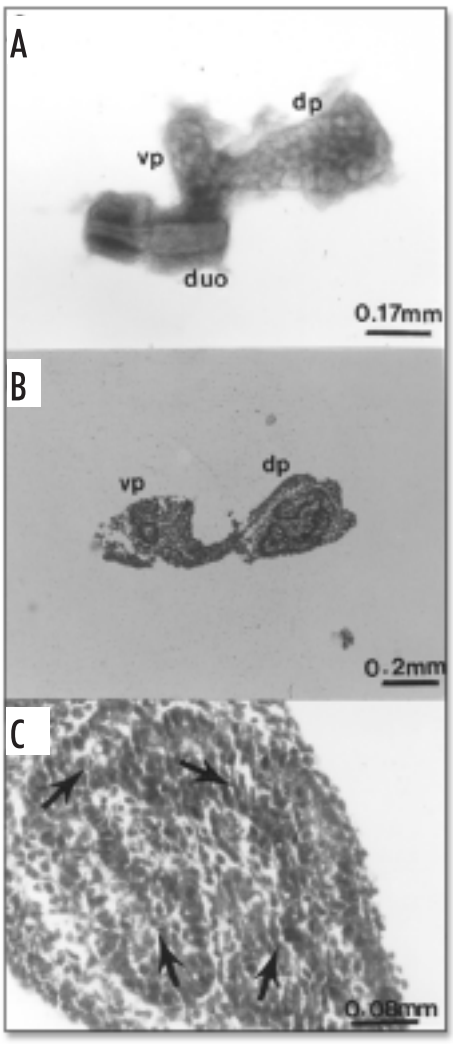

Figure 7. (A) Photograph and (B and C)photomicrographs pancreatic anlagen freshly dissected from E12.5 rat embryos. (A) A section of duodenum (duo) from an E12.5 Lewis rat embryo. The dorsal pancreas anlage (dp) and ventral pancreas anlage (vp) are labeled; (B) A H&E stained section of the dp and vp with the duodenum removed; (C) high-power view of the dorsal pancreas shown in (A and B). Arrows delineate a condensing cord of tubulo-acinar cells. Magnifications are shown. Reproduced with permission.[35]

mesenchyme by breaking down the extracellular matrix. After migrating into the mesenchyme the endocrine cells coalesce into islets[26] known to be polyclonal in origin.[27]

In pigs (~120 day gestation), the dorsal and ventral anlagen are formed on E20-29. The pig pancreas develops much more slowly than the rat pancreas. Cells moderately immunoreactive for insulin can be detected at ~4 weeks of gestation. At 10 weeks of gestation, insulin-staining cells appear in the duodenal portion of the pancreas derived from the ventral bud. Beginning at 13 weeks of gestation, cells that are intensely immunoreactive for insulin are distributed throughout the parenchyma. However, it is not until ~10–13 days after birth, that the cells cluster together in small islets.[28]

ORGANOGENESIS OF THE ENDOCRINE PANCREAS

Both digested and cultured and whole fetal pancreas from early and late gestation embryos or neonates has been used for isotransplantation into sub renal-capsular, intra-peritoneal and other sites.[29-38] No matter whether dispersed or intact developing pancreas is transplanted, there is a selective survival of the endocrine portion (islets) of pancreatic anlagen grafts. In studies that compared directly, host beta cell mass post-transplantation of whole versus digested pancreas, it was found that mass was larger if whole pancreas were transplanted.[31]

In studies that compared directly, the results of transplantation of tissue obtained from embryos of different ages and from neonates, it was found that expansion of transplanted beta cell mass was higher for tissue originating from mid-term embryos than from late-term embryos or neonates.[30] Unfortunately, in studies using comparable techniques and whole pancreas[29] it was found that use of tissue taken from embryos earlier than ~4 days post-formation of the fetal pancreas was limited by the failure of such tissue to engraft.

Experimental diabetes in rodent hosts has been treated successfully using embryonic pancreas transplants.[29,30] It was proposed that intra-peritoneal transplantation is advantageous relative to sub renal-capsular transplantation for diabetes control in that the former: (1) involves more limited surgery; (2) provides a large surface area for implantation; and (3) recapitulates an orthotopic site physiologically, in that secreted insulin enters the portal system (via the superior mesenteric vein) rather than the systemic venous system (via the renal vein).[29] Brown et al. showed that a partial reversal of streptozotocin-diabetes in rats into which fetal pancreases were isotransplanted beneath the renal capsule, was rendered complete following shunting of the venous drainage from the transplants to the liver.[32] Levels of circulating insulin in transplanted rats fell following imposition of the shunt as a result of increased extraction of insulin passing into the liver as well as diminished secretion by the transplanted anlagen. Unfortunately, the advantages of the intra-peritoneal site were rendered of limited utility by the finding that whole anlagen transplanted intra-peritoneally, failed to vascularize.[29]

In studies that compared directly the fates of E17-18 pancreatic anlagen isografts and allografts, allotransplanted recipients showed only a transient recovery from the alloxan diabetes (3–13 days) followed by a return to the diabetic state at the time of graft rejection.[30]

We have transplanted pancreatic anlagen from embryos into the peritoneal membranes of rodents using techniques developed for the transplantation of metanephroi.[35,36]

Rat to Rat Isotransplantation. Shown in Figure 7A is a photograph of a section of duodenum (duo) from an E12.5 Lewis rat embryo. The dorsal pancreas (dp) and ventral pancreas (vp) are labeled. Shown in Figure 7B is a H&E-stained section the dorsal and ventral pancreatic anlage with the duodenum removed. A condensing cord of tubulo-acinar cells (arrows) is discernable in the dorsal anlage (Fig. 7C). However, the tissue is otherwise undifferentiated.

By two weeks post-transplantation of whole pancreatic anlagen into the omentum of a Lewis rat, the tissue has undergone differentiation and insulin-positive islets of Langerhans can be delineated.[35]

Figure 8 illustrates a pancreas anlage 15 weeks post-transplantation.[37] Shown in Figure 8A is an H&E-stained section and in Figure 8B, a control serum stained section. The corresponding anti-insulin antibody stained-section is shown in Figure 8C. Figure 8D shows an enlarged islet of Langerhans, stained with anti-insulin antibodies. Arrows delineate islets. The 'organ' that differentiates post-transplantation of pancreatic anlagen is a novel one, consisting of islet tissue within stroma surrounded by peritoneal fat.

Electron microscopy was performed to delineate whether beta cells contained insulin granules. Figure 9A shows a cell within an islet. Its cytoplasm is packed with neurosecretory granules containing

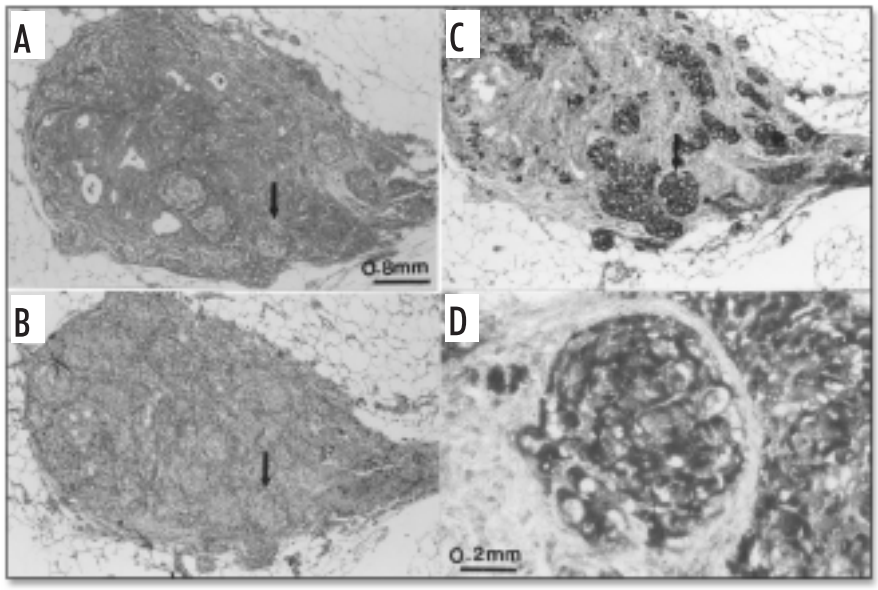

Figure 8. Photomicrographs of adjacent sections of pancreatic anlagen obtained 15 weeks post-implantation into the peritoneum of a host rat: (A) A H & E-stained section; (B) A control antibody-stained section; (C and D) Anti-insulin antibody-stained sections; (D) An islet of Langerhans. Islets are delineated in (A–C) (arrows). Magnification is shown for (A–C) (A), and for (D). Reproduced with permission.[37]

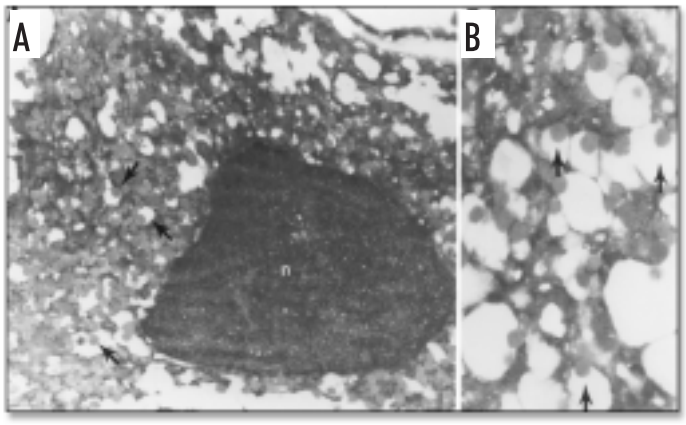

Figure 9. Electron micrographs of beta cell in a pancreatic anlagen obtained 15 weeks post-implantation into the peritoneum of a host rat. (A) 15,000X; (B) 25,000X. Eccentric dense bodies within cytoplasmic vacuoles are delineated (arrows). n, nucleus. Reproduced with permission.[35]

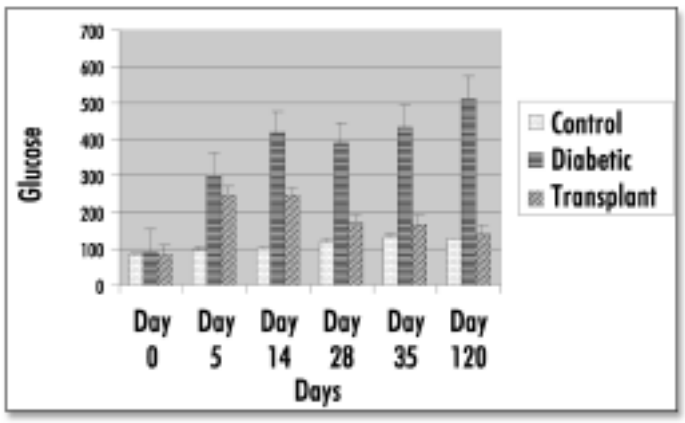

Figure 10 (Left). Levels of glucose (mg/dl) measured over time in control rats (Control) , rats rendered Diabetic using streptozotocin (Diabetic) and streptozotocin-diabetic rats into which 10 pancreatic anlagen were transplanted after glucose measurements were made on day 5 (Transplant). Data are expressed as mean ± SEM. Reproduced with permission.[35]

eccentric dense cores which represent crystallized insulin (arrows). A higher magnification of the vacuoles is shown in Figure 9B.[35]

After measurement of baseline blood glucose levels (day 0), streptozotocin-diabetes was induced in Lewis rats. A control group received vehicle instead of streptozotocin. On day 5 post-streptozotocin or vehicle, levels of blood glucose were measured. In some streptozotocin diabetic rats, 10 pancreatic anlagen were transplanted into the peritoneum (transplant). Other rats underwent sham-surgery (diabetic). Glucose levels were measured again at 8 AM on days 14, 28 and 35-post administration of vehicle or streptozotocin.

As shown in Figure 10, levels of glucose were elevated in diabetic rats compared to controls on days 5, 14, 28, and 35. In contrast, by day 35, glucose levels were not different between controls and transplanted (previously hyperglycemic) rats. Normoglycemia has persisted at 4 months (120 days).

Glucose tolerance tests were performed on a second group of control rats, diabetic rats, and transplanted (previously hyperglycemic) rats, at 18 weeks post-transplantation of 10 pancreatic anlagen into the transplanted group. The k values for the rate of glucose disappearance (%/min) in control rats and in diabetic rats were 2.92 ± 0.44 and 0.48 ± 0.26 respectively.[35] K values for transplanted rats were 2.82 ± 0.46, not significantly different from controls and increased significantly relative to diabetic animals.[35]

A number of growth factors participate in the processes of islet differentiation and growth.[10] To determine whether induction of normoglycemia in diabetic hosts can be accelerated if pancreatic anlagen are incubated with two of them prior to implantation, we added vascular endothelial growth factor (VEGF) and hepatocyte growth factor (HGF) in the media in which anlagen are stored prior to placement into hosts.[36] Rats in transplant, diabetic and control groups had levels of glucose measured during the next 40 weeks.

In contrast, to the case for the transplant group receiving non-growth factor incubated tissue, in which it took ~30 days for levels of glucose to normalize (Fig. 10), levels of glucose in the transplant group, elevated significantly relative to the control group at 1 week post-implantation, were no longer elevated by 2 weeks post-transplantation in rats that received VEGF and HGF-treated tissue. Levels of glucose in transplants and controls remained indistinguishable for 40 weeks. In contrast, the levels of glucose in the diabetic group were elevated significantly relative to controls at all times at which measurements were made and glucose tolerance remained abnormal.[36]

The rate of weight gain over time is reduced in diabetic relative to non-diabetic rats. An early response to successful transplantation of rat pancreatic anlagen into diabetic rats is a restoration of the non-diabetic pattern of weight gain.[38] Normalization of glucose levels in the transplant group occurred in the context of a normalization of rat weight.[36]

As was the case for non-growth factor treated anlagen,[35] the VEGF and HGF-treated tissue differentiated into structures consisting of islets of Langerhans surrounded by stroma with no exocrine tissue present. Both insulin and glucagon could be detected in islet cells.[36]

Rat to Mouse Xenotransplantation. E 12.5 Lewis rat pancreatic anlagen were transplanted into the omentum of C57Bl/6J mice. Some host mice were treated with co-stimulatory blocking agents. Other mice were treated with injections of vehicle.[35]

In mice that received vehicle, no development of anlagen was observed at 4 weeks post-transplantation (Fig. 11A). In contrast, Figures 11B-E show a developed E12.5 Lewis rat pancreas anlagen 4 weeks post-transplantation into the omentum of an adult C57Bl/6J mouse that received co-stimulatory blockade. Figure 11B shows an H&E-stained section, Figure 11C a control serum-stained section, Figure 11D an anti-insulin antibody-stained section, and Figure 11E a section stained using the combined Gomori method. Islets are delineated (arrows).

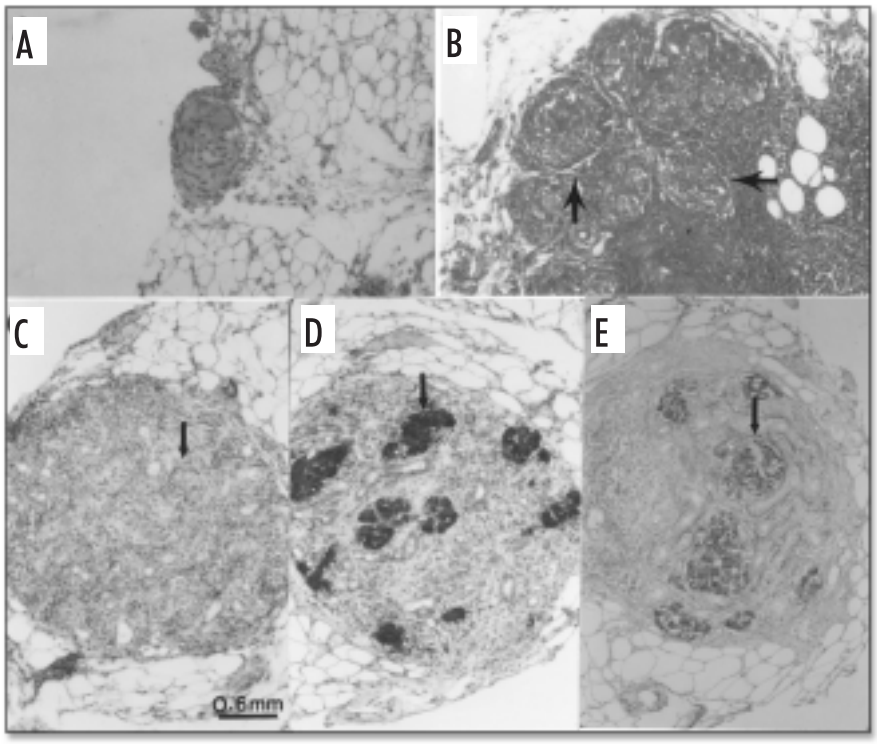

Figure 11. Photomicrographs of adjacent sections of rat pancreatic anlagen obtained 4 weeks post-implantation into the peritoneum of host mice that received no co-stimulatory blockade (A) or were treated with co-stimulatory blocking agents (B–E): (A and B) H& E-stained sections; (C) A control-stained section; (D) An anti-insulin antibody-stained section; (E) A combined Gomori-stained section. Islets are delineated (arrows). Magnification is shown (C). Reproduced with permission.[35]

Pig to Rat Xenotransplantation. To establish the feasibility of pig to rodent xenotransplantation of pancreatic anlagen, we implanted 5 VEGF and HGF-treated pancreatic anlagen from E29 pig embryos into adult Lewis rats. Remarkably, unlike the case for rat-to-mouse pancreatic anlagen transplantation,[35] pig pancreatic anlagen engrafted post-implantation into non-immunosuppressed immunocompetent rat hosts.[36]

As was the case for experiments in which rat anlagen were transplanted into rats, levels of glucose in the transplant group, elevated significantly relative to the control group at 1 week post-implantation, were no longer elevated at 2 weeks post-transplantation or at later times (3–20 weeks) in rats that received pig pancreas implants (Fig. 12). Weight gain and glucose tolerance were normalized[36] as for rat to rat transplants. In contrast, levels of glucose in the diabetic group were elevated significantly relative to controls at all times at which measurements were made and glucose tolerance remained abnormal.[36]

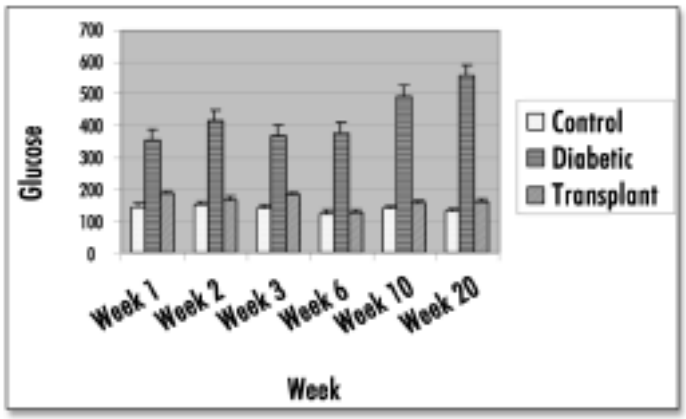

Figure 12. Levels of glucose (mg/dl) measured over time, in each of 5 control rats (Control) , rats rendered diabetic using streptozotocin and sham-transplanted (Diabetic) and streptozotocin-diabetic rats into which 5 VEGF and HGF-treated pig pancreatic anlagen were transplanted (Transplant). Data are expressed as mean ± SEM. Reproduced with permission.[35]

Figure 13A illustrates a section of a duodenum (duo) obtained from an E29 domestic pig embryo. The dorsal pancreas (dp) and ventral pancreas (vp) are labeled. An anti-insulin antibody-stained section of another E29 pig pancreas shows ducts that contain some insulin-positive cells (arrows) surrounded by undifferentiated cells (Fig. 13B). Figure 13C depicts a section of rat omentum, 3 weeks post transplantation of pig pancreatic anlagen. The site of an implant is delineated (arrow). Figure 13D shows an H&E-stained section of rat omentum obtained 3 weeks post-transplantation of 5 pig pancreatic anlagen. Scattered bits of tissue are found amidst omental fat. Figures 13E and 13F show higher power anti-insulin antibody and contrrol stained sections of omentum respectively. Positive staining for insulin is observed in Figure 13E. Unlike the case post-transplantation of rat pancreas anlagen,[35,36] no defined islets can be identified at this time in the implanted embryonic pig pancreas tissue.

Using RT-PCR that distinguishes rat from pig insulin RNA, we determined that tissue containing pig insulin mRNA (presumably porcine pancreatic tissue) had engrafted in the omentum of rat hosts, but not elsewhere. Using an ELISA that distinguishes rat from pig insulin, we showed that pig insulin, but not rat insulin was present in the omentum of transplanted rats.[36]

Figure 14 shows tissues from rats into which pancreatic anlagen had been transplanted 20 weeks previously. Relative to an age-matched sham-transplanted diabetic control (Fig. 14A) the omentum in transplanted rats contains an increased quantity of fat (Fig. 14B),

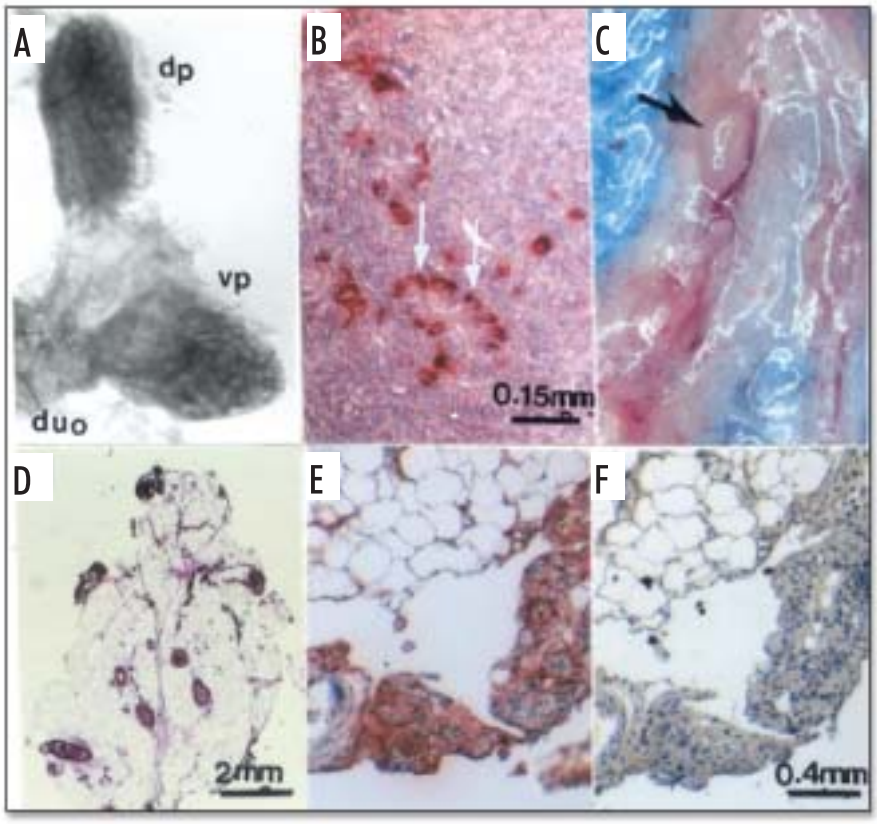

Figure 13. (A) Photographs and (B) photomicrograph of pancreatic anlagen freshly dissected from E 29 pig embryos and photograph (C) and photomicrographs (D–F) of pig pancreatic anlagen 3 weeks post-implantation in the omentum of a diabetic rat: (A) A section of duodenum (duo) from an E29 Lewis rat embryo. The dorsal pancreas anlage (dp) and ventral pancreas anlage (vp) are labeled; (B) An anti-insulin antibody-stained section of the pancreas. Arrows delineate insulin positive cells in ducts; (C) Rat omentum. The site of a pancreas implant is delineated (arrow). (D) An H and E-stained section of the omentum from a transplanted rat; (E) anti-insulin antibody stained section of transplanted rat omentum; (F) control antibody stained section of transplanted rat omentum. Magnifications are shown in A (for A and B), (D) for (C and D) and F (for E and F). Reproduced with permission.[36]

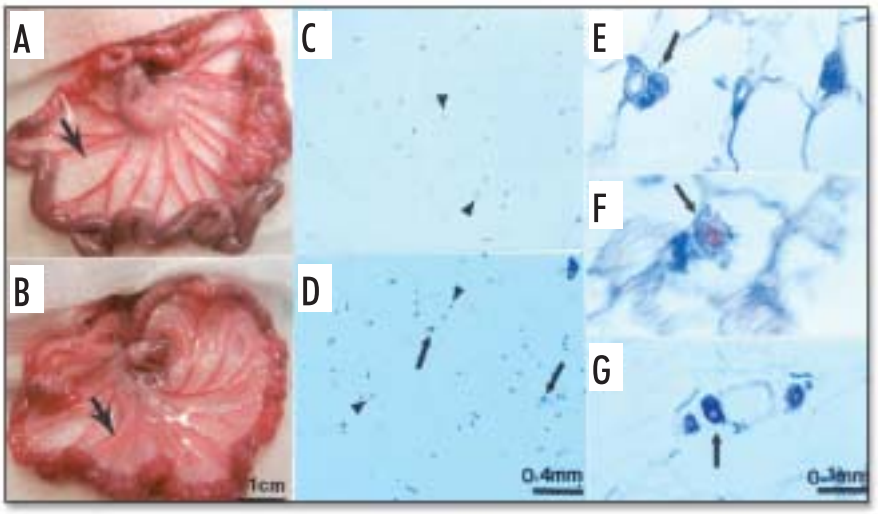

Figure 14. (A and B) Photographs and (C–G) photomicrographs of omentum from diabetic rats into which into which: (A) sham transplantation was performed 20 weeks previously; (B and D–G), 5 pig pancreatic anlagen had been transplanted 20 weeks previously (B and D–G); or (C) into which 10 rat pancreatic anlagen had been transplanted 20 weeks previously. (A) Omentum from a rat that underwent sham transplantation; (B) Omentum from a rat into which 5 pig pancreatic anlagen had been transplanted 20 weeks previously; arrows delineate fat; (c) H&E-stained section of omentum from a Lewis rat into which 10 Lewis rat pancreatic anlagen had been transplanted 20 weeks previously (D) H&E-stained section of omentum from a rat into which 5 pig pancreatic anlagen had been transplanted 20 weeks previously. Arrowheads delineate flattened nuclei, arrows, rounded nuclei; (E–G) Sections of omentum from a Lewis rat into which 5 pig pancreatic anlagen had been transplanted 20 weeks previously: (E) A control antibody-stained section; (F) An anti-insulin antibody-stained section; (G) A combined Gomori-stained section. Arrows delineate cells. Magnification is shown in B (for A and B), D (for C and D) and in G (for E–G). Reproduced with permission.[36]

consistent with the findings of others who transplanted pancreatic anlagen to non-omental sites.[33,34] Omentum from a rat into which a pancreatic anlagen from an E12.5 Lewis rat embryo had been implanted 20 weeks previously contains adipocytes with characteristic flattened nuclei (Fig. 14C), as does omentum from a Lewis rat into which a pancreatic anlage from an E29 pig embryo had been implanted 20 weeks previously (arrowheads, Fig. 14D). However, omentum from the rat into which the embryonic pig pancreas had been transplanted is more cellular than that into which the rat anlagen had been transplanted and also contains cells in clusters with larger rounded nuclei (arrows, Fig. 14D). These cells have the morphology of engrafted transplanted beta cells (-polygonal with large nucleus and granular cytoplasm), do not stain with control antibody (arrow, Fig. 14E), are insulin positive (arrow, Fig. 14F), and contain Gomori positive granules (arrow, Fig. 14G) characteristic of beta cells. Some of these cells are also glucagon-positive.[36] The presence of individual islet cells in the rat omentum post-xenotransplantation of pancreatic primordial from pig (Fig. 14E–G) differs from what is observed (whole islets within stroma) following rat to rat allotransplantation (Fig. 8) or rat to mouse xenotransplantation (Fig. 11). While we do not know for certain why this is the case, it may reflect the inability of individual pig islet cells to coalesce into islets[26] in the setting of a rat extracellular matrix.

PERSPECTIVES AND CHALLENGES

The applicability of human organ transplantation to treat end-stage disease is limited by the number of organs available to transplant. The use of porcine organs in lieu of those from human donors could provide a physiologically compatible alternative. Unfortunately pig to primate (human) transplantation of vascularized organs such as the kidney presents formidable and thus far unsolved immunological challenges.[1-3]

In contrast to xenotransplantation of whole organs, cell and tissue grafts from pig to primate might be feasible for replacement of organ function, since they are less susceptible to hyperacute and acute vascular rejection.[1-3] From this immunological perspective, cell/tissue transplantation (pig to primate) is possible for a number of organs. For example, porcine cellular transplants such as pancreatic islets, can be transplanted into humans without triggering hyperacute or acute vascular rejection.[8] In addition, pig neural cells are not subject to hyperacute or acute vascular rejection post-transplantation into humans.[39]

As delineated by Cascalho and Platt,[2] cellular transplantation has limitations. First, it is difficult to imagine how functions of structurally complex organs can be enhanced or recapitulated by cells alone. An example of such a function is glomerular ultrafiltration in kidneys. Second, cellular transplantation might prove ineffective in diffuse diseases. As an example, transplanted myocytes would not be expected to restore contractile function in the setting of dilated cardiomyopathy. Finally, since differentiated cells and stem cells from mature individuals have a limited proliferative potential, such cellular grafts may not be capable of repairing or replacing large masses of defective tissue. In the case of pancreatic islets, limited ability for beta cells to replicate post-implantation is reflected by a declining pool of functioning endocrine tissue over time.[9]

One possible approach to replacing complex organ functions and overcoming the limited potential for growth or division of mature cells is through organogenesis or the growing of new organs post-transplantation of primitive tissues, embryonic primordia or stem cells.[2] As reviewed above, organogenesis can be accomplished for kidney and for the endocrine pancreas via transplantation of renal or pancreatic anlagen. Organogenesis of other organs has been accomplished. For example, fragments of fetal liver obtained from E18–19 rat embryos or E90 pig embryos differentiate into hepatocytes and prolong survival post-transplantation into rats poisoned with D-galactosamine that induces lethal acute hepatic failure,[40] and functional tissue-engineered intestine can be grown in situ by transplanting 'organoid units' (mesenchymal cell cores of large or small bowel surrounded by polarized epithelia) onto a polymer scaffold in the omentum of recipient rats.[41]

The delineation of successful strategies for organogenesis of the kidney, endocrine pancreas, liver, intestine or other organs will take time. To understand that this is the case, one need only contemplate the chronology of events that led to the implementation of an organ replacement strategy in wide-use today, hemodialysis.[42] The first successful dialysis of animals was carried out in 1903, and the first human dialysis was performed in 1926. During the ensuing decades, a number of different experimental approaches to hemodialysis of humans were employed. Yet, it was not until the mid 1950s that the first commercially available dialysis apparatus was marketed and not until the 1970s that hemodialysis was widely implemented.[42]

Reflecting upon the chronology delineated above, any accounting for the progress to date in renal organogenesis must consider that we are one hundred years into hemodialysis, but only one or two decades into growing new kidneys.

Knowledge derived from a multiplicity of fields must be applied to organogenesis to render it clinically applicable. In the case of hemodialysis, the development of artificial membranes and anticoagulants were prerequisite for its routine implementation.[42] Similarly, technologies from both within and without the wide and expanding base of knowledge that has rendered possible our current therapies for organ failure will be required for advances in growing new organs. Today, many of these technologies are at the cutting edge of biomedicine. No doubt, during the coming decades the use of several will move into mainstream clinical practice.

References

1. Samstein B, Platt J. Physiologic and immunologic hurdles to xenotransplantation. J Am Soc Nephrol 2001; 12:182-93.
2. Cascalho M, Platt JL. Xenotransplantation and other means of organ replacement. Nat Rev Immunol; 2001 1:154-60.
3. Edge ASB, Gosse ME, Dinsmore J. Xenogeneic cell therapy: current progress and future developments in porcine cell transplantation. Cell Transplant 1998; 7 525-39.
4. Soin B, Smith KG, Zaidi A, Cozzi E, Bradley JR, Ostlie DJ et. al. Physiological aspects of pig-to-primate renal xenotransplantation. Kidney Int 2001, 60:1592-7.
5. Hammerman MR. Tissue engineering the kidney. Kidney International 2003; 63:1195-204.
6. Shapiro JAM, Lakey JRT, Ryan EA, Korbutt GS, Toth E, Warnock GL et al. Islet transplantation in seven patients with type 1 diabetes mellitus using a glucocorticoid-free immunosuppressive regimen. New Engl J Med 2000; 343:230-8.
7. Soria B, Skoudy A, Martin F. From stem cells to beta cells : new strategies in cell therapy of diabetes mellitus. Diabetologia 2001; 44:407-24.
8. Groth CG, Korsgren O, Tibell A, Tollemar J, Moller E, Bolinder J, et al. Transplantation of porcine fetal pancreas to diabetic patients. Lancet North Am Ed 1994; 344:1402-4.
9. Robertson RP. Pancreatic islet transplantation for diabetes: Successes, limitations and challenges for the future. Mol Genet Metab 2001; 74:200-5.
10. Bonner-Weir S. β-Cell turnover: Its assessment and implications. Diabetes 2001; 50:S20-4.
11. Horster MF, Braun GS, Huber SM. Renal embryonic epithelial induction nephrogenesis and cell differentiation. Physiol Rev 1999; 70:1157-91.
12. Netter FH. Anatomy structure and embryology In : Becker EL Churg J eds. The Netter Collection of Medical Illustrations, Volume 6, Kidneys Ureter and Bladder, Pittsburgh PA. Novartis 1997: 2-35.
13. Wolf AS, Palmer SJ, Snow ML, Fine LG. Creation of a functioning mammalian chimeric kidney. Kidney Int 1990; 38:991-7.
14. Rogers SA, Lowell JA, Hammerman NA, Hammerman MR. Transplantation of developing metanephroi into adult rats. Kidney Int 1998; 54:27-37.
15. Dekel B, Burakova T, Arditti FD, Reich-Zeliger S, Milstein O, Aviel-Ronen S, et al. Human and porcine early kidney precursors as a new source for transplantation. Nat Med 2003; 9:53-60.
16. Rogers SA, Liapis H, Hammerman MR Transplantation of metanephroi across the major histocompatibility complex in rats. Am J Physiol 2001; 280:R132-6.
17. Hammerman, MR Transplantation of developing kidneys. Transplant Rev 2002; 16:62-71.
18. Hammerman MR Implantation of renal rudiments In: Polak J, Hench L, Kemp P eds Future Strategies for Organ Replacement. Imperial College Press London UK 2002:199-211.
19. Miller SB, Hansen VA, Hammerman MR. Effects of growth hormone and IGF I on renal function in rats with normal and reduced renal mass. Am J Physiol 1990; 259:F747-51.
20. Rogers SA, Hammerman MR. Prolongation of life in anephric rats following de novo renal organogenesis Organogenesis 2004; In Press.
21. Rogers SA, Talcott M, Hammerman MR Transplantation of pig metanephroi. ASAIO J. 2003; 49:48-52.
22. Rogers SA, Hammerman MR. Transplantation of metanephroi after preservation in vitro. Am J Physiol 2001; 281:R661-5.
23. Rogers SA, Hammerman MR. Transplantation of rat metanephroi into mice. Am J Physiol 2001; 280:R1865-9.
24. Hammerman MR. Renal Organogenesis from Transplanted Metanephric Primordia. J Am Soc Nephrol 2004; 15:1126-32.
25. Hammerman MR. Xenotransplantation of developing kidneys. Am J Physiol 2002; 283:F601-6.
26. Johansson KA, and Grappin-Botton A. Development and diseases of the pancreas. Clin Genet 2002; 62:14-23.
27. Deltour L, Leduque P, Paldi A, Ripoche MA, Dubois P and Jami J. Polyclonal origin of islets in aggregation rat chimeras. Development 1991; 112:115-21.
28. Alumets J, Hakanson R, and Sundler F. Ontogeny of endocrine cells in porcine gut and pancreas. Gastroenterology 1983; 85:1359-72.
29. Brown J, Danilovs JA, Clark WR, and Mullen YS. Fetal pancreas as a donor organ. World J Surg 1984; 8:152-7.
30. Hegre OD, Leonard RJ, Erlandsen SL, McEvoy RC, Persons JA, Elde RP, et al. Transplantation of the islet tissue in the rat. Acta Endocrinologia 1976; 205:257-78.
31. Hegre OD, Schmidt RV, McEvoy RC. Syngeneic transplantation of the fetal rat pancreas IV. Dissociated versus whole organ implantation. Metabolism 1979; 28:157-62.
32. Brown J, MullenYS, Clark WR, Molnar G, and Heinsinger, D. Importance of hepatic portal circulation for insulin action in streptozotocin-diabetic rats transplanted with fetal pancreases. J Clin Invest 1979; 64:1688-94.
33. Du Toit D, Muller C, Page B, Louw J. Foetal rat pancreatic transplantation: Posttransplantation development of foetal pancreatic iso- and allografts and suppression of rejection with Mycophenolate Mofetil (MMF) and Cyclosporine-based immunesuppression. Microsc Res Tech 1998; 43:347-55.
34. Adams GA, Squires EC, Maestri M, Lee L, Alfrey EJ, Dafoe DC. Regimens of IGF-1 treatment in fetal pancreas transplantation. J Surg Res 1997; 68:73-8.

35. Rogers SA, Liapis H, Hammerman MR. Intraperitoneal transplantation of pancreatic anlagen ASAIO J 2003; 49:527-32.
36. 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:E502-9.
37. Hammerman MR. Applications of Organ Precursor Cell Therapy: Can lessons from embryonic kidney transplantation be applied to the endocrine pancreas? Curr Opin Nephrol Hypertens 2004; 13:23-9.
38. Hegre OD, Leonard RJ, Schmitt BA, Lazarow A. Isotransplantation of organ-cultured neonatal pancreas: reversal of alloxan diabetes in the rat. Diabetes 1976; 25:180-9.
39. Deacon T, Schumacher J, Dinsmore J, Thomas C, Prather P, Kott S, et al. Histological evidence of fetal pig neural cell survival after transplantation into a patient with Parkinson's disease. Nat Med 1997; 3:350-3.
40. Hagihara M, Shimura T, Takebe K, Munkhbat B, Tsuii K. Effects of iso and xeno fetal liver fragments transplantation on acute and chronic liver failure in rats. Cell Transplant 1994; 3:283-90.
41. Grikscheit TC, Ochoa ER, Ramsanahie A, Alsberg E, Mooney D, Whang EE, et al. Tissue-engineered large intestine resembles native colon with appropriate in vitro physiology and architecture. Ann Surg 2003; 238:35-41.
42. Morrin PA. The development of nephrology. J Postgrad Med 1994; 40:109-17.