Renal Organogenesis from Transplanted Metanephric Primordia

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Abstract. One novel solution to the shortage of human organs available for transplantation envisions growing new organs in situ via xenotransplantation of developing primordia from animal embryos. It has been shown that renal primordia (metanephroi) transplanted into animal hosts undergo organogenesis in situ, become vascularized by blood vessels of host origin, and exhibit excretory function. Metanephroi can be stored for up to 3 d in vitro before 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. This is a review of studies exploring the therapeutic potential for renal organogenesis posttransplantation of kidney primordia.

The applicability of kidney allotransplantation to treat end-stage renal disease is limited by the number of organs available to transplant. Humans and pigs are of comparable size and share a similar renal physiology, and pigs are plentiful and can be bred to be pathogen-free; therefore, it has been proposed that pigs represent an ideal substitute donor. Unfortunately, the transplantation of whole vascularized organs, such as the kidney originating from pigs, into the group of primates that includes humans, the great apes, and old-world monkeys is rendered problematic because of the processes of humoral rejection (hyperacute and acute vascular rejection) that occur across this xenogeneic barrier (1,2,3).

In contrast to xenotransplantation of whole vascularized organs from pig to primates, cell and tissue grafts might be feasible, because they are less susceptible to humoral rejection (4). Cellular transplants, such as pancreatic islets or neurons from embryonic pigs, can be transplanted into humans without triggering hyperacute or acute vascular rejection (4). It has been proposed that metabolic and synthetic liver functions in humans could be augmented by porcine hepatocyte transplantation and that contractile functions of skeletal and cardiac muscle might be enhanced after integration of transplanted porcine myocytes (3,4).

Cellular transplantation has limitations. It is difficult to imagine how functions of structurally complex organs, such as glomerular filtration and reabsorption in kidneys, can be enhanced or recapitulated by cellular transplants. One potential approach to replacing such functions is through organogenesis or the growing of kidneys in situ after transplantation of renal primordia (3).

Renal Organogenesis

Metanephroi are the primordia 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 to 28 of embryonic pig development (E21–28) when outgrowths of the mesonephric ducts, designated ureteric buds, collect intermediate mesoderm (metanephric blastema) about their distal ends. Numerous outgrowths arise from the distal end of the ureteric bud, which push radially into the surrounding mass of metanephric blastema. The metanephric blastema differentiates into all tubular structures of the adult nephron with the exception of the collecting system, which derives from ureteric bud (5).

The major vessels supplying the kidney originate from lateral branches of the abdominal aorta that terminates in a plexus of arteries in close proximity to the renal pelvis, the renal artery rete (6). During its development, the renal anlage attracts the major portion of its vasculature from the developing aorta (7). In that its blood supply originates from outside the developing organ, the kidney is chimeric. Its ability to attract its own vasculature in situ establishes the potential for a transplanted metanephros to attract a vasculature from a suitable vascular bed.

Advantages of Transplanting Metanephroi to Achieve Organogenesis In Situ

The literature provides four reasons why the use of transplantation of metanephroi to achieve organogenesis in situ might be advantageous relative to transplantation of developed kidneys. First, if developing metanephroi are obtained at sufficiently early stage, antigen-presenting cells (APC) that me-
diate direct host recognition of alloantigen or xenoantigen would be expected to be absent from the renal primordia because they would not have yet developed in the donor and migrated into the metanephros (8,9). Second, donor antigens such as MHC class I and II may not be expressed on developing metanephroi to the extent they are expressed in adult kidney (10,11). Third, the immune response to transplanted fetal tissue differs from that to adult tissue in terms of the elicitation of a T helper 2–biased response when that target organ is of fetal origin (12). Fourth, to the extent that the renal vasculature originates from outside the kidney, one would expect a transplanted metanephros to be supplied by blood vessels of host origin (13).

The first studies to address the question of APC depletion in metanephroi were performed by Foglia et al. (9), who transplanted metanephroi obtained from outbred Sprague Dawley rat embryos aged embryonic day (E)15 to E21 beneath the renal capsule of adult Sprague Dawley hosts. Fetal renal allograft growth and survival was age-dependent in that the growth and differentiation in situ over a 15- to 30-d period, was best for metanephroi obtained from E15 embryos and worsened progressively for renal primordia obtained on E16 to E21. The developed E15 metanephroi showed maturation of renal elements and no sign of rejection when examined 10 d posttransplantation, whereas E20 metanephroi had a poor renal architecture and a dense lymphocytic infiltrate after a comparable period of time (9). In contrast to metanephroi obtained on E15, liver tissue harvested on E15 and transplanted beneath the renal capsule of hosts underwent little growth and prompt rejection (9).

Velasco and Hegre (14) transplanted metanephroi or liver tissue from E15, E17, E18, and E19 Fisher rat embryos (RT1 v1) beneath the renal capsule of MHC (RT1) incompatible Wistar Furth adult rats (RT1 b). All fetal hepatic grafts were rejected by 10 d posttransplantation. In contrast, the degree of rejection of the metanephroi was age-dependent, those from E15 embryos showing minimal or moderate rejection and those from older embryos showing more intense rejection. If liver and metanephroi from E15 embryos were co-transplanted at different sites into Wistar Furth rats, metanephroi underwent a more severe rejection than if they were implanted without liver.

APC populate liver well before E15 in rats but are not present in the circulation until several days later (8). It was speculated that the absence of APC in metanephroi obtained from E15 embryos together with their presence in liver tissue obtained concurrently could explain the differential fate of metanephroi transplanted with or without liver. Under the former conditions, but not the latter, direct presentation of donor antigens to host T cells could take place (14).

Statter et al. (11) transplanted renal tissue originating from E14 adult C57Bl/6 mice (H-2 b) beneath the renal capsule of adult congenic B10.A hosts (H-2 b). Expression of donor-and host-specific class I (H2K b) and class II (A β b) transcripts in donor tissue was low at E14 and increased progressively in renal tissue from older mice. After transplantation, surviving kidney grafts showed enhanced expression of class I and class II transcripts; however, neither class I nor class II protein could be detected in transplanted metanephroi, in contrast to its presence in transplanted adult renal tissue (11).

Dekel and co-workers (10,12,15) have carried out a series of investigations in which human adult or embryonic kidney tissue is transplanted beneath the kidney capsule of immunodeficient rats (severe combined immunodeficiency [SCID/Lewis and SCID/nude chimeric rats]). Human adult human kidney fragments transplanted beneath the renal capsule of such rats survive for as long as 2 mo posttransplantation. The overall architecture of the transplanted kidney tissue and the normal structure of individual cells in glomeruli are preserved. The intraperitoneal infusion posttransplantation of allogeneic human peripheral blood mononuclear cells (PMBC) results in rejection of adult human grafts.

Human fetal kidney fragments transplanted beneath the renal capsule of immunodeficient rats display rapid growth and development. Glomeruli and tubular structures are maintained for as long as 4 mo posttransplantation. In contrast to the case for transplanted adult human kidney fragments, infusion of allogeneic human PMBC into host results in either minimal human T cell infiltration or T cell infiltrates that do not result in rejection and do not interfere with the continued growth of the human fetal renal tissue (10,15). Fetal human grafts have reduced expression of tissue HLA class I and II relative to the adult human grafts, consistent with a reduced effectiveness in inducing an alloantigen-primed T cell response (12).

Dekel et al. (12) have shown that transcript levels for interferon-gamma and interleukin-2 (IL-2) in grafts of fetal human kidneys grafted under the renal capsule of immunodeficient rats are markedly reduced posttransplantation relative to levels in adult human kidney tissue grafted to the same site. Peak levels of these cytokines appear late after PMBC infusion. Concomitant with these findings, IL-4 mRNA is upregulated during the early phase post-PMBC infusion, and IL-10 mRNA is expressed throughout the post-PMBC infusion interval. In addition, levels of mRNA coding for chemokines RANTES and MIP1 beta, their receptor, CCR5, and the cytolytic effector molecule, Fas ligand, are suppressed in the fetal grafts relative to levels in adult grafts. Thus, fetal kidney induces the down-regulation of Th1 cytokines, chemokines, and Fas ligand and the sparing of Th2 cytokines in the fetal renal grafts. These findings suggest that the human immune response of kidney rejection is dependent on whether the target organ is of fetal or adult origin. An allogeneic immune system appears to mount a T helper 2–biased response when the target organ is of fetal origin, resulting in enhanced survival of transplanted fetal tissue relative to adult tissue against which a T helper 1–biased response is mounted.

Isotransplantation/Allotransplantation of Embryonic Kidneys to Enhance Renal Function

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 (16,17). However, such incorporation and a consequent enhancement of renal function have never been demonstrated for
isotransplants (16), allotransplants (17), or xenotransplants (15). In addition, space limitation beneath the renal capsule has proven to be an impediment to the growth of transplants (17). Transplantation of metanephroi (outbred rat to outbred rat) can be carried out with no host immunosuppression. This is not possible if fully developed kidneys are transplanted across the same allogeneic barriers (17).

In contrast to the case for rat metanephroi 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 (17). A metanephros in a retroperitoneal dissection from an E15 rat embryo is shown in Figure 1A. The ureteric bud is delineated by an arrowhead. If transplanted with its ureteric bud attached, the metanephros enlarges and becomes kidney-shaped within 3 wk (Figure 1B). The ureteric bud differentiates into a ureter (Figure 1B, arrowhead).

In contrast to transplanted developed kidneys (17), metanephroi transplanted into non-immunosuppressed hosts have a normal kidney structure and ultrastructure postdevelopment in situ and become vascularized via arteries that originate from the superior mesenteric artery of hosts (18). Figures 2a and 2b show hematoxylin and eosin (H&E)–stained sections of an E15 rat metanephros consisting of branched utereric bud (ub) and undifferentiated metanephric blastema (mb). H&E–stained sections of a renal anlage six wk posttransplantation into the omentum of a host rat show developed cortex with a labeled glomerulus (g), proximal tubule (p) with a brush border membrane (arrowhead), and distal tubule (d) (Figure 2c). A developed medulla with a labeled collecting duct (cd) is shown in Figure 2d. An artery (a) supplying the transplanted metanephros is shown in Figure 2e. Electron microscopy of a developed renal anlage (19) reveals normal renal structures (Figure 3). Developed metanephroi transplanted onto the omentum clear inulin infused into the host’s circulation following ureteroureterostomy between transplant and host (Figure 2f), a procedure that can be readily carried out at if metanephroi are implanted in close proximity to the host ureter (17). If incubated with the appropriate growth factor mix before implantation, clearance is enhanced and metanephroi secrete a concentrated urine (UV/GFR < 1) (18).

Using inbred congenic rats (PVG-RT1\(^C\) and PVG-RT1\(^av\)), we have shown that metanephroi can be transplanted across the RT1 locus into non–immune-suppressed hosts. A state of peripheral immune tolerance secondary to T cell ‘ignorance’ permits the survival of transplanted metanephroi. Most likely the ignorance results from the absence of APC originating from the donor in the embryonic renal tissue and the consequent absence of direct presentation of transplant antigen to host T cells (presentation by donor dendritic cells to host T cells) (20), as was shown previously for sub-renal capsular transplants (14).

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

Figure 1. (a) Photograph of retroperitoneal dissection from an embryonic day 15 (E15) rat embryo showing metanephros (m) and ureteric bud (arrowhead). (b) Photograph of a developed metanephros (m) in the omentum of an adult host rat 3 wk posttransplantation. Arrowhead shows developed ureter. Magnification is shown. Reproduced with permission from reference 18.

Figure 2. (a–d) Photomicrographs and (e and f) photographs. (a and b) E15 metanephros. A branched segment of utereric bud (ub) and metanephric blastema (mb) are labeled in panel b. (c and d) H&E–stained section of cortex from a developed metanephros. Glomerulus (g), proximal tubule (p), brush border (arrowhead), and distal tubule (d) are shown. (d) H&E–stained section of medulla from developed metanephros. Collecting duct (cd) is shown. (e and f) An artery originating from the omentum (a) is shown supplying a developed metanephros. (f) Anastomosis is shown (arrow) between host and implanted developed metanephros (m) ureters. Magnifications shown for panel a, b, c and d (d), and for e. Reproduced with permission from references 17 and 22.
branched ureteric bud (arrowheads). The ureteric bud is labeled (ub) (Figure 4A).

Two weeks posttransplantation into the omentum of an adult host (Figure 4B), Yucatan minipig metanephroi are enlarged and have the characteristic lobular (arrows) pig kidney-like structure (21). The ureter is labeled (u). Figure 4C shows 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 4F. A ureteric bud (ub) is delineated in Figure 4E. A proximal tubule lumen is shown in Figure 4F (arrow).

Theoretically, metanephroi could be harvested immediately before implantation into humans; however, it would be more practical if, like most human renal allografts, metanephroi could be stored in vitro for a period of time before transplantation.

To determine whether metanephroi can be stored in vitro before transplantation, we transplanted metanephroi from E15 rat embryos into the omentum of non-immunosuppressed uninephrectomized (host) rats either directly or suspended in ice-cold University of Wisconsin (UW) preservation solution for 3 d before 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 d.

By 4 wk posttransplantation, metanephroi that had been preserved for 3 d had grown and differentiated such that glomeruli, proximal and distal tubules, and collecting ducts with normal structure had developed. At 12 wk posttransplantation, inulin clearances of preserved metanephroi are comparable to those of metanephroi that had been implanted directly, consistent with the viability of preserved metanephroi (22).

The studies outlined above demonstrate that metanephros isografts and allografts can be implanted into adult animals such that differentiation, growth, vascularization, and function occur posttransplantation. As delineated above, metanephros xenografts undergo differentiation and growth post-transplantation into immune-deficient rodents (10,12,15). To delineate conditions under which metanephros xenotransplantation can be carried out in humans, we and others have performed cross-species metanephros transplantation into immune-competent animals.

**Xenotransplantation of Embryonic Kidneys**

We transplanted metanephroi from an E15 Lewis rat embryo across a concordant xenogeneic barrier into the peritoneum of 10-wk-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. In
contrast, in mice that receive co-stimulation blocking agents hCTLA4Ig, anti-CD45RB, and anti-CD154, the transplanted rat metanephros undergoes differentiation and growth \textit{in situ} (13).

To gain insight into the origin of the vasculature (donor versus host) of metanephroí transplanted in the omentum, using our rat→mouse model, we stained developing rat metanephroí using mouse specific antibodies directed against the endothelial antigen CD31. The vasculature of transplanted developed rat kidneys transplanted into mice is largely of mouse origin including glomerular capillary loops (Figure 5, A and B). In contrast, glomerular capillary loops in rat metanephroí transplanted into rats to not stain for mouse CD31 (Figure 5, C and D) (13).

Using a highly disparate model (pig to rodent) we have transplanted E28 Yorkshire pig metanephroí, consisting only of branched ureteric bud and undifferentiated metanephric blastema (21) into the peritoneum of Lewis rats (23) or C57Bl/6J mice (21). The histologic appearance E28 metanephroí from Yorkshire pig embryos is indistinguishable from that of metanephroí from E28 Yucatan miniature pig embryos (21). Five weeks posttransplantation, no trace of the metanephroí transplanted as described (21,23) 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: 1 mg/d intravenously on days 3, 2, and 1 before transplantation, on the day of transplantation and on days 2, 7, 10, and every 4\textsuperscript{th} day thereafter posttransplantation; anti-CD11a: 1 mg intravenously on the day of transplantation, on days 1, 2, 4, and 7, and every 7\textsuperscript{th} day thereafter posttransplantation; antiTCR-2\textBeta: 50 \textmu g intravenously on days 7 to 2 before transplantation, 100 \textmu g on day 1 before transplantation, and 50 \textmu g/d daily thereafter posttransplantation; anti CD28: 0.5 mg intravenously on the day of transplantation; CRH: 1 \textmu g/d subcutaneously on the day of transplantation and daily thereafter. Figure 6A illustrates a pig metanephros (pm) in the rat omentum 5 wk posttransplantation. An 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 an H&E–stained section of the cortex, and Figure 6D an 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 \textit{et al.} have successfully transplanted metanephroí 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, metanephroí 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). Exactly as was the case in our studies of rat metanephroí transplanted into mice (13), Dekel \textit{et al.} (15) found mouse CD31 expression in external vessels as well as developing glomeruli and small capillaries of pig metanephros xenografts (15), consistent with a host origin for the vasculature of the developed metanephros cellular transplants (15).

In addition, Dekel \textit{et al.} (15) transplanted adult pig kidney tissue or E27–28 pig metanephroí 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 wk post-implantation into non CTLA4-Ig–treated hosts showed rejection of tissues. In CTLA4-Ig–treated hosts, most E27–28 metanephroí 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).

The investigations outlined above demonstrate that metanephros xenografts can be implanted into adult animals such that differentiation, growth and vascularization occur posttransplantation. While the results of these studies are encouraging, they must be considered in the perspective of challenges that remain before this technology can be employed clinically.

**Perspectives and Challenges**

At the present time, the means by which kidney function can be replaced in humans include dialysis and renal allotransplan-
Dialytic therapies are lifesaving, but often poorly tolerated. The success of organ transplantation is such that the major problem facing the field today is neither the technical hurdles nor the medical complications, but rather the shortage of available organs. The use of pigs in lieu of human subjects as donors (xenotransplantation) is a potential solution for the organ shortage (1).

There are risks associated with xenotransplantation of pig metanephroi. These include risks of xenotransplantation per se, such as the possibilities that microbes endemic to the donor species might infect human subjects and that xenotransplantation could lead to the generation of novel pathogens created by recombination of donor retroviruses with elements of the human genome (1), and risks associated with the use of embryonic tissue, such as the possibility that abnormal or unregulated development will occur in hosts (15). The risks dictate that studies conducted to test the use of porcine metanephroi in humans proceed with due caution.

The clinical applicability transplantation of metanephroi will require that a number of challenges be met (24,25). From a tactical perspective: (1) a level of function in transplants sufficient to sustain life long-term will need to be attained in animal models; (2) regimens to control acute rejection must be developed that can be employed in humans; (3) successful pig to primate transplantation must be demonstrated; and (4) a xenograft donor must be identified, from which the use of organs obtained results in a high likelihood for engraftment, low likelihood for rejection, and absent potential for teratogenesis.

Finally, therapeutic strategies must be optimized for use of the embryonic transplants: (1) optimal times for transplantation must be determined, such at the point at which dialysis is initiated; (2) the timing for posttransplantation procedures such as ureteroureterostomy must be defined; and (3) parameters for determining whether additional transplantsations of metanephroi are possible or useful must be established.

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References


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