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Experimental Models of Cardiac Transplantation - design determines relevance

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Abstract

Purpose of review—Experimental models have contributed enormously to basic immunology. However, the use of reductionist experiments has produced results that are not always successfully translated into the clinic. Recently, incorporation of more realistic clinical parameters in experimental designs has produced new insights relevant to cardiac transplantation.

Recent findings—Experiments in mice have provided crucial insights into the concept that T cell responses to pathogens generate memory cells with cross-reactive specificities for histocompatibility antigens. These memory T cells are resistant to current immunosuppressive strategies. Memory T cells infiltrate grafts within hours after transplantation and grafts subjected to clinically relevant periods of cold ischemia are more susceptible to injury by this cellular infiltrate. Early immune responses now can be investigated with improved “humanized” mice. Mice with multiple knock in genes for human cytokines support development of human monocytes, macrophages and NK cells in increased numbers and with better function.

Summary—Better and more clinically relevant experimental designs are providing animal models tailored to address clinic exigencies.

Keywords

Memory T cells; Heterologous immunity; Humanized mice

Introduction

Animals have been indispensable in the evolution of cardiac transplantation into a clinically practical procedure. In the pioneering era, dogs were integral to the development of the surgical procedures for cardiac transplantation (1). Once the surgical technique was translated to humans, survival outcomes were unacceptable until Cyclosporin A was discovered to have powerful immunosuppressive effects on skin and cardiac allografts in mice, rats and pigs (2, 3). With the clinical application of Cyclosporine, longterm survival of cardiac transplants became a clinical reality (1). More recent therapeutic agents as diverse as

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Eculizumab (monoclonal antibody to C5) and Belatacept (analogue to CTLA4Ig fusion protein) also originated with experiments in mice (4–7). In spite of these successes, experimental models testing tolerance inducing strategies have been criticized for producing results that cannot be effectively translated into the clinic. A review of recent findings indicates that the fault lies not with the experimental animal but with the experimental design.

Advantages of mouse models

Development of microsurgical techniques that permitted heterotopic transplantation of hearts first in rats and then in mice led to a surge of immunological data. Mice in particular have many apparent advantages in addressing mechanistic questions (8)*. The availability of pathogen-free, genetically inbred stains of mice decrease variables inherent in many large animal models. The capability to delete or insert selected genes permits dissection of complex immune responses. The use of genetically defined mice together with an increasing array of monoclonal antibodies and molecular markers has delineated subsets of lymphocytes and more recently macrophages that respond to cardiac transplants. An enormous network of cytokines, chemokines, co-stimulatory ligands and receptors have also been identified in mouse models of transplantation.

Shortcomings of reductionist mouse models

Experimental studies are designed to be highly reductionist to eliminate as many confounding variables as possible. As a result, early studies in mice used healthy recipients with no pre-existing disease, transfusions, pregnancies or previous transplants. In addition, the donor mice were not subjected to trauma and ischemic times were minimized. Treatment modalities tested under these pristine conditions frequently resulted in longterm graft survival and even tolerance. Not surprisingly, these treatments were usually less successful when tested in human patients (9). Yet the effects of all of these variables can and have been modeled in mice (10–14)*. Recent advances in understanding these parameters are discussed in the next section.

Some of the characteristics that make mice attractive experimentally have been recognized more recently as shortcomings. For example, the evident advantage of inbred strains of mice is counterbalanced by the fact that common laboratory strains are derived from a few original lines of mice (15, 16). The genetic diversity of these inbred mice is extremely limited when compared to mice caught in the wild (17). Reichenbach and colleagues (18)* have begun to explore cardiac allograft survival in more genetically heterogeneous mice. A much wider spectrum of rejection was observed for these allografts, ranging from very acute (1–4 days after transplantation) through acute to chronic rejection (>75 days). The very acute rejections were dependent on complement and neutrophils, but no antibodies were detected. Although this mode of rejection does not have an obvious clinical counterpart, further studies may reveal cryptic antibodies or other mediators of potential clinical relevance. The chronic rejection observed in these more outbred mice might be of greater interest. Current models of chronic rejection often take advantage of genetically well-matched donor and recipient strains of mice. This strategy avoids the confounding variable

of immunosuppressive treatments, but is unrealistic relative to the large majority of clinical transplants, particularly hearts, which are not matched on the basis of histocompatibility.

Genetically modified mice are another major resource that have yielded valuable insights, but that also have caveats. Genetically engineered mice are frequently backcrossed onto the commonly used and fertile C57BL/6 or Balb/c mouse strains. These strains have immunologically divergent T cell and macrophage responses (19, 20)*. C57BL/6 mice have a propensity for Th1 responses characterized by high IFN-γ and low IL-4 production. In contrast, Balb/c mice are biased towards Th2 responses characterized by low IFN-γ and high IL-4 production. Similarly, C57BL/6 mice are predisposed towards inflammatory macrophage (M1) responses, and Balb/c mice towards wound healing macrophage (M2) responses.

Not only are there divergences in immune responses between strains of mice, there are even greater differences between the immune systems of mice and humans, which have been the subject of recent reviews (21, 22)*. These range from cellular expression of signaling molecules, mediators and receptors to cellular composition of lymphoid organs. For example, lymphocytes constitute 30–50% of peripheral blood leukocytes in humans, compared to 75–90% in mice, and the majority of circulating lymphocytes in humans are T cells as opposed to mice where B cells predominate (23).

Finally, there are limitations specifically related to cardiac transplants in mice. The small size of mice prohibits performing fully functional orthotopic cardiac allografts. Instead heterotopic transplants are performed in which the ascending aorta of the heart is anastomosed to the abdominal aorta and the pulmonary artery to the vena cava (24). This results in good perfusion of the coronaries, but a very limited workload for the heart. With time, mural thrombi form in the left ventricle and the heart atrophies. In addition, the structure and location of the coronary arteries differ between humans and mice. In humans, the major branches of the coronary arteries are located in the epicardial fat. In mice, only a short segment of the coronary arteries close to the aortic root are epicardial and the remainder of the arteries are intramural. The epicardial and intramyocardial segments of coronary arteries have embryological, anatomical and physiological differences that influence pathological processes. This is evident in “myocardial bridging”, an anatomical variant in which a segment of coronary dips into the myocardium. These intramural bridge segments of coronaries develop less atherosclerosis than the adjacent epicardial segments (25, 26)*. The proliferative neointimal lesions characteristic of chronic allograft vasculopathy in humans also develop primarily in the epicardial portions of the coronary arteries with limited involvement of intramural branches (27). Moreover, the pathological lesion found in intramural coronary branches is often characterized by medial necrosis and fibrosis. Similarly, in murine cardiac allografts, the small epicardial segments of coronary usually develop the more severe arterial pathology than the intramural arteries (28).

Addressing the shortcomings of reductionist mouse models

One of the major distinctions between the healthy young animals commonly used in experimental transplantation and patients with terminal organ failure who receive transplants

is the number and diversity of memory T cells engendered by previous antigenic stimulation. Medawar (29) established that rejection of skin grafts in rabbits was an immune response in part based on evidence of a memory response as demonstrated by accelerated rejection of second grafts from an individual donor. Compared to naïve T cells, memory T cells possess unique survival advantages which allow them to persist long-term, have lowered activation thresholds, expand much more quickly and efficiently, and express effector functions much more rapidly, features which are advantageous in protective immunity against infection, but are detrimental in the setting of transplantation (30). Additionally, memory T cells have been demonstrated to survive lymphoablative therapies that very effectively eliminate naïve T cells (31–33) and are resistant to many traditional costimulatory blockade therapies (10, 34–36). Seminal work performed by Heeger and colleagues demonstrated that high numbers of donor-reactive memory T cells in the peripheral blood of renal transplant patients prior to transplant are associated with increased incidence of acute rejection episodes and with poorer renal allograft function at 1 year (37, 38). Furthermore, the detection of such alloreactive memory cells by IFN-γ ELISPOT assays has been demonstrated to be predictive of post-transplant renal function and even the development of chronic allograft nephropathy (39, 40).

Not all patients with demonstrable memory T cells have an obvious history of exposure to alloantigens through previous transplant, pregnancy, or blood transfusion. Another less obvious stimulus to memory T cells is exposure to pathogens. Braciale TJ et al (41) demonstrated that some cytotoxic T cell clones from mice specific for type A influenza viruses, also had cytotoxic reactivity to major histocompatibility antigens. Generation of allospecific memory T cells by cross-reactions with pathogens is termed heterologous immunity. Elegant work done by Adams and colleagues (42) revealed that infection of mice with certain strains of virus not only generate a viral-specific memory T cell response, but may also induce a heterologous alloimmune memory response to a previously unseen donor antigen. Importantly, the ability to generate such a donor cross-reactive memory T cell response was found to be dependent on the viral strain during primary infection and a critical threshold of viral infection. Subsequent memory T cell generation could mediate resistance to tolerance induction with mixed-chimerism and costimulatory blockade. In addition to virally induced heterologous immunity, several other approaches have been used to study the impact of donor-reactive memory T cells on allograft outcome in rodent transplant models by priming recipients directly with donor antigen or the adoptive transfer of donor-antigen primed memory T cells (11, 42–45). Such strategies, manipulating rodent recipients to induce high pre-transplant numbers of donor-specific memory T cells, have clearly demonstrated that memory T cells are able to directly mediate aggressive rejection of transplanted organs that is resistant to costimulatory blockade therapies and resembles the increased acute allograft rejection observed clinically.

The antithesis of these observations is that the numbers of these memory T cells in unsensitized naïve rodent recipients housed in SPF environments in research facilities are insufficient to directly mediate allograft rejection and this less vigorous rejection can be obviated using costimulatory blockade and other tolerance inducing strategies. Important studies by Schenk and colleagues demonstrated that even naïve adult laboratory mice possess a repertoire of endogenous memory T cells, naturally generated from environmental

exposures, a proportion of which are alloreactive (11). Despite never having been directly primed with donor antigens, such endogenous memory CD8 T cells within naive recipient mice rapidly infiltrate cardiac allografts within hours of reperfusion and are activated by donor class I MHC to proliferate and produce IFN-γ (11). However, in keeping with prior observations, further studies revealed that the large numbers of early infiltrating memory CD8 T cells within the allograft and their expression of effector mediators appeared insufficient to directly mediate graft rejection (46). The stark contrast between the aggressive costimulatory-blockade resistant rejection of transplanted organs following induction of high numbers of donor-reactive memory T cells by recipient priming and the relative inability of naturally generated endogenous memory T cells to mediate allograft rejection raise questions about the robustness of endogenous memory T cell repertoires in unprimed mice. Additionally, these data suggest that the priming strategies currently used to generate and study costimulatory blockade resistant heterologous memory T cell responses in mice disproportionately biases the T cell response to strong reactivity to donor antigens to a degree rarely seen naturally in human patients.

Recent work has begun to address the sufficiency of the endogenous memory T cell repertoire in recapitulating the impact of endogenous heterologous immunity seen in clinical transplantation. Despite the universality of ischemia-reperfusion injury in the transplantation of solid organs and the detrimental impact of prolonged organ preservation time on graft function and survival (47, 48), organ preservation and surgical operational times are typically minimized in the setting of rodent models of cardiac transplantation, features which may not be clinically representative and which may optimize results testing allograft-prolonging strategies such as costimulatory blockade. In support of this, recent work has demonstrated that increasing the duration of cold ischemic graft storage in a clinically relevant manner generates an inflammatory environment within the allograft following reperfusion that promotes endogenous memory CD8 T cell activation to sufficient levels that increased effector functions and directly provoked allograft failure in naïve unprimed mice (14)**. Importantly, the rejection mediated by these memory CD8 T cells was found to be resistant to costimulatory blockade therapy in a manner similar to that seen clinically, but without prior recipient priming to bias the endogenous memory T cell repertoire to strong donor reactivity.

Therapies directed at preventing the rapid entry of endogenous memory T cells into transplants have been found to be effective in mice (14, 49, 50) and may have clinical application particularly in heart transplant patients whose immunosuppression is significantly more intense than renal transplant patients.

Improved “humanized” mouse models

Recognition of the many differences between the immune systems of mice and men led to the development of “humanized” mice. The ideal goal of humanized mice is to establish a functional human innate and adaptive immune system in an experimental animal. With time this goal has been slowly approached beginning with studies in 1988 of transferring normal human cells to severely immunodeficient mice (51). The limited numbers and function of engrafted human cells in early models was increased by selecting mice with additional

immune deficiencies, implanting human thymic tissue and increasing the numbers of human cells transferred as detailed in reviews of this field (52, 53).

Pober, Tellides and colleagues have used increasingly sophisticated versions of humanized mice as recipients of interpositional grafts of human arteries. Initially, their experiments focused on the role of inflammatory mediators, such as IFNγ, IL-1 and VEGF, in neointima formation (54–57), more recently they have developed a model to test the effects of ischemia-reperfusion (58). Wood and colleagues have used humanized mice to examine the modulatory effects of in vitro expanded human T regulatory cells on neointimal formation in transplanted human arteries (59, 60).

Recent models have probed genes for mouse cytokines that do not support cells of the human innate immune system. Using gene knock in technology, these mouse genes have been replaced by human cytokine genes that are then expressed in physiologically appropriate levels and tissues. Previously Flavell and colleagues reported that the strategy of knocking in of human genes for single cytokines effectively increased lineages of hematopoietic cells (61, 62), more recently they have demonstrated that simultaneous expression of 4 human cytokines genes (GM-CSF, M-CSF, IL-3, and thrombopoietin) augmented the numbers and function of monocytes, macrophages and NK cells (63).**

The development of mice equipped with a more complete innate immune system should enhance studies of pathology in arterial transplants.

Conclusion

Animal models and mouse models in particular continue to contribute to our understanding of transplant immunology. The clinical relevance of these models depends upon appropriately designed experiments. To this end, new models have tested the effects of cold ischemic time on injury from memory T cells generated by heterologous immunity. These models have identified potential new therapeutic interventions. More sophisticated methods of creating humanized mice have expanded possibilities of probing the innate and adaptive human immune responses to transplants.

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Abbreviations

GM-CSF	Granulocyte/Monocyte colony-stimulating factor
M-CSF	Macrophage colony-stimulating factor
IL	interleukin
NK cells	Natural Killer cells

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Key points

(3–5 key points/phrases that summarize your article)

(1) Experimental design determines the clinical relevance of animal models

(2) Mice continue to contribute critical information about transplantation

(3) Variables that affect both innate and adaptive immune responses need to be considered in animal models.