Animal Models of Chronic Allograft Injury: Contributions and Limitations to Understanding the Mechanism of Long-Term Graft Dysfunction

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Advances in immunosuppression have reduced the incidence of acute graft loss after transplantation, but long-term allograft survival is still hindered by the development of chronic allograft injury, a multifactorial process that involves both immunologic and nonimmunologic components. Because these components become defined in the clinical setting, development of animal models enables exploration into underlying mechanisms leading to long-term graft dysfunction. This review presents animal models that have enabled investigation into chronic allograft injury and discusses pivotal models currently being used. The mechanisms uncovered by these models will ultimately lead to development of new therapeutic options to prevent long-term graft dysfunction.

Keywords: Animal models, Transplantation, Chronic allograft injury, Graft rejection.

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Solid organ transplantation has become the standard of care for patients with end-stage organ failure. Despite the dramatic advances of modern immunosuppression in reducing acute graft loss, the development of chronic allograft injury remains the major obstacle to long-term allograft survival (*1*, *2*). Part of the difficulty in improving long-term outcomes has been that multiple factors contribute to a common histologic pattern of injury (*3*, *4*).

Traditionally, when immunologic factors are believed to be the cause of chronic allograft injury, the overall process is termed chronic rejection, with involvement of cellular or humoral (antibody mediated) components, or both (*3*, *5*, *6*). One manifestation of this process is characterized by progressive arteriosclerosis as a result of the proliferation of the intimal smooth muscle cells, leading to ischemic damage and fibrosis. Key components of this process include alloreactive $CD4^+$ T cells and antibodies directed against the allograft (*7*). It is also believed that indirect allorecognition plays a role in chronic rejection, with the recipient's antigen-presenting cells processing donor-derived peptides through major histocompatibility complex (MHC) molecules (*8*, *9*). Nonimmunologic mechanisms such as ischemia-reperfusion injury, hypertension, and drug toxicity also contribute to chronic allograft injury (Table 1) (*6*, *10–14*).

Although allograft vasculopathy may be a common feature of chronic rejection in many solid organ transplants, each organ undergoes distinct histologic injury. Renal tissue undergoing chronic allograft injury is notable for the development of transplant glomerulopathy and tubular atrophy. Chronic allograft injury in cardiac tissue occurs after endothelial cell injury propagates the development of coronary allograft vasculopathy by means of increased intravascular macrophages, interstitial edema, and neutrophilic infiltration. Meanwhile, lung transplants undergoing chronic allograft injury endure epithelial damage with subsequent epithelial and submucosal mononuclear cell infiltration and fibrotic thickening. This leads to progressive occlusion of small airways (bronchiolitis obliterans) with collagen-rich granulation tissue. In liver allografts, endarteritis and fibrotic changes resulting in loss of bile ducts mark chronic injury (*15*, *16*).

Animal models have been of great importance to the advancement of transplant immunology. Because factors driving chronic allograft injury become defined clinically, we rely on animal models to allow us to decipher underlying mechanisms. The relative ease of genetic, physiologic, and pharmacologic manipulation over a short-time frame has permitted understanding of the mechanisms of graft injury.

Although invaluable, animal models are not a perfect system. These models compress a chronic process into an

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TABLE 1. Factors associated with chronic allograft injury in transplantation (particularly in renal transplant)

Recipient factors
Nonimmunologic factors
Age
Female sex
African American race
Cause of renal disease
Diabetes mellitus
Blood pressure
Calcineurin inhibitors
Immunologic factors
Rejection episodes (cellular and humoral)
Human leukocyte antigen matching
Panel reactive antibodies
Compliance with treatment
Infections (cytomegalovirus disease and BK virus nephropathy)
Donor factors
Living vs. deceased
Age
Female sex
Vascular disease
Glomerular disease
Cause of death
Nephron mass
Ischemic time
Delayed graft function

acute time frame, leading to distortions when applying insights gained back to the clinical process. Hence, it is critical to consider the limitations of animal models and to understand the underlying mechanisms while interpreting results obtained from these models.

In this review, we provide historical perspectives on important animal models that gave insight into chronic allograft injury and discuss the most relevant models being used to advance our understanding. Although not exhaustive, we attempt to define the key strengths and weaknesses of various models. In some cases, only a single example of a pertinent model will be highlighted. Our overall intent is to inform investigators of animal models available for the study of chronic allograft injury and the rationale behind using these models.

The identification of important animal models was conducted by performing a PubMed search with the following terms: chronic allograft nephropathy, chronic allograft injury, and chronic rejection, limiting our search to animals, English language, and publication dates 1960 to 2009. This yielded approximately 950 articles, which were manually evaluated for pertinence to the aim of our review.

KIDNEY

In the clinical scenario, chronic allograft injury in the kidney is notable for progressive decline in renal allograft function and nonspecific pathologic findings (tubular atrophy and fibrosis, transplant glomerulopathy, and occasionally fibrointimal proliferation of intrarenal arteries) (*17–19*). Experimental analyses investigating chronic damage seen in renal allografts have mostly taken place in rodent models.

Rat Models

The most commonly used chronic rejection model for kidney transplantation is the rat Fischer 334 (F344) to Lewis (LEW) model, which was initially described by White et al. (*20*) in 1969. These inbred strains are considered haploidentical and differ only at minor histocompatibility (non-MHC) loci. The MHC of the rat is located on chromosome 20, where class I loci consist of RT1.A, E, G and C, and class II loci consist of RT1.B. and D. F334 ($RT1^{1v1}$) rats have a variant haplotype that differs from LEW ($RT1^{l}$) rats in the class I E/C region but is identical to LEW in the immunodominant RT1.A and B regions. In this model, the renal allograft demonstrates slow deterioration of kidney function associated with parenchymal changes and complete graft failure after 48 weeks (*21*).

The histologic changes seen in this model include mononuclear cell infiltration with glomerular basement membrane thickening and mesangial expansion by 4 weeks after transplantation, with extension of this process and significant glomerular damage, proteinuria, and some tubular atrophy approximately 10 weeks. Ultimately, extensive interstitial fibrosis, vascular intimal thickening, and tubular atrophy are seen at 28 weeks (Fig. 1). Furthermore, C4d deposition has been demonstrated, suggesting that antibody-mediated rejection (AMR) plays a role in eliciting chronic allograft injury in this model (*21*, *22*).

As originally described, the F344-to-LEW model was hindered by frequent acute or subacute rejection episodes, with only 25% of renal allografts developing chronic allograft injury. Diamond et al. (*23*) added a 10-day course of cyclosporine A (CSA) to prevent episodes of acute rejection, which permitted longer graft survival. This modification to the

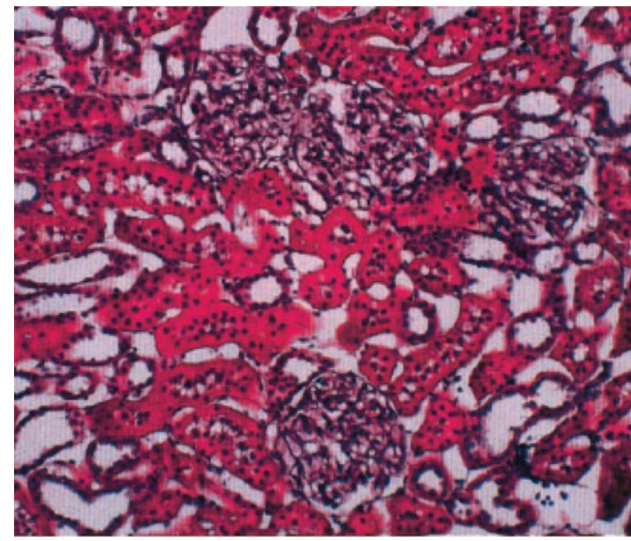

FIGURE 1. Cross-section of kidney allograft by Periodic Acid-Schiff staining 24 weeks after Fischer into Lewis transplant. Tubular atrophy, interstitial fibrosis, glomerulosclerosis, and immune cell infiltrates are present (courtesy of Dr. Yang).

F344-to-LEW model established the first reproducible model for chronic allograft nephropathy. Unfortunately, CSA caused glomerulosclerosis, obliterative arteriolopathy, and interstitial fibrosis after 12 months of daily therapy (*24*), despite the relative resistance of rat kidneys to CSA-induced injury (compared with humans), unless salt-depleted (*25*, *26*). Hence, some long-term graft deterioration may arise from not only alloantigen-dependent factors but also as a consequence of alloantigen-independent factors such as calcineurin inhibitor (CNI) toxicity (*27*). Chronic injury models using CSA were applicable during the period when CSA was used as the primary immunosuppressive agent in the clinical setting. This trend has changed, and likewise, use of CSA in chronic injury models may be deemed by some as less relevant now than in the past. This underlines the ever-evolving nature of transplantation in both the clinical and research settings.

Nevertheless, the F344-to-LEW model has enabled investigators to extract key elements in our understanding of chronic allograft injury. The reliability of measuring creatinine and proteinuria in this model is well established (*23*), making it a robust clinical model that can be elegantly manipulated to investigate impact of clinical variables on chronic injury.

By using the F344-to-LEW model, Azuma et al. blocked the CD28-B7 costimulatory pathway of T-cell activation early after transplantation with CTLA4Ig and prevented both acute rejection and chronic allograft injury. This finding pushed investigators to explore mechanisms that would promote allograft tolerance in transplant recipients, a shift from the previous focus of chronic allograft injury prevention through immunosuppression (*28*). Chandraker et al. (*29*) further used this model to help to confirm that chronic allograft rejection can be averted by intervening with costimulatory blockade even late after transplantation, demonstrating that there may be a window of opportunity during which progression of chronic rejection can be halted despite initial graft injury, and that ongoing T-cell alloantigen recognition and activation are key mediators of chronic rejection.

A similarly important finding was made using another model, in which Wistar Furth (WF, $RT1^u$) kidneys were transplanted into fully MHC-mismatched LEW rats, with subsequent administration of CSA and transfer of either T helper (Th) 1 or Th2 subset clones. Serum creatinine and proteinuria data collected from this model clearly showed that Th1 clones promote the progression of chronic allograft injury, whereas Th2 clones regulate alloimmune responses and protect allografts from progressive chronic rejection (*30*). This key finding may provide new insight into the role of Th2 cells in preventing chronic rejection and further establishes our understanding of tolerogenic principles.

To address the concerns that CNIs could potentially interfere with mechanistic studies investigating the development of chronic allograft injury, a minor mismatch model has been introduced in which LEW kidneys are transplanted into MHC-identical congenic WF.1L rats. Congenic strains are inbred strains that are derived from their origin by selective matings, such that they differ from the originating strain at only one independently segregating genetic locus. The WF.1L strain is derived from backcrossing LEW to WF rats, selecting 1/L progeny at F6, and mating brothers/sisters for many generations. Allografts in this model develop graft interstitial inflammation with mononuclear cell infiltration, glomerulosclerosis, tubular atrophy, and expression of cytokines representative of chronic injury (tumor necrosis factor-α, interferon [IFN]-γ, and interleukin [IL]-2) at day 90 and subsequent proteinuria at day 120 (*27*). However, vasculopathy (a salient feature of chronic allograft injury in cardiac allografts) is unusual in this kidney allograft model, although some argue that in the clinical setting, chronic rejection in the kidney may affect the glomeruli, interstitium, and tubules, with only 43% of patients demonstrating significant vasculopathy, suggesting the presence of different alloimmune mechanisms of injury (*31*).

In addition to helping to uncover alloantigen-dependent factors that lead to chronic changes, rat models have been further modified to investigate common nonimmunologic factors that affect graft survival in the long term. In exploring these models, we gain appreciation for the investigative efforts that have been made to understand the multifactorial cause of chronic allograft injury. An exploration of several of these models follows.

As noted earlier, the ease of manipulating clinical variables has made the F344-to-LEW model invaluable in studying the impact of alloantigen-independent factors in chronic allograft injury. The effect of hypertension in the recipient LEW rat was studied by Schindler et al. (*32*), who demonstrated higher immunogenicity of the allograft in this setting. Kusaka et al. (*33*) showed that clipping the contralateral native kidney 4 weeks after transplantation resulted in significant intimal thickening of allograft arteries, which progressed to luminal obliteration with extensive perivascular and interstitial fibrosis by 24 weeks. In some of the earliest studies investigating alloantigen-independent factors using the F344-to-LEW rat kidney transplant model, Azuma and Tilney (*34*) demonstrated that initial injury to the renal allograft, caused by ischemia and episodes of acute rejection, strongly influenced chronic rejection in allografted organs.

Other alloantigen-independent factors have been studied using this model of chronic rejection. Tullius et al. (*12*) studied the contributions of donor age and ischemia-reperfusion injury by using rat donors of different ages and varying the time period between harvesting and transplantation. They demonstrated that functional deterioration and structural changes progressed in parallel to increasing donor age and prolonged ischemia time, seeing the greatest impact of expanded ischemia on grafts from older donor animals. Further elucidating the importance of alloantigen-independent factors, Pratschke et al. (*11*) illustrated increased late graft failure in organs from brain-dead rats, whose increased immunogenic profile was clearly demonstrated by Takada et al. (*35*). Other studies have demonstrated the importance of nephron mass in chronic allograft injury, where the presence of one native kidney in addition to the allograft led to decreased glomerulosclerosis and proteinuria (*14*, *36*).

Various pharmacologic interventions have been studied extensively in this model, including the use of angiotensin-converting enzyme inhibitors to reduce chronic changes and ameliorate the degree of proteinuria, and the use of statins, which reduce macrophage and T-cell infiltration in the allograft (*37*, *38*). Among the immunosuppressive agents, mycophenolate mofetil was able to suppress chronic rejection when given immediately or 8 weeks after grafting (*39*). Rapa-

mycin has also shown similar suppressive capacity (*40*). Notably, the combination of rapamycin and mycophenolate led to significant reductions in the Banff sum score during a 50-week period (*41*).

An important observation in the F334-to-LEW kidney model is that the reverse strain combination does not result in the development of chronic injury in renal allografts (yet cardiac allografts do develop chronic allograft injury). It has become clear that strain-dependent development of chronic allograft injury is a common phenomenon in animal transplant models. Although not understood, the reasons could lie in strain-specific variations in antigen processing and the repertoire of T cells available to recognize the foreign antigens (*42*). Furthermore, it is possible that certain organs from some strains may be more susceptible to nonspecific injury while undergoing transplantation, lowering the threshold for chronic allograft injury in the long term.

Transplants between major histoincompatible rats can progress to chronic allograft injury, provided the recipient is treated with immunosuppression. Dark Agoutis (DA, AG-B4, $RT1^{av1}$) into Brown-Norway (BN, $RT1^{n}$) kidney transplantation has been shown to be an adequate model to study chronic allograft injury with the use of a triple immunosuppressive drug regimen (azathioprine, CSA, and steroids) (*43*). This model reflects a more clinically relevant drug combination and offers the advantage of rapid onset of chronic allograft injury in 40 to 60 days despite treatment, but unfortunately it obscures the distinction between rejection and CSA toxicity. In fact, a persistent criticism against major histoincompatible models of chronic injury is that the use of immunosuppressive agents is a must, and many transplant immunologists question whether these agents are merely delaying acute rejection, rather than promoting development of chronic injury.

Mouse Models

Although rat kidney transplantation models have provided the foundation for our understanding of chronic allograft injury, attempts have been made to develop other renal allograft models. Establishing mouse models in transplantation has become preferred because of the ease of developing inbred laboratory mouse lineages and the availability of multiple immunologic and immunomanipulative reagents against mouse cell markers. In mice, MHC proteins are encoded on the *H-2* gene locus on chromosome 17. These are subdivided into MHC class I, encoded by regions K and D, and class II, encoded by regions Aa, Ab, Ea, and Eb (*44*). In general, mouse kidney transplantation models have not been overwhelmingly successful because of unpredictable outcomes (particularly, high rate of tolerance after kidney transplantation) and variable degrees of development of the classic lesion of arteriosclerosis with fibrointimal hyperplasia (*45*, *46*). This may be partly due to a low level of expression of MHC molecules on endothelial cell surfaces in mice at baseline, which is subsequently up-regulated during injury (*47*–*49*). Given the technical difficulty of kidney transplantation in mice, allografts can experience varying degrees of nonspecific injury, leading to unpredictable levels of MHC molecule up-regulation. Furthermore, $CD8^{+}$ T cells in mouse kidney transplants tend to down-regulate their T-cell receptors, further driving the mechanism for spontaneous survival of renal transplants in mouse models (*50*).

Despite the limited utility of mouse renal transplant models in the investigation of chronic allograft injury, some groups have developed models to understand immune mechanisms underlying kidney allograft rejection. Halloran and coworkers (*51*) reported the use of one model in which the evolution of lesions signifying kidney rejection can be studied across MHC and non-MHC disparities by assessing histopathologic changes during a 21-day period in common strain combinations. They were able to demonstrate distinct early alloantibody-independent and late alloantibody-mediated injury processes using this model.

Non-Human Primate Models

Nonhuman primate kidney transplant models have been proposed, including by Knechtle and coworkers. In their model of chronic allograft injury, rhesus monkey recipients underwent $CD3^{+}$ T-cell depletion before undergoing kidney transplantation from class I- or class II-mismatched donors. The recipients experienced prolonged renal allograft survival with predictable development of chronic allograft nephropathy (*52*). Similarly, macaque monkeys have been used in a class II-mismatched model with suboptimal CSA immunosuppression to characterize chronic allograft injury (*53*). These unique animal models can help to provide relevance to the human clinical scenario, but inaccessibility and cost may limit them from becoming adopted.

HEART

Rat Models

The development of models for chronic allograft injury using heterotopic cardiac transplantation has further refined our understanding of chronic injury in transplantation. Rat cardiac transplantation models were explored extensively by investigative groups several decades ago (*54*, *55*), but limitations to these models included restricted availability (only noncommercially available colonies of inbred rats) or necessity of aggressive immunotherapy to prevent acute rejection. However, Adams et al. (*56*, *57*) demonstrated chronic rejection using heart transplantation in the LEW-to-F344 strain combination without the requirement of immunosuppression. In this non-MHC mismatch model, medium- and long-surviving LEW cardiac allografts in Fischer rats undergo a rejection process that involves diffuse concentric intimal proliferation in large- and medium-sized arteries, the pathognomonic finding in chronically rejected cardiac allografts. Long-surviving cardiac allografts develop subendothelial accumulations of mononuclear cells, followed by diffuse fibrotic intimal thickening. The histologic similarities of the LEW-to-F344 cardiac transplant model to the appearance of graft arteriosclerosis seen in human cardiac allografts undergoing chronic rejection has led to this model being among the most commonly reported model of chronic graft vascular disease (CGVD).

Alternatively, the Piebald Viral Glaxo (RT1c)-to-August Copenhagen Irish (ACI, RT1a) heart transplantation model, with a short postoperative course of low-dose CSA, has also been used to further understand CGVD. Poston et al. introduced this MHC class II fully mismatched model after pointing out that no heart transplantation models were available that reliably demonstrated progressive coronary artery narrowing despite adequate immunosuppression, arguing

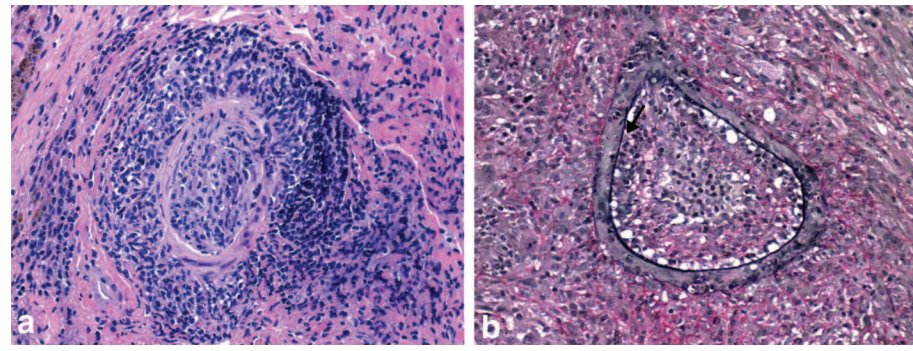

FIGURE 2. Representative photomicrograph of a transplanted heart from bm12 into B6 at 100 days after transplantation. (a) Hematoxylin-eosin staining demonstrating advanced allograft vasculopathy with surrounding inflammatory infiltrate. (b) Elastic Van Giesen staining showing intimal hyperplasia and the elastica interna (*arrow*), which sets the limit between intima and media (courtesy of Dr. Sayegh).

that without development of coronary vasculopathy in the setting of immunosuppression, the criteria for CGVD were not being met. They raised concern that the vascular changes seen in medium- and long-surviving allografts in the strong-responder LEW-to-F344 model are secondary to a muted acute or subacute (and not chronic) rejection process (*58*). The Piebald Viral Glaxo-to-ACI model exhibits more isolated chronic vascular disease when compared with several other rat models of heterotopic heart transplantation but notably does not induce myocardial rejection. In this model, rapamycin, but not CSA, is capable of reducing the degree of CGVD and opens the door for investigators to study chronic injury in a model that is relatively resistant to T-cell–directed immunosuppressants (like CSA) but susceptible to novel agents whose mechanism of actions lies beyond reduction in T-cell–mediated immunity. Alternatively, the reduced degree of vasculopathy seen in this model may be explained by rapamycin's antiproliferative effect on vascular smooth muscle (*59*).

Guttmann and coworkers developed a congenic rat cardiac transplantation model that demonstrated progressive chronic allograft injury without the need for immunosuppression. In this WF.1L-to-LEW model, cardiac allografts survive for an indefinite period but develop intense large-vessel vasculitis and occlusive vasculopathy with myointimal thickening (*60*). This group's development of the WF.1L strain and their subsequent findings served as the basis for the use of the reverse-strain combination in rat kidney transplantation (described earlier) and allows for the examination of a purely indirect pathway of injury.

Techniques for tolerance induction such as donor-specific blood transfusions and costimulatory blockade have been used for some time, but recently, investigation into histologic lesions of these long-term surviving recipients has revealed chronic rejection (*61*, *62*). To investigate this further, Soulillou and coworkers (*63*) used a tolerance induction protocol by donor-specific blood transfusion before LEW.1W-to-LEW.1A congenic rat cardiac transplantation and demonstrated increased levels of IgG antibodies against donor MHC class I and II molecules, and C4d deposition in graft capillaries, suggesting an antidonor humoral response in long-term surviving allografts. With the advent of increasingly effective immunosuppressive agents, the rate of acute cellular rejection has diminished, and some attention is being shifted toward the role of AMR in the clinical setting. This model provides a useful means for researchers to understand AMR further and to test therapeutic strategies aimed at blocking it.

Mouse Models

Murine models of chronic allograft injury include those involving different strains with minor histoincompatibilities, which usually leads to a smoldering immunologic response within the allograft and ultimately, chronic injury. The most used surgical technique is the method described by Corry et al. (*64*), which involves ligation of the donor heart pulmonary veins and inferior vena cava and placement of the allograft in the recipient's abdomen, with subsequent anastomosis of the donor pulmonary trunk and aorta to the abdominal aorta and inferior vena cava of the recipient. Limitations of any heterotopic cardiac transplantation models include the absence of pumping function (although myocardial contractility and coronary blood flow is preserved) and the occurrence of perioperative pericardial inflammation, which can affect the epicardial vessels (*16*).

An established MHC class II-mismatched mouse model of CGVD that has been used involves heterotopic, revascularized cardiac transplantation from B6.C.*H-2-bm12* (bm12) into a wild-type C57BL/6 (B6, *H-2*^{b}) mouse (*65–67*). Bm12 mice are a variant strain of C57BL/6 mice, in which a spontaneous mutation has occurred in the I-A^{b} locus, designated *I-A*^{bm12}. In this single MHC class II mismatch model, the majority of bm12 cardiac allografts survive up to 100 days and develop significant vasculopathy, notable for intraluminal accumulation of mononuclear leukocytes (at 4 weeks posttransplant), intimal lesions (by 8 weeks), and accumulation of smooth muscle cells signifying fibroproliferative arteriosclerotic lesions (by 12 weeks; Fig. 2). The limited alloreactive T-cells activation and emergence of a population of regulatory T cells allow long-term allograft survival with the development of significant vasculopathy (*68*).

This model has been used predominately to explore the role of immunologic factors on graft injury. For instance, Nagano et al. (*66*) convincingly demonstrated the requirement of IFN-γ for the development of persistent coronary arteriosclerosis (but not necessarily for parenchymal graft rejection) by comparing histologic and molecular changes in bm12 hearts transplanted into wild-type versus IFN-γ–deficient B6 mice. More recently, Yuan et al. (*67*) demonstrated an accelerated graft rejection in T-bet-deficient mice because of up-regulation of IL-17–producing CD4 Th17 cells. Others have illustrated the importance of chemokine receptors (*69*) and T-cell trafficking (*70*) in the development of coronary vasculopathy and chronic allograft rejection using this model.

In a model introduced to assess the impact of differences in class I versus class II mismatches in the development of chronic allograft injury, bm1 hearts (which have a 3-amino acid mutation on one α helix of the class I $H2K^b$ molecule) are transplanted into B6 mice. The bm1-to-B6 cardiac transplantation model capitalizes on an isolated class I mismatch between these two strains of mice, in which allograft rejection is mediated primarily by $CD8^+$ T cells. Mean allograft survival reaches 20 to 30 days, regardless of whether donor hearts are revascularized or simply implanted through split ear cardiac grafting, in which a 1- to 2-day-old neonatal heart is placed in a pouch created in the pinna of the recipient (*71–73*). Of note, the reverse strain combination has been shown to yield allograft survival beyond 60 days, although allograft vasculopathy does develop (*74*).

As in rat models, heterotopic cardiac transplantation between fully MHC-mismatched mouse strains have been described as models for studying chronic rejection with the use of postoperative immunosuppressants to prevent acute rejection. In one model, anti-CD40L is administered after B6-to-BALB/c ($H2^d$) heterotopic cardiac transplantation. If there is no manipulation, cardiac allografts are promptly rejected; however, if treated with anti-CD40L, acute rejection is ameliorated, and recipients develop chronic rejection. In this model, approximately 50% of cardiac allografts survive 100 days, but with severe evidence of histologic chronic allograft changes (*75–78*).

As described earlier, the role of AMR is gaining the attention of investigators, as an "active humoral" type of chronic rejection has recently been defined in humans (*79*). For instance, anti-human leukocyte antigen class I antibodies have been implicated in promoting the development of chronic rejection (*80*). To further investigate the pathogenesis of AMR, Jindra et al. (*81*) have suggested a BALB/c-to-B6.RAG1-knockout cardiac transplant model in which the recipients are reconstituted with antidonor MHC class I antibodies. The histologic changes mimic those seen in human allografts undergoing AMR, and the analysis of molecular markers in this model provides information to understand mechanisms driving AMR. Others have used a mouse heart transplant model to demonstrate that a minimum threshold of antibody exposure may be necessary to cause chronic allograft injury but that C4d levels may be undetectable if not sampled at the appropriate time (*82*).

AORTA

Orthotopic aortic allografting has also been tried as a model for chronic vascular rejection after its success as a vascular repair model. Rat abdominal aortae were isografted or allografted from BN- to-LEW and between WF and DA rats without immunosuppression. After a short, spontaneously reversible acute episode of rejection, these allografts developed vascular wall changes that are similar to those seen in human tissue transplants undergoing chronic rejection. Persistent perivascular inflammation, gradual loss of smooth muscle cells in the media, fragmentation of the internal elastic lamina, and appearance of proliferating smooth muscle cells in the intima have been observed, and some investigators have demonstrated the effectiveness of vitamin D analogues in reducing these stigmata of transplant vasculopathy (*83–85*). ACI-to-LEW rat combinations have also yielded similar outcomes (*86*), and other investigators have even reported success in various rabbit models of aortic transplantation for uncovering the role of accelerated atherosclerosis on chronic allograft vasculopathy (*87*).

To use the more extensively developed molecular tools available for mice, several groups have developed mouse models of aortic transplantation using bypass of recipient aorta by donor thoracic aorta between C3H ($H\text{-}2^k$) and C57Bl/10J ($H\text{-}2^b$) strains. These models demonstrate intimal thickening 2 months after transplantation (*88,89*). Although aortic transplant models have shown promising results, a major shortcoming is that the absence of supporting parenchyma with associated donor lymphoid tissue, the effect of the inflammatory response at the anastomotic site, and the severe smooth muscle cell death in the media do not translate well into the process of chronic rejection involving a whole harvested organ. Nevertheless, these novel models could help in understanding the endothelial milieu during chronic allograft injury (*16*).

LIVER

As mentioned previously, chronic allograft injury in the liver is associated with bile duct atrophy, obliterative arteriopathy, and interstitial fibrosis. The incidence of chronic rejection in liver transplants is much lower than other solid organs, ranging from 3% to 5% at 5 years. This difference might be related to the unique immunologic properties of the liver allograft, including its capacity for regeneration, MHC molecule expression, and special reticuloendothelial system (*90*). Liver animal models have been seldom reported, but Gao et al. (*91*) performed liver transplants from DA-to-BN rats under low-dose CSA treatment and noted chronic allograft liver injury between 30 and 60 days after surgery. Notably, they described the development of bile duct proliferation, which is not consistent with the chronic hepatic injury seen in humans, and may raise questions as to the overall ability to translate findings in this model to the clinical scenario.

LUNG

Rodent Models

Chronic lung rejection after transplantation in humans is characterized by pulmonary artery intimal proliferation, interstitial fibrosis, and most notably, obliterative bronchiolitis (OB). Animal models using orthotopic lung allografts have been generally used to study early postoperative problems, such as ischemia-reperfusion, airway dehiscence, and acute rejection. There is some difficulty associated with the reliable development of obliterative airway disease (OAD) in pulmonary tissue. For instance, several rat models manifest more intense vascular and parenchymal inflammation but develop only modest airway injury (*92*, *93*). One orthotopic rat lung transplant model reported in the literature demonstrated the development of OAD, but this was contingent on the occurrence of a several-week period of acute rejection preceding the appearance of chronic lung allograft damage (*92*). This has led some investigators to blame high rates of acute rejection, and the failure to develop OAD, as the pitfall of rodent lung transplant models (*94*).

Although rodent models of chronic lung allograft injury do exist, most models use heterotopically placed tissues as surrogates for whole-lung allografts (*95–99*). OAD was originally investigated in such a murine model by Hertz et al. using a BALB/c-to-C3H trachea transplant model. In this model, tracheas transplanted into the subcutaneous tissue developed subepithelial inflammation, epithelial necrosis, and fibroproliferation, mimicking human OB by day 21 (*95*). Using this model, the same group found that administration of CSA reduced the development of OAD in a dose-dependent fashion, but epithelial injury and cellular inflammation still occurred (*100*). Attempts at performing orthotopic tracheal transplants in BALB/c-to-B6 mice have been described, but interestingly, these allografts do not reliably develop OAD (*101*). However, this same model has been applied by others to demonstrate the development of OAD after retransplantation of the BALB/c trachea allografts (shown to obtain recipient-derived epithelium after orthotopic transplantation) back into BALB/c mice, suggesting that airway epithelium plays a crucial role in OAD development (*102*).

The rodent model for chronic injury in lung allografts was further refined by Morris and coworkers (*97*) by using BN-to-LEW rats for tracheal transplantation into recipient omentum. Progressive cellular graft infiltration and eventual complete luminal obliteration are noted, mimicking OB in lung transplant patients. This model has been used in understanding the role of chemokines, cytokines, and molecular signaling pathways in the development of OB after lung transplantation (*98*, *103*, *104*). Unfortunately, drawbacks of these surrogate models for lung transplantation include the elimination of the air-epithelium interface and the failure to reestablish blood flow to the allografted tissue.

Further complicating the study of chronic injury in lung allografts using rodent models is that immunologic differences between humans and rodents seem to be amplified in pulmonary tissue. For instance, the bronchial epithelial surfaces of humans (and large animals), but not rodents, have constitutive expression of class II antigens. Differences such as these must be taken into account when planning experimental work in pulmonary transplantation.

Despite noteworthy difficulties in developing models of chronic injury in lungs, rodent models of airway transplantation have recently provided a rich backdrop to investigate the role of AMR in chronic injury. Mohanakumar and coworkers (*105*) have described a murine model in which the introduction of anti-MHC class I antibody into native lungs led to the development of fibrosis and distal airway occlusion similar to chronic airway rejection in human lung transplantation. They demonstrated an important role of IL-17 in this model and introduced the question of whether approaches to prevent autoimmunity, in addition to alloimmunity, should also be considered for the treatment of chronic rejection in lung allografts.

Swine Models

Miniature swine lung transplantation models have been reported (*106*, *107*), including one model between strains MHC-matched for both class I and II. In this model, lung recipients received a short course of CSA and subsequently developed OB and other chronic changes by the third to fourth month (*107*). The mean graft survival was 228 days. This model of chronic lung allograft injury has the advantage of high frequency of OB in recipients, immunologic similarity between human and swine immune systems (including epithelial and endothelial MHC antigen expression), and orthotopic placement of the lung allograft. However, the difficulty in obtaining animals with only minor histoincompatibilities, prolonged waiting time, frequent development of concomitant acute rejection, and technical and surgical difficulties in performing orthotopic swine lung transplantation are notable disadvantages that should be considered by investigators hoping to study lung transplantation in animal models.

CONCLUSIONS

In this review, we have summarized some of the most relevant animal models that have been used in the study of chronic allograft injury and have presented key findings to illustrate how these models have impacted our understanding of the pathogenesis of chronic allograft injury. Although the precise mechanisms underlying chronically occurring allograft injury are unknown, animal models allow investigators to identify individual immunologic and nonimmunologic components to understand chronic allograft injury, with the ultimate goal of translation into clinically beneficial therapies.

Possible Future Models

As the field of transplant immunology progresses, so must the models and means by which we study the mechanisms controlling rejection. Increasing ability to generate transgenic animals allows improved control over the immunologic systems in our models and permits precise determination of immune mechanisms in vivo. For instance, the use of a new T-cell receptor transgenic mouse (named 4C) in a cardiac transplant model allows investigation of chronic rejection in the absence of indirect pathway and alloantibodies and demonstrates that chronic allograft vasculopathy can occur through the CD4 direct pathway alone (*108*). In this model, 4C T cells (direct allospecificity against I-A^d) are adoptively transferred into C57BL/6 *Rag1*$^{-/-}$ mice that then receive a BALB/c heart transplant. The resulting chronic graft damage that occurs allows appreciation of the $CD4^+$ direct pathway's ability to mediate chronic transplant vasculopathy, which had not previously been considered of significant importance. Indeed, other novel animal models will continue to be developed, which will promote increasingly sophisticated studies in isolating the mechanisms underlying chronic allograft injury.

Clinically, because the rate of acute cellular rejection has decreased with the use of more powerful immunosuppressive medications and the doses of CNIs used have been decreased in kidney transplantation, there has been increasing recognition of the role of AMR. The effects of antiallograft antibodies have not been elucidated fully but are believed to play a role in late allograft loss (*109–111*). However, models for studying AMR are somewhat lacking. In this review, we have recognized the work of several investigators whose recently introduced models have shown promising results in uncovering the role of antibody-mediated chronic rejection.

Understanding the pathogenesis and molecular pathways that dictate long-term allograft failure will allow investigators to target preventive and therapeutic strategies in

humans. As shown from the relevant findings discussed in this review, investigations of chronic allograft injury in animal models have established a long list of mechanisms that now require further research to evaluate new avenues for therapy.

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