COMPOSITION AND METHOD FOR IMPROVING FUNCTION OF EMBRYONIC KIDNEY TRANSPLANTS

This invention was made with Government support from the National Institute of Health Grant/Contract No. P50 DK45181. The U.S. Government may have certain rights to this invention.

Background of the Invention:

The metanephric kidneys originate during the fifth week of gestation in humans, during day 12 of embryonic rat development, and during day 20-28 of embryonic pig development, when outgrowths of the mesonephric ducts, so-called ureteric buds, collect about their distal ends, intermediate mesoderm (metanephric blastema) located caudal to the mesonephros. The outgrowths 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 of the tubular structures of the adult nephron with the exception of the collecting system. The origin of the glomerular blood vessels, is in part, extrametanephric.

Studies of metanephric organ culture have shown that kidney development *in vitro* is dependent upon the expression of a number of polypeptides within the developing organ. Blocking the expression or action of any of transforming growth factor alpha (TGF-α), hepatocyte growth factor (HGF), insulin-like growth factor I (IGF I) or insulin-like growth factor II (IGF II), inhibits metanephric growth and development *in vitro* (Hammerman M.R. (1995), *Seminars in Nephrology 15*:291-299). Vascular endothelial growth factor (VEGF), is

also produced by developing kidneys. Blocking VEGF activity *in vivo* inhibits renal vascularization (Kitamano *et al.* (1997), *J. Clin. Invest. 99*:2351-2357). Exposure of developing metanephroi to vitamin A stimulates glomerulogenesis *in vitro* (Vilar *et al.* (1995), *Kidn. Intl. 49*:1478-1487).

Once renal development is complete in a mammal, no new nephrons form. The loss of renal functional mass that occurs following insults to the adult kidney is compensated, in the short term, by hypertrophy and hyperfunction of the remaining nephrons. However, these compensatory changes are often transient and under some circumstances maladaptive in that they may lead to further loss of renal function.

End-stage chronic renal failure afflicts more than 300,000 individuals in the United States alone, most of whom are treated using dialysis, a treatment with considerable morbidity. Another treatment is renal allotransplantation, which is limited by the number of available organs for transplantation. A possible solution to the lack of organ availability is the use of renal xenografts. The clinical renal xenografts performed to date have utilized primate donors, because the closer species are phylogenetically, the more easily xenografts are accepted. The clinical experience with the use of primates as kidney donors dates from the 1960s. However, the results of xenografting of kidneys has been unsatisfactory, and this technique has remained an experimental one for three decades.

Another possible solution to the lack of organ availability is the transplantation of developing kidneys (metanephric allografts or xenografts). The allotransplantation of developing metanephroi into adult animals has been attempted by several investigators. Woolf *et al.* (1990), *Kidn. Intl. 38*:991-997) implanted pieces of sectioned metanephroi originating from embryonic day (E)13-E16 mice into tunnels fashioned in the cortex of kidneys of newborn outbred mice. Differentiation and growth of donor nephrons occurred in the host kidney. Glomeruli were vascularized, mature proximal tubules were formed and extensions of metanephric tubules into the renal medulla were observed. Glomerular filtration was demonstrable in donor nephrons using fluorescently-labeled dextran as a marker of filtration into the proximal tubules. However, connection of donor nephrons to the collecting system

of hosts, that would be required for plasma clearance to occur, could not be demonstrated. In contrast to the case in newborn mice, metanephric tissue transplanted into kidneys of adult mice neither grew nor differentiated, but was extruded as a mass under the renal capsule resembling a poorly-differentiated tumor. It was concluded that the neonatal kidney, which has a rim of undifferentiated cortex (the nephrogenic zone) can facilitate the differentiation of an embryonic implant, but that this ability is lacking in the fully-differentiated adult kidney.

Abrahamson *et al.* (1991), *Lab. Invest 64*:629-639, implanted metanephroi from E17 rat embryos beneath the renal capsule of five adult rat hosts. Within 9-10 days post-implantation, every graft became vascularized, new nephrons were induced to form and glomerular and tubular cytodifferentiation occurred. Glomeruli from transplanted metanephroi were identifiable because they were approximately two-thirds the diameter of those within host kidneys. Intravenous injection of antilaminin IgG into hosts resulted in labeling of glomerular basement membranes of grafted kidneys, confirming perfusion of the grafts by the host's vasculature.

Robert *et al.* (1996), *Am. J. Physiol. 271*:F744-F753, grafted metanephroi from E12 mouse embryos into kidney cortices of adult and newborn ROSA26 mouse hosts. ROSA26 mice bear an ubiquitously expressed β-galactosidase transgene that can be identified by staining in histological sections, permitting differentiation of transplanted from host tissue. Grafts into both newborn and adult hosts examined 7 days post-transplantation were vascularized by components originating from both donor and host.

Koseki *et al.* (1991), *Am. J. Physiol* C550-C554, transplanted rat nephrogenic mesenchymal cells that had been transfected with a Lac Z reporter gene by a retrovirus, underneath the capsule of kidneys of neonatal rats. Transplanted mesenchymal cells were integrated into functioning host nephron segments.

Armstrong *et al.* (1993) *Exp. Nephrol. 1*:168-174, previously reported the formation of cysts in metanephroi transplanted under the kidney capsule of mice. They suggested that the presence of cysts in developed donor metanephroi, coupled with their inability to

demonstrate any connection between the donor and host collecting systems, raised the possibility that transplanted metanephroi become obstructed in the subcapsular site.

Barakat and Harrison (1971), *J. Anat. 110*:393-407M, transplanted sections of embryonic rat metanephroi into a subcutaneous site in the abdominal wall of closely related or unrelated adult rats. Lymphocytic infiltration of the graft and replacement of the graft by fibrosis occurred in both related and unrelated adult hosts, but was more rapid in the unrelated hosts.

Growth factors have been used for the purpose of reducing transplant rejection and improving transplant function. U.S. Pat. No. 5,135,915 to Czarniecki *et al.*, describes immersing grafts in a formulation comprising transforming growth factor for a period of a few minutes up to several days prior to transplantation. The pretreatment with TGF-ß purportedly reduces transplant rejection. U.S. Pat. No. 5,728,676, to Halloran describes the administration of insulin-like growth factor (IGF) before, during, or after organ transplantation for the purpose of inhibiting transplant rejection. In a canine renal autotransplantation model, it was found that storing the removed kidneys in a preservation solution supplemented with IGF-I for a period of 24 hours prior to transplantation back into the dog, significantly improved renal function for the first 5 days following transplantation (Petrinec *et al. (1996), Surgery 120(2)*:221-226).

Summary of the Invention:

Methods and compositions are disclosed for use with embryonic metanephric tissue so as to increase the functioning renal mass obtained upon transplantation into a recipient. The invention includes isolated embryonic metanephric tissue in combination with a growth factor containing composition comprising one or more growth factors for metanephric development. Such growth factors include, but are not limited to, insulin-like growth factor I (IGF-I), insulin-like growth factor II (IGF-II), vascular endothelial growth factor (VEGF), transforming growth factor alpha (TGFα), transforming growth factor beta (TGFβ), hepatocyte growth factor (HGF), fibroblast growth factors (FGFs), platelet-derived growth factor (PDGF), nerve growth factor (NGF), vitamin A, growth hormone (GH), retinoic acid

(RA), tamm horsfall glycoprotein (THG), epidermal growth factor (EGF), leukemia inhibitory factor (LIF), angiopoetins 1 and 2, and bone morphogenetic proteins (BMPs).

The invention also includes embryonic metanephric tissue which has been pretreated with a growth factor containing composition. Such tissue has a developmental potential which is different from embryonic metanephric tissue which has not been so treated. More particularly, the renal development or function of metanephric tissue treated with one or more growth factors is enhanced as compared to metanephric tissue which has not been so treated.

The invention also includes uses of growth factor containing compositions in the preparation of a medicament for the growth and development of embryonic metanephric tissue prior to the implantation into a recipient, i.e., *ex vivo*. Such compositions comprise at least one growth factor for metanephric development. Such growth factors include, but are not limited to insulin-like growth factor I, insulin-like growth factor II, vascular endothelial growth factor, transforming growth factor alpha, transforming growth factor beta, hepatocyte growth factor, fibroblast growth factors, platelet-derived growth factor, nerve growth factor, vitamin A, growth hormone, retinoic acid, tamm horsfall glycoprotein, epidermal growth factor, leukemia inhibitory factor (LIF), angiopoetins 1 and 2, and bone morphogenetic proteins (BMPs).

An alternative embodiment of the invention uses such growth factor containing compositions in the preparation of a medicament for enhancing growth and development of embryonic metanephric tissue for use after tissue implantation into a recipient. In some aspects of the invention, the growth factor compostition does not include IGF I.

In vivo methods are also disclosed for treatment of metahephric tissue after transplantation into a recipient. The methods comprise contacting the tissue with a growth factor composition containing one or more growth factors other than IGF-I. At the time of transplantation, some aspects of the invention, use growth factor compositions which do not include VEGF.

In alternate embodiments, the growth factor composition includes one or more growth factors, but may exclude IGF-I and/or VEGF. However, in such circumstances, further embodiments include the combination of two or more growth factors wherein the second growth factor is selected from IGF-I and/or VEGF.

Detailed Description of the Invention:

The methods described in published European Application No. 0 853 942 A2 are applicable to the present invention and are incorporated herein by reference. To summarize, the EPO publication describes the transplantation of metanephric tissue from an embryonic mammalian donor to an allogenic or xenogenic mammalian recipient. The recipient can be at any developmental stage, including juvenile and adult. A preferred recipient is a human with reduced renal function. The metanephric tissue is taken from the donor at a suitable stage of development, typically within 1 to 5 days after the metanephros begins formation, and is implanted into the recipient either within the omentum, preferably adjacent a host kidney, or under the renal capsule of a host kidney. The metanephric tissue grows and becomes vascularized, in large part by the recipient to form a chimeric kidney. The chimeric kidney develops to form mature structures, including a ureter, which can externalize urine formed by the chimeric kidney after connection to the host's excretory system. In experiments where rat metanephric allografts were transplanted into the abdominal cavity of non-inbred adult rat hosts, the metanephric tissue developed into functioning chimeric kidneys which were not rejected by the host for as long as 32 weeks after transplantation. In contrast, when developed rat kidneys from adult donors are transplanted into non-inbred rat hosts, they are rejected within 7 days. It is believed that the vascularization of the metanephric tissue by the recipient facilitates the acceptance of the transplant.

In addition, EPO Appl. No. 0853942 discloses the use of insulin-like growth factor I (IGF-I) for the purpose of enhancing the growth and development of embryonic metanephric tissue which has been implanted into a recipient. In particular, IGF-I was continuously infused during ureteroureterostomy. In addition, vascular endothelial cell growth factor (VEGF) is disclosed for the purposes of promoting angiogenesis by its administration to the site of the implant at the time of implantation.

The present invention is directed to the discovery that growth factor treatment of the metanephric tissue before, during, and/or after transplantation, enhances the development and functioning of the chimeric kidney. In addition, the removal of host renal tissue prior to transplantation of the donor metanephric tissue further enhances development of the chimeric kidney.

As used herein, the phrase "growth factor" for metanephric development refers to any molecule that promotes the growth, proliferation, and/or differentiation of metanephric tissue. Thus, the phrase encompasses growth factors, ligands that bind to growth factor receptors, vitamins, and other molecules that assist in the development of metanephric tissue. The phrase also encompasses molecules that stimulate endogenous production of growth factors for metanephric development either by the donor metanephric tissue or by the transplant recipient. For example, growth hormone is known to stimulate IGF-I production. Whether a particular growth factor assists in metanephric development can be readily determined by routine experimentation using the procedures described herein.

Presently preferred growth factors include tamm horsfall glycoprotein (THG), ligands of the EGF-receptor such as transforming growth factor alpha, epidermal growth factor (EGF), and amphiregulin; growth hormone, insulin-like growth factors (IGFs), particularly IGF-I and IGF-II; fibroblast growth factors, particularly FGF 2, platelet-derived growth factor, leukemia inhibitory factor (LIF), angiopoetins 1 and 2, particularly angiopoetin 1, and bone morphogenetic proteins (BMPs), particularly BMP 7, vitamin A and derivatives thereof such as retinoic acid; vascular endothelial growth factor (VEGF); hepatocyte growth factor (HGF), nerve growth factor (NGF), cytokines such as TGF-ß and other members of the TGF-ß family (see Atrisano *et al.* (1994), *J. Biochemica et Biophysica Acta 1222*:71-80), and growth hormone (GH) (see Hammerman, M.R. (1995), *Seminars in Nephrology*). Other growth factors include sodium selenite, and transferrin, prostaglandin E_1 (PGE_1). It is intended that each of the terms used to define metanephric growth factors includes all members of a given family. For example, the fibroblast growth factor family consists of at least 15 structurally related polypeptide growth factors (Szebenyi and Fallon (1999) Int. Rev. Cytol., 185:45-106). Using known procedures, it can readily be determined whether a particular factor serves as

growth factor for the developing metanephroi. For example, in cultures of metanephroi, antibodies can be used to block the action, if any, of a certain factor. An inhibition of development compared to controls indicates whether the factor acts as a growth factor for metanephroi development. As another method, comparisons of development of metanephroi in culture with and without supplements can be used to determine whether a certain factor acts as a growth factor (see Hammerman *et al.* (1993), *Pediatr Nephrol 7(5)*:616-620); Hammerman MR (1995), *Seminars in Nephrology 15*:291-299; Schofield PN and Boulter CA (1996), *Exp'l Nephrology 4*:97-104; Pugh *et al.* (1995), *Kidney Int 47(3)*:774-781); Humes *et al. (1991), Lab Invest 64(4)*:538-545); and Vilar *et al.* (1996), *Kidney Int. 49(5)*:1478-1487).

The effect, if any, of a putative growth factor also can be determined by testing transplanted metanephroi which have been (1) pretreated with the factor; (2) contacted with the factor during transplantation; (3) contacted with the factor after transplantation; and, (4) at the time of ureteroureterostomy. Renal development and/or renal function can then be compared to transplanted metaneophroi which have not been treated with the factor. Renal development may be judged by kidney weight, vascularization and formation of kidney tissue, *e.g.*, glomeruli, tubules, renal papilla and ureter. Kidney function can be determined by inulin or creatinine clearance. When treated with a "growth factor" metanephroi tissue is distinguished from non-treated renal metaneophroi by its enhanced renal development and/or ability to contribute to renal function after transplantation.

The metanephros can be treated with a single growth factor that enhances development, or combinations of growth factors can be administered either sequentially, or as a growth factor cocktail. Preferred growth factor cocktails comprise any combination of two or more of the following growth factors: retinoic acid (RA), tamm horsfall glycoprotein (THG), epidermal growth factor (EGF), nerve growth factor, fibroblast growth factors, particularly FGF 2, platelet derived growth factor (PDGF), leukemia inhibitory factor (LIF), angiopoetins 1 and 2, particularly angiopoetin 1 and bone morphogenetic proteins (BMPs), particularly BMP 7, IGF-I, IGF-II, TGF-α, TGF-ß, HGF, and/or VEGF. Transferrin, PGE_1, sodium selenite and growth hormone may also be used in some embodiments. The growth factors are dissolved in any physiologically-acceptable solution in which the metanephroi can be immersed.

Various cell culture media can be used, such as a 50:50 mixture of Dulbecco's modified Eagles medium and Hams F12 (DMEM:HF120). Physiological saline is another suitable solution, particularly if the growth factors are administered to the transplant recipient during or after the transplantation procedure. The growth factors are usually used at concentrations ranging from about 1 fg/ml to 1 mg/ml. Concentrations between about 1 to 100 ng/ml are usually sufficient for most growth factors. Simple titration experiments can be performed to readily determine the optimal concentration of a particular growth factor.

In a preferred embodiment, embryonic metanephric tissue is treated with a growth factor composition comprising one of more of the following:
10^{-7} M IGF I; 10^{-7} M IGF II; 10^{-8} M TGFα; 10^{-9} M HGF; 5-25 µg/25 µl VEGF; 1 µM RA; 0.5 µg/25 µl FGF 2; 0.25 ng/25 µl NGF; 5 µg/25 µl THG; 50-100 ng/ml LIF; or 1 µg/25 µl EGF. In alternative embodiments, the growth factor composition includes one or more of the above growth factors, but specifically excludes IGF I and/or VEGF. In other embodiments, the growth factor composition includes two or more growth factors and the second growth factor is IGF I and/or VEGF.

In a preferred embodiment, the growth factor composition is comprised of the group consisting of IGF I, IGF II, TGFα, HGF and VEGF. In an alternative embodiment, the composition is the same except that IGF I and/or VEGF are excluded.

In a preferred embodiment, the growth factor composition is comprised of the group consisting of IGF I, IGF II, TGFα, HGF, VEGF and RA. In an alternative embodiment, the composition is the same except that IGF I and/or VEGF are excluded.

In a preferred embodiment, the growth factor composition is comprised of the group consisting of IGF I, IGF II, TGFα, HGF, VEGF and NGF. In an alternative embodiment, the composition is the same except that IGF I and/or VEGF are excluded.

In a preferred embodiment, the growth factor composition is comprised of the group consisting of IGF I, IGF II, TGFα, HGF, VEGF, RA and NGF. In an alternative embodiment, the composition is the same except that IGF I and/or VEGF are excluded.

In a preferred embodiment, the growth factor composition is comprised of the group consisting of IGF I, IGF II, TGFα, HGF, VEGF, RA, FGF 2 and NGF. In an alternative embodiment, the composition is the same except that IGF I and/or VEGF are excluded.

In a preferred embodiment, the growth factor composition is comprised of the group consisting of IGF I, IGF II, TGFα, HGF, VEGF, RA, FGF 2, NGF and THG. In an alternative embodiment, the composition is the same except that IGF I and/or VEGF are excluded.

In a preferred embodiment, the growth factor composition is comprised of the group consisting of IGF I, HGF, VEGF and EGF. In an alternative embodiment, the composition is the same except that IGF I and/or VEGF are excluded.

In a preferred embodiment, the growth factor composition is comprised of the group consisting of IGF I, IGF II, HGF, VEGF, FGF 2 and EGF. In an alternative embodiment, the composition is the same except that IGF I and/or VEGF are excluded.

In a preferred embodiment, the growth factor composition is comprised of the group consisting of IGF I, TGFα, HGF and VEGF. In an alternative embodiment, the composition is the same except that IGF I and/or VEGF are excluded.

The methods of the invention are used to treat embryonic metanephric kidney so as to enhance growth and development of embryonic tissue after transplantation into a recipient. Embryonic tissue can be treated prior to transplantation, during transplantation, after transplantation, or a combination of treatments can be used.

In a preferred embodiment, embryonic metanephric tissue is pretreated prior to transplantation. By "pretreated" herein is meant contacting isolated metanephros in a solution containing one or more growth factors. That is isolated metanephros are treated *ex vivo* such that pretreated tissue has a developmental potential which is different from tissue which has not been in contact with a solution containing one or more growth factors.

In a preferred embodiment, embryonic metanephric tissue is treated with a composition containing one or more growth factors during or after transplantation. Such treatment includes contacting transplanted metanephric tiusse with one or more growth factors at the time of transplantation, after transplantation and/or at ureteroureterstomy.

Treatment at the time of transplantation includes bathing the metanephri *in vivo* in a solution containing one or more growth factors. In some of these embodiments, the growth factor composition excludes VEGF. However, VEGF can be combined with one or more other growth factors in such embodiments.

Treatment at a time after transplantation includes infusing a solution containing one or more growth factors into a host, e.g., using an osmotic pump. Treatment at the time of urerteroureterostomy is preferably by bathing the metanephri tissue during the surgical procedure. In a preferred embodiment, the growth factor containing composition is administered after urerteroureterostomy, but before closing of the surgical wound. In some of these embodiments, the growth factor composition excludes IGF I. However, IFG I can be combined with one or more other growth factors in such embodiments.

Prior to the transplantation procedure, metanephric tissue is harvested from one or more suitable mammalian donors at an appropriate stage of fetal development. Preferably, the metanephric tissue is harvested soon after the metanephric kidney begins formation and prior to the presence of blood vessels that either originate within the metanephros or from inside or outside the metanephros. If the embryonic renal tissue is harvested too early in development, it may, once implanted into the recipient, differentiate into non-renal tissues such as hair and gut. Tissue harvested too late in the development of the metanephric kidney, for example,

tissue having visible blood vessels, may contain more antigen-presenting cells and cell-surface antigens and thus present more of threat of rejection by the recipient. Preferably, the harvested metanephroi contains metanephric blastema, segments of ureteric bud, and nephron precursors, and does not contain glomeruli.

The preferred developmental stage for harvesting the metanephros will vary depending upon the species of donor. Generally, the metanephros is preferably harvested 1 to 5 days after the metanephros forms. Preferably, the metanephros is harvested from 1 to 4 days after the metanephros forms, and more preferably from about 2 to 4 days after metanephros formation. In rats, the metanephros forms on day 12.5 of a 22-day gestation period, and on day 11 of a 19 day gestation period in mice. In these species, a suitable time frame in which to harvest the donor metanephros of mice or rats is typically between the second and fourth day after the metanephros begins formation. Preferably the metanephros is harvested within 3 days after formation of the metanephros begins.

In species having a longer gestation period, the time-frame during which the metanephros is preferably harvested following its formation, can be longer. Generally, the time frame in which the metanephros is harvested will be less than about one fifth of the total gestation period of the donor, preferably less than about one seventh of the total gestation period of the donor, and more preferably, less than about one tenth of the total gestation period of the donor. Table 1 shows the time-course (in days) of metanephros development and gestational period in some vertebrates.

TABLE 1

	Metanephros Formation (days)	Gestational Period (days)
Human	35-37	267
Macaque	38-39	167
Pig	20-30	114
Guinea Pig	23	67
Rabbit	14	32
Rat	12.5	22
Mouse	11	19
Hamster	10	16

Pigs are preferred xenogeneic donors for humans because of their comparable organ size, and availability. Additionally, the digestive, circulatory, respiratory and renal physiologies of pigs are very similar to those of humans. In the case of renal function, the maximal renal concentrating ability (1080 mOsm l^{-1}), total renal blood flow (3.0-4.4 ml min^{-1} g^{-1}) and glomerular filtration rates (126-175 ml min^{-1} 70 kg) of the miniature pig are virtually identical to those of humans (see Sachs DH (1994), *Veterinary Immunology and Immunopathology 43*:185-191). The use of metanephroi from transgenic pigs that have been "humanized" to reduce the potential for transplant rejection may provide further advantages (e.g. Pierson *et al.* (1997), *J. Heart Lung Transplant 16*:231-239). Pig metanephroi are harvested at about the 10 mm stage. This occurs between approximately embryonic day 20 and embryonic day 30. Human tissue could be used as an allogeneic source for transplantation.

Metanephroi are removed surgically under a dissecting scope and suspended in a suitable holding medium, such as a 1:1 mixture of Dulbecco's modified Eagles Medium and Hams F12 medium (Rogers *et al. (1991), J. Cell Biol. 113*:1447-1453), and placed under sterile conditions, until they are transplanted. It is preferred to use the whole metanephros, with renal capsule intact, for transplantation. One or more metanephroi may be used per recipient, depending upon the increase in nephron mass that the recipient needs.

If the metanephroi are to be treated with growth factors prior to transplantation, the growth factors can be added directly to the holding medium. When used as a pretreatment, the growth factor composition exerts a positive effect in a surprisingly short period of time. Significant improvement in the development of the implant can be achieved when the metanephric tissue is contacted with the growth factor composition *in vitro* for less than 24 hours. Preferably, the metanephric tissue is contacted with the growth factor composition *in vitro* for less than 8 hours, and preferably less than 2 hours. Preferred *in vitro* temperatures are 0-10°C. Optimal results can usually be achieved when the metanephric tissue is contacted with the growth factor composition for as little as about 20 to 60 minutes prior to implantation into the transplant recipient.

To transplant the metanephric tissue, surgery is performed on the recipient to expose one or both kidneys. Surgical procedures for renal transplantation are well known in the art (e.g. Cohn *et al.(1982), Am. J. Physiol 24*:F293-F299). The donor metanephroi can be implanted directly under the renal capsule of the recipient's kidney, or into a fold of the omentum where it forms a chimeric kidney that functions independently of the recipient's kidney. The omentum is a membranous structure which connects the bowels. It is a preferred site for the implant, particularly if the implanted metanephric tissue is intended to replace a malfunctioning or non-functioning kidney which may be removed, either at the time of transplantation or after the donor metanephric tissue develops sufficiently to form a functioning chimeric kidney. Implantation of the metanephric tissue into the omentum is also preferred if it is desired to treat the tissue with growth factors after implantation. The preferred temperature for post-transplantation treatment with growth factors is 37°C. The omentum is a more accessible site for the growth factor treatment compared to underneath the renal capsule of the recipient's kidney. An osmotic pump that provides a steady supply of growth factors could be placed in the omentum next to the implanted tissue. Alternatively, the recipient could receive periodic injections of the growth factors in the vicinity of the transplant or the growth factors could be delivered in a manner such that they are present in the recipient's blood that circulates through the transplant.

While a donor metanephros can be placed adjacent to any portion of the omentum, it is preferable to implant it in an omental fold which will retain the developing kidney at the site of implantation. It is most preferable to implant the metanephros at an omental fold located near one of the recipient's kidneys, particularly near the ureter, so that the developing ureter of the metanephros can be readily connected to the recipient's excretory system.

When implanted into the recipient's kidney, an incision, large enough to receive the donor tissue is made in the fibrous renal capsule that surrounds the recipient kidney. The location of the incision can be anywhere in a viable portion of the recipient kidney, but most conveniently will be at an external border of the kidney that is easily accessible during surgery. The donor tissue is placed between the capsule and the cortex of the recipient kidney.

The implanted metanephroi are allowed to grow and differentiate within the recipient under conditions that allow the metanephric tissue to vascularize and develop to form mature, functioning nephrons. Suitable conditions may include the use of pre or post-operative procedures to prevent rejection of the implant in addition to the use of growth factors that facilitate the development and functioning of the metanephric tissue. In some cases of allogeneic transplantation, there may be no host rejection of the transplanted metanephros. However, in the case of xenogeneic transplantation, rejection prevention measures are typically taken. This is usually done by immunosuppressing the recipient after the transplantation. Cyclosporine A (CSA) treatments can provide sufficient immunosuppression to prevent rejection of the donor tissue. CSA treatment protocols to prevent transplant rejection are known in the medical field. Local immunosuppression techniques are described by Gruber (1992), *Transplantation 54*:1-11. In U.S. Pat. No. 5,560,911, antibodies directed against idiotypes on naturally occurring human anti-animal antibodies are disclosed for use in inhibiting xenograft rejection. The anti-idiotypic antibodies are injected into the xenograft recipient in order to bind to the idiotypes expressed on anti-xenograft antibodies. Anti-idiotypic antibodies that bind human anti-pig antibodies, to prevent rejection of transplanted pig tissues by a human patient are exemplified. Anti-lymphocyte globulins are also known for prevention of transplant rejection (Lacy *et al.* (1981), *Diabetes 30*:285-291). As an

alternative to immunosuppression, the implanted metanephros can be treated prior to transplantation to reduce its antigenicity. Exemplary approaches to the reduction of immunogenicity of transplants by surface modification are disclosed by Faustman WO 92/04033 (1992). For xenografts into human transplant recipients, transgenic animals that have been humanized to reduce organ transplant rejection may be used. Finally, agents thought to induce tolerance to transplanted tissue can be administered to recipients such as CTLA4-Ig (Lin *et al.* (1993), *J. Exp. Med. 178*:1801-1806).

Metanephric kidneys transplanted using the techniques described herein grow, and become vascularized in large part by the recipient, to form chimeric kidneys. It is believed that the vascularization by the recipient may facilitate the acceptance of transplanted xenogeneic tissue. When implanted into the recipient's kidney, the metanephroi become imbedded into the parenchyma of the recipient kidney. The donor metanephroi begin to form various mature structures that are distinguishable from the structures in the adjacent recipient renal tissue, including mature glomeruli and tubules, renal papilla, and ureter. After a sufficient period of development, it is evident that the glomeruli are capable of filtering plasma. Hence, implantation of the metanephric tissue contributes to an increase in the nephron mass of the recipient.

Filtering glomeruli are evidenced by the detection of urine within the donor metanephroi. This can be done by measuring the levels of urea nitrogen and/or creatinine in fluid aspirated from the donor tissue. Such fluid may be contained within one or more cysts associated with the donor tissue (see Example 1). Urine is defined herein as fluid having a concentration of creatinine and/or urea nitrogen that is higher than the concentration of the corresponding components found in the recipient's plasma. The concentration differential varies, and will be reduced with increased hydration of the recipient. However, generally, the concentration of creatinine in the donor metanephroi will be at least twice the concentration found in the recipient's plasma. The concentration of urea nitrogen in the donor metanephroi will generally be at least fifty percent greater than the concentration of urea nitrogen in the recipient's plasma.

In order to facilitate the externalization of the urine that forms within chimeric kidney, a standard ureter to ureter anastomosis procedure can be used to hook up the ureter that forms from the implanted metanephros with the ureter of a kidney of the recipient. During this procedure, the chimeric kidney can be further treated with growth factors by direct administration of a growth factor-containing composition to the chimeric kidney as it is exposed during the procedure. When the metanephroi are implanted into the omentum of the recipient, externalization of urine can be achieved by linking the ureter directly to the recipient's ureter or bladder. These procedures, and other procedures known in the art for the externalization of urine are summarized in *Adult and Pediatric Urology*, 3rd Ed., Gillenwater, *et al.*, Eds. ¶. 987-994 and 2369-2375 (1996). In some cases, post-transplantation surgery may be unnecessary as the intrarenal transplanted donor kidneys may incorporate into the collecting system of the host.

The following exemplifies some of the growth factor compositions that have been used. Particularly preferred growth factor combinations for pretransplantation use are set forth below. The table includes the concentrations actually used in working Example 8.

PRE-TRANSPLANTATION GROWTH FACTOR COMPOSITIONS										
Comp.	IGFI	IGFII	TGFα	HGF	VEGF	RA	FGF2	NGF	THG	EGF
1	10^{-7}	10^{-7}	10^{-8}	10^{-9}	.2-1 mg/ml	-	-	-	-	-
2	10^{-7}	10^{-7}	10^{-8}	10^{-9}	.2-1 mg/ml	1 μM	-	-	-	-
3	10^{-7}	10^{-7}	10^{-8}	10^{-9}	.2-1 mg/ml	-	-	20 ng/ml	-	-
4	10^{-7}	10^{-7}	10^{-8}	10^{-9}	.2-1 mg/ml	1 μM	-	20 ng/ml	-	-
5	10^{-7}	10^{-7}	10^{-8}	10^{-9}	.2-1 mg/ml	1 μM	20μg/ml	20 ng/ml	-	-
6	10^{-7}	10^{-7}	10^{-8}	10^{-9}	.2-1 mg/ml	1 μM	20μg/ml	20 ng/ml	1.0 μg/ml	-

Particularly preferred growth factor combinations for post-transplantation use are set forth below. The table includes the concentrations actually used in working Example 9.

POST-TRANSPLANTATION GROWTH FACTOR COMPOSITIONS										
Comp.	IGFI	IGFII	TGFα	HGF	VEGF	RA	FGF2	NGF	THG	EGF
7	10^{-7}	-	-	10^{-9}	.2-1 mg/ml	-	-	-	-	4.0 µg/ml
8	10^{-7}	10^{-7}	-	10^{-9}	.2-1 mg/ml	1 µM	-	20 ng/ml	-	4.0 µg/ml
9	10^{-7}	-	10^{-8}	10^{-9}	.2-1 mg/ml	-	-	-	-	-

All cited references are incorporated herein by reference.

In order that the invention described herein may be more fully understood, the following examples and are set forth. It should be understood that these examples are for illustrative purposes only and are not to be construed as limiting the scope of the invention in any manner.

Example 1

Allogeneic transplantation of metanephroi into host kidney

Transplantation Methods:

Whole metanephroi, with renal capsules intact, were removed surgically under a dissecting scope from E15 Sprague-Dawley rat embryos (Harlan, Indianapolis IN), and suspended in saline solution on ice under sterile conditions. Within 45 minutes after removal, four metanephroi per recipient were implanted under the capsule of normal kidneys of 6 week old outbred normal (NL) female Sprague-Dawley rats. Some of the recipient rats had undergone contralateral nephrectomy (UNX) or unilateral nephrectomy and one-half contralateral kidney infarction (1 1/2 NX) using previously described procedures (Rogers *et al.*, *supra*; Rogers *et al.*, (1998) *Kidney Int. 54*:27-37). Transplanted metanephroi were approximately 700 um in diameter and, as would be expected for this stage of development, contained segments of ureteric bud and some developing nephron precursors, but no glomeruli. When noted, recipient rats received Cyclosporine A (CSA) beginning post-transplantation (5 mg/kg body weight per day injected subcutaneously) in vehicle (peanut oil). As a control, vehicle only was injected.

Structural Development of Metanephroi:

Four or six weeks later, kidneys were removed from the rats. When recipient kidneys were examined post-transplantation, cysts containing clear fluid surrounded the sites where metanephroi were transplanted under the capsule of NL, UNX or 1 1/2 NX rats. Structures resembling small kidneys approximately 7 mm (7000 um) in diameter were present under the cysts and were embedded into the larger recipient kidney. Thus, the diameter of the transplanted metanephroi had increased 10-fold reflecting a 1000-fold increase in volume. Histological examination of fixed, paraffin-embedded, and sliced sections of the tissue mass stained with hematoxylin and eosin revealed that the structures were integrated into the parenchyma of recipient kidneys, and that clusters of lymphocytes were present at the transplant-recipient interface.

Metanephroi transplanted into kidneys of vehicle-treated rats that had undergone UNX underwent growth, development and vascularization in vivo. They contained mature glomeruli and tubules that could be distinguished from glomeruli and tubules in adjacent recipient renal tissue by their smaller size and different staining characteristics in paraffin sections stained with hematoxylin and eosin. Blood vessels were present in transplanted metanephroi. Some were identifiable as arteries. Glomeruli in transplanted metanephroi contained red blood cells, distinguishing them from glomeruli of rat metanephroi grown in organ culture, in which vascularization does not occur. Cysts were present within the parenchyma of transplanted metanephroi that contained structures resembling a renal papilla. Other structures were lined with transitional epithelium characteristic of the ureter. Lymphocytes accumulated around the periphery of transplanted metanephroi, but there was no evidence of rejection of tubular or vascular elements. Similar growth, development and vascularization of metanephroi transplanted into rats that had undergone UNX or into kidneys of NL rats were observed.

Metanephroi transplanted into kidneys of CSA-treated 1 1/2 NX rats were examined 4 weeks post-transplantation. Results of such transplantations were similar to those into kidneys of vehicle-treated 1 1/2 NX rats except no peripheral lymphocytes were observed.

Integration of Transplanted Metanephroi into Recipient Renal Tissue:

To determine whether transplanted metanephroi became integrated into recipient kidneys, kidneys of NL rats 6 week post-transplantation were examined. To clear blood from the organ, kidneys were perfused using a modified Ringers solution injected into the aorta distal to the renal arteries following occlusion of the aorta proximal to the kidneys and transection of the inferior vena cava. This results a blanching of the kidney as blood is replaced by perfusate. Normally, the entire kidney blanches as described by Bortz *et al.* (1988), *J. Cell Biol. 107*:811). However, following perfusion of kidneys that contained a transplanted metanephros, blood remained in the transplanted structure relative to the recipient kidney. Most likely, this reflects a reduced perfusion in chimeric blood vessels (derived from transplant and host kidneys) that have been shown to supply transplanted metanephroi relative to perfusion in those supplying the host kidney (Robert *et al.*(1996), *Am. J. Physiol. 271*:F744). Blood could be traced into the papilla of the recipient kidney.

Kidneys were stained using tetragonobolus purpurea lectin (TPL), as described by *Rogers et al. (1993), Am. J. Physiol. 264*:F996, which is expressed in collecting ducts of developing rat kidneys prior to birth and for several weeks following birth, but not in collecting ducts of kidney from adult rats. In adult rat kidney, TPL is expressed in distal tubules and medullary thick ascending limbs of Henle's loop. In recipient kidney tissue, TPL was expressed in cortex within distal tubule and medullary thick ascending limb as would be expected. However, TPL was also expressed in a population of collecting ducts which radiate from the transplanted metanephros into the papilla of the recipient kidney together with blood vessels, evidencing that the collecting system and blood supply of the transplanted metanephros become incorporated into the papilla of the recipient kidney.

Testing of Chimeric Kidney Function:

Levels of urea nitrogen and creatinine were measured in aspirated cyst fluid, and in blood from the aorta, and urine from the bladder of the 1 1/2 NX vehicle-treated rats using previously described methods (Rogers *et al.*, *supra*). Levels of urea nitrogen were increased 2.6-fold and 15-fold, respectively, in cyst fluid and bladder urine relative to blood, and levels of creatinine were increased 12-fold and 28-fold, respectively, as shown in Table 2 (All

measurements were made at the time of sacrifice; comparisons were made using the multiple comparison procedure described by C.W. Dunnett (1955), *J. Am. Statistical Assoc. 50*:1096). Thus, both urea nitrogen and creatinine were concentrated in cyst fluid relative to blood, indicating that the cyst fluid was urine. The concentrations of urea nitrogen and creatinine in the cyst fluid were significantly less than the concentrations in bladder urine, indicating that the cyst urine did not originate from leaked bladder urine. This is consistent with reports that the ability of a 4 week-old kidney (transplanted kidney) to clear the blood of urea nitrogen and creatinine relative to a 10 week-old kidney (recipient kidney), is reduced [Aperia *et al.* (1975), *Am. J. Physiol. 228*:1319].

TABLE 2

Plasma Creatinine/ureaN*	**Cyst Fluid Creatinine/ureaN***	**Bladder Urine Creatinine/ureaN***
	1 1/2 NX rats (n=7)	
1.14±.08/ 53.8±6.3	13.4±2.2/ 136±16	32.3±5/ 800±72
Creatinine	***UreaN (urea nitrogen)***	
Plasma<cyst fluid, p<0.01	Plasma<cyst fluid, p<0.01	
Cyst fluid<bladder urine, p<0.01	Cyst fluid<bladder urine, p<0.01	
*mg/dl		

Example 2

Xenogeneic transplantation of metanephroi into host kidney

Metanephroi from N.I.H. Swiss mouse metanephroi (E14) were transplanted underneath the renal capsule of 1 ½ NX Sprague Dawley rats. Following implantation, host rats were treated with cyclosporine A (CSA) (5 mg/kg body weight injected subcutaneously once per day), or vehicle (peanut oil). Four weeks post-transplantation, all that remained of the metanephroi implanted into the kidneys of rats that did not receive CSA treatment was a mass of fibrotic

tissue. However, in CSA-treated recipients metanephroi grew, vascularized and developed. The presence of a urothelial-lined cavity containing a renal papilla in the transplanted metanephroi indicated that glomerular filtration occurs in the donor renal tissue.

Example 3

Allogeneic transplantation of metanephroi into host omentum

Metanephroi were dissected from E15 sprague-Dawley rats as previously described in Example 1 and implanted into 6 week old outbred UNX Sprague Dawley rats and into rats that had no native renal tissue removed, in omental folds near the recipients' kidneys. Recipient rats received no immunosuppression post-transplantation.

After 6 weeks, transplanted metanephroi were removed and examined. They had assumed a kidney-like shape in situ, had intact ureters and were approximately one-third the diameter of native kidneys. Sections of transplanted metanephroi were prepared and stained with hematoxylin and eosin. Both cortical and medullary tissue were present. Cortices contained well-developed glomeruli containing red blood cells, proximal tubules with well-developed brush border membranes, and distal tubules. Medullas contained well-developed collecting ducts. Ureters were lined with transitional epithelium. Rare accumulations of lymphocytes were observed, but there was no evidence of rejection of tubular or vascular elements.

In contrast to finding in rats that underwent unilateral nephrectomy at the time of implantation, little or no growth of metanephroi occurred when they were implanted in rats that had no native tissue removed.

Example 4

Connection of implanted metanephros to bladder and demonstration of inulin clearance in transplanted metanephroi

Metanephroi were dissected from E15 sprague-Dawley rat embryos as previously described in Examples 1 and 3 and implanted within 45 minutes in the omentum of anaesthetized 6 week old female UNX Sprague Dawley (host) rats. During the same surgery, the host rats had undergone unilateral nephrectomy using methods described by Miller *et al.(1990), Am. J. Physiol 259*:747-751.

Six weeks following transplantation, end-to-end ureteroureterostomy was performed using microvascular technique (interrupted 10-0 suture) between the ureter of an implanted metanephros and the ureter of the kidney that had been removed. Four weeks later all remaining native renal tissue (the contralateral kidney) was removed from host rats, following which inulin and creatinine clearances were measured on conscious rats after placement of an indwelling bladder catheter and intravenous line as described by Miller *et al.* (1992), *Proc. Natl. Acad. Sci. 89*:11876-11880. Baseline measurements for inulin were performed on urine and blood samples obtained prior to beginning the inulin infusions. These "background" values were subtracted from measurements performed after beginning the inulin infusion. Infusion of inulin was begun only following removal of all remaining native renal tissue and drainage of all urine remaining in the bladder (10-20 µl). Only the implanted metanephros remained connected to the bladder. As a control, an attempt was made to measure clearances in rats that had undergone bilateral nephrectomy, but had no transplanted metanephros connected to the bladder. However, in contrast to the case in rats with a transplanted metanephros connected to the bladder (results discussed below), no urine appeared in the bladder catheter over a 3 hour collection time in rats that had no transplanted metanephros.

Plasma creatinines at the time of measurements (following removal of all native renal tissue) were 1.3 ± 0.06 mg/dl, approximately 3-times normal. Inulin and creatinine clearances were 0.11 ± 0.02 and 0.65 ± 0.18 µl/min/100g body weight respectively (mean ± SE). The mean body weight of rats was 238 ± 3.0g. The mean weight of metanephroi was 71 ± 15 mg. The

mean volume of urine collected during 3 hours was 49 ± 13 µl. Inulin and creatinine clearances in a group of 5 normal rats of similar size were 0.92 ± 0.14 and 0.84±0.12 ml/min/100 g respectively.

To define the relationship between the length of time post implantation and inulin clearance by metanephroi, clearances were measured at 12-16, 20-24 and 32 weeks post-implantation. The data, which is summarized in Table 3, demonstrate that metanephroi cleared inulin from the host's circulation for the full length of the experiment, as long as 32 weeks after implantation. The magnitude of clearances did not change significantly as a function of time. The weights and appearance of the transplanted metanephroi also did not change as a function of time in hosts.

TABLE 3

Time after implantation (weeks)	Inulin clearance (µl/min/100g rat weight)	Weight (mg)
12-16 (n=7)	0.23 ± 0.6	72 ± 3.8
20-24 (n=5)	0.31 ± 0.13	88 ± 9.7
32 (n=3)	0.34 ± 0.6	76 ± 23

Data are expressed as mean ± SEM.

Example 5

Further reduction of host renal mass at the time of implantation or IGF-I administration to host to enhance metanephros development

The weights of transplanted metanephroi into the omentum of rats that had undergone unilateral nephrectomy with partial contralateral renal infarction at the time of implantation were increased more than 2-fold (145 vs 71 mg) and inulin clearances expressed per gram of kidney weight were increased more than 12-fold compared to values obtained in rats that underwent unilateral nephrectomy without partial contralateral renal infarction. Urine volumes (145 ± 24 µl / 3 hours) were also significantly increased ($p<0.005$, Student's t test). These observations, coupled with the finding that growth and development of transplanted metanephroi do not occur if no native renal mass is removed at the time of implantation, indicate that the stimulus that results in compensatory renal growth following reduction of renal mass (see Miller *et al.*(1990), *Am. J. Physiol. 259*:747-741) may also enhance the growth and development of transplanted metanephroi.

In further experiments, metanephroi were surgically dissected from E15 Sprague-Dawley rat embryos and implanted within 45 minutes in the omentum of anaesthetized 6 week old female Sprague Dawley (host) rats. During the same surgery, host rats underwent unilateral nephrectomy, using the same procedures as described in Example 4. Four weeks following transplantation, end-to-end ureteroureterostomy was performed using microvascular technique between the ureter of a metanephros implanted in the omentum and the ureter of the kidney that had been removed. Three days following ureteroureterostomy, recombinant human IGF I (Genentech Inc., South San Francisco, CA) was administered by Alzet pump infusion (60 µg/day/animal) into some of the rats. Eight to 12 weeks later, all remaining native renal tissue (the contralateral kidney) was removed from host rats. For rats receiving IGF I treatment, growth factor treatment was ceased two days prior to removal of the contralateral kidney. An indwelling bladder catheter and intravenous line (see Rogers *et al.* (1998), *Kidney Inter'l 54*:27-37) were placed into the rats. Inulin clearances were then measured on conscious rats. Baseline measurements for inulin were performed on urine and blood samples obtained prior to beginning the inulin infusions. These "background" values were subtracted from measurements performed after beginning the inulin infusion. Infusion of inulin was begun only following removal of all remaining native renal tissue and drainage of all urine remaining in the bladder (10-20 µl). Only the implanted metanephros remained connected to the bladder.

Inulin clearances at 12-16 weeks post-transplantation were measured in metanephroi from IGF I treated rats and rats that received no IGF I. As shown in Table 4 below, administration of the IGF significantly increased inulin clearances. Weights of metanephroi were not significantly higher in IGF I treated rats. The appearances of of the transplanted metanephroi were similar in IGF I-treated and non-treated animals. See also Rogers et al. (1999) Dev. Genet. 24:293-298.

TABLE 4

	Implant (N=7)	Implant/ IGF I Treatment
Weight (mg)	72 ± 10	87 ± 11
Urine volume (µl/hr)	31 ± 9	47 ± 10
Inulin Clearance		
(µl/min/100g)	0.23 ± 0.06	0.62 ± 0.13*
(µl/min/g KW)	8.67 ± 2.83	19.8 ± 4.12*
(µl/min/rat)	0.60 ± 0.18	1.70 ± 0.41*

*Implant / IGF I treatment > Implant, $p < 0.05$ Student's test
Data are expressed as mean ± SEM

Example 6

Use of Single Growth factors to enhance development of chimeric kidney

To ascertain whether exogenous vascular endothelial growth factor (VEGF) enhances the growth and development of metanephroi transplanted into the omentum adult rat metanephroi were exposed to VEGF prior to transplantation into the omentum (pre-treatment) or at the time of ureterouretostomy between the transplanted metanephros and the host (post-treatment).

Post treatment exposure was done four weeks following transplantation of metanephroi into the omentum of UNX rats. At this time, end-to-end ureterostomy was performed between the ureter of the transplanted metanephros and the ureter of the kidney that had been removed

from the host. For 45 minutes after ureteroureterostomy, some metanephroi were bathed in 25 µl of a 50:50 mixture of Dulbecco's modified Eagles medium: Hams F12 (DMEM:HF12) containing recombinant human VEGF (Genentech Inc. South San Francisco CA) or DMEM:HF12 containing no additions.

Treatment with VEGF did not affect the weights of transplanted metanephroi measured at 12-16 weeks following transplantation, compared to the weights of untreated metanephroi. However inulin clearances and urine volumes were increased significantly in metanephroi pre-treated with VEGF, and increased further in post-treated metanephroi (Table 5).

TABLE 5

	Implant (N=7)	VEGF-pretreatment (N=6)	VEGF-post-treatment (N= 3)
Weight (µg)	72 ± 10	75 ± 8.5	81 ± 17
Urine volume (µl/hour)	31 ± 9.1	74 ± 7.5[1]	184 ± 26[4,5]
Inulin Clearance			
(µl/min/100g)	0.23 ± 0.06	0.70 ± 0.09[1]	1.6 ± 0.37[3,6]
(µl/min/g KW)	8.67 ± 2.8	27.8 ± 4.9[2]	50 ± 1.8[3,6]

Implant < VEGF: [1] $p < 0.05$; [2] $P < 0.01$; [3] $P < 0.01$; [4] $P < 0.001$
VEGF pre-treatment < VEGF post-treatment: [5] $p < 0.01$; [6] $p < 0.001$;
Data are expressed as mean ± SEM

Our findings are consistent with the proposed role for VEGF in vascularization of the developing kidney (7), and establish the potential for the pharmacological use of VEGF to enhance the growth and function of transplanted metanephroi.

Example 7

Use of Multiple Growth Factors treatments to enhance development of chimeric kidney

For 45 minutes prior to implantation into 1½ NX host rats, metanephroi taken from day 15 embryonic rats were incubated at 4°C in 25 microliters of a 50:50 mixture of Dulbecco's modified Eagles medium:Hams F12 (DMEM:HF12) with or without growth factors. The growth-factor containing solution contained 10^{-7} M IGF-I, 10^{-7} M IGF-II, 10^{-8} M TGF-α, 10^{-9} M HGF and 25 µg VEGF. The metanephroi were implanted into the recipient rats using the methods described in Example 4. Ureteroureterostomies were performed 4 weeks later. Twelve weeks after ureteroureterostomies were performed, inulin clearances were measured in host rats after removal of all remaining native renal tissue. Following measurement of inulin clearance, metanephroi were removed from the hosts and weighed.

As shown in Table 6, weights of metanephroi that had been incubated in DMEM:HF12 containing growth factors were not different from weights of metanephroi that were incubated in DMEM:HF12 without growth factors. However, inulin clearances and urine volumes were increased 3-4 fold in metanephroi that had been incubated with the growth factors compared to values in metanephroi that had been incubated without growth factors.

TABLE 6

Group	Inulin clearance (µl/min/100g rat weight)	Weight (µl)	Urine volume (µl/hour)
UNX+ (n-5)	0.43 ± 0.16***	84 ± 24	34 ± 7.1
UNX+ and growth factors	1.35 ± 0.11	71 ± 8.9	145 ± 8.6**

*UNX+ and growth factors > UNX+, $p < 0.001$, Multiple comparison procedure (C.W. Dunnett, *supra*).
Data are expressed as mean ± SEM.

In another experiment, metanephroi were exposed to growth promoting agent/growth factor mixtures either prior to transplantation into the omentum (pre-treatment) or 4 weeks post-transplantation at the time of ureterouretostomy between the transplanted metanephros and the host (post-treatment).

Pretreatment with 10^{-7} M IGF I; 10^{-7} M IGF II; 10^{-8} M TGF-α; 10^{-9} M HGF and 10^{-7} M VEGF followed by post-treatment with 10^{-7} M IGF I; 10^{-8} M TGF-α; 10^{-9} M HGF and 10^{-7} M VEGF did not affect the weights of transplanted metanephroi measured at 12-16 weeks following transplantation, compared to the weights of untreated metanephroi (implant). However inulin clearances (expressed as µl/min/100) were increased significantly (more than 100-fold) to about 6% of normal (Table 7).

TABLE 7

	Implant (N=7)	Growth Factors (N=4)	Normal (1 kidney) (N=3)
Weight (µl)	73 ± 3.9	86 ± 18	1580
Urine volume (µl/hour)	31 ± 9.1	621 ± 62	-
Inulin Clearance			
(µl/min/100g)	0.24 ± 0.06	27 ± 8.2	460
(µl/min/g KW)	8.67 ± 2.8	1031 ± 253	375

Example 8

Growth Factor Pretreatments with Increased Inulin Clearance

In other experiments, metanephri were incubated as described previously (e.g., Example 7) in one of the following growth factor containing solutions:

1. 0.25 ng/25 µl NGF
2. 1 µM RA
3. 0.5 µg/25 µl FGF2
4. 5 µg /25 µl THG
5. 10^{-7}M IGF I, 10^{-7}M IGF II, 10^{-8}M TGFα, 10^{-9}M HGF and 25 µg/25 µl VEGF

5-

6. 10^{-7}M IGF I, 10^{-7}M IGF II, 10^{-8}M TGFα, 10^{-9}M HGF, 5-25 μg/25 μl VEGF and 1 μM RA

7. 10^{-7}M IGF I, 10^{-7}M IGF II, 10^{-8}M TGFα, 10^{-9}M HGF, 5-25 μg/25 μl VEGF and 0.25 ng/25 μl NGF

8. 10^{-7}M IGF I, 10^{-7}M IGF II, 10^{-8}M TGFα, 10^{-9}M HGF, 5-25 μg/25 μl VEGF, 1 μM RA and 0.25 ng/25 μl NGF

9. 10^{-7}M IGF I, 10^{-7}M IGF II, 10^{-8}M TGFα, 10^{-9}M HGF, 5-25 μg/25 μl VEGF, 1 μM RA, 0.5 μg/25 μl FGF2 and 0.25 ng/25 μl NGF

10. 10^{-7}M IGF I, 10^{-7}M IGF II, 10^{-8}M TGFα, 10^{-9}M HGF, 5-25 μg/25 μl VEGF, 1 μM RA, 0.5 μg/25 μl FGF2, 0.25 ng/25 μl NGF and 5 μg/25 μl THG

Pretreatment of metanephri with any of the above growth factor containing solutions increased inulin clearance compared to metanephri that had been incubated without growth factors.

Example 9

Growth Factor Pretreatment/Post-treatment Combinations with Increased Inulin Clearance

In other experiments, metanephri were pre-treated prior to transplantation and post-treated following transplantation. In one experiment, metanephri were pretreated with 10^{-7}M IGF I, 10^{-7}M IGF II, 10^{-8}M TGFα, 10^{-9}M HGF, 5-25 μg/25 μl VEGF, 1 μM RA, 0.5 μg/25 μl FGF2 and 0.25 ng/25 μl NGF, followed by post-treatment at the time of ureterouretostomy with 10^{-7}M IGF I, 10^{-9}M HGF, 5-25 μg/25 μl VEGF and 1 μg/25 μl EGF. In a similar experiment, metanephri were pretreated with 10^{-7}M IGF I, 10^{-7}M IGF II, 10^{-8}M TGFα, 10^{-9}M HGF, 5-25 μg/25 μl VEGF, 1 μM RA, 0.5 μg/25 μl FGF2, 0.25 ng/25 μl NGF and 5 μg/25 μl THG, followed by post-treatment at the time of ureterouretostomy with 10^{-7}M IGF I, 10^{-9}M HGF, 5-25 μg/25 μl VEGF and 1 μg/25 μl EGF. In both experiments, pretreatment followed by postreatment increased inulin clearance compared to metanephri that had been incubated without growth factors.

In another experiment, metanephri were pretreated with 10^{-7}M IGF I, 10^{-7}M IGF II, 10^{-8}M TGFα, 10^{-9}M HGF, 5-25 μg/25 μl VEGF, 1 μM RA, 0.5 μg/25 μl FGF2 and 0.25 ng/25 μl NGF. Post-treatment consisted of exposing the transplanted metanephri at the time of ureterouretostomy to a growth factor solution containing 10^{-7}M IGF I, 10^{-9}M HGF, 5-25 μg/25 μl VEGF and 1 μg/25 μl EGF followed by infusion three days later with a solution containing 10^{-7}M IGF I, 10^{-7}M IGF II, 10^{-9}M HGF, 5-25 μg/25 μl VEGF, 0.5 μg/25 μl FGF2 and 1 μg/25 μl EGF.

In a similar experiment, metanephri were pretreated with 10^{-7}M IGF I, 10^{-7}M IGF II, 10^{-8}M TGFα, 10^{-9}M HGF, 5-25 μg/25 μl VEGF, 1 μM RA, 0.5 μg/25 μl FGF2, 0.25 ng/25 μl NGF and 5 μg/25 μl THG. Post-treatment consisted of exposing the transplanted metanephri at the time of ureterouretostomy to a growth factor solution containing 10^{-7}M IGF I, 10^{-9}M HGF, 5-25 μg/25 μl VEGF and 1 μg/25 μl EGF followed by infusion three days later with a solution containing 10^{-7}M IGF I, 10^{-7}M IGF II, 10^{-9}M HGF, 5-25 μg/25 μl VEGF, 0.5 μg/25 μl FGF2 and 1 μg/25 μl EGF.

In both experiments, pretreatment followed by postreatment increased inulin clearance compared to metanephri that had been incubated without growth factors.

Example 10

Transplantation of pig metanephroi

The techniques for harvesting metanephroi, transplanting metanephroi into a recipient and for reducing host renal mass are similar to those employed on mice and rats, as described in Examples 1-6, except that microsurgical techniques are not necessary. Rather, surgeries are performed in an operating room using standard surgical techniques appropriate to large animals (see Cohn *et al.*, *supra*).

Metanephroi are surgically dissected under a dissecting microscope from pig embryos at an appropriate stage of embryonic development, which is approximately gestation week 4-6. If

developed metanephric vasculature is evident and/or a significant number antigen-presenting cells are present, then less developed metanephroi are obtained. If the metanephroi do not sufficiently differentiate upon implantation, then metanephroi are obtained at a later developmental stage. Within 45 minutes of removal from the donor animals, the metanephroi are implanted the omentum of anaesthetized adult (host) pigs. During the same surgery, one kidney is removed (UNX) from the host pig, or a unilateral nephrectomy and partial contralateral renal infarction (1 ½ NX) is performed.

In light of the fact that allogeneic transplantation of metanephroi into adult rats using the above-described methods does not result in transplant rejection, it is expected that rejection of the pig metanephroi by the host pig will not occur. However, if rejection does occur, immunosuppression protocols can be devised using routine procedures.

After the implanted metanephroi have developed to the extent that ureters have formed, end-to-end ureteroureterostomy is performed using surgical technique (interrupted suture) between the ureter of a metanephros implanted in the omentum and the ureter of the kidney that had been removed. At the same time, or up to about 6 weeks later depending upon the size and developmental state of the transplanted metanephros, all remaining native renal tissue (i.e. the remaining contralateral kidney) is removed from the host so that only the implanted metanephros remains connected to the bladder.

Baseline measurements for inulin are performed on urine and blood samples obtained prior to beginning the inulin infusions. These "background" values are subtracted from measurements performed after beginning the inulin infusion. At various time points following the removal of the contralateral kidney, inulin and creatine clearances are measured after placement of intraarterial, intravenous, and suprapubic bladder catheters into the pigs as in previous rat studies (Rogers *et al., supra*). The chimeric kidney that results from the procedure is considered functional if inulin clearance is greater than or equal to 10% of normal. Additional parameters are considered to evaluate the growth and development of metanephroi including the diameter of transplanted metanephroi and the presence or absence of mature glomeruli and tubules.

Techniques and methods developed in the pig allotransplantation procedures are applied in clinical trials for xenotransplantation of pig metanephroi into humans with end-stage chronic renal failure. In addition, immunosuppression methods are used.

Example 11

Growth factor treatment for increasing inulin clearance of transplanted pig metanephroi

Using methods described in Example 8, pig metanephroi are implanted into adult pigs. Growth factors are administered to the host or to the metanephroi prior to implantation and/or post implantation to increase the glomerular filtration rate of the implanted metanephroi.

In one growth factor treatment protocol, porcine somatotropin (GH) (Monsanto Chemicals, St. Louis, MO), which stimulates IGF I production in pigs, is administered to the host 2 mg/day IM beginning at the time of transplantation or after ureteroureterostomy. In another, IGF-I is administered to the pigs.

As another growth factor treatment, immediately after dissecting metanephroi from embryonic pigs, the metanephroi are incubated *in vitro* for up to 24 hours in DMEM:HF12 containing 10^{-7} M IGF-I, 10^{-7} M IGF-II, 10^{-8} M TGF-α, 10^{-9} M HGF and 1 mg/ml VEGF.

In further protocols, the *in vitro* and/or *in vivo* growth factor treatement utilizes one or more of the following growth factors: insulin-like growth factor I, insulin-like growth factor II, transforming growth factor alpha, transforming growth factor beta, acidic fibroblast growth factor, basic fibroblast growth factor, vascular endothelial growth factor, platelet-derived growth factor, nerve growth factor, transferrin, prostaglandin E_1, sodium selenite, vitamin A, and growth hormone.

The use of growth factor treatment in combination with reduction of native renal tissue, as described in Example 1 can be used to further enhance the function of the implanted metanephroi.

WHAT IS CLAIMED IS:



1. Isolated embryonic metanephric tissue which has been obtained from a donor at a suitable stage of embryonic development for use in a method of increasing the functioning nephron mass of a recipient in combination with a growth factor composition comprising at least one growth factor for metanephric development.

2. The isolated embryonic metanephric tissue according to claim 1 wherein said growth factor is selected from the group consisting of insulin-like growth factor I, insulin-like growth factor II, vascular endothelial growth factor, transforming growth factor alpha, transforming growth factor beta, hepatocyte growth factor, fibroblast growth factors, platelet-derived growth factor, leukemia inhibitory factor, angiopoetins 1 and 2, bone morphogenetic proteins, nerve growth factor, vitamin A, and growth hormone.

3. Embryonic metanephric tissue which has been pretreated with a growth factor composition comprising at least one growth factor for metanephric development wherein said pretreated metanephric tissue has enhanced renal development or function in recipients as compared to metanephric tissue which has not been pretreated with said growth factor composition.

4. The embryonic metanephric tissue of claim 4 wherein said growth factor is selected from the group consisting of insulin-like growth factor I, insulin-like growth factor II, vascular endothelial growth factor, transforming growth factor alpha, transforming growth factor beta, hepatocyte growth factor, fibroblast growth factors, platelet-derived growth factor, leukemia inhibitory factor, angiopoetins 1 and 2, bone morphogenetic proteins, nerve growth factor, vitamin A, and growth hormone.

5. Use of a growth factor composition in the preparation of a medicament for enchancing the growth and development of embryonic metanephric tissue prior to implantation into a recipient said composition, wherein said composition comprises at least one growth factor for metanephric development.

6. The use of claim 5 wherein said growth factor is selected from the group consisting of insulin-like growth factor I, insulin-like growth factor II, vascular endothelial growth factor, transforming growth factor alpha, transforming growth factor beta, hepatocyte growth factor, fibroblast growth factors, platelet-derived growth factor, leukemia inhibitory factor, angiopoetins 1 and 2, bone morphogenetic proteins, nerve growth factor, vitamin A, and growth hormone.

7. Use of a growth factor composition in the preparation of a medicament for enchancing the growth and development of embryonic metanephric tissue after implantation into a recipient said composition, wherein said composition comprises a growth factor for metanephric development other than insulin-like growth factor I.

8. The use of a growth factor composition according to claim 7 wherein said growth factor is selected from the group consisting of insulin-like growth factor II, transforming growth factor alpha, transforming growth factor beta, hepatocyte growth factor, fibroblast growth factors, platelet-derived growth factor, leukemia inhibitory factor, angiopoetins 1 and 2, bone morphogenetic proteins, nerve growth factor, vitamin A, and growth hormone.

9. A method for the *ex vivo* treatment of embryonic metanephric tissue comprising contacting embryonic metanephric tissue obtained from a donor at a suitable stage of embryonic development with a growth factor composition comprising a growth factor for metanephric development.

10. The method of claim 9 wherein said growth factor is selected from the group consisting of insulin-like growth factor I, insulin-like growth factor II, vascular endothelial growth factor, transforming growth factor alpha, transforming growth factor beta, hepatocyte growth factor, fibroblast growth factors, platelet-derived growth factor, leukemia inhibitory factor, angiopoetins 1 and 2, bone morphogenetic proteins, nerve growth factor, vitamin A, and growth hormone.

* C L M S *

11. A method for the *in vivo* treatment of metanephric tissue after transplantation into a recipient comprising contacting said metanephric tissue with a growth factor-containing composition comprising one or more growth factors for metanephric development other than insulin-like growth factor I.

12. The method of claim 11 wherein said growth factor-containing composition comprises one or more growth factors selected from the groups consisting of insulin-like growth factor II, vascular endothelial growth factor, transforming growth factor alpha, transforming growth factor beta, fibroblast growth factors, vascular endothelial growth factor, platelet-derived growth factor, leukemia inhibitory factor, angiopoetins 1 and 2, bone morphogenetic proteins, nerve growth factor, vitamin A, and growth hormone.

13. A method for the *in vivo* treatment of metanephric tissue at the time of transplantation into a recipient comprising contacting said metanephric tissue with a growth factor-containing composition comprising one or more growth factors for metanephric development other than vascular endothelial growth factor.

14. A method for the *in vivo* treatment wherein said growth factor-containing composition comprises one or more growth factor selected from the groups consisting of insulin-like growth factor I, insulin-like growth factor II, transforming growth factor alpha, transforming growth factor beta, fibroblast growth factors, vascular endothelial growth factor, platelet-derived growth factor, leukemia inhibitory factor, angiopoetins 1 and 2, bone morphogenetic proteins, nerve growth factor, vitamin A, and growth hormone.

