

Experimental rat models of chronic allograft nephropathy: a review

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Abstract: Chronic allograft nephropathy (CAN) is the leading cause of late allograft loss after renal transplantation (RT), which continues to remain an unresolved problem. A rat model of CAN was first described in 1969 by White et al. Although the rat model of RT can be technically challenging, it is attractive because the pathogenesis of CAN is similar to that following human RT and the pathological features of CAN develop within months as compared with years in human RT. The rat model of RT is considered as a useful investigational tool in the field of experimental transplantation research. We have reviewed the literature on studies of rat RT reporting the donor and recipient strain combinations that have investigated resultant survival and histological outcomes. Several different combinations of inbred and outbred rat combinations have been reported to investigate the multiple aspects of transplantation, including acute rejection, cellular and humoral rejection mechanisms and their treatments, CAN, and potential targets for its prevention.

Keywords: interventions, therapy, late allograft loss, renal transplantation

Introduction

Despite the advances in immunosuppressive agents, chronic allograft nephropathy (CAN) remains the leading cause of late allograft loss following renal transplantation (RT).¹ Experimental research has been undertaken in animal models to understand the pathogenesis and molecular biology of CAN and to identify potential therapeutic targets for its prevention and treatment.^{2,3}

In humans, CAN develops slowly over months and years before being fully established. In rat models of CAN, impairment of renal function and histopathology similar to that described in humans can be produced in a few months, allowing investigators to study its pathology within a reasonable period of time. The factors implicated in the pathogenesis of CAN in the rat mimic the pathogenesis of CAN in human clinical RT; particularly to RT from a live kidney donor source, since the cold-ischemia time is short in both situations. Thus, the rat provides an important model of RT in which to study the pathology and potential treatment of CAN.⁴ The aim of this paper is to review the published literature on the models of CAN and to explore their advantages and disadvantages and the interventions undertaken.

Literature search

The literature search was carried out in PubMed and relevant websites using the words “renal transplantation”, “chronic allograft nephropathy”, “rat”, “animals”, “transplant fibrosis”, and “prevention”. Relevant references were compiled in the

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EndNote (X6.0.1, Bld 6599; Thomson Reuters, New York, NY, USA) software.

Advantages of rat models

RT techniques have been described in pigs, dogs, rabbits, rats, and mice.⁵⁻⁹ Compared with larger animals, the advantages of using the rat include 1) improved long-term survival of renal transplants due to the use of inbred strains, 2) lower cost of experimental animals, 3) simple animal maintenance which does not require sophisticated facilities, 4) less critical requirement for aseptic surgery, and 5) well established techniques of vascular and ureteric anastomoses for RT.^{10,11}

Strains of rats

The first description of RT in rats was presented by Bernard Fisher and Sun Lee at the American College of Surgeons Meeting in Chicago in 1961 and subsequently published in 1965.^{8,12} RT was performed using inbred Lewis (LEW) rats, and therefore no immunosuppressive regimens were required. In subsequent studies, the following rat strains were used as either donor or recipient: Fisher 344 (inbred (^{ln})), Sprague Dawley (SD) (outbred (^{ou})), Wistar Furth (WF) (outbred (^{ou})), LEW (inbred (^{ln})), brown Norway (BN) (inbred (^{ln})), and Dark Agouti (DA) (inbred (^{ln})). Either inbred or outbred strains may be used depending upon the aim of the individual study.^{10,13-23}

The following combinations such as Fisher 344-to-LEW, DA-to-BN, DA-to-WF, DA-to-LEW, SD-to-WF, and LEW to-BN have been used to induce CAN, with both advantages and disadvantages to each model.²³⁻³³ However, since the Fisher 344-to-LEW model of RT is the most commonly employed in experimental studies, the establishment and application of this combination is described in detail.

Fisher 344-to-LEW model of CAN Establishment of the model

White and Mullen³⁴ described in 1969 for the first time RT using the Fisher 344 (F344) rat as donor and the LEW rat as recipient to produce a model of chronic rejection. Although the LEW-to-LEW isograft maintains relatively well preserved renal function, F344 allografts develop clinical and histopathological features of CAN within a few weeks to months after RT.³⁵ Paul et al³⁹ demonstrated that RT between F344-to-LEW rats survives spontaneously, without the use of immunosuppressive drugs. The F344-to-LEW model is useful because the rat strains differ partially at major histocompatibility complex (MHC) (class I and class II) and various

non-MHC loci, thereby making a weakly histocompatible combination that allows RT without immunosuppression, which cannot be done in humans. LEW rats receiving an F344 graft develop raised glomerular pressure and proteinuria after 1 week post-RT, which gradually evolves into CAN.³⁶ The histological changes occur predominantly in the glomeruli showing mesangial expansion, glomerular fibrosis, intimal thickening of blood vessels, dilatation of capillaries, splitting of capillary basement membrane, and deposition of eosinophilic materials in capillary loops. Classical changes of CAN in the vascular and tubulointerstitium occur simultaneously or later, but similar to lesions seen in human RT.¹² Vass et al³⁷ observed marked increase in the number of perivascular and interstitial lymphatic vessels in the allografts, which correlated with the extent of interstitial fibrosis.

The same investigators also demonstrated that LEW rats with long-standing F344 RT produce antibodies against both glomerular and tubular basement membranes.³⁸ Subsequently, antibodies directed against mesangial and endothelial cells have been detected in the sera of the allografts with long-standing RTs. Western blot analysis, followed by immunoprecipitation, has identified the antibody-binding sites on mesangial cells to be biglycan and decorin.³⁹ It is recognized that both biglycan and decorin appear to play roles in the tissue repair process and bind transforming growth factor (TGF)- β , a profibrotic cytokine which may play a major role in CAN. Antibodies binding to biglycan and decorin appear to have the effect of generating more "free" TGF- β to interact with its receptors and interfere with the stimulation of matrix metalloproteinases. TGF- β inhibits the proliferation of mesangial cells and may play a role in renal fibrosis by stimulating mesangial cells to produce matrix proteins. Fibroblasts are also stimulated by TGF- β to produce matrix protein.⁴⁰

In CAN, the alteration of the extracellular matrix compartment and proliferation of various cell types are regulated by the metzincin superfamily of metallo-endopeptidases, including matrix metalloproteinases (MMPs), a disintegrin and metalloproteinase (ADAM) and meprin. Berthier et al⁴¹ observed that members of metzincin families (MMP, ADAM, and meprin) and of tissue inhibitors of metalloproteinase (TIMPs) are differentially regulated in CAN in the F344-to-LEW model. Messenger ribonucleic acid (mRNA) levels of MMPs (MMP-2/-11/-12/-14), their inhibitors (TIMP-1/-2), ADAM-17, and TGF- β 1 significantly increase in CAN. In contrast, mRNA levels of MMP-9/-24 and meprin α/β downregulate significantly.^{41,42}

Cyclosporin

Diamond et al²⁴ from Boston, USA, used cyclosporin (5 mg/kg/day) for the first 10 days post-RT to prevent acute rejection and studied the changes in renal function, morphology, and immunohistology between F344-to-LEW allografts and LEW-to-LEW isografts over the course of 24 weeks. A higher urine albumin excretion was demonstrated in the allografts as early as 4 weeks after RT, which rose dramatically by 20 weeks. Serum creatinine was elevated in allografts after 16 weeks, while renal blood flow and glomerular filtration rate (GFR) measured by p-aminohippurate and inulin clearances, respectively, were significantly lower in the allografts at 24 weeks. The frequency of glomerulosclerotic lesions was significantly increased in allografts and correlated with albumin excretion. There was significantly greater infiltration of the glomeruli with cluster of differentiation (CD)8⁺ and pan-T-cells in the allografts.²⁴ Grenz et al⁴³ demonstrated significant improvement in GFR in the same model when theophylline was given (10 mg/kg, subcutaneously) (GFR of allografts 0.23 ± 0.05 mL/min/g kidney weight [n=10] compared with 0.50 ± 0.09 mL/min/g in rats receiving theophylline [n=9, $P < 0.01$]) without affecting the interstitial infiltration of the graft by monocytes/macrophages and T-cells. Likewise, serum cytokines interleukin (IL)-2, IL-6, IL-10, tumor necrosis factor (TNF)- α , and erythropoietin plasma levels were not different among the allograft groups.⁴³ In both studies, cyclosporin given for the first 10 days prevented acute rejection and allowed development of CAN in the allografts.

Alloantibodies, cytokines, and adhesion molecules

Hancock et al⁴⁴ from Boston examined the host immune mechanisms responsible for the initiation and progression of chronic rejection in the same model. Peak levels of immunoglobulin (Ig)M and IgG alloantibodies were demonstrated at 2–4 weeks by flow cytometry, with a gradual decline to baseline thereafter. Immunohistology showed early and progressive deposition of IgM, IgG, C3, and fibrin in the vessel walls and glomeruli. In addition, by 12 weeks, extensive infiltration by activated IL-2 receptor positive (IL-2R⁺) macrophages and CD4⁺ T-cells could be demonstrated in the glomeruli and blood vessels, in conjunction with staining for cytokines such as TNF- α , IL-1, and IL-6. The persistent and dense intraglomerular expression of IL-6 indicated its potent mitogenic effect on mesangial cells leading to glomerulosclerosis.

There was also increased expression of intercellular adhesion molecule-1 (ICAM-1) in the graft vascular endothelium,

which peaked at 16 weeks and showed increased binding in vitro to naïve host lymphocytes.⁴⁴ The authors concluded that an antibody-mediated host response followed by a cellular response was responsible for the release of cytokine production and upregulation of adhesion molecules which induced the scarring process leading to graft loss.

Prevention of chronic rejection

Manipulation of the cellular immune response

Several experiments have been carried out in the F344–LEW model to abrogate the alloantigen-driven histological and immunological events in order to delay or prevent alloantigen recognition. T-cell activation requires two steps: namely, binding of T-cell receptor to the antigen presented by the antigen-presenting cells (APCs) (signal 1) followed by co-stimulation (signal 2) through binding of CD28 molecule of the T-cell to CD80 (B7-1) and CD86 (B7-2) on the APC. Blockade of the co-stimulation pathway (CD28–B7) produces a state of anergy, a failure of T-cell activation, which is also produced by CD152 (cytotoxic T-lymphocyte antigen-4; CTLA-4) combined with CD28 and CD86. Alternatively, CD40 ligand on T-cells can combine with CD40 on the APC to initiate the co-stimulation signal leading to activation of these cells.

Blockade of the co-stimulation pathway by the infusion of CTLA-4-immunoglobulin, which binds to B7 molecules on the surface of the APC, leads to significant reduction of proteinuria, glomerulosclerosis, intimal hyperplasia, and mononuclear cellular infiltration.⁴⁵ Similarly, administration of an anti-CD28 monoclonal antibody significantly preserves graft morphology and recipient survival in this model by blocking the T-cell co-stimulation pathway.⁴⁶

Blockade of chemokines

Chemokines are a family of small-size (8–10 kDa) chemotactic cytokines which, in transplantation, induce the recruitment and activation of T-cells and monocytes or macrophages. So far, more than 50 chemokines and 20 chemokine receptors have been identified. Members of the chemokine family are divided into four groups (C, CC, CXC, and CX3C) based upon the position of their first two cysteine residues. They are further classified as inflammatory or hemostatic.

In F344-to-LEW rat allografts, BX-471, a chemokine receptor type 1 (CCR1) antagonist, prevents the infiltration of T-cells and macrophages, decreases cell proliferation (ED1, CD8, and Ki67), and reduces expression of acute phase reactive proinflammatory genes (heme oxygenase-1, osteopontin) and molecules associated with fibrosis (plasminogen activator inhibitor-1, TGF- β , and biglycan).

There is a significantly lower number of smooth muscle actin-positive interstitial myofibroblasts and a reduction in the deposition of collagen.⁴⁷

Similarly, Met-RANTES (Regulated on Activation, Normal T-cell Expressed), a chemokine receptor antagonist (CCR5), blocks the effects of RANTES in F344-to-LEW allografts, reducing the infiltration of lymphocytes and macrophages, accompanied by decreased mRNA expression of IL-2, IL-1 β , TNF- α , and RANTES resulting in reduced glomerulosclerosis, tubulointerstitial fibrosis, and proteinuria.⁴⁸

Immunosuppressive agents

Immunosuppressive drugs have reduced the incidence of acute rejection and improved graft survival. However, the majority are associated with side effects such as the nephrotoxicity of calcineurin inhibitors (CNIs), which are known to contribute to the development of CAN.⁴⁹ Mycophenolate mofetil (MMF), in contrast, acts by inhibiting de novo DNA synthesis, cell surface molecule expression, and antibody production. MMF reduces CAN and improves allograft survival when given immediately or 8 weeks after RT.⁵⁰ In the same model, Luo et al⁵¹ examined the effects of tacrolimus with MMF on renal fibrosis and observed progressive deterioration of renal function and CAN morphology in the vehicle and tacrolimus groups, while serum creatinine and inflammatory infiltration (Banff score) showed a significant decrease in the MMF group after 8 weeks post-transplantation compared with those in the other groups. Furthermore, expression levels of connective tissue growth factor and α -smooth muscle actin in the MMF group were significantly reduced, and the downregulated expression of E-cadherin was abated.⁵¹

Sirolimus and its derivative, everolimus, significantly reduce infiltration of the allograft with macrophages and lymphocytes and reduce expression of TGF- β , ICAM-1, and VCAM (vascular cell adhesion molecule)-1, reducing interstitial fibrosis and tubular atrophy (TA) and prolonging allograft survival.⁵²⁻⁵⁴

Ko et al,⁵⁵ in the same model, observed greater protection against vasculopathy in the sirolimus-treated group, but sirolimus induced proteinuria. The effects were likely to be related to inhibition of vascular endothelial growth factor signaling.⁵⁵ In the same model, Luo et al⁵¹ concluded that sirolimus ameliorated fibrogenesis in kidney allografts by inhibiting the epithelial mesenchymal transition (EMT) process, whereas cyclosporine did not have this effect.

Oxidative stress

Oxidative stress is increased in the F344-LEW rat transplant model and is associated with EMT and chronic interstitial fibrosis. Nicotinamide adenine dinucleotide phosphate (NADPH) oxidase (Nox) is an important source of reactive oxygen species generation in the kidney. Inhibition of NADPH-2 (Nox-2) by diphenyleneiodonium or apocynin reduces fibronectin and phosphor-Smad2 and increases E-cadherin levels, thereby inhibiting EMT and fibrogenesis.⁵⁶ In the same model, L-arginine decreases proteinuria and glomerulosclerosis.⁵⁷ However, vitamin E supplementation alone does not prevent CAN.⁵⁸ Both in human RT recipients and in F344-to-LEW allografts, MMF significantly downregulates Nox-2 activation and EMT through the nuclear factor- κ B pathway in tubular epithelial cells.⁵⁹

Angiotensin antagonists

Angiotensin II increases the expression of cytokines and adhesion molecules thereby stimulating the synthesis of extracellular matrix and increasing glomerular capillary pressure. Administration of an angiotensin converting enzyme inhibitor (trandolapril) or angiotensin II receptor blocker (losartan) in this model leads to diminished expression of chemokine MCP-1 (monocyte chemoattractant protein-1), preservation of glomerular and tubulointerstitial structures, reduced proteinuria, and improved graft survival.^{60,61} Combination of losartan and MMF completely prevents the development of proteinuria, largely reduces glomerular and tubulointerstitial injury, and suppresses intragraft cell infiltration, thereby producing a better outcome in comparison with transplants receiving a single agent.⁵⁰

Lipid lowering agents

In the F344-to-LEW renal allograft model, pravastatin reduces macrophage and T-cell graft infiltration and decreases intragraft expression of TGF- β , causing an attenuation of the recipient anti-donor IgG alloantibodies and upregulation of the anti-apoptotic gene *Bag-1*.⁶²

Mineralocorticoid receptor antagonist agent

The mineralocorticoid hormone aldosterone is involved in development and/or progression of renal diseases via interaction with a non-epithelial mineralocorticoid receptor (eg, reducing neointima formation). Lahmer et al⁶³ examined the effects of the aldosterone synthase inhibitor fadrozole and the mineralocorticoid receptor antagonist spironolactone compared with vehicle in the F344-to-LEW rat model of CAN. Fadrozole- and spironolactone-treated animals

demonstrated a higher degree of proteinuria, pathologically elevated potassium values, higher tubulointerstitial damage and glomerulosclerosis, and markedly increased heart weight/bodyweight as compared with vehicle. The positive effects of inhibition of aldosterone as described in cardiac models could not be detected in kidney recipients.⁶³

DA-to-BN model of CAN

DA-to-BN rat model, which requires triple immunosuppression with cyclosporin (5 mg/kg/day), azathioprine (2 mg/kg/day), and methylprednisolone (2 mg/kg/day), has been extensively studied by Soots et al.²³ This model developed an early acute rejection 5–7 days after RT and chronic rejection within 40–60 days after RT, with chronic allograft damage index (CADI) score of 7.9 ± 3.1 compared with syngenic transplants, which showed no evidence of acute or chronic rejection (CADI: 1.7 ± 2.0). In the same study, combinations of other strains of rats such as DA-to-albino Oxford and piebald-viral-Glaxo (PVG)-to-BN were examined. The DA-to-albino Oxford combination demonstrated a prolonged acute rejection, but no characteristic chronic changes. The PVG-to-BN combination did not show changes of either acute or chronic rejection. The grafts were monitored with ultrasound-guided fine-needle aspiration cytology. This study concluded that the experimental model of DA-to-BN of RT resembles the CAN seen in human clinical RT.²³

The same authors have used the DA-to-BN model to demonstrate a significant rise in the collagen/DNA ratio in cytomegalovirus (CMV)-infected grafts compared with uninfected animals, correlating with the development of fibrosis. The expression of type I and III collagen mRNA and presence of myofibroblasts was associated with interstitial fibrosis in CMV-infected grafts. It was concluded that CMV accelerated both atherosclerosis and the development of CAN in rat allograft kidneys.^{64,65} Soots et al⁶⁶ examined the effect of flushing the donor kidney with Euro-Collins[®] solution (Fresenius Aktiengesellschaft, Oberursel, Germany) containing sodium nitroprusside solution in this model and observed significant reduction in CADI score of TA and interstitial fibrosis with the use of sodium nitroprusside.⁶⁶

DA-to-WF model of CAN

Yilmaz et al⁶⁷ studied chronic rejection in the DA-to-WF where the allografts, given cyclosporin (5 mg/kg/day, subcutaneously) for 1 week, developed chronic rejection 3 months post-transplantation. Allografts receiving cyclosporin (5 mg/kg/day, subcutaneously) for 12 weeks did not show changes of chronic rejection. In this model,

prolonged use of cyclosporin prevented the development of acute rejection. Syngenic DA-to-DA RTs did not show any evidence of chronic rejection.^{67,68} Savikko et al⁶⁹ investigated the role of platelet-derived growth factor (PDGF) in the same model and demonstrated that administration of the imanitib (10 mg/kg/day, orally), a PDGF inhibitor, for the first 30 days after RT completely prevented development of CAN at 90 days in the treated group. An immunosuppressive drug, FK778, inhibits de novo pyrimidine biosynthesis and several receptor tyrosine kinases. Rintala et al⁷⁰ investigated its effects on acute and chronic rejection as well as post-transplant PDGF and TGF- β expression in combination therapy with CNIs. FK778 in combination with CNIs significantly reduced both early and late PDGF and TGF- β expression and development of CAN.

DA-to-LEW model of CAN

Pascher et al³¹ have described the development of CAN using DA rats as donors to LEW recipients in a variety of immune-modulating models. One model, considered to be a high-responder immunologically, was used in experiments which involved the use of carbon monoxide to reduce immunogenicity and thereby the development of CAN.⁷¹ Transplanting kidneys from DA to LEW rats, without immunosuppression, leads to death of rats from acute rejection some 7 days after transplantation.⁷² Denecke et al¹¹ had examined the effect of low-dose cyclosporine in the same model and observed induction of donor hypo-responsiveness to secondary renal allografts, significantly reduced alloantibody titers in the primary recipients, and dose-dependent and transferable “pro-tolerogenic” effect of low-dose cyclosporine treatment.¹¹

SD-to-WF model of CAN

The SD-to-WF combination is an accelerated model of CAN, where histological evidence of CAN appears as early as 4 weeks post-RT. Teng et al⁷³ examined the expression of the cytoprotective genes *A20*, *heme oxygenase (HO)-1*, *Bcl-2*, and *Bcl-XL* in this model using three different immunosuppressive regimens. All animals received cyclosporin 10 mg/kg/day for 10 days, but two further groups were maintained on either cyclosporin 6 mg/kg/day or MMF 20 mg/kg/day. At the end of 8 weeks, CAN was evident in all groups, but the expression of *A20* in grafted kidneys was significantly higher in the MMF than in the cyclosporine group, helping to explain the mechanism by which MMF ameliorates transplant arteriosclerosis in experimental chronic rejection. There was no significant difference between the cyclosporin and the

MMF groups in the expression of *HO-1*, *Bcl-2*, and *Bcl-XL*.⁷³ Similar results were observed when rapamycin was compared with tacrolimus in this model.⁷⁴

Fractalkine is a unique chemokine that functions both as a potent chemoattractant molecule (soluble form)¹ and as an adhesion molecule (membrane anchored form) for cells expressing the fractalkine receptor CX3CR1, such as monocytes, NK (natural killer) cells, and subsets of CD8⁺ T-cells, involved in chronic transplant arteriosclerosis. Cao et al³² demonstrated increased expression of the fractalkine receptor CX3CR1 in the SD-to-WF model of RT. Fractalkine/CX3CR1 was mostly expressed in the tubulointerstitium and tubular epithelial cell basolateral membrane. A proportion of the vessel showed positive staining for fractalkine/CX3CR1, occasionally in glomerular parietal wall cells, was significantly lower in MMF than cyclosporine-treated animals.³²

LEW-to-BN model of CAN

Transplanting kidneys from LEW-to-B (RT¹ⁿ) rats shows interstitial mononuclear cell infiltration, tubulitis, and glomerulitis, in addition to early phase of arteritis at 30 days. By 80 days, TA is seen in 25%–50% and interstitial fibrosis in up to 25% of renal cortex. There is focal, diffuse, segmental, or globular glomerulosclerosis. In a study by Neto et al,³³ all recipients had received tacrolimus (0.5 mg/kg/day) for 7 days. Cardinal et al⁷⁵ demonstrated that the administration of molecular hydrogen dissolved in water to this model slowed the progression of CAN, reduced oxidant injury and inflammatory mediator production, and improved overall survival. Inflammatory signaling pathways, such as mitogen-activated protein kinases, were less activated in renal allografts from hydrogen water-treated rats as compared with regular water-treated rats.⁷⁵

WF-to-LEW model of CAN

Solini et al⁷⁶ developed a model of CAN using a fully MHC-mismatched rat strain combination, with WF rats as kidney donor and LEW rats as recipients. The two strains differ for class I, class II, and non-MHC genes. Cyclosporin (5 mg/kg/day, intramuscularly) needed to be given for the first 10 days to prevent acute rejection. At 120 days, the allografts developed features of CAN and donor-specific antibodies and chronic antibody-mediated rejection.⁷⁶ A few studies have been carried out in this model, which include gene transfer of CTLA-4 Ig into donor kidney, leading to prevention of progressive proteinuria and CAN, and transfer of donor-specific T helper-2 clones into recipient rats to regulate alloimmune response and prevention of CAN.^{77,78}

Conclusion

We reviewed the relevant published literature that described RT in rat models of CAN employing combinations of strains and the outcomes of various interventions. We believe that the review will help researchers to understand the application of various rat models of CAN in understanding the molecular mechanisms and development of novel treatments for CAN.

Disclosure

The authors report no conflicts of interest in this work.

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