The 'Mighty Mouse' Model in experimental cardiac transplantation

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Tissue rejection remains a major problem in organ transplantation. The use of experimental animal models continues to enhance our understanding of the rejection process and offers strategies for its prevention. The popular mouse model of heterotopic cardiac transplantation has been used for over three decades to help investigators understand the pathogenesis of graft rejection and in turn, how novel drugs can attenuate the immune response to transplanted organs. Also, since the genetic blueprint of mice is well-known, specific genes can be modified to study their affect on graft acceptance and tolerance. This review briefly outlines what is known about the unique physiology, haemodynamics and dynamic morphology of the transplanted mouse heart, with particular emphasis on insights gleaned from hi-resolution ultrasound. Current applications and methods to assess rejection are also discussed.

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Introduction

Experimental Animal Models of Transplantation

THE TRANSLATION FROM THEORETICAL FRAMEWORKS to clinical transplantation as an established and effective therapy for end-stage organ failure has been possible through extensive scientific insight gained from experimental animal models (1). The French surgeon Alexis Carrel was the first to develop surgical procedures for experimental transplantation and his inaugural work on establishing vascular procedures for kidney and heart transplantation in dogs was recognized with the Nobel prize award in 1912. Poor outcomes in clinical organ transplant surgery made in the late 1930's were followed by a renewed interest in the early 1960's due to the production of a novel immunosuppressant, 6-mercaptopurine and subsequently, cyclosporine in 1972. The improved survival rates of transplant patients led to increasing numbers of interested clinicians and researchers in the field, and naturally led to the development of more sophisticated animal models to investigate basic lines of enquiry in transplantation. These include new pharmacological agents capable of mitigating immune responses towards allografts, effective preservation solutions, elucidating immunological mechanisms of rejection and tolerance, developing novel sources of grafts (xenografts), and the establishment of post-transplant monitoring protocols (1-9).

Heterotopic Vascularized Cardiac Grafts in Rodents

Heterotopic transplantation was used as early as the 1940's and 50's in large animals but it wasn't until work by Abbott *et al*. in 1964 that the heterotopic heart transplantation (HHTX) was perfomed in rodents; the authors used rats and utilized end-to-end anastomosis of the donor thoracic aorta to the recipient abdominal aorta and donor pulmonary artery to recipient inferior vena cava (IVC) (10). Abbott's procedure resulted in restricted blood flow to the lower extremities and consequently, paraparesis and paraplegia (1). This procedure was revised by Ono and Lindsey to allow for greater blood flow to the lower extremities and employed end-to-side anastomoses of the donor vessels to the recipient aorta and IVC, resulting in 90% graft and recipient survival (11). Heterotopic cervical transplantation was performed in rats in 1971 (12) and required the anastomosis of the re-

cipient external jugular vein and common carotid artery to the donor aorta and pulmonary artery, though thrombosis occurred in 30% of subjects (1). In 1973, Corry et al. were investigating the role of MHC molecules in cardiac graft rejection and concurrently reported performing the HHTx protocol in mice using the same technique established by Ono and Lindsey in the rat (13). Blood flow directionality in this model has been verified using ultrasound biomicroscopy (UBM) and is illustrated in **Figure 1** (14). Briefly, blood from the recipient abdominal aortic circulation flows retrograde into the donor graft ascending aorta. In the presence of a competent aortic valve this will direct blood into the coronary arteries to perfuse the cardiac graft; the venous blood then drains into the right atrium via the coronary sinus. Blood then fills the right ventricle which is ejected through the pulmonary artery and enters into the recipient IVC (15). Thus, the model represents a left ventricular bypass with omission of normal pulmonary circulation for blood reoxygenation. Rat HHTx graft blood flow volume comprises about 5% of total blood volume per minute (16).

In experimental cardiac transplantation, rats and mice are the most commonly used animals, though canine, swine, primates and other rodents have also been employed (1). Vascularized heterotopic (abdominal) heart grafting is the method of choice in rodent transplantation and new surgical techniques

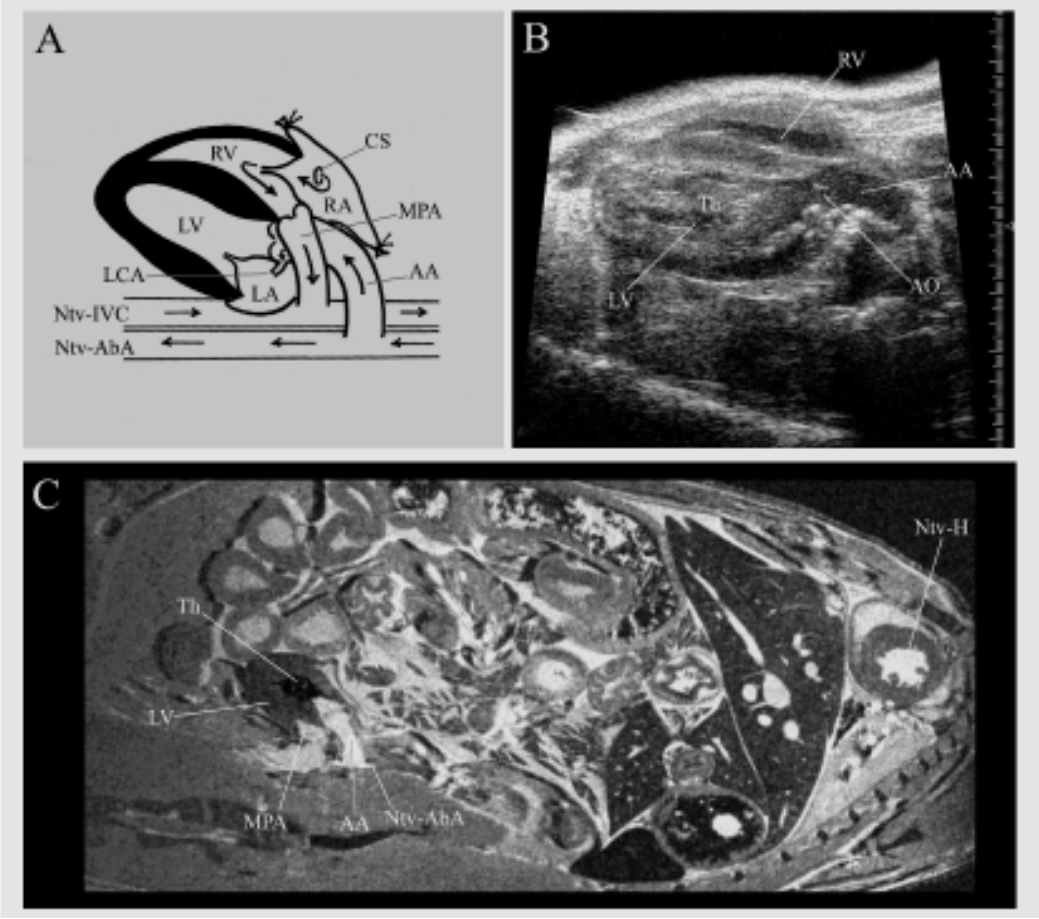

Figure 1 | Schematic Illustration and Images of Anastomoses and Blood Flow in Heterotopically Transplanted Cardiac Grafts in Mice. A. The cardiac graft is heterotopically implanted in the abdomen with the ascending aorta (AA) anastomosed to the native abdominal aorta (Ntv-AbA) and main pulmonary artery (MPA) to the native inferior vena cava (Ntv-IVC). The pulmonary veins and vena cava of the graft are ligated. **B.** High-frequency ultrasound biomicroscopic (UBM) image of an isograft on day 1 post-transplantation, showing the longitudinal section of the flow channel from aortic anastomosis, AA to the left ventricle (LV). **C.** An MRI of an isografted, fixed mouse on day 50 post-transplantation, illustrating the native heart (Ntv-H) and the cardiac graft in an oblique sagittal section. *AO*, aortic orifice; *CS*, coronary sinus; *LCA*, left coronary artery; *RA*, right atrium; *RV*, right ventricle; *MPA*, main pulmonary artery; *LA*, left atrium; *LV*, left ventricle; *Th*, thrombus. Reprinted from Zhou *et al.*, *Ultrasound Med Biol*, 33(**6**)870-9 (2007) with permission from Elsevier (RB is co-author).

are being developed to improve survival and reduce complications (17, 18). Xenotransplant models also exist for various inter-species combinations (e.g., rat -> mouse; pig -> monkey) (8, 19, 20).

Vascularized vs. Non-Vascularized Grafts

Primarily or fully vascularized allografts perfused by blood from the recipient circulation, such as kidney, liver, and heart, have many advantages over non-vascularized transplants such as skin allografts, in investigating mechanisms of graft susceptibility to rejection and tolerance induction (21). It is well documented that non-vascularized grafts are subject to non-specific ischemic degeneration that can lead to inflammation and necrosis, even in isografts, which can render them susceptible to subsequent immune rejection (22). However, vascularity alone has been shown to be insufficient to explain the predisposition for non-vascularized grafts to reject faster than vascularized ones (23). One explanation is the presence of tissue-specific antigens. Work by Steinmuller et al. have shown that in hemopoietic chimeras, allogeneic hemopoietic cells survived indefinitely but would not accept skin grafts from the same donor, suggesting that the skin expressed antigens not found on the hemopoietic cells (24). Other important factors to consider are the number and type of antigen presenting cells present in the graft; skin has an abundant number of Langerhans cells — professional antigen presenting cells — which have the capacity to migrate from the graft and efficiently stimulate a large number of alloreactive T cells (25). Finally, the physical size of the graft may also be a determinant in allograft rejection susceptibility; a larger graft or several grafts implanted to the same recipient have a greater total number of cells and thus, require more time for immunological destruction before graft failure ensues (26).

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Advantages and Disadvantages of Using the HHTx Murine Model in Transplant Research

Since the HHTx rodent model does not possess venous return to the graft, only limited comparisons can be made with the physiology of the native heart. The use of orthotopic grafts in rodents have been limited in the past due to lack of availability of cardiopulmonary bypass systems for these small animals, particularly mice, however, newer surgical techniques appear to enable transplanted hearts to have normal blood flow direction that approximate the native mouse heart (27). Furthermore, there are numerous studies employing the murine model in gaining translational insight into long-term graft changes that are clinically relevant, such as the development of graft vasculopathy, though these will not be discussed further (28-30).

Though the mouse abdominal HHTx model is a non-physiologic, non-loaded heart model due to the unique directionality of intra-cardiac blood flow (31), it is still valuable in investigating a wide variety of transplant-related issues (32, 33). The benefits of the model include: lower maintenance and surgical costs, limited number of personnel required, availability of a plethora of genetically inbred lines, and isolation and characterization of the MHC molecules in congenic inbred murine lines (1). Studies on MHC molecules form the foundation for understanding immune-mediated responses to allografts. The mouse model also provides important information on graft ischemia-reperfusion injury, rejection pathogenesis, specific reagents for detailed examination of the immunopathology of allograft rejection, immune modulating agents and immunosuppressants, and the physiologic changes that take place post-transplant in vascularized organs (1, 7, 34-38).

Experimental Applications of the HHTx Mouse Model

Organ Preservation

Rodent heterotopic models of transplantation have been previously used to investigate the efficacy of novel preservation (39) and cold cardioplegia solution (40) as well as to determine the extent of myocardial injury subsequent to hyperkalemic cardioplegia and prolonged hypothermic ischemia (41). McGregor et al. have demonstrated, for example, the beneficial effects of St. Thomas' cardioplegia solution in augmenting myocardial protection (40). The University of Wisconsin solution (UW lactobionate) has also been evaluated in HHTx rodent models to determine the degree of cardiac injury (39, 42). Other solutions studied include 0.9% saline, lactated Ringer's and cold solution (43). First glimpses into ischaemic times were also gleaned from rodent studies and continue to offer beneficial information translatable to clinical practise (44-46).

Graft Surveillance and Rejection

HHTx rat and mouse models are widely applied for immunologic and histological studies (35, 47-49). Investigation of effector mechanisms of graft-infiltrating cells by utilizing rat and mouse models of adoptive transfer followed by HHTx surgery has led to a wealth of information regarding immunopathological mechanisms. A sample of the literature includes: determining the critical role of CD4+ and CD8+ T lymphocytes in stimulating graft rejection and their mechanistic roles (50-52); the potential therapeutic effect of antagonism of chemokine (CCR5) and chemokine receptors (CXCR3) in inhibiting both acute and chronic allograft rejection (48); understanding the association of increased Fas ligand mRNA expression with myocardial apoptosis and ischemia-reperfusion injury and the eventual progression to chronic rejection (53); and studying the post-transplant shift in expression of genes classified as defense, communication, and metabolism during the graft response to transplantation injury and rejection (54). For further reading on this large topic, a recent review by Wehner *et al*. is worth consulting (55).

Pharmacological Testing and Immunosuppression

A number of pharmacological agents have been tested in rodent HHTx models for safety and efficacy prior to studies in larger animals and validation to clinical trials. A few examples include Cyclosporin A (7), pioglitazone (35), sirolimus/rapamycin (5), and mycophenolate mofetil (56). Other studies, such as Mitsuhashi *et al.'s*, have used gene therapy to induce xenograft tolerance and provided the first demonstration of permanent survival of alphaGal+ hearts following transplantation with autologous bone marrow transduced with porcine GalT-expressing lentivirus (57). Co-stimulatory blockade antibodies (58), genetically engineered dentritic cells (59) and other agents (60) have also been used to induce cardiac allograft tolerance in the mouse and rat. Finally, several monoclonal antibodies targeting molecular epitopes expressed on vascular endothelium and activated T cells have been utilized to induce tolerance and/or improve long-term graft viability; these include intercellular adhesion molecule-1 (61), lymphocyte function associated antigen and vascular cell adhesion molecule (62).

Xenotransplantation

Cordant and discordant xenograft rejection have been studied in several common species combinations, such as mouse->rat, hamster->rat, guinea pig->rat and hamster->guinea pig, to examine the efficacy of immunosuppressive drugs (63), total lymphoid irradiation, antibodies (64), and inflammatory mediators (65) in settings of accelerated and hyperacute rejection.

Gene Knock-out and Transgenic Models

With the advent of the genomics and proteomics era, specific gene knock-out and transgenic models have now become available, and are currently being produced in the mouse model (50, 66-68). These developments include transgenic single T-cell receptor (TCR)/peptide/major histocompatibility complex molecule models (50), transcriptional regulator class II transactivator deficient mice (66), CD40 ligand knock-out mice

(67), and IL-4 deficient mice (68), to name a few. The vast array of possibilities, which are plentiful in literature, have paved the way for molecular biologists, clinical biochemists, and transplant scientists to elucidate specific gene-protein pathways and the components of the immune system that are implicated in the rejection process.

Dynamic Graft Gene Expression

Changes in gene expression post-transplant have been assessed in transplanted mice using DNA microarrays. Work by Christopher et al. used dendrograms and self-organizing maps to determine the differences between isograft and allograft gene expression profiles (classified broadly into defence, communication and metabolism genes) and confirmed by real-time PCR (54, 69). The authors' results, which are beyond the scope of this review, demonstrated the temporal pattern of gene expression of grafts in response to transplantation injury and rejection.

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Assessing HHTx Graft Rejection in the Mouse and Rat

Palpation and Histology

Two common methods for assessing graft function in rat and mouse HHTx models employ finger palpation of cardiac contractions (13, 70) and histology (71, 72). The finger palpation method uses a subjective scale where an observer scores the force of contraction from '0' (cessation of graft contraction) to '4' (vigorous graft contraction). Several criticisms of this method of assessment include its subjectivity, insensitivity in detecting early changes post-transplant (PTx), and poor evaluation of long-term graft survival (73). The 'gold standard' for diagnosing rejection clinically and experimentally remains histology (46, 74, 75). Biopsies however are limited by their invasiveness, the patchy nature of cellular infiltration and tissue necrosis (76-78) and are not feasible for serial studies in mice and rodents due to the small size of the rodent heart (14).

Electrocardiography (ECG)

Electrocardiography has been performed in anaesthetized animals with needle electrodes inserted subcutaneously in the abdomen and limbs of the rat (71) and mouse (79). Early attempts reported an accurate determination of graft rejection though high graft heart rates (350-450 BPM) and poorly defined S-T segments precluded consistent interpretation (80). More recently, analysis of total power of heart rate variability was found to be significantly higher in acutely rejecting rat HHTx grafts when compared to isografts (81) using an abdominally-implanted telemetric ECG radio-transmitter. In mouse HHTx studies, allografts were rejected in 10-14 days and showed a rapid fall in palpated heart rate, measured heart rate, and ventricular QRS complex voltage (73). The major limitation of ECG is the possibility that the trace can be affected by the location of abdominal electrodes. This complication is compounded by the lateral and horizontal movements of the graft within the abdomen (73) and interference from surrounding structures such as the intestines and peri-graft effusion (14).

Nuclear Magnetic Resonance (NMR) and Related Applications

NMR imaging has been used for pre-clinical investigations evaluating myocardial metabolism during rejection in rat HHTx grafts (82). Haug et al. found that rejecting grafts showed relatively low levels of phosphocreatinine and high levels of inorganic phosphate versus isograft controls (82, 83). More recent efforts have used magnetic resonance imaging (MRI) to measure reduction in MR signal intensity in allografts versus isografts following injection of dextran-coated ultrasmall super-

paramagnetic iron oxide particles, which was confirmed to be due to macrophage uptake of these particles. The reduction in intensity was also reversible with immunosuppression (84). Other MR-based approaches have been investigated to assess morphological and physiologic changes in rat HHTx grafts, such as the degree of edema, ventricular mass and volume changes, SV, %EF, and LV chamber dimensions, but have not yet been validated for wide-scale experimental use (85). NMR-based approaches have several limitations, however, which include the need for highly technical protocols, very costly equipment, its invasiveness, and the inherent inability in obtaining serial data in the same animal subject in perfusion studies. MRI is also a less desirable option for mice, whose haemodynamic status can be significantly altered by the imaging procedure and its utilization of deep or prolonged anaesthesia (83, 86).

Molecular and Biochemical Markers of Cardiac Graft Rejection

There is a large body of work focused on scintigraphy in detecting cardiac allograft rejection in mouse and rat HHTx models. Previous studies have employed various isotopes and targets to study the effects of acute rejection on radiotracer uptake. These include 123iodine-labeled and ^{111}In anti-ICAM-1 mAbs (87, 88), ^{111}In-labeled anti-MHC class II antigen mAbs (88), and 111In-antimyosin mAbs (89), and most have documented increased accumulation of the respective isotope in association with histologic rejection. The requirements for costly antibodies and radiological materials as well as the lack of consensus on specific molecular targets important for detecting rejection however have precluded these approaches for routine use. Work by Suzuki et al. and others have focused on assessing upregulation of cytokines (e.g., IL-2, IL-4) and their transcripts (e.g., IFN mRNA) in infiltrating cells during rejection in murine HHTx grafts (7, 90), though the majority of studies suggest that these techniques be used as an adjunct to histology.

Ultrasound and Related Applications

The current state of conventional echocardiography and the novel high-frequency ultrasound biomicroscopy (UBM) modality to image acutely rejecting HHTx cardiac grafts non-invasively is summarized in **Table I**. The recent development of gas-filled, lipid-based antibody-conjugated microbubbles for binding cellular and biochemical targets important in graft rejection has proposed myocardial contrast echocardiography as an exciting and promising method of detection in experimental and clinical settings (91). Recently, ultrasound imaging of rat HHTx grafts following injection of microbubbles targeted to ICAM-1 illustrated the ability of adhered bubbles to induce significantly increased myocardial video-intensity in rejecting versus control grafts (76). Microbubbles are currently being tested for UBM applications (92).

Diastolic and Systolic Dysfunction

Previous studies on murine HHTx grafts indicate that both the left and right atria do not participate in atrial filling and thus are not subsequently involved in ventricular filling during diastole (14, 77). However, the relaxation time constant (TE) of the LV pressure fall has been used to evaluate LV diastolic dysfunction during allograft rejection in rats (93) and was reported to be prolonged in rejecting grafts versus control; the authors suggested this finding resulted from impaired myocardial relaxation due to decreased myocardial blood flow and increased LV end-diastolic pressure. Other studies have utilized maximum rate of LV pressure rise (dP/tmax) though the data in rat HHTx allografts has yielded conflicting results (94, 95). The validity of assessing peak systolic and diastolic LV pressures in rat HHTx grafts indicate that both quantities decrease significantly in allografts on day 5 PTx, but are unchanged in isografts (93). However, these measurements require implantation of a balloon in the graft to measure pressure-volume relations.

Though clinically parameters of systolic function have proved to be of limited use (96), inferior to diastolic changes (97), and not rejection-specific, significant decreases in % ejection fraction and stroke volume have

Table 1 | Summary of Experimental Echocardiography in HHTx Animal Models and Applicability to the Mouse Model.

Standard 2-D Clinical Echocardiographic parameters used and/or are associated with acute cardiac graft rejection	Echo parameters used to evaluate heterotopic (abdominal acute graft rejection)	Directionality and mechanism of change in heterotopic acute graft rejection
Graft mass J Am Soc Echocardiogr. 2002; 15:917-25.	**Heart mass (HETERO-RAT)** J Thorac Cardiovasc Surg. 1994 Nov;108(5):928-37, Am J Physiol Heart Circ Physiol 284: H2061-H2068, 2003	Increased wet/dry weights in allografts on day 5 post-Tx vs. isografts due to ischaemic injury and PMN infiltration; 38% decrease in isograft mass @ day 7
Aortic regurgitation Am J Cardiol. 1994;73:1197-201.	**Aortic regurgitation (HETERO-MOUSE)** Ultrasound Med Biol. 2007 Jun;33(6):870-9	Found to be consistently elevated in isografts; No parallel study on allografts
Mitral regurgitation Echocardiogaphy. 1992 Mar;9(2):169-74	**Mitral regurgitation (HETERO-MOUSE)** Ultrasound Med Biol. 2007 Jun;33(6):870-9	Found to be consistently elevated in isografts; No parallel study on allografts
% Ejection fraction Transplant Proc. 38, 636-638 (2006)	**%Ejection Fraction, %Fractional Shortening (HETERO-MOUSE)** Am Soc Echocardiogr 2002; 15;1315-20	%EF and %FS decreased significantly during acute rejection in allografts but differences vs. isografts may not be significant except at severe rejection
Stroke volume Transplant Proc, 38, 636-638 (2006)	**Stroke work, cardiac output (HETERO-RAT)** J Thorac Cardiovasc Surg. 1991 Mar;101(3);446-9.	Biopsy-proven rejecting day 3 post-Tx allografts had diminished CO and SW after isoproterenol injection when compared to isografts; due to systolic dysfunction
Posterior wall thickness Arq Bras Cardiol. 1989 Sep;53(3);151-5.	**Posterior wall thickness (HETERO-MOUSE)** J Am Soc Echocardiogr 2002 Oct; 15(10 Pt 2); 1315-20.	Increases in PWTh isografts early post-Tx due to I/R injury; gradual increases in PWTh post-Tx are associated with histological evidence of myocyte necrosis, cell infiltration
Heart rate variability J Heart Lung Transplant. 1998 Jun;17(6);578-85.	**Heart rate variability (HETERO-RAT)** J Heat Lung Transplant 1999; 18:499-509.	Total power of HR variability in allografts increased significantly from 1.5 to 6 days post-Tx; Allograft peak-to-peak amplitudes of QRS complex and HR were significantly decreased on day 5.5 or later
Isovolumic relaxation time J Heart Lung Transplant. 2005 Feb;24(2):160-5	**Relaxation time (HETERO-RAT)** World J Surg 2001; 25(5): 545-552.	Prolonged relaxation time in histologically rejecting grafts starting at 3 days post-Tx; may be related to histological rejection and/or decreased MBF and possibly ischaemia
LV peak systolic and end-diastolic pressure	**LV peak systolic, end-diastolic pressure (HETERO-RAT)** World J Surg 2001; 25(5): 545-552.	Significant decrease in LVPSP at day 5 post-Tx in allografts; stable in isografts LVEDP increased significantly on day 5 post-Tx in allografts, stable in isografts; etiology not discussed
Tricuspid early peak flow velocity Circulation. 1992 Nov;86(5 Suppl):II259-66	**Tricuspid Inflow (HETERO-MOUSE)** Ultrasound Med Biol. 2007 Jun;33(6):870-9	Small and variable in waveform and not measured post-Tx in isografts; No parallel study on allografts
Intraventricular septal thickness Eur Heart J. 1989 May; 10(5):400-8	**Intraventricular septal thickness (HETERO-DOG)** J Heart Lung Transplant. 1999 Jun; 18(6):510-6	Significant increase in IVS thickness prior to sacrifice (5-7 days) in dog allografts; Etiology not discussed
LV end-systolic volume Circulation. 1987 Nov;76(5);998-1008	**Mean LV filling volume (HETERO-RAT)** J Heart Lung Transplant. 1998 Jun; 17(6):608-16	Decreased LV filling volume in day 5 post-Tx allografts; Edema and ischaemia possible causes
Myocardial echogenicity Circulation. 1997 Jan 7;95(1):140-50.	**Myocardial echogenicity (HETERO-GOAT)** J Heart Transplant. 1987 Jan-Feb;6(1):1-7.	All myocardia increased in brightness at day 3 but decreased thereafter; caused by myocardial edema due to ischaemia
Left ventricular chamber size J Heart Lung Transplant. 1993 Nov-Dec; 12(6 Pt 1):1009-17	**Left ventricular end-diastolic diameter (HETERO-MOUSE)** Am Soc Echocardiogr 2002;15:1315-20	Mild decreases in LVEDD in isografts but significant decrease seen in day 5 allografts when compared to day 1 post-Tx and isografts; not measured past day 5 post-Tx
Myocardial blood (coronary) flow Circulation 79:59, 1989	**Myocardial blood (coronary)flow (HETERO-RAT)** J. Heart Transplant. 8:48, 1989	Significant decrease in myocardial coronary flow on day 3 post-Tx but no changes after day 5 in allografts; histologic rejection and increased edema assoc. w/ reduced MBF
DP/tmax values (maximum rate of rise of LV pressure) Circulation. 1989 Jan;79(1):66-75	**DP/tmax values (max rate of LV pressure rise) (HETERO-RAT)** J Heart Lung Transplant. 1999 Jun; 18(6):524-31	Significant changes in DP/tmax values in day 5 allografts vs. day 1 values and isografts; Isografts remained stable during period of observation

been associated with histologic rejection (98). In rat HHTx allografts, diminished cardiac output and stroke work after isoproterenol treatment was found on day 3 PTx (99). Furthermore, studies in HHTx murine grafts indicate a diminishing trend in %EF and %FS in allografts from days 3 to 5 PTx (77). In a recent study employing UBM-Doppler by Zhou et al., left ventricular %FS was found to increase significantly in the long-term, from day 14 to 50 in isografts (14). No parallel study has been reported for allografts.

...after 7 days PTx in murine allografts, coronary blood flow velocities were significantly diminished, whereas it significantly increased from day 1 to 5 and remained consistent after 2 weeks post-implantation in isografts.'

Coronary Perfusion

Studies in rat HHTx models have shown a marked decreased in myocardial tissue blood flow as assessed by the hydrogen-clearance method in day 3 and 5 allografts, whereas it remained stable in isografts (93). Using UBM-Doppler and M-mode analyses, Zhou et al. showed that after 7 days PTx in murine allografts, coronary blood flow velocities were significantly diminished, whereas it significantly increased from day 1 to 5 and remained consistent after 2 weeks post-implantation in isografts (14), thus proposing a novel, non-invasive method for detecting allograft rejection relatively early in mice. The same study also characterized a previously undocumented Doppler waveform of coronary flow in murine grafts, showing the complex interaction between the recipient and donor cardiac cycles.

Valvular Dysfunction

Tricuspid regurgitation is common in clinical orthotopic grafts (due to elevated pulmonary vascular resistance) (100) but does not seem to be applicable in the mouse model. The right atrium and ventricle are under conditions of low pressure due to non-loading conditions and atrophy (73), inevitably resulting in right atrial and ventricular collapse. Furthermore, Zhou et al. previously showed in murine HHTx isografts that tricuspid and pulmonary UBM-Doppler waveforms are small in amplitude and variable in waveform, thus precluding these measurements as reliable parameters of evaluating rejection (14). The study also characterized mitral and aortic regurgitation in isograft control studies, which showed high regurgitant jets PTx, with consistently high values (>200 cm/s) achieved in isografts after two weeks post-implantation.

Graft Morphology

Serial echocardiography detected a significant increase in LV posterior wall thickness and conversely, a decrease in LV end-diastolic diameter in rejecting murine allografts from days 0-5 PTx versus isografts (77). Unfortunately, the poor resolution at lower frequencies and the high inter- and intra-observer variability have limited interpretation and applicability of these findings. Furthermore, this observation should be taken cautiously, since ventricular atrophy due to the non-loading nature of the graft can result in decreased myocardial wall thicknesses in isografts (101, 102). Utilizing high frequency echocardiography (UBM, ~30 MHz), Zhou et al. found LV anterior wall thicknesses were consistent throughout the post-transplantation period in isografts, but posterior wall thicknesses gradually decreased and the LV chamber dimensions were reduced (14), likely due to atrophy and low filling pressures. It is anticipated that a comparison study will be published for allografts.

Conclusion

The heterotopic mouse model is an indispensable tool in experimental cardiac transplantation. It combines the advantages of murine studies with a well-established model of fully-vascularized organ transplantation. It is likely that in the boom of the genomics and proteomics era, our understanding of molecular and biochemical mechanisms of graft tol-

erance and acceptance will be enhanced with the increasing variety of genetically inbred murine strains. Consequently, the need for a reliable method for assessing graft status following transplant will likely continue to be explored, though mouse echocardiography seems to be most promising since it can be performed quickly, is non-invasive, and provides a wealth of information on graft physiology, haemodynamics and morphology.H

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About the Author

Ramy obtained both his Honours Bachelor of Science (2004) and Master of Science (2007) from the University of Toronto, Canada, the latter from work at The Hospital for Sick Children, Toronto. He completed his medical training at Sydney Medical School, Australia (2010) and is a junior doctor at St George Hospital, a large and busy teaching centre in Sydney's South East. He has published a small number of publications in the area of murine (Ultra Med and Biol, Jun 2007;33(6):870-9) and clinical cardiac transplantation (Hypothesis 4(1):19-27 (2006)) as well as authored one book in the field entitled 'Seeing The Transplanted Mouse Heart Like Never Before' (ISBN Number: 3836487381). He's presented at national conferences and has dozens of abstracts. Ramy's current interest lies in obesity medicine and, to that end, is completing a retrospective study on the optimal metabolic rehabilitation of Type II Diabetics at Concord Repatriation General Hospital, a tertiary teaching hospital of the University of Sydney. Ramy recently married a beautiful Australian and enjoys Sydney's beaches whenever he can.

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