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REVIEW

Systematic review of mouse kidney transplantation

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Abstract

A mouse model of kidney transplantation was first described in 1973 by Skoskiewicz *et al.* Although the mouse model is technically difficult, it is attractive for several reasons: the mouse genome has been characterized and in many aspects is similar to man and there is a greater diversity of experimental reagents and techniques available for mouse studies than other experimental models. We reviewed the literature on all studies of mouse kidney transplantation to report the donor and recipient strain combinations that have been investigated and the resultant survival and histological outcomes. Some models of kidney transplantation have used the transplanted kidney as a life-supporting organ, however, in many studies the recipient mouse's native kidney has been left *in situ*. Several different combinations of inbred mouse strains have been reported, with varying degrees of injury, survival or tolerance because of haplotype differences. This model has been exceptionally useful as an investigational tool to understand multiple aspects of transplantation including acute rejection, cellular and humoral rejection mechanisms and their treatment. Furthermore, this model has been used to investigate disease mechanisms beyond transplant rejection including intrinsic renal disease and infection-associated pathology.

Introduction

A rodent model of kidney transplantation was first described in rat (*Rattus norvegicus*) in 1965 by Lee *et al.* [1], and this was followed in 1973 when Skoskiewicz *et al.* reported experimental kidney transplantation in mouse (*mus musculus*) [2]. The model is significantly different from human transplantation; however, the physiological outcome for the kidney is the same. A functioning kidney transplant requires establishment of vascular flow through arterial and venous anastomoses between donor vessels and recipient systemic circulation, with urinary tract drainage either via anastomosis to the recipient urinary tract or other excretory outlet.

Technical aspects inevitably vary and have been developed to overcome the small size of the vessels. In human transplantation, the site of kidney implantation is most commonly heterotopic with extra-peritoneal placement of the kidney in the iliac fossa. The donor renal artery on a patch of aorta, often called a 'Carrel patch', is anastomosed to the external iliac artery and donor renal vein to the external iliac vein [3]. The donor ureter is then anastomosed to the native bladder. The most common experimental method in the mouse is to create an arterial anastomosis by end-to-side anastomosis of the donor aorta to the intra-abdominal recipient aorta, such that the donor aorta acts as a conduit to the renal artery [4]. The donor renal vein is anastomosed end-to-side to the recipient vena cava. However, some believe an easier technique is to use more accessible vessels and in rodents the common carotid artery has been used as the source of inflow [5,6]. Use of both right and left kidneys from a single donor to two recipients has been described [7]. The urinary tract anastomosis can be performed between donor and recipient bladder or donor ureter to recipient bladder, in the rat model uretero-cutaneous drainage has been described, although this carries inherent risk of infection [5]. A novel method to investigate the rejection process in the kidney without undertaking the complex heterotopic transplantation has been described where nonvascularized cortical fragments of donor kidney

tissue are implanted under the recipient kidney capsule [8]. Furthermore simultaneous kidney and cardiac transplantation has been described in mice as a method to mirror simultaneous transplantation in patients with cardiomyopathy and renal disease [9].

During the process of organ transplantation the cells are subjected to ischaemia. The longer the ischaemia the greater the injury it receives, with a spectrum from reparable damage to ultimate cell death caused by ischaemia reperfusion injury. In mouse studies, warm ischaemic time less than 35 minutes results in survival, dependent on the transplant, of 70–80% at 3 days [10]. However, survival as low as 50% at 1 week have been reported [11]. Furthermore histological tubular injury becomes severe as cold ischemia reaches 4 hours in the mouse model [12]. The benefits of using a true transplantation model of ischaemia reperfusion injury compared with *in situ* clamping of the renal artery and vein as a model is that the entire process of transplantation is recreated.

A rodent model of kidney transplantation has been useful as an investigational method to understand multiple aspects of transplantation including ischaemia reperfusion injury, acute rejection, cellular and humoral rejection mechanisms and the treatment of these. Although the mouse model is technically difficult and estimated to take over 40 procedures before an acceptable operative time is achieved [13], it is attractive for several reasons. The mouse genome has been characterized and in many aspects is similar to man [14]. In addition, there is greater diversity of experimental reagents and techniques available for mouse experiments making this an excellent experimental system. We reviewed the literature on all studies of mouse kidney transplantation to report the donor and recipient strain combinations that have been investigated and the resultant survival and histological outcomes.

Methods

A review of the literature was performed in accordance with methodology defined by the Cochrane Collaboration for identifying scientific articles [15]. Search of MEDLINE (1946 to present) and EMBASE (1980–2013 week 01) databases was performed in January 2013 using Ovid SP interface (Ovid Technologies, Inc., Wolters Kluwer Health) by employing an optimally sensitive strategy [16]. The search terms, such as 'renal transplant', 'kidney transplant', 'mouse', 'murine', 'animal model', were used in combinations using Boolean search terms. Duplicate reports were filtered using OVIDSP search function. Article titles in English and European foreign languages were considered; however, there were no manuscripts that required translation. Additional references were obtained by visually scanning reference lists from studies, hand-searching key journals and conference proceedings and by citation searching. All abstracts were obtained and assessed for relevancy to the systematic review topic; for articles relevant to the systematic review, complete manuscripts were obtained in hard or electronic copy. Review articles and un-published or ongoing studies were not included.

Results

The transplant model

Following systematic review, there were 62 articles describing studies into rejection and the basic science of kidney injury [2,8,10–12,17–73], a further eight studies reported technical surgical aspects of mouse kidney transplantation [7,13,74–79]. The original study described an initial nephrectomy at the time of transplantation followed by a second nephrectomy before closure of the abdomen. However, many reported removing the second kidney from 3 to 7 days after transplantation to create a truly transplant dependent model [2,10–12,17,18,20,22–50]. This avoids an initial period of acute renal failure. In other studies, the contralateral kidney was left *in situ* such that the mouse was not dependent on the transplant for survival [8,19,21,51–73]. The benefit is that the mouse undergoes a single procedure and reduces the morbidity and the risk of death from a second surgical procedure. In addition, the mouse does not suffer from the adverse affects of gradual renal failure. Importantly histological outcomes at an early time-point have been compared between mice dependent on the allograft and those with a single native kidney and no difference was observed [62]. However a transplant dependent model allows functional assessment, serum urea or creatinine and survival as an outcome (Table 1) [2,10,11,17,20,22–37,39,41,42,45,46,48]. For studies using the model that is not dependent on the transplanted kidney, a time-point for histological analysis was used [8,19,21,51–73]. Older studies are largely descriptive of the histological outcome describing abnormalities in the glomeruli, tubules, vasculature and infiltration of inflammatory cells with parenchymal changes such as oedema and fibrosis [2,10,17–21,51–53,58,73]. Semi-quantitative histology scores have been used by some authors to measure changes such as degree of tubulitis, glomerulitis and arteritis [55,57,59–63,65,66,69–72].

Strain combination

Inbred mice are considered syngeneic with specific genotypes being homozygous at virtually all loci, with phenotypes that are identical. Nomenclature of inbred strains is designated by the parent strain consisting of capital letters and combinations of letters and numbers, or numbers only. Related inbred strains, strains that have a common origin

Table 1. MHC mismatch and mouse survival in transplant-dependent studies. Where two strains are denoted a F_1 hybrid progeny was the recipient or donor, haplotype given in brackets.

Author	Donor strain	Recipient strain	Survival
Skoskiewicz 1973 [2]	B10.BR (H-2^k)	C57BL/6J × A/Jax (H-2^b × H-2^a)	Chronic rejection with survival 59–336 days.
	C57BL/6 (H-2^b)	C3H × DBA/2 (H-2^k × H-2^d)	Survival 26–130 days.
Russell 1978 [10, 17]	B10.D2N (H-2^d)	C57BL/6J × A/Jax (H-2^b × H-2^a)	Survival 42–390 days, 'spontaneous acceptance' in some. 30% died in 30 days. 20% died between 30–100 days.
	C57BL/6 -H(zl) (H-2^{ba})	C57BL/6J × A/J (H-2^b × H-2^a)	Survival similar to B10.D2N donor.
Benson 1985 [18]	B10.BR (H-2^k)	C57BL/6J (H-2^b)	'High percentage survived for many weeks'.
Inoue 1991 [20]	NZW (H-2^z)	NZW × NZB (H-2^z × H-2^b)	All mice died within 10 months regardless of transplant.
Jevnikar 1993 [11]	C57BL/6 (Ins I-E^b) (H-2^b)	C57BL/6 × C3H (H-2^b × H-2^k)	8.3 month mean survival.
	C57BL/6 (H-2^b)	C57BL/6 × C3H (H-2^b × H-2^k)	11.4 months mean survival.
Ogasa 1995 [22]	C57BL/6	BALB/c	20% accepted >100 days. Some rejected within 20 days.
Zhang 1995 [24] Lazarovits 1996 [26], 1996 [27]	(H-2^b)	(H-2^d)	Reversal of acute rejection with anti-CD45RB therapy on day 4 with survival >60 days.
Zhang 1996 [25]	BALB/c (H-2^d)	CBA (H-2^k)	33% accepted >100 days
	C57BL/6 (H-2^b)	C3H/HeN (H-2^k)	50% accepted >100 days
Qi 1999 [28]	BALB/c (H-2^d)	C57BL/6 (H-2^b)	Mean survival 7.4 ± 0.8 days. Survival improved by cyclosporine 37.5 ± 6.6 days or rapamycin 37.9 ± 3.7 days.
Gorczynski 2000 [29]	BALB/c (H-2^d)	C3H (H-2^k)	14–20 day survival. Portal vein injection of dendritic cells expressing Il-10 and TGF-beta increased survival up to 50 days.
Bickerstaff 2001 [30] 2008 [33] Cook 2008 [34]	DBA/2 (H-2^d)	C57BL/6 (H-2^b)	80% survival over 60 days with some survival up to 200 days. Pre-sensitized rejection within 19 days, mean survival 8.6 ± 4.3 days.
Lin 2006 [36]	C57BL/6 (H-2^b)	B10.BR (H-2^k)	100% graft loss by day 9.
	B10.BR (H-2^k)	C57BL/6 (H-2^b)	Approximately 90% lost allograft at day 8.
	C57BL/6 (H-2^b)	BALB/c (H-2^d)	100% graft loss by 9 days. Cell-mediated rejection
	C57BL/6 (H-2^b)	C3H/HeN (H-2^k)	100% graft loss by 9 days
	C3H/HeN (H-2^k)	C57BL/6 (H-2^b)	Approximately 80% graft loss by 9 days, one survivor greater than 30 days
Han Lee 2006 [35]	C3H/HeJ (H-2^k)	C57BL/6 (H-2^b)	Survival over 60 days. Death within 14 days if infected with polymavirus in transplant kidney.
Brown 2007 [37] 2008 [38]	DBA/2 (H-2^d)	C57BL/6 (H-2^b)	Long-term acceptance with median survival 100.5 days.
	BALB/c (H-2^d)	C57BL/6 (H-2^b)	Acute rejection with median survival 8 days.
Lutz 2007 [39]	BALB/c (H-2^d)	C57BL/6 (H-2^b)	Survival up to 100 days. Reduced survival with blockade of the inducible co-stimulatory molecule pathway.

Table 1. continued

Author	Donor strain	Recipient strain	Survival
Bickerstaff 2008 [32]	A/J ($H\text{-}2^a$)	C57BL/6 ($H\text{-}2^b$)	Survival 60–120 days.
	A/J ($H\text{-}2^a$)	C57BL/6 [$CCR5^{-/-}$] ($H\text{-}2^b$)	Rejection within 21 days with a mean survival of 13.29 ± 4 days.
	A/J ($H\text{-}2^a$)	C57BL/6 [$CCR5^{-/-}$ / $\mu MT^{-/-}$] ($H\text{-}2^b$)	70% of the recipient's accepted allograft beyond 60 days.
Gueler 2008 [40]	C57BL/6 ($H\text{-}2^b$)	BALB/c ($H\text{-}2^d$)	Vehicle treatment 100% mortality in 11 days. Recipient pre-treatment with antibody against C5aR resulted in 75% survival over 12 weeks.
Meng 2008 [41]	BALB/c ($H\text{-}2^d$)	C57BL/6 ($H\text{-}2^b$)	Mean survival time 52 days. Anti-CD154 prolonged graft mean survival time >100 days.
DiLillo 2011 [42]	DBA/2 ($H\text{-}2^d$)	C57/BL6 [huCD19Tg] ($H\text{-}2^b$)	Mean survival 54 ± 8 days with 20% surviving >100 days. Survival better after anti-CD19 84 ± 9 days and 67% surviving > 100 days, but not anti-CD20
Wang 2011 [45]	C57BL/6 ($H\text{-}2^b$)	B10.BR ($H\text{-}2^k$)	Six of the seven kidney allograft recipients survived >150 days.
	C57BL/6 [$RAG^{-/-}$] ($H\text{-}2^b$)	B10.BR ($H\text{-}2^k$)	No difference in median survival time compared to control donor mice.
Albrecht 2012 [46]	C3H/HeJ ($H\text{-}2^k$)	C57BL/6 ($H\text{-}2^b$)	Survival greater than 60 days.
	C3H/HeJ × C57BL/6 ($H\text{-}2^k \times H\text{-}2^b$)	C57BL/6 ($H\text{-}2^b$)	Less MHC disparity no affect on rejection or survival with concomitant mouse-polyomavirus infection.
	C57BL/6 [$K^bD^b\beta_2$ $m^{-/-}$] or ($H\text{-}2^b$)	C57BL/6 ($H\text{-}2^b$)	MHC class I-deficient donor kidneys did not affect rejection by mouse-polyomavirus infected recipients.
	C3H/HeJ × C57BL/6 ($H\text{-}2^k \times H\text{-}2^b$)	C57BL/6 [aly/aly] ($H\text{-}2^b$)	Loss of adaptive immunity resulted in 100% survival of allograft >60 days with concomitant mouse-polyomavirus infection.
Li 2012 [47]	BALB/c ($H\text{-}2^d$)	NOD.Cg-Prkdc^{scid} IL2rg^{tm1Wjl}/Szj ($H\text{-}2^{g7}$)	CMV reactivation following transplant. Eleven of fifty recipients died between 9–40 days after transplant.
Zarjou 2012 [48]	BALB/c ($H\text{-}2^d$)	C57BL/6 ($H\text{-}2^b$)	Approximate 78% survival up to 60 days.

and separated before the twentieth generation are given symbols that indicate this relationship. Mouse major histocompatibility molecules (MHC) are similar to man with corresponding allelic sets within each MHC polypeptide molecule (Table 2).

Several inbred mouse strains have been used in kidney transplantation studies, including C57/BL6, BALB/C, CBA/J, C3H and DBA/2 mice. The alloimmune response between donor and recipient occurs as a result of differences at MHC class I and II loci. Subsequently, the rejection of allografts between mouse strains is variable, with tolerance believed to occur between some strains (Fig. 1). Outbred mice have not been reported in transplantation studies reflecting their genetic variability, unknown haplotype expression and hence the contribution to the rejection response. It is of note that there is disparity between survival results reported by different groups, specifically BALB/C kidney transplantation into C57/BL6 recipients has been described to result in rapid rejection with mouse survival as low as 7.4 days [28], compared with some mice surviving over 100 days [39]. Whether this is a phenomenon because of sub-strain difference is not apparent and it may represent an opportunity to study the genomic basis of rejection; alternatively, in those with short survival, surgical factors may have been involved.

Recognition of alloantigens

Various aspects in the alloimmune response have been elucidated using mouse studies. Recognition of allograft antigens have been described to occur via three mechanisms; the direct, semi-direct and indirect pathway.

Table 2. Allelic Designations for haplotype (H-2) complex of mice used in transplantation studies. Pure strain mice are homozygous for the H-2 region and for a particular set of alleles that occur in a strain a superscript letter has been given, e.g. for C57/BL6 mice the haplotype is H-2^b. Thus for each of the nucleotide sequences for each individual MHC allele a similar superscript is used, however some of these overlap between strains.

	MHC Class I			MHC Class II			
Haplotype	H-2K	H-2D	H-2L	I-Aa	I-Ab	I-Ea	I-Eb
H-2^a	k	d	d	k	k	k	k
H-2^b	b	b	b	b	b	b	–
H-2^d	d	d	d	d	d	d	d
H-2^k	k	k	–	k	k	k	k
H-2^z	u	z	z	u	u	u	u
H-2 ^{g7}	d	b	–	d	g7	–	–

– Null; no allele expressed at this MHC loci.

The direct pathway describes presentation of donor peptide associated with MHC molecules on the surface of donor cells to recipient effector cells, such as to the recipient T-cell receptor. This process may evolve during the initial ischaemia reperfusion phase when perivascular lymphocytic infiltration occurs, and recipient lymphocytes may interact with donor antigen-presenting cells, these being resident dendritic cells, 'passenger' leukocytes or nonlymphoid cells such as tubular epithelium [80]. Alternatively, the direct pathway may result from migration of donor dendritic cells from the allograft to the recipient spleen and this has been observed in a mouse cardiac transplantation model and following mouse kidney transplantation [38,81]. Other studies have failed to identify dendritic cell migration from kidney to spleen [45]. Whether this is specific to immunological response in this strain combination is unknown, alternatively it may reflect temporal differences. The semi-direct pathway describes recipient's antigen-presenting cells acquiring intact donor MHC molecules and presenting these to the recipient's responder cells. Evidence of this has been shown *in vivo* following mouse kidney transplantation where donor MHC class II was identified within the recipient spleen and expressed by the recipient's own dendritic cells, in addition the recipient's B cells and macrophages appeared to acquire donor MHC [38]. In the indirect pathway, donor MHC alloantigen is processed and presented to effector cells as peptides by recipient antigen-presenting cells. The potential importance of this was demonstrated where donor kidneys deficient in MHC class I induced a lower number of CD8+ T cells to infiltrate the allograft compared with wild type kidneys [57].

Acute rejection

Mononuclear cell infiltration and active cellular rejection appear as early as 3 days following transplantation in multiple strain combinations and reported in a broad range of studies (Fig. 2) [2,8,10,17–19,21–24,26,27,30–35,37–42,44–46,48,52–58,62–66,68–70]. Investigating a strain combination that develops T cell mediated rejection has revealed that infiltrating effector T cells exhibit RNA transcription of perforin 1, granzyme A and B, interferon-γ and lymphotoxin-B [64]. However, the rejection involving cytotoxic T cells did not appear to be dependent on contact-mediated mechanisms as selective gene disruption in the recipients of perforin or granzymes-A or B did not affect rejection [66], furthermore injury did not appear to occur through Fas–Fas ligand cytotoxic mechanisms as knockout of Fas expression in the donor kidney had no effect [63,69].

Humoral rejection: antibody

The original study by Skoswiewicz *et al.* identified the production of cytotoxic antibodies between fully MHC mismatched mice, where the incubation of recipient serum with donor-strain splenocytes induced cell death [2]. However, as in human transplantation, the contribution of the different MHC classes is unlikely to be the same as no cytotoxic antibodies have been identified with some combinations despite complete MHC class I and partial MHC class II mismatch [2,10,17,61].

Donor-specific antibody determination by flow cytometry is a commonly described method of identifying surface bound immunoglobulin on donor-derived cells, usually splenocytes or thymocytes, when incubated with recipient serum [30,32,33,44,52]. Both MHC class I and class II appear to be targets for allospecific IgG [52]. MHC class I deficient mice are able to generate allospecific antibodies against MHC class II [53]. The presence of circulating donor-reactive alloantibody has also been reported to occur in mice that accept kidney allografts, indeed secondary challenge with subcutaneous injection with donor splenocytes failed to stimulate a delayed-type hypersensitivity response [30]. Not only IgG but IgM has been noted to be

1. Normal [37,44,62]
2. Antibody mediated changes [6,30,32,33,35,44,87]
3. Borderline changes
4. T-cell mediated changes [8,31,32,35–37,39,41,42,44,45,48,52–54, 56–58,62–64,68,70]
5. Interstitial fibrosis and tubular atrophy [37,46,48,57,65,71,72]

Figure 1 Summary of relevant histological studies. Studies have reported histological changes in the mouse kidney allograft that mirror the Banff classification system [86].

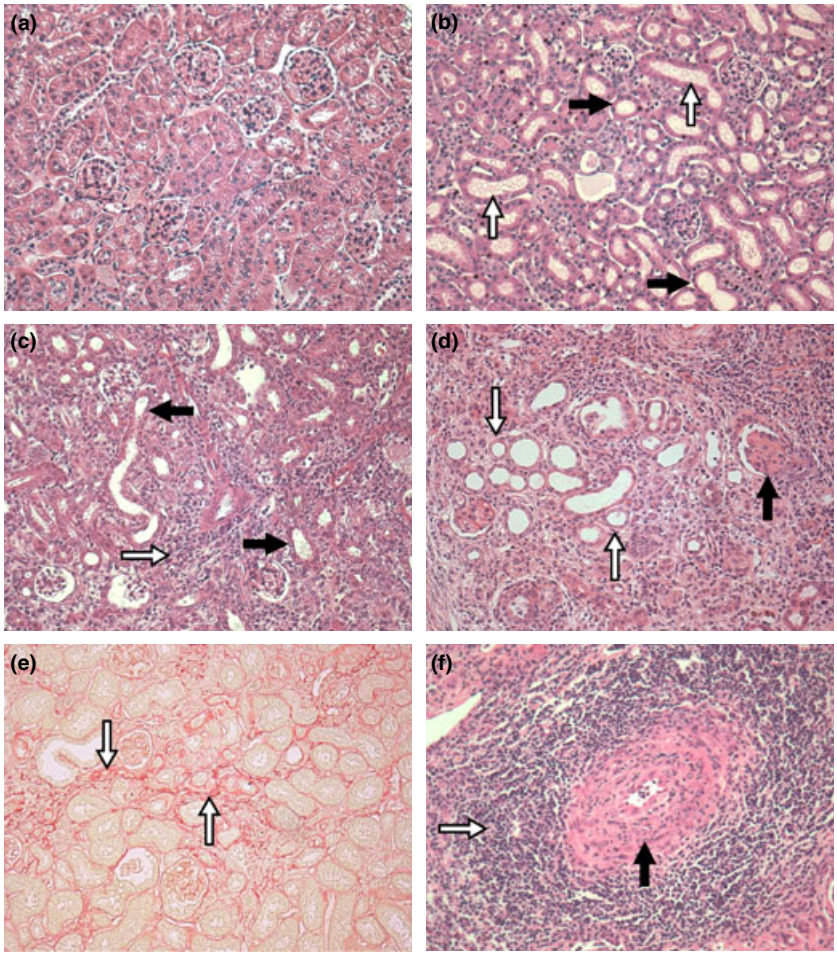

Figure 2 Histology of the transplanted kidney (×200 magnification, Periodic acid-Schiff unless stated otherwise). (a) Syngeneic kidney transplant at 2 weeks showing viable glomeruli and tubules with normal kidney histology. (b) Ischaemia and reperfusion of the kidney results in acute tubular injury which resolves over three to 5 days. Dilated tubules with intra-luminal casts (hollow arrow ⇧) are easily identified along with epithelial flattening (block arrow ⬆). (c) Acute rejection is characterized by interstitial mononuclear cell infiltrate (hollow arrow ⇧) and inflammatory changes such as tubulitis (block arrow ⬆), occurring in combinations such as BALB/C → FVB/NJ by 2 weeks [70]. (d) Chronic damage to the allograft results in tubular atrophy (hollow arrow ⇧) with evidence of glomerulosclerosis (block arrow ⬆), occurring in combinations such as C57/BL6^{BM12} → C57/BL6 [72]. (e) Deposition of collagen as a marker of interstitial fibrosis can be identified by picrosirius red staining, which identifies fibrillar collagen (hollow arrow ⇧). This is easily observed in an allograft over 8 weeks after transplantation in the strain combination C57/BL6^{BM12} → C57/BL6 [72]. (f) Chronic damage to the allograft may be associated with intimal hyperplasia and vasculopathy (block arrow ⬆) and associated with perivascular mononuclear cell infiltration (hollow arrow ⇧), occurring in combinations such as C57/BL6^{BM12} → C57/BL6 at 8 weeks following transplantation [72].

deposited in glomeruli by 30 days after transplantation [21]. Acute antibody-mediated rejection can be induced by presensitization of the recipient with donor strain skin graft, this resulted in development of rejection within 19 days, compared with 75% of nonsensitized recipients surviving over 60 days [33].

Humoral rejection: complement

The importance of systemic and locally produced complement in rejection has been probed using this model. Complement activation by antibody within the kidney has been identified by C3d deposition in peritubular capillaries, glomeruli and mesangium [32,33]. C3d is the final degradation product of the third complement component and directly binds to the antigen. C4 has also been observed on peritubular capillary walls [6,35]. Similarly C4d, which is a well-recognized marker of antibody mediated rejection in humans, has been identified in peritubular capillaries [44].

Using several transgenic strain combinations, individual components of the complement system have been knocked-out. Blockade of endothelial C5a receptor, using a monoclonal antibody, significantly improved survival in a donor–recipient strain combination that results in acute

rejection [40]. Furthermore knockout of C3 synthesis in donor kidney cells resulted in a weakened response to donor antigens and rejection was less vigorous with defective T-cell priming [31]. However, loss of C4 expression in the donor or recipient did not affect rejection suggesting that in multiple strain combinations complement activation occurs through a C4-independent pathway [36].

Chronic allograft damage

The first studies in mice showed variable rejection, with some reporting acceptance in allografts; however, it was observed that 'glomerular damage seems to be slowly progressive over the duration of survival of the allografts' and the mechanism for this was unknown [10]. Skin grafts following kidney transplantation strengthened the evidence for regulation of the rejection response where secondary skin grafts developed slow rejection and scarring compared with skin transplanted to naïve mice resulting in acute rejection [2,10,17]. In human kidney transplantation, chronic damage is characterized by interstitial fibrosis and tubular atrophy, similarly in a mouse model with minimal MHC class II disparity, this pathology develops and can be easily identified and quantified (Fig. 2) [72,82]. Chronic damage of the renal allograft is also characterized by transplant arteriosclosis with fibrointimal hyperplasia affecting intrarenal blood vessels [48,65]. Furthermore in this model of transplant vasculopathy, a perivascular infiltrate of macorphages and cytotoxic T cells was found [48,57].

Tolerance

There is a recent evidence that infiltrating inflammatory cells may not necessarily be destructive to the allograft and may be subjected to regulation or tolerance. Following transplantation, a perivascular infiltration of mononuclear cells has been identified in several strain combinations [30,33,34,44,72]. Furthermore cuffs of leukocytes around vessels and small aggregates of mononuclear cells within the cortex have been associated with accepted kidney allografts [32]. In MHC disparate allografts that have been described to spontaneously develop rejection or tolerance, the number of graft-infiltrating Foxp3+ regulatory T cell was shown to strongly correlate with renal function [37]. In the accepted allografts prominent lymphoid sheaths around arteries contained CD3+ Foxp3+ cells, CD4+ cells, dendritic cells and B cells [34,44]. Furthermore depletion of Foxp3+ cells using a transgenic model resulted in widespread CD8+ interstitial mononuclear inflammation, tubulitis and endarteritis [44].

Further evidence of tolerance is suggested in experiments that measure donor skin tumour growth on a recipient mouse. When skin is transplanted with a donor strain kidney, the tumour grew to a larger size with delayed rejection, and indeed without rejection in some mice, compared with those who had not received a kidney transplant [19]. Inhibition of all T cells accessing the allograft may be detrimental as demonstrated in a strain combination that has been reported to support long-term acceptance. In this model, acute rejection can be induced by transplantation in to the same strain recipient with C-C chemokine receptor-5 (CCR5) knockout, CCR5 is an important receptor for T cell attraction [32]. Compared with control mice the knockout T-cell infiltration was similar 10 days after transplantation; however, a possible explanation could be that the regulatory T cells that are dependent on this signalling pathway were unable to enter the allograft. Furthermore CCR5 knockouts developed higher titres of donor-specific antibody suggesting T-cell attraction into the graft may be required to abrogate the generation of an antibody response. In addition, blockade of T-cell activation by inhibiting the inducible co-stimulatory molecule pathway resulted in worse graft function and survival compared with control kidneys [39].

Key mechanisms: cellular components

Various experiments have been performed that elucidate the key roles played by specific cells of the immune system. In mismatched donor and recipients, the presence of CD4+ and CD8+ T cells in the transplanted kidney have been identified in several studies [44,52,56]. In long-surviving allografts, CD8+ T cells have been shown to down-regulate their T-cell receptors, which can be reversed *ex vivo*, suggesting an *in vivo* regulatory process within the allograft [56]. Transplantation of MHC mismatched kidneys into hosts lacking mature B cells and an inability to produce immunoglobulin did not appear to alter cellular infiltration and tubulitis, however, the allografts did have reduced arteritis, venulitis and oedema [62]. Furthermore nude mice, that have a greatly impaired immune system because of absent T cells, do not mount a rejection response against a MHC mismatched kidney allograft [62].

In mouse, donor–recipient strain combinations where rejection is acute, inhibition of the alloimmune response by manipulation of MHC expression has been attempted. MHC class I deficient donors resulted in better renal function but no difference in histological injury compared to those with intact MHC class I molecules, whereas MHC class I deficient recipients had no difference in function or histological rejection [52,53,57]. In addition, the cellular rejection response characterized by infiltration of T cells and macrophages was not influenced by MHC class I deficiency in the donor kidney [53]. Kidney allograft rejection may be associated with *de novo* expression of MHC class II molecules in tubular cells [18], as well as in the perivascular areas and glomeruli [52]. Using scintigraphy the peak

MHC class II expression has been shown to occur at 6 days following transplantation in rejecting allografts [55]. Specifically, donor MHC class I and II appear to be localized to the basolateral aspect of the epithelium [59,60,63]. However, when tubular epithelial cells are induced to express high levels of MHC class II by gene insertion, the rejection process did not appear to be worsened [11]. The normal mouse kidney contains considerably fewer MHC class II cells, such as dendritic cells, than are seen in the human [83], however, following transplantation there is marked up-regulation of MHC class II molecules [18]. The mouse allograft also mirrors findings in human transplantation where older donor kidneys have been associated with worse function [84]. Transplantation of old donors aged 18 months compared with young 3 month old donors resulted in a more rapid emergence of epithelial changes and markers of senescence, however, there was no difference in MHC class I or II expression following transplantation [71].

Our own studies have focused on the importance of monocytes and particularly the contribution of renal macrophages to the rejection process. Following transplantation diphtheria toxin induced knockout of recipient macrophages reduced arteritis, tubulitis and micro-vascular injury [70]. Furthermore in a mouse model of interstitial fibrosis and tubular atrophy it appears that macrophage mediated fibrosis in the allograft acts through galectin-3, a beta-galactoside-binding lectin, as galectin-3 knockout recipients have reduced fibrosis [72].

Key mechanisms: cytokines

The importance of cytokines in mediating processes of rejection has been investigated using mouse kidney transplantation. Particularly interferon-γ has been identified as a key component by acting through the transcription factor IRF-1 [61]. MHC-induction by donor kidney cells appears to be dependent on interferon-γ and associated with inflammatory cell infiltration, however, in interferon-γ receptor knockout donors the allograft succumbs to early thrombosis, congestion and necrosis suggesting interferon-γ may also have a protective role [59]. Furthermore interferon-γ induces transcription of mRNA associated with MHC function, factors in antigen presentation and complement components [67].

Development of therapeutic agents

The model has allowed the study of novel immuno-suppressive agents. Indeed administration of rapayamcin and cyclosporine increased mouse survival in a strain combination characterized by acute rejection [28]. Although the majority of studies using the mouse model of kidney transplantation have been to investigate the rejection process induced by allograft disparity syngeneic-grafts have also been utilized as a model of ischaemia reperfusion in transplantation [49]. For example, the pretreatment of donor mice with an antibody against Toll-like receptor 2 prior to syngeneic transplantation improved graft function [49].

Targeting specific cells for depletion in the mouse has allowed testing of potential therapeutic monoclonal antibodies. B cell depletion by CD19 monoclonal antibody treatment significantly reduced kidney allograft rejection and abrogated allograft specific IgG development, whereas CD20 monoclonal antibody treatment did not [42]. Reversal of acute rejection with an antibody against CD45RB, an isoform of CD45 expressed by multiple leukocytes, also improved survival in a transplant dependent model and this was associated with a reduction in TNF-α expression [22–24, 26,27]. Rejection has also been shown to be modulated by monoclonal antibody blockade of CD154, a co-stimulatory molecule, by differentially targeting T-effector and T-regulatory cell subsets this resulted in a regulated intra-graft induction of chemokines and was associated with improved survival and graft histology [41]. Therefore, it is apparent that the mouse models are a useful testing ground for drugs and therapeutic molecules as well as to dissect rejection mechanisms.

Difference between kidney and other transplantation models

The seminal work by Billingham *et al.* first investigated transplantation and rejection through skin grafts in mice [85], however, there is gathering evidence that this experimental model may not adequately reflect the immune response to vascularized solid organs. Transplantation of other solid organs been described in addition to the kidney transplant model and the kinetic and severity of the rejection response are different in all these organs. In an extensive study by Zhang *et al.* the rejection pattern after liver, kidney, heart and small bowel transplantation in three different mouse strain combinations was investigated [25]. The majority of liver allografts were spontaneously accepted, defined by survival greater than 100 days despite complete MHC disparity, whereas a mixed pattern of acute rejection and acceptance occurred in kidney recipients. In this study, all the cardiac and intestinal allografts were rapidly rejected within a mean time of less than 10 days and no spontaneous acceptance [25]. Several separate groups have directly compared heart transplantation with kidney transplantation in the same strain combination and found that kidney allografts have a significantly prolonged survival compared with a heart allograft [30,45]. An example is the combination of a DBA/2 donor into C57/BL6 recipient where kidney transplantation led to 80% survival over

60 days whereas cardiac transplantation resulted in rejection within 7–10 days and skin rejection in 15–17 days [30]. Furthermore in a donor-recipient strain combination where rejection occurs, 84.4% of CD8+ T cells isolated from cardiac allografts were α/βTCR+ compared with 44% in the kidney allografts suggesting a down regulation of this receptor in the latter model [56]. An explanation for the differences between the rejection process elicited by kidney or cardiac transplantation is likely to be explained by the different antigen recognition pathway by the recipient as following kidney transplantation there appeared to be a greater migration of donor MHC class II cells to the spleen compared with the following cardiac transplantation [38].

Other applications of kidney transplantation

This model has been utilized for studies other than those investigating rejection such as to explore disease models by separating intrinsic renal abnormalities from that that of circulating factors. For example, in a mouse model of X-linked hypophosphatemic rickets, a genetic metabolic disturbance, kidney transplantation was performed with wild-type mice as both kidney recipient and donor with the phenotype strain. This allowed the exclusion of an intrinsic renal abnormality to be the reason for the phenotype and the identification of a humoral factor to be causative, as a wild type kidney did not correct the hypophosphataemia [73]. Similarly, to dissect the contribution of circulating humoral factors to the kidney phenotype in a model of glomerulonephritis New Zealand White mice kidneys, which do not develop an abnormal kidney phenotype, were transplanted into F1-hybrid New Zealand White and Black mice, which spontaneously develop glomerulonephritis; this experiment resulted in the native kidney and the allograft-developing glomerulonephritis [20].

The model has also been used as a means to develop *in vivo* imaging techniques. To examine the ischaemia reperfusion injury in syngeneic grafts real-time immunofluorescence-based microscopy has been trialled, allowing imaging of functional and structural changes within the kidney caused by inflammation [50]. Potentially, this may lead to novel techniques to monitor rejection in human transplantation.

Three studies have used the mouse kidney transplant model to investigate the effect of transplant-associated viral infections [35,46,47]. In mice infected with polyomavirus the allograft developed worse histological injury with increased numbers of CD8+ T cells compared with virus-free recipients [35]. Similarly, acute infection of the donor with BK virus worsened rejection between MHC disparate allografts, and acute infection of the recipient on day 1 resulting in 100% mortality [46]. A model of a donor-derived viraemia has shown latent cytomegalovirus can be reactivated following transplantation resulting in disseminated primary infection when transplanted in to mice that are deficient in T cells, B cells and natural killer cells [47].

Conclusion

We have reviewed all the current studies that have described a mouse kidney transplant and documented the strain combinations and the resultant survival and histology where available. We believe this review will serve as a reference point for all researchers interested in developing a mouse kidney transplant model and identify which strain combinations have been examined previously. The strength of this mouse model of intra-abdominal kidney transplantation is that it directly mirrors that of human transplantation, therefore the rejection dynamics are analogous and findings translatable. This model will continue to reveal important facets to transplantation and allow the development of novel treatments for ischaemia reperfusion injury and rejection, with potentially the keys to understanding and inducing immunological tolerance.

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