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Chapter 3

Kidney Transplantation in Mice

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Abstract

Kidney transplantation is the treatment of choice for patients with end-stage renal failure. However, long-term function of the transplanted organ is commonly hindered by chronic allograft dysfunction (CAD). Mechanisms of CAD remain largely unknown. Investigations employing small animal models of kidney transplantation continue to be of paramount importance in enhancing our knowledge of transplant immunobiology, allowing for the design of therapeutic strategies to improve clinical outcomes. With enormous advances in molecular biology and genetic engineering over the past decade, murine models of kidney transplantation have become an increasingly powerful tool for understanding the biological processes of kidney allograft rejection at the molecular level, and for determining significant targets for effective interventions. However, kidney transplantation in mice is a highly challenging surgical procedure. Although techniques therein have been described for many years, few centers in the world have utilized this model due to its technical complexity. The technique of mouse kidney transplantation described in this chapter is based on more than 3000 successful kidney transplantations with a 90% survival rate; it has been used by investigators in multiple research centers around the world to explore novel molecular pathways involved in the immunological and physiological processes inherent in kidney transplantation.

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Keywords: Mouse kidney transplantation, microsurgery, allograft rejection, renal function, complications, survival, acute allograft rejection, chronic allograft rejection, spontaneous acceptance

Introduction

Kidney transplantation is the gold standard of treatment for patients with end-stage renal failure and has become a routine procedure in clinical practice. However, transplant recipients inevitably face allograft rejection, mediated by their immune system in response to transplanted foreign tissues. To control this response, allograft recipients require life-long immunosuppression, which often results in an increased susceptibility to infection, risk of malignancy, and cardiovascular complications (Ferguson 2011). These adverse effects contribute substantially to morbidity and mortality among transplant recipients, and limit the efficacy and durability of transplantation as a therapy. Despite the development of increasingly effective immunosuppressive drugs and therapeutic regimens, acute allograft rejection, especially acute humoral rejection mediated by preexisting donor specific antibodies, is still a significant and potentially devastating complication. Moreover, chronic allograft dysfunction (CAD) as a result of acute and chronic alloimmune-mediated injury still develops in a majority of transplant recipients regardless of continuous immunosuppression, and remains a major barrier to long-term renal graft survival. Further improvement of clinical success in transplantation relies on thoroughly understanding the biological processes involved in allotransplantation. In the past thirty years, rodent organ transplantation models have provided a vital tool for gaining novel insights into the cellular and molecular mechanisms underlying allograft rejection. With advances in microsurgery and genetic bioengineering, mouse models of organ transplantation offer greater advantages over other rodent models, including abundant availability of genetically modified mouse strains and biological reagents. Nevertheless, mouse kidney transplantation (MKT) is a highly challenging microsurgical procedure. It is currently performed in only a few transplant centers in the world, due to the complexity of vascular anastomosis (e.g. the diameter of the mouse renal artery is less than 0.4 mm) and urinary tract restoration. In addition, adult mice are approximately 10 times smaller than rats, have extremely small blood volume (~2 ml/25g mouse), and relatively larger body surface area. These factors make them highly susceptible to anesthesia complications and hypovolemic shock. Mastery of this specialized microsurgical technique requires extensive training and skill on the part of the microsurgeon(s), as well as specialized equipment and instruments. The learning curve for mouse kidney transplantation is usually much longer than that of other mouse models (e.g. heart, skin Tx) and rat kidney transplantation. It may take 8−12 months of intense training, or more than 200 transplants, to achieve a 90% success rate.

Mouse kidney transplantation was pioneered by Skoskiewicz (Skoskiewicz 1973) and his colleagues in the early 1970s. Though the technique they described is similar to the rat model reported by Lee in 1963 (Fisher 1963; Fisher 1965; Lee 1967), it had a much higher incidence of surgical complication, with a 30−50% mortality rate (Russell 1978a; Coffman 1993). Likely due to its technical difficulties, use of the MKT model was extremely limited over the next couple of decades after the initial reports. With the emergence of molecular biology and

genetic engineering technologies during the early 1990s, there was a renewed interest in MKT. Since then, several modified techniques have been reported and contributed to its improvement (Table 1). With refined technique, MKT has become a reliable and reproducible model, serving as a useful tool for studying the immunological mechanisms of allograft rejection at the molecular level. Over the last 15 years, more than 3000 kidney transplants have been performed in mice with a high success rate, using the modified microsurgical techniques developed by Zhang et al. (Zhang 1995). This chapter describes the surgical techniques of MKT procedures that are currently used in the laboratory, and discusses important aspects of MKT including critical points to technical success, renal function, postoperative complications, and applications.

Table 1. Surgical techniques for MKT

Reference	Technique features
(Skoskiewicz 1973)	First report of successful mouse kidney transplants, vascular anastomosis using donor aorta patch and vena cava patch, and donor bladder dome to recipient bladder dome anastomosis
(Kalina 1993)	Anastomosis for renal artery and vein using cuffs of donor suprarenal aorta and vena cava, two surgeons
(Zhang 1995)	Vascular anastomoses using donor renal vein to recipient vena cava, and donor infrarenal aortic cuff to recipient aorta; donor ureter attached to small bladder patch was sutured to recipient bladder
(Han 1999)	Direct insertion of the donor ureter to the recipient bladder
(Wang M 2003)	Technique for combined heart and kidney transplantation in mice
(Martins 2006a).	Anti-reflux Urinary Reconstruction, two layer suturing
(Tian 2010).	Method of harvesting both left and right kidneys from one donor, then transplanting them to two different recipients

Anatomy of Mouse Urinary System

Kidney

The kidneys are paired, bean-shaped organs lying retroperitoneally against the dorsal body wall on either side of the spine. They are not attached to the body wall, but are held loosely in place by adipose tissue. The left kidney is normally located posterior to the right kidney. The upper pole of the left kidney is usually at a lower level (the 13^{th} rib) than that of the right kidney (12^{th} rib), which allows for easier technical exposure and isolation of the left kidney.

Ureter

The ureter extends from the kidney to the bladder, passing dorsal to uterine horn (or vas deferens). The ureters enter the dorsal wall of the bladder neck separately, lateral to the entrances of the male vasa deferentia or the female uterine. An intramural part courses

through the bladder muscles in a slightly oblique direction, serving as a barrier to reverse movements of urine.

Bladder

The oval-shaped bladder is in the posterior abdominal cavity in the midline of the body, ventral to the colon. It varies in size with the amount of urine contained. It narrows into a neck that is continuous with the urethra.

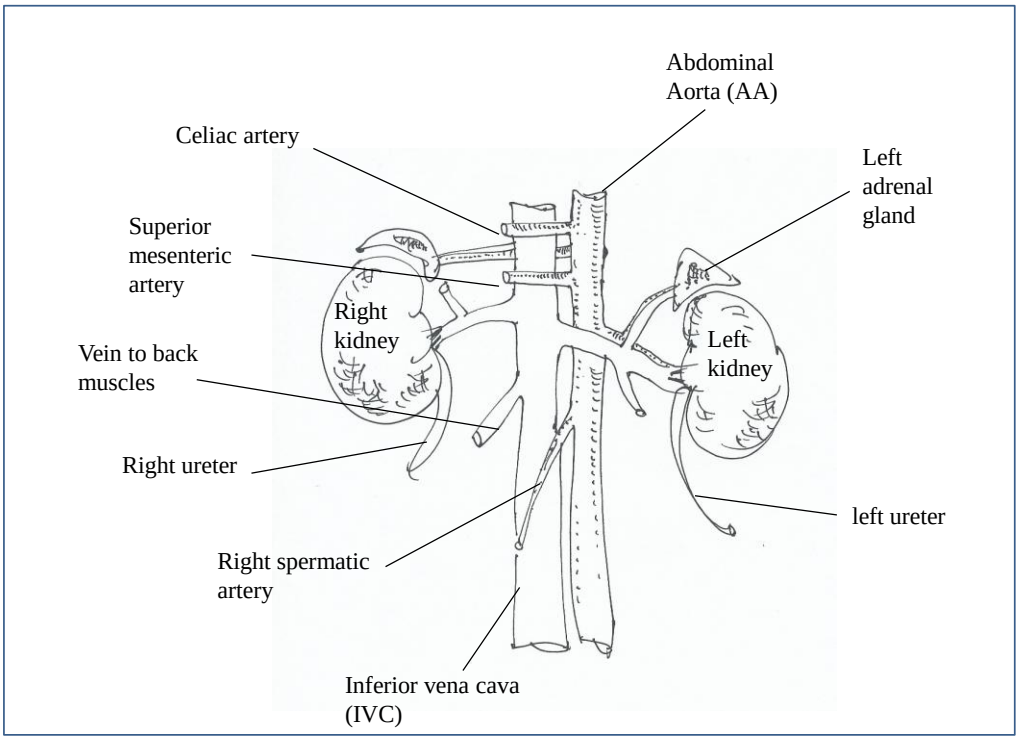

Figure 1. Anatomy of mouse urinary system.

Vascular Supply

The paired renal arteries extend from the abdominal aorta below the superior mesenteric artery to the kidneys and adrenals. They are asymmetrical, the right being more anterior and higher than the left, and are usually dorsal to the inferior vena cava and the renal veins. The paired genital arteries (spermatic or ovarian) usually arise from the aorta posterior to the renal arteries with varying pattern; they may branch from the renal arteries or from the renal aortic junction. The lumbar vessels (arteries and veins) are segmental pairs arising from the dorsal surface of the aorta and vena cava, and supply the dorsal musculature (Figure 1).

Surgical Techniques

General

Three technical models have been reported for MKT. They are: 1) one-stage procedure in which the recipient has a bilateral nephrectomy immediately after grafting; 2) two-stage procedure in which one of the recipient's native kidneys is left intact during renal grafting but is then removed on the fourth to seventh postoperative day (POD); and 3) one of the recipient's native kidneys is kept intact throughout the experiment. The first two are life-sustaining models in which the recipient is exclusively dependent upon renal graft function, whereas the last is a non-life-sustaining model. As the native kidney remains in the recipient, renal graft function is therefore not reflected by recipient survival.

Decision on which models to use depends on both specific research purposes and the microsurgeon’s experience and preference. The two-stage procedure is recommended for those in the learning period. This period is accompanied by a high mortality rate in the first few days following transplant, as the incidence of severe ischemic injury is usually very high since the warm ischemia is extended. The two-stage procedure allows sufficient time for the kidney graft to recover from any ischemic and reperfusion injury, and provides an opportunity to directly observe or biopsy the graft during the second surgery. However, because the animals are exposed to two major surgeries, complications or an unanticipated mortality may occur after the second procedure, particularly for some transgenic animals. It is also not feasible to assess graft function when the native kidney remains in the recipient. Our experience demonstrates that the one-stage procedure can be performed by a skilled microsurgeon with a 90% success rate.

Animals

Male mice weighing 25 to 30g are most suitable as donors and recipients. CD1 outbred mice are ideal for training purposes because of their low cost. No benefit is gained from using larger mice, as their vessels are not of commensurately larger caliber, and dissection is more difficult because of extensive fatty tissue.

Preoperative Care and Anesthesia

Food and water are not restricted for donors or recipients. Isoflurane inhalation (2−3%) using an inhalation anesthesia system is the preferred method for anesthesia. Alternatively, appropriate anesthesia can be achieved by either ketamine HCl (100 mg/kg i.p.) in combination with xylazine HCl (10 mg/kg i.p.) or sodium pentobarbital (60 mg/kg, i.p.) in case the inhalation system is unavailable. Buprenorphine (buprenex) is given subcutaneously at a dose of 0.05 mg/kg during the induction phase of anesthesia prior to the first incision. Animals are kept on a warming blanket or under a heating lamp during surgery. The depth of anesthesia is frequently examined (10−15 min.), and respiratory functions, heart rate and toe pinch response are monitored. A supplemental dose (of one-half the initial dose) of the

injectable anesthetic is given if the animal has an elevated respiratory rate or positive pedal reflex. Guidelines for aseptic technique are strictly followed throughout the operation.

Donor Operation

The abdomen is entered via a long midline incision. The left kidney is exposed by moving the intestine laterally to the right side and using a mosquito clamp to retract the stomach. Manipulation of the kidney is avoided and 0.5% xylocaine applied around the renal pedicle in case of renal vasospasm, the latter of which is usually unnecessary with an experienced microsurgeon.

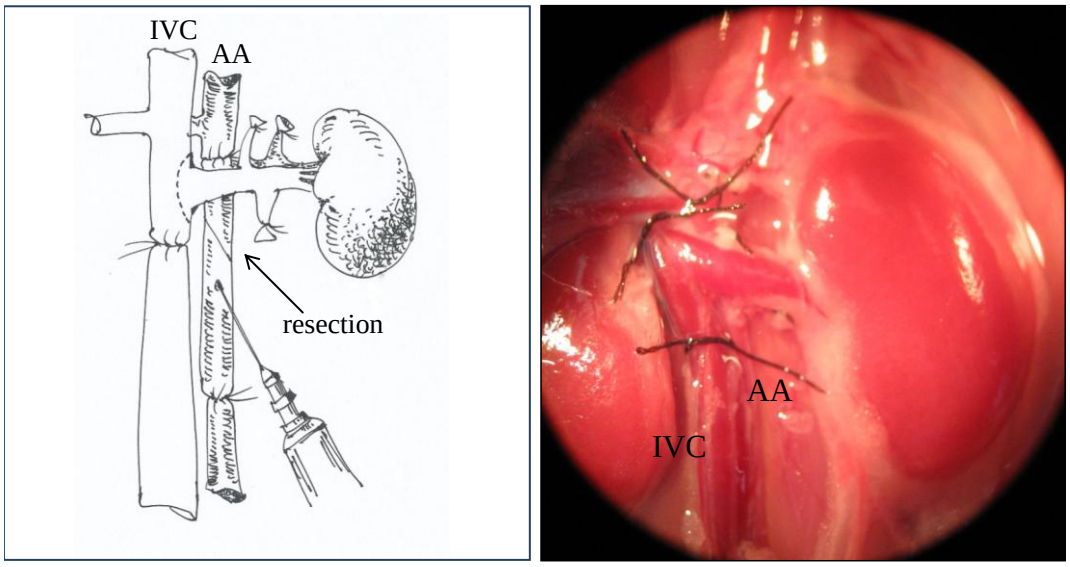

Figure 2. A sketch (left) and a live image (right) showing the donor procedure: the preparation and perfusion of the donor kidney with heparinized cold Ringer's solution through the aorta prior to graft removal (AA: aorta, IVC: inferior vena cava).

Isolation of the Renal Vessels

The left kidney is isolated by ligating and dividing the adrenal and testicular vessels with 8-0 silk sutures. These ties serve as markers to orient the graft and to avoid twisting the renal vein during the recipient surgery. After ligating and dividing a few lumbar branches, the aorta and inferior vena cava (IVC) are mobilized at their junction with the left renal artery and vein. Four 8-0 silk sutures are tied around the aorta and IVC above and below the renal artery and vein (Figure 2).

Dissection of Ureter

Two methods are currently used for restoration of urinary tract continuity: anastomosis between donor ureter-bladder patch to recipient bladder (Zhang 1995) and direct insertion of donor ureter into recipient bladder (Han 1999). To prepare the donor ureter-bladder patch, the left ureter is dissected free from the renal hilus to the bladder. To minimize bleeding, the aorta

is first ligated below the renal vessel. The ureterovesical junction is exposed using caudad retraction on the bladder dome. To enable urinary reconstruction using a bladder-to-bladder anastomosis, a small, elliptical patch of bladder containing the left ureterovesical junction is excised. The right ureter and urethra are not included with the bladder. A generous amount of connective tissue is left around the ureter and bladder patch to minimize risks of compromising the blood supply causing ureter-bladder patch ischemia. To prepare the donor ureter for direct insertion, the left ureter is dissected free from the renal hilus to the bladder; the distal 3−5 mm of donor ureter is cleared of surrounding fat tissue, while leaving periureteral blood vessels intact. There are pros and cons to using either method. Based on the authors' experience, the method of direct insertion is associated with increased risk of urinary complication (e.g., leakage).

Perfusion and Harvest of the Graft

After ligating the aorta above the renal artery, a 30-gauge needle is introduced into the infrarenal aorta, and the graft is slowly perfused *in situ* with 0.5−1 ml of cold, heparinized sodium chloride or Ringer's lactate solution (heparin concentration = 100 U/ml). The renal vein is transected at its junction with the IVC. The aorta is divided obliquely, approximately 2 mm below the renal artery. The kidney and its vascular supply, along with the ureter attached with or without the bladder patch, is removed *en bloc* and stored in Ringer's lactate solution at 4°C. Fluid replacement is not provided during the donor surgery, which usually takes less than 35 minutes (Figure 2).

Recipient Operation

Working through a midline incision, the intestine is covered with wet gauze and carefully retracted to the left side. The recipient's native right kidney is removed first. After ligating the lumbar branches, the infrarenal aorta and IVC are carefully isolated and cross-clamped with two 4 mm microvascular clamps. Retracting with an 11-0 nylon suture through a full thickness of aorta, an elliptical aortotomy (approximately one-fifth the diameter of vessel) is made with a single cut using iris scissors. A longitudinal venotomy (0.18 mm) is made in the IVC by first puncturing it with a 30-gauge needle and then snipping with iris scissors at a slightly lower level than the aortotomy (0.08 mm). Larger openings are avoided as they may cause narrowing at the anastomotic site resulting in anastomotic thrombosis. Both the aorta and IVC are flushed thoroughly with heparinized saline to clear intraluminal blood or clots.

Venous Anastomosis

Two stay sutures are placed at both apices of the venotomy. The donor kidney is then removed from the ice, and placed in the right flank of the mouse. After ensuring that the orientation of the donor renal vein is correct, an end-to-side anastomosis between donor renal vein and recipient IVC is performed using continuous 10-0 or 11-0 nylon sutures. The posterior wall is sutured within the vessel lumen without repositioning the graft. The anterior wall is then closed externally using the same suture. The venous wall in the mouse is extremely thin and fragile and it usually collapses after cutting; saline irrigation is used during anastomosis to keep the vessel walls apart, thus providing better visualization for suturing. Once the venous anastomosis is completed, the vein is gently stretched before tying the

sutures to avoid narrowing at the anastomotic site. Using only four or five sutures for each side, the venous anastomosis is completed within 10 minutes.

Arterial Anastomosis

The arterial anastomosis between the donor aortic cuff and recipient aorta is performed in the same fashion as the venous anastomosis, except that only two or three stitches are required for each side. Since the aortic diameter is smaller than the vein, there is a high risk of thrombosis. Techniques used to avoid this complication include: making a small elliptical aortotomy, penetrating the full thickness of the aortic wall, using as few stitches as possible, and carefully handling the arterial wall. Adventitial stripping is avoided because full-thickness sutures are required to ensure a leak-proof anastomosis. The graft is rinsed with cold saline several times during the procedure. A small quantity of microfibrillar collagen (Avitene®) is placed around the arterial anastomosis before releasing the clamps to help accelerate hemostasis. Gentle pressure is applied to the anastomotic site with a dry cotton swab for 1−2 minutes after revascularization. The kidney graft is perfused instantly with successful anastomosis. Any excessive (loose) Avitene should be removed. Occasionally, vasospasm may appear to linger for 1−2 minutes and then spontaneously disappear. Persistent vasospasm usually indicates a technical error and formation of thrombosis, for which neither topically-applied xylocaine nor aggressive anticoagulation has an appreciable reversal effect.

Urinary Tract Reconstruction

The two methods currently utilized for restoration of urinary tract are donor ureter-bladder patch to recipient bladder anastomosis, and donor ureter to recipient bladder insertion. Although the technique for ureter-bladder patch to bladder anastomosis is still a preferred method for many microsurgeons, ureter to bladder insertion may be a quicker, easier procedure to perform, and be associated with fewer complications.

A) Ureter-bladder patch to bladder anastomosis: after ensuring correct orientation of the ureter, the dome of the recipient bladder is divided. Bleeding is controlled by cautery. Two stay sutures of 10-0 nylon are placed 180° apart through the recipient bladder and donor bladder patch, which are placed on gentle traction by mosquito clamps. Each side of the bladder is anastomosed with four or five interrupted 10-0 sutures (Figure 3A and C). The time for the bladder-to-bladder anastomosis requires ~ 25 minutes.

B) Ureter to bladder insertion: two small holes are made by piercing through the lateral walls of the bladder using a 25-gauge needle or a pair of microsurgical forceps. The end of the ureter is then grasped and pulled through both holes, leaving 2−3 mm outside of the bladder. The donor ureter is fixed proximally to the exterior wall of the bladder by three stitches through the periuretal connective tissue using 10-0 nylon. The distal end of the ureter is severed and the remaining 1 mm of ureter is allowed to retract into the bladder cavity. The second bladder hole is closed and secured with one or two stitches using a 10-0 suture (Figure 3B and D). The time for connecting the ureter to the bladder is around 15 minutes.

Contralateral Nephrectomy

Contralateral nephrectomy is performed following bladder anastomosis in the one-stage procedure. The abdomen is closed in two layers with a continuous 5-0 synthetic absorbable vicryl suture for the inner muscular layer, and 5-0 nylon suture for skin. Completion of the recipient surgery, as shown in Figure 3, requires approximately 80 minutes for an experienced microsurgeon (Figure 4).

In the two-stage procedure, contralateral nephrectomy is performed on the fourth or seventh postoperative day. The recipient is anesthetized as described in the previous section. The left kidney is removed after ligating the left ureter, renal vein and artery via a midline incision. The abdomen is closed in two layers as described above.

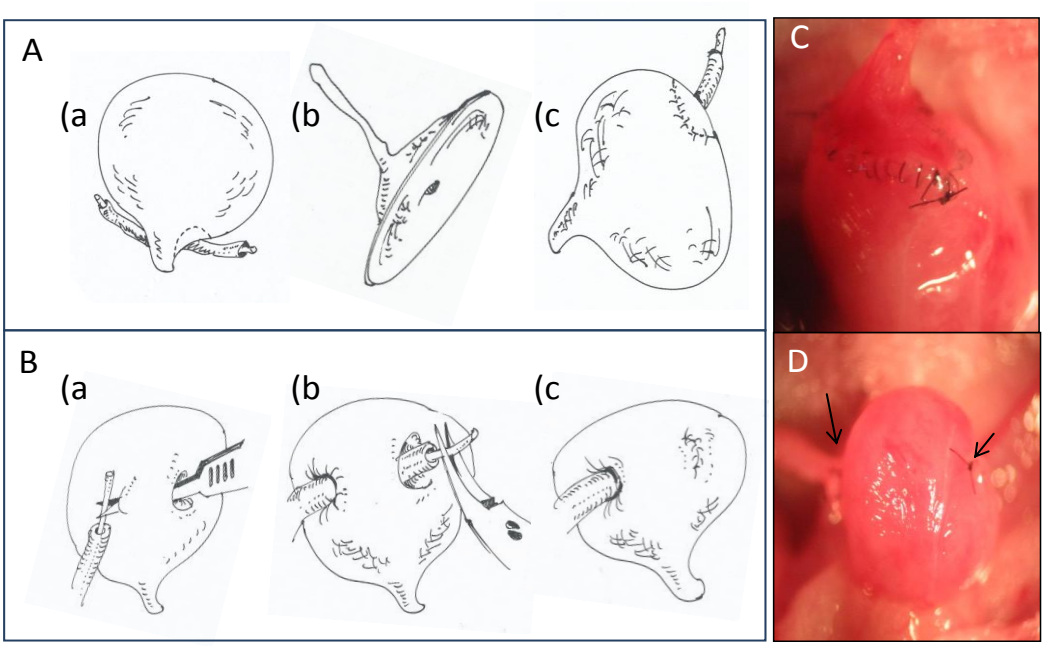

Figure 3. The stepwise techniques of urinary tract reconstruction (A–B), and live images showing the completed reconstruction.
A: Ureter-bladder patch to bladder anastomosis: (a) resection of the bladder patch with the ureter during the donor surgery; (b) a small elliptical patch of bladder containing the left ureterovesical junction; (C) completed bladder-to-bladder anastomosis.
B: Ureter to bladder insertion: (a) the end of the ureter is pulled through the holes; (b) the distal end of the ureter is severed and allowed to retract into the bladder cavity; (c) the bladder hole is closed by 1−2 stitches using a 10-0 suture.

Fluid Replacement and Postoperative Care

After closing the abdomen, 1.5 ml saline is given subcutaneously. Intravenous fluid replacement during the surgery is not necessary, and whether it promotes rapid recovery and improves the success rate of MKT remains to be determined. The animals are kept 1−2 per cage on a warming blanket, with only part of the cage placed directly on the blanket, or under a heating lamp after surgery until they wake. They are checked every hour for the first three

hours, and then at least twice a day for two days, for their general health condition and any signs of surgical complications (e.g. wound bleeding, disheveled appearance, edematous limbs, abdominal mass, marked decrease in activity, dehydration, or infection).

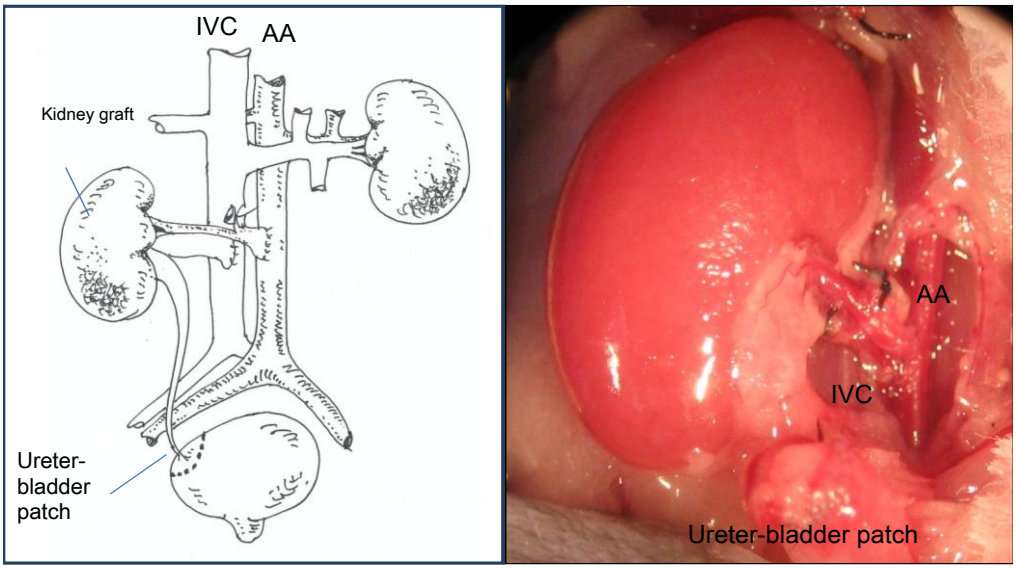

Figure 4. The completed recipient surgery.

Mice usually recover from anesthesia within 1−2 hours post operation and are given regular food and water *ad libitum*. Incidence of infection is negligible with aseptic techniques and antibiotic prophylactics are usually not required, but a single intramuscular injection of a second or third generation cephalosporin or Baytril in drinking water (100 mg in 400 mL of water) may be administered to transplant recipients after surgery for four days.

Surgical Complications

Hypovolemic Shock

Mice are vulnerable to body fluid and blood loss, and dehydration occurs inadvertently after transplant surgery. If untreated, this often leads to hypovolemic shock and early postoperative mortality post-transplant. Hypovolemic shock is a common cause of postoperative death within the first 72 hours for microsurgeons in training, and occurs more frequently in the one-stage procedure than in the two-stage procedure. Dehydration is manifested by: 1) turgid or sunken eyes; 2) continued presence of skin tent; and 3) piloerection. It may be reversible by administering subcutaneous warmed fluids (such as normal saline) twice a day, 1.5 to 2 ml each time, for the first 24 hours or more depending on degree of dehydration. Hypovolemia can be avoided by: minimizing blood loss during donor and recipient surgeries; keeping warm ischemic and cross-clamping times as short as possible; administering sufficient crystalloid to the recipient if surgery lasts for more than 1.5

hours; maintaining the recipient at warm temperature during surgery; and using the two-stage surgical procedure.

Vascular Complications

A major technical difficulty associated with MKT is the vascular anastomosis due to the extremely small renal vessel diameter in mice. Consequently, uncontrolled arterial bleeding and thrombosis are commonly observed early on during the learning curve, and can occur at both arterial and venous anastomotic sites. Thrombosis at the arterial anastomotic site is the most common vascular complication (25–50%) (Zhang 1995; Martins 2006b) and the most common cause of intraoperative graft failure or death within two to five days after MKT. To reduce the risk of thrombosis, it is critical to avoid a larger aortotomy and venotomy that would inevitably cause excessive stretching and narrowing at the anastomotic site. There are several advantages for using a segment of donor infrarenal aorta rather than a Carrel patch of aorta to restore arterial blood flow (Russell 1978b; Zhang 1995). First, this technique avoids extensive dissection at the renal artery and aortic junction, which may cause vasospasm. Second, it provides a large lumen and sufficient length for anastomosis. Third, it is easier to prepare and requires less time to suture than a Carrel patch. Warm ischemia can be minimized by using continuous inverted sutures on the posterior wall of the arterial or venous anastomosis without repositioning the graft. Anticoagulant is not required during or after surgery.

Ureteric Complications

Ureteric reconstruction is the most problematic and least standardized procedure in both clinical and experimental kidney transplantation. Various techniques, including end-to-end anastomosis of the ureter with or without stenting (Oesterwitz 1982), ureter-to-bladder anastomosis (Fisher 1965; Lee 1967) and bladder-to-bladder anastomosis (Fabre 1971), have been used in rat kidney transplantation. Due to the small size of the mouse ureter, it is not feasible to perform a ureter-to-ureter anastomosis. Currently, ureter-to-bladder implantation and a bladder-to-bladder anastomosis are the only practical methods for reestablishing ureteric continuity in MKT. Unfortunately, both techniques are prone to complications, some of which are described below.

Urinary Leakage

Urinary leakage is a potentially severe complication resulting in urine peritonitis and death within one to two weeks after surgery. It can occur with both techniques of urinary tract reconstruction. With ureter-bladder patch anastomosis, urinary leakage is likely due to necrosis of the donor bladder patch. During the donor surgery, the vesical-prostatic vascular plexus is inevitably damaged, leaving the renal-ureter-bladder axis as the sole blood supply to the donor bladder patch (Silber 1979). Measures to minimize the incidence of donor bladder necrosis include careful preservation of ureter-bladder patch blood supply, and use of a small bladder patch. With ureter insertion, urinary leakage occurs due to detachment of the inserted ureter (Han 1999; Tian 2010). This can be avoided by positioning the kidney graft lower to

reduce tension between the inserted ureter and bladder. In addition, great care should be taken to ensure the donor ureter is securely fixed to the bladder wall.

Hydronephrosis and Chronic Pyelonephritis

Hydronephrosis and chronic pyelonephritis can impair kidney graft function and cause late death. Contributing factors may include: 1) the presence of vesical calculi produced by sutures (D'Silva 1990); or 2) denervation of the donor bladder (Silber 1979). Sun Lee's group demonstrated that regardless of their chemical or physical composition, any suture exposed to urine can provide the initiating stimulus for calculi formation and cause obstructive uropathy. In addition, the higher incidence of hydronephrosis in allografts than in isografts suggests that there is an immunologic etiology, such as chronic rejection, in ureteric obstruction in the kidney allograft (Miyazawa 1995).

Clinical Course and Allograft Rejection of MKT

Kidney Graft Survival

Mice usually recover from surgical distress within a few hours. They appear no different from normal mice 24 hours after surgery, despite 10−15% weight loss over the initial three to five days. Signs of illness (e.g., weight loss >25%, lethargy, diarrhea, unkempt appearance) within four days often signifies severe, irreversible surgical complications. These animals should be euthanized and excluded from the study. All isografts with life-sustaining kidney transplants survive indefinitely with adequate renal function and normal histology. Survival of allografts is largely dependent upon donor-recipient strain combinations (Russell 1978a; Russell 1978b; Zhang 1996).

Assessment of Kidney Graft Functions

Previous studies have shown that mouse kidney allografts transplanted across complete MHC disparities can survive for extended periods of time; therefore, animal survival does not reflect the rejection process. Kidney graft function allows for a more precise quantitation of the intensity of immunological injury. For life-sustaining renal grafts, both blood creatinine (Cr) and urea nitrogen (BUN) levels, as well as creatinine clearance (CLcr) (Wang 2010), are commonly used to determine renal function after transplantation. More sophisticated but less commonly used methods, such as inulin and para-aminohippuric acid clearances, have also been reported (Coffman 1993). Urine and plasma creatinine can be measured by Beckman-Coulter auto-analyzer using the Jaffe method (Peake and Whiting 2006), or I-STAT Portable Clinical Analyzer (Bickerstaff 2008; Bickerstaffa 2008). The I-STAT handset analyzer (Abaxis, United City, CA) offers greater convenience for sequential monitoring of Cr and uses a less than 0.2 ml whole blood sample, which can be obtained weekly or biweekly via the lateral tail veins or retro-orbital sinus without sacrificed recipients. Conventional units (mg/dl) can be converted to SI units of micromol/L by multiplying the conventional units by 88.4 (Kratz 1998).

CLcr is a measure of glomerular filtration rate (GFR) that is most frequently used as a parameter to evaluate renal function in clinical practice. It is found that CLcr is useful in evaluating renal function for long-term surviving allografts with chronic rejection. CLcr (μl/min) is measured by collecting total urine volume over a 12-hour period with mouse metabolic cages, and calculated with the traditional equation $U \times V/P$: where U is urine Cr levels, V is the volume of urine by minute, and P is the plasma levels of Cr (Lavender 1969).

Normal mice excrete a drop or two of urine at a time, highly concentrated with a total output of less than 1 ml/24 hr. Mouse kidneys contain approximately 4.8 times as many glomeruli as found in rats, and have a higher GFR. Normal mouse plasma Cr is less than 0.2 mg/dl, CLcr is about 110 μl/min. Changes in CLcr levels are usually consistent with progressive renal graft dysfunction and tissue damage.

Characteristics of Kidney Allograft Rejection

It is shown that in MHC fully mismatched kidney allografts, allorecognition by T cells occurs in perivascular sites as early as the first postoperative day (POD1), but parenchymal damage begins at day three, coinciding with the emergence of interstitial T- and dendritic cell infiltrates (Einecke 2009).

Significant functional changes start between POD5 and POD7, manifested by increased Cr and decreased CLcr levels, correlating with histological changes featured by a range of focal to diffuse lymphocytic infiltration.

However, progression and pattern of kidney allograft rejection varies among different strain combinations. For example, the majority (>70%) of untreated BALB/c recipients of B6 or C3H allografts, also referred to as “high responder,” fail between POD14 and POD30 due to mixed acute cellular and vascular rejection; less than 30% can survive to POD100 (Wang 2008). In contrast, most untreated B6 allografts (>90%) of BALB/c or C3H allografts survive to more than 100 days with chronic rejection (Figure 5A). The untreated allografts surviving to POD100 usually sustain a normal or slightly elevated plasma Cr and CLcr (Figure 5B) that coincide with a markedly increased urine output (3–4 ml/24 hr).

This finding may be attributed to impaired urinary concentrating capacity and/or augmented compensatory response (Kaufman 1974) that follows loss of renal mass due to chronic allograft rejection. Histological features of acute cellular and vascular rejection include lymphocytic infiltration, hemorrhage, edema, tubulitis, vasculitis, and glomerular and tubular necrosis; typical chronic rejection is indicated by focal perivascular monocyte infiltration, interstitial fibrosis, tubular atrophy, and intimal proliferation (Figure 6). The explanation for differential responses observed among the various strain combinations remains unclear.

Previous studies suggested that the distinct cytokine profiles expressed by different mouse strains play an essential role in regulating the pattern of rejection (Wang 2003). Interestingly, a recent study by Melk et al. (Melk) suggested that donor age may influence allograft survival, as old donor kidneys display abnormal parenchymal susceptibility to transplant stresses, more rapid emergence of epithelial changes, and enhanced induction of senescence marker p16(INK4a).

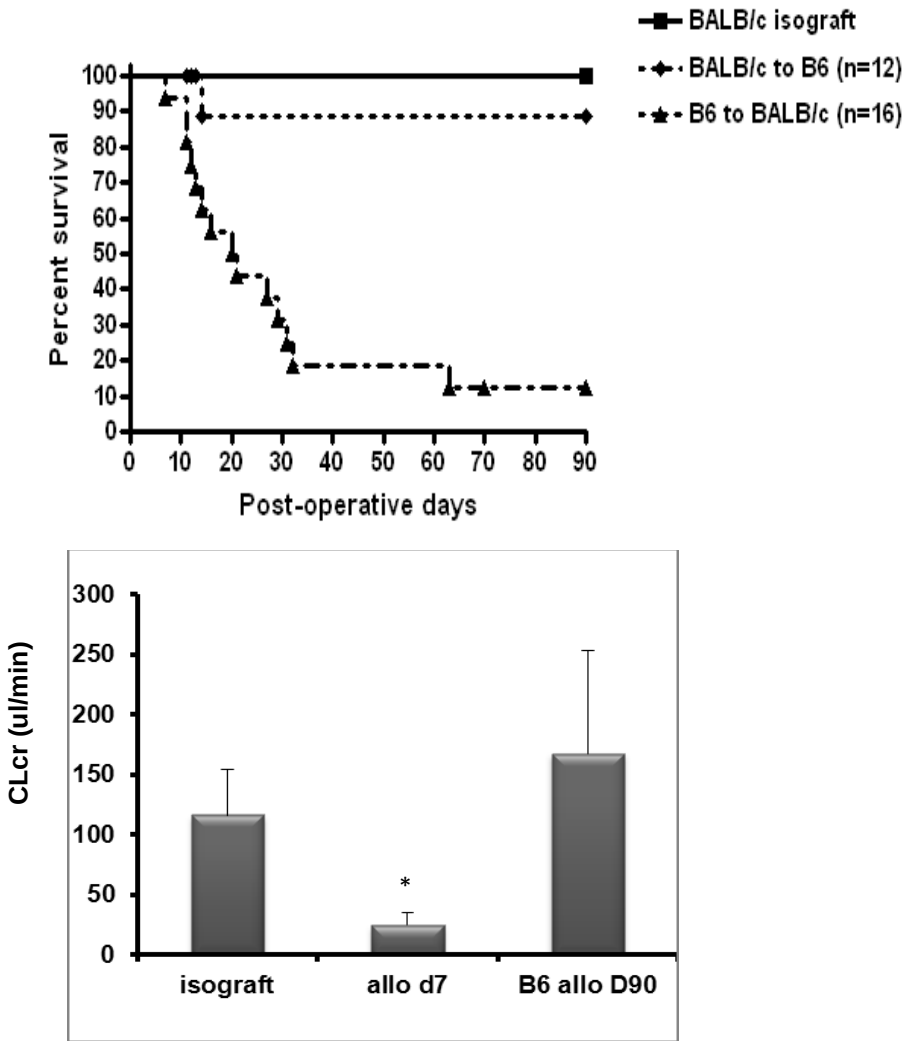

Figure 5. Pattern of kidney transplant survival (A) and renal graft function (B) as expressed by CLcr (μl/min) (*p<.0.01 vs. other graft).

Kidney allografts with either a single MHC Class I or Class II disparity survive a long period without immunosuppression, while hearts are rejected in the same strain combination (Corry 1973; Skoskiewicz 1973; Russell 1978a). Even with fully mismatched MHC, some mice develop spontaneous acceptance, which does not occur in analogous heart grafts. The distinct biological features of hearts and kidneys may account for the differential survival (Corry 1973; Cook 2008; Steger 2008; Brown 2011; Wang 2011) of the respective allografts. The kidney encompasses an abundant collection of largely independent nephrons with parallel functions. Damage to a portion of kidney tissue may not significantly impact the function of other nephrons in the undamaged portions of the kidney. In addition, the kidneys are known to have enormous compensatory capacity and great flexibility in adjusting to varying demands. Therefore, life may be sustained by partial kidney function, at least temporarily. On the other hand, cardiac function relies on coordinated contractions of cardiac

muscle cells in the heart controlled by its conduction system. Hence, tissue damage in heart grafts, if concentrated in appropriate locations such as the conduction system, can lead to rapid irreversible loss of function and cessation of beating. Taken together, it is likely that the immune response sufficient to reject heart allografts may cause only localized and partially reversible damage to kidney allografts. In some fully MHC-mismatched strain combinations, such as B10.BR to B6 and DBA to B6, long-term survival of kidney allografts has been associated with increased Foxp3 expression and altered B cell response when compared with rejection of heart allografts in the same strain combination (Wang 2011). It has also been shown to correlate with development of TGF-beta-dependent immune regulation involving "regulatory" dendritic cells and IDO (Steger 2008). Other suggested explanations include organ specific immunogenicity and the size or mass of the organ (He 2004) to be transplanted. However, precise mechanisms by which they contribute to the discrepancy in graft survival observed in different organs remains to be further investigated.

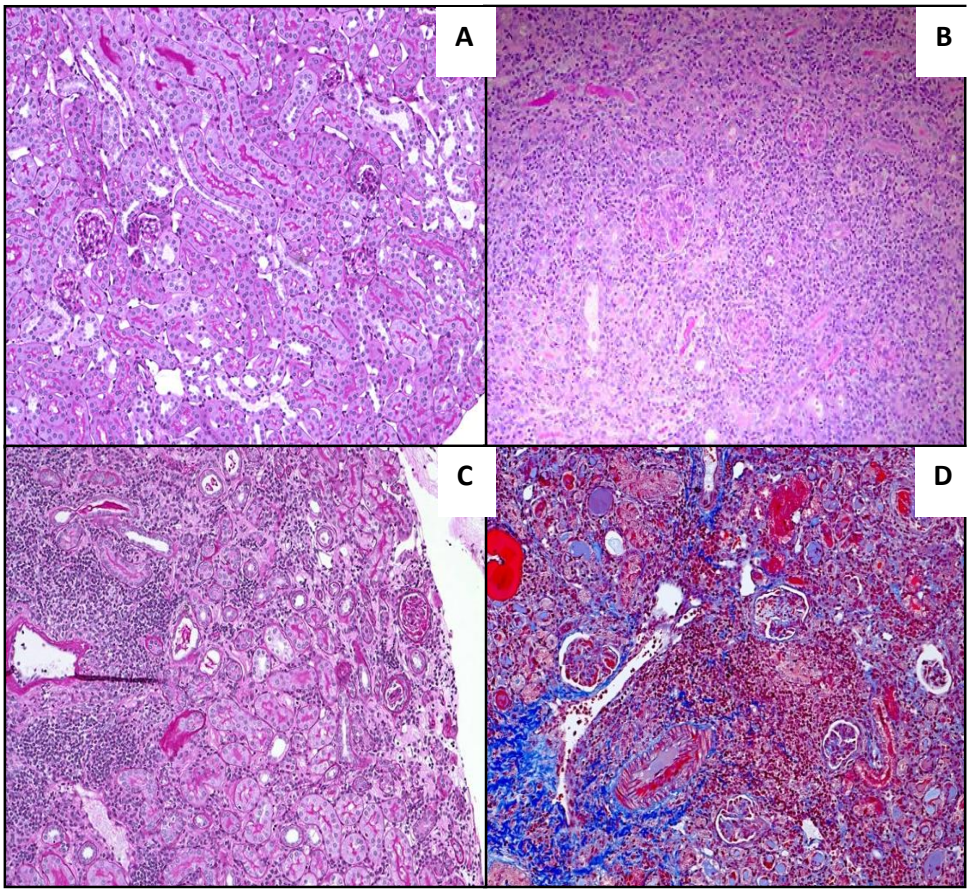

Figure 6. Histologic features in BALB/c isografts and B6 to BALB/c allografts. The grafts were fixed in formalin and stained with PAS (A−C) or trichrome (D). A: POD90 isograft; normal histology; B: allograft on POD14, early phase rejection; C: allograft on POD90, late phase rejection; D: allograft on POD90 with trichrome staining showing increased collagen deposition.

Applications

MKT provides a functional graft that mimics human kidney transplant physiology and biology. The MKT model is unique, as it provides several alternatives for designing research protocols. Life supporting MKT can be used to determine the impact of novel molecular pathways on kidney allograft function and graft survival, and to explore therapeutic targets. Conversely, non-life supporting MKT is primarily used for short-term immunological and histological studies. The "high responder" strain combination can be used to study acute rejection, while long-term surviving renal allografts may provide a useful tool to study chronic rejection and tolerance. Due to the variation in survival in the mouse kidney transplant model, renal function may be an alternative end-point for assessment of graft rejection. To minimize this variation, a large sample size is recommended.

Table 2. Recent studies on kidney allograft rejection using MKT

Reference	Factor studied	Key Findings
(Franceschini 2003)	prolyl-hydroxylase inhibitor (PHI)	PHI improves renal function and reduces the severity of chronic rejection via inhibition of matrix synthesis
(Halloran 2004)	perforin or granzymes A and B	T-cell-mediated kidney transplant rejection is associated with but not mediated by perforin or granzyme A or B
(Cheng 2006)	Connective tissue growth factor (CTGF)	CTGF is a biomarker and mediator of kidney allograft fibrosis and chronic rejection
(Wyburn 2006)	IL-18	IL-18 expression increases, but abrogation of IL-18 fails to prevent rejection
(Du 2007)	NO/iNOS	endogenous NO through renal NOS2 is protective of kidney allografts via Fas
(Sis 2007)	IFNγ /IFNγR	$IFN\gamma R^{-/-}$ kidney allografts rapidly fail with massive ischemic necrosis, IFNγ prevents early perforin-granzyme-mediated destruction
(Gueler 2008)	C5aR	Complement 5a receptor inhibition improves renal allograft survival
(Qi 2008)	$CD11b^{+}$ cells	macrophage-mediated endothelial cell cytotoxicity leads to loss of the renal microvasculature
(Wang 2008)	$Foxp3^{+}$ regulatory T cells (Treg)	Reduction of intragraft Treg infiltrates is associated with progression of renal allograft rejection
(Bickerstaffa 2008)	CCR5	Recipients with CCR5 deficiency reject cardiac allografts with features consistent with AHR
(Melk)	p16(INK4a), senescence marker	Increase in p16(INK4a) expression is associated with old allografts and corresponded with more rapid emergence of epithelial changes
(Wang 2010)	MyD88, Trif, TLR2, TLR4	MyD88-and TRIF-dependent TLR signaling in recipients contribute to chronic graft dysfunction

Studies carried out with genetically engineered inbred mice have contributed tremendously to our understanding of transplant immunobiology and genetics, and have provided the foundations upon which many clinical interventions have been developed. Table 2 shows a summary of key findings from recent studies on kidney allograft rejection using MKT.

With advances in immunosuppression agents, the frequency of acute rejection episodes post kidney transplantation has decreased (Sekijima 2010). However, acute antibody-mediated rejection (AMR) is increasingly being recognized as a significant cause of renal allograft dysfunction and graft loss. Studies (Bickerstaff 2008; Rother 2008) using a presensitized MKT model have demonstrated that recipient presensitization with donor skin grafts results in donor specific AMR of subsequent renal allografts, leading to graft loss in eight to ten days after transplantation. The clinic course and pathohistological features of this model closely resemble AMR encountered in human kidney transplant recipients with preformed anti-HLA antibodies. This presensitized MKT model provides a valuable tool for studying the pathogenesis of AMR and screening potential therapeutic interventions (Gloor 2010). In addition to delineating and exploring new mechanisms underlying kidney allograft rejection, MKT has also been used to study ischemia/reperfusion injury (Sorensen 2011), transplant infectious diseases (e.g., CMV and BK infection) (Hummel 2001; Han Lee 2006; Zhang 2008), transplant genomics, and proteomic and biomarker screening (Cheng 2006; Wu 2008).

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