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Rat heart-aorta cluster transplantation: a novel model to study transplant rejection

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Abstract The purpose of this study was to develop a microsurgical cluster model of heart plus entire thoracic aorta transplantation and to compare it to the isolated model of heart transplantation as a tool to study transplant rejection. Thirty-six syngeneic (DA × DA and Lew × Lew) and allogeneic (DA × PVG and DA × Lew) cluster heart-aorta transplants were compared to 43 syngeneic and allogeneic isolated heart grafts. Graft survival, recipient survival and histological data on myocardial and aortic tissues were assessed. There was no statistically significant difference in graft survival between the two models studied ($P > 0.05$). In the cluster transplants, the aortic component was spared the severity of acute rejection noted for the myocardial counterpart. In conclusion, the results demonstrated that the cluster model was technically feasible and highly reproducible. Additionally, it was possible to apply this model to the study of experimental allograft rejection using novel immunosuppressants. The success of the cluster model in strongly mismatched transplant strain combinations underscores its potential for application in slower rejection combinations, making it particularly suited for chronic rejection studies. The inherent capacity for sampling a broader range of vessel sizes in one animal makes the cluster model more suitable than the isolated models of aorta or heart for application to experimental protocols.

Key words Aorta transplantation, rat · Heart-aorta transplantation, rat · Rat, heart-aorta transplantation · Rejection, heart-aorta transplantation, rat

Introduction

Pressure to identify alternative and efficient treatment modalities that reduce the need for retransplantation for chronic rejection in human allografts is gaining momentum in the face of a limited supply of donor organs. Chronic rejection is becoming especially important in the case of vital organs such as the heart and liver, where bridge-to-graft options are scarce. By way of example, 5 years post-transplantation, human cardiac allografts are predisposed to a 50% chance of significant coronary artery disease. The appearance of arteriosclerosis and accompanying intimal proliferation in the coronary vessels are hallmark features of this post-

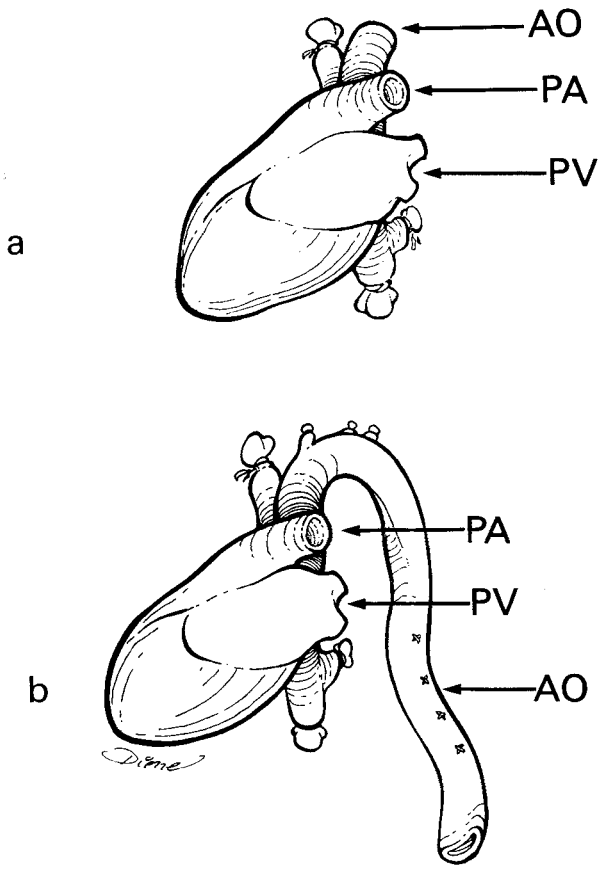

Fig. 1 a, b Donor organ procurement in: **a** isolated heart model; **b** cluster heart-entire thoracic aorta model (*AO* aorta, *PA* pulmonary artery, *PV* pulmonary veins

transplant lesion, which is the commonest cause of late graft loss resulting in sudden death in grafted patients [3]. Complementing the situation in clinical heart transplantation, kidney transplants under cyclosporin immunosuppression are subject to a 25 % chance of chronic rejection (the second leading cause of graft loss for this organ group) 5 years post-transplantation [1].

Since the rat aortic allograft model closely resembles human transplant vasculopathy, it will continue to be used in the search for newer explanations of the disease process. Novel immunomodulating agents proposed for the treatment of chronic rejection will also undergo evaluation using such a model. The isolated models of heart [6, 8, 9, 15, 16, 19, 20, 22] and aorta [4, 14, 21, 23, 27] transplantation have been routinely used to study acute and chronic rejection under immunosuppression. With numerous recent introductions of novel immunosuppressants in transplantation, these models are becoming increasingly popular in research. Chronic rejection, in particular, has been studied using these isolated models of transplantation.

The purpose of the present study was to develop a microsurgical cluster model of heart plus the entire thoracic aorta transplantation and to demonstrate its feasibility and reproducibility as a tool to study transplant rejection. In the context of graft survival and histological changes in the component units of the cluster (heart and aorta), the cluster model will be compared to the isolated heart model. If the cluster model proves technically feasible and reproducible, without substantially deviating from the isolated heart in terms of graft survival and histology, then this model could provide substantially more data on the changes in a variety of arterial vessels, ranging from the larger calibered aorta to the myocardial microvessels in the cluster graft, in one animal. With the onus to refine experimental protocols and reduce the numbers of animals used in preclinical investigational research without sacrificing the rigor of experimental designs and the quality of the resulting data, the cluster model, if feasible, would be an attractive option for replacing the isolated models in the preclinical investigation of novel immunosuppressive agents.

Materials and methods

Rats

Inbred strains of male DA (RTA^a), Lewis (RT1^l) (Lew) and PVG (RT1^c) rats (200–250 g) were obtained from Harlan and Olac (Bicester, UK) and Charles River (UK) and maintained in our facility under appropriate conditions. They were allowed unrestricted access to food and water and a period of acclimatization before being subjected to surgery. The animals were handled and cared for humanely according to the "Principles of Laboratory Animal Care" (NIH Publication No. 85–23, revised 1985) and prevailing Home Office guidelines in the UK. In addition, the procedures performed in this study were licensed by the Home Office governing body. Donor operations were carried out under terminal enflurane anesthesia, while recipient surgery was performed under recovery anesthesia consisting of a mixture of enflurane (2 l/min) and oxygen (2 l/min) using a Boyle's anesthetic outfit. All surgical procedures were conducted using an aseptic technique. For the donor operation, a x 2.5 magnification was adequate, whilst the vascular anastomoses in the recipient were performed under a x 6.5–x 10 magnification using an operating microscope.

Donor operation (Fig. 1)

The donor's peritoneal cavity was opened by a transverse subcostal incision followed by division of the portal hilar vessels and the infrahepatic vena cava (IVC). The entire diaphragm was first divided and then the costal flap was reflected and fixed on the neck using a hemostat. The aorta was then divided at the diaphragmatic level in order to achieve thorough exsanguination and the suprahepatic IVC was clamped at the diaphragm. These steps achieved a rapid exsanguination of the ventricular and atrial cavities and provided adequate exposure for the rest of the donor procedure. Next, the superior vena cavae were dissected, ligated and divided

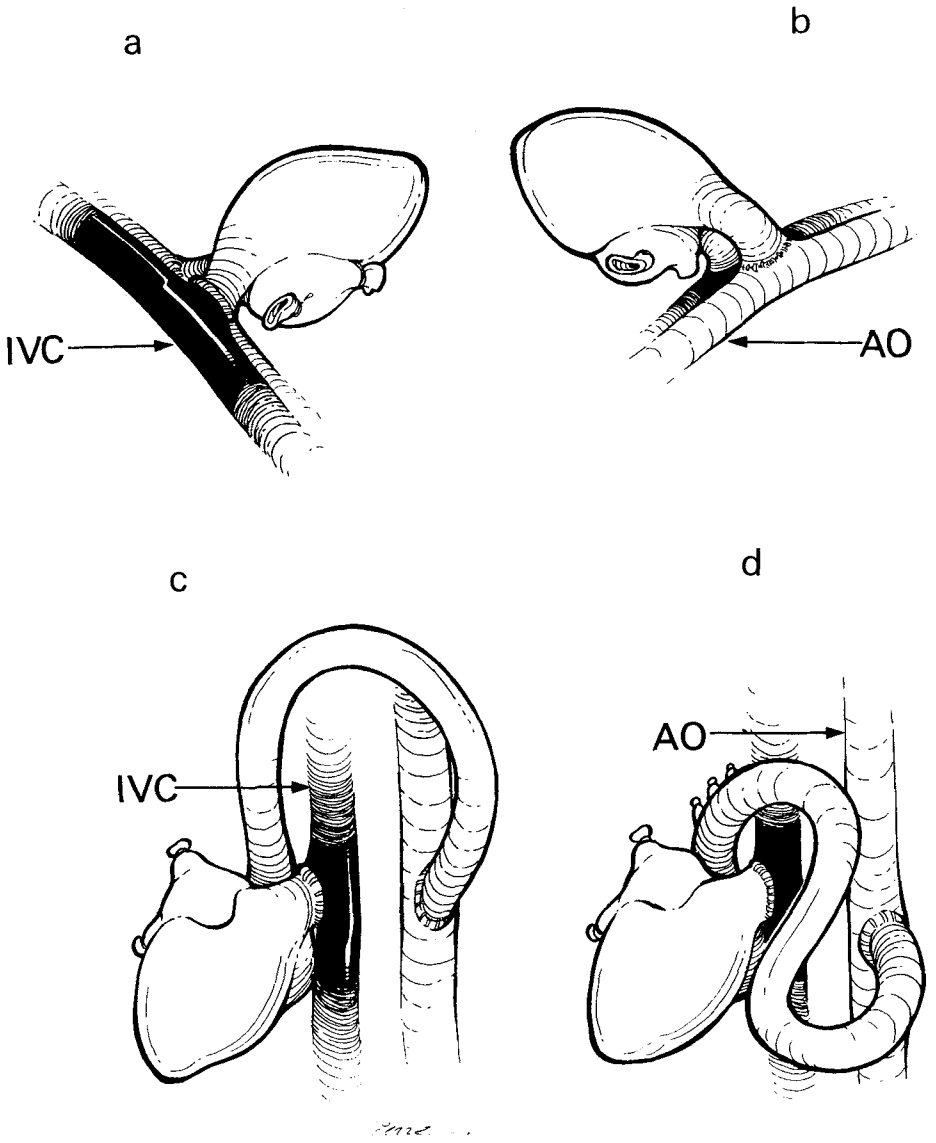

Fig. 2 a–d Recipient transplantation. Isolated heart model: **a** view of pulmonary arterial *(PA)* anastomosis to recipient's inferior vena cava *(IVC)*; **b** view of aortic anastomosis to recipient's aorta *(AO)*. Cluster heart-aorta model: **c, d** variations in the orientation of the transplanted aortic segment

as in the technique for isolated heart procurement. Once this was achieved, the root of the pulmonary trunk (PT) was dissected bluntly and the en bloc heart-lungs-aortic unit was perfused via the IVC with 4–5 ml of 4 °C heparinized lactated Ringer's solution. The IVC was then tied and divided at the end of the perfusion, followed by the division of the PT. At this point, both lungs were excised separately after ligating their individual hilae, which left the heart-aortic unit in situ. A cold sponge was placed over the heart and the branches of the aortic arch were identified, dissected and divided serially. This dissection and serial ligation was carried distally to the descending thoracic aorta (AO) with a view to identifying and ligating as many lumbar branches as possible. A x 6.5 magnification greatly aided this step of the procurement. Once the entire AO was freed, the heart-thoracic-aortic unit was excised from the thorax by dividing the trachea and stripping the entire preparation from the esophagus. The cut end of the donor AO of the en bloc unit was flushed of blood and stored at 4 °C in normal saline until transplantation. Figure 1 shows the procured cluster graft in comparison to the isolated heart.

Recipient operation (Fig. 2)

The abdomen was entered via a midline incision, and the small bowel was retracted to the left side of the animal after dividing the ligament of Treitz. Minimal gauze-aided blunt dissection of the recipient's abdominal infrarenal AO and IVC was followed by the application of a single Lee vascular clamp to both vessels. The arteriotomy was positioned slightly lower than the venotomy, thus ensuring a smoothly contoured aortic loop at the end of transplantation. End-to-side vascular anastomoses were performed between the donor's AO and PT and the recipient's AO and IVC, respectively, using 9-0 or 10-0 monofilament nylon. Prior to releasing the Lee vascular clamp, a small Acland vascular clip was placed on the transplanted AO just above the aortic anastomosis. This step permitted a controlled reperfusion of the aortic segment and heart by helping to identify and tie hitherto unligated lumbar arteries. Any such vessels were treated accordingly using 9-0 nylon. Within 2 min of declamping, reperfusion of the cluster graft resulted in a smoothly configured aortic loop and sinus rhythm. The abdomen

Table 1 Experimental design and graft survival (*CMC* carboxymethyl cellulose, *Lef* leflunomide, *CyA* cyclosporin, *FK* FK 506)

(A) Isolated heart model					
Graft type	Drug/dose	Schedule (days)	Route	Survival (days)	Median/range (days)
Syngeneic					
DA × DA (n = 4)	CMC	0–29	Oral	> 100	> 100
Lew × Lew (n = 5)	CMC	0–29	Oral	> 100	> 100
Allogeneic					
DA × Lew (n = 5)	CMC	0–9	Oral	4, 5, 5, 5, 5	5/4–5
DA × Lew (n = 20)	Lef 5 mg/kg	0–9	Oral	20, 20, 21	20/20–21
	Lef 10 mg/kg	0–9	Oral	14, 14, 14, 15	14/14–15
	Lef 20 mg/kg	0–29	Oral	35, 36, 44, 46	40/35–46
	CyA 15 mg/kg	0–29	Oral	37, 37, 38, 40, 44	38/37–44
	FK 1 mg/kg	0–29	Oral	41, 45, 45, 48	45/41–48
DA × PVG (n = 9)	Lef 10 mg/kg	0–9	Oral	7, 16, 16, 16	16/7–16
	FK 0.3 mg/kg	0–9	i.m.	18, 25, 27, 28, 31	27/18–31
(B) Cluster heart-aorta model					
Syngeneic					
DA × DA (n = 5)	CMC	0–29	Oral	> 100	> 100
Lew × Lew (n = 5)	CMC	0–29	Oral	> 100	> 100
Allogeneic					
DA × Lew (n = 5)	CMC	0–9	Oral	4, 4, 5, 5, 5	5/4–5
Da × Lew (n = 19)	Lef 5 mg/kg	0–9	Oral	19, 19, 19, 21	19/19–21
	Lef 10 mg/kg	0–9	Oral	14, 14, 14, 14	14
	Lef 20 mg/kg	0–29	Oral	35, 36, 36, 40	36/35–40
	CyA 15 mg/kg	0–29	Oral	35, 37, 38, 38	37.5/35–38
	FK 1 mg/kg	0–29	Oral	42, 45, 45	45/42–45
DA × PVG (n = 2)	Lef 10 mg/kg	0–9	Oral	16	16
	FK 0.3 mg/kg	0–9	i.m.	26	26

was closed in two layers after ensuring that the aortic loop was not kinked or obstructed. Figure 2 c, d schematically depicts the most frequently encountered orientation of the aortic loop. In comparison, Fig. 2 a, b shows the conventional isolated heart model as viewed from the graft's aortic and pulmonary arterial sides.

Experimental design and immunosuppression (Table 1)

Table 1 shows the different strain combinations used in the development of the cluster model. The experimental design served to compare graft survival of the isolated heart versus the cluster heart-aorta model in the presence and absence of immunosuppression. Grafts were exchanged across weaker (DA × PVG) and stronger (DA × LEW) histocompatibility barriers in an effort to take into account the MHC disparity present in the clinical population. Syngeneic exchanges served as controls between DA and Lew rats separately. The two models in this study were compared using a monotherapy immunosuppression protocol. Cyclosporin (CyA), FK 506 (FK) and leflunomide (Lef) were administered for a period of 10 or 30 days, beginning on the day of transplantation. A shorter period of immunosuppression, i.e. 10 days, and a control situation of no immunosuppressive cover provided the avenue to study aortic rejection (if it presented) separately and the effects of a rejecting cardiac graft on the accompanying aorta. Immunosuppression was given for as long as 30 days post-transplantation in an effort to prolong cardiac survival long enough so as to allow aortic rejection to occur. Using this strategy we hoped to create a set of conditions to simultaneously study graft vessel disease in the aorta and coronaries. All immunosuppression was withdrawn from the 11th or 31st day onward, respectively, and grafts were monitored for rejection.

Immunosuppressants

Cyclosporin (Sandoz Pharmaceuticals, Basel, Switzerland) and leflunomide (HWA 486; a gift from Hoechst, Wiesbaden, Germany) were suspended by agitation in 1% carboxymethyl cellulose (CMC) and given orally. FK 506 (Fujisawa Pharmaceuticals, Munich, Germany) was dissolved in normal saline and administered orally or intramuscularly.

Postoperative care, observations, management and endpoints

Ten millilitres of dextrose-saline was injected subcutaneously post-transplantation and the rats were transferred to a temperature-regulated warming cage and allowed to recover. Immediately upon transfer to their holding cages, they were permitted free access to food and water. Surviving rats were weighed and observed for rejection on a daily basis by palpating the transplanted heart. Donor and recipient operation times and graft ischemic times were recorded in every instance. Isolated heart grafts possessing a barely palpable ventricular impulse or complete cessation were considered rejected. The same endpoint was also used to assess heart graft survival for the cluster heart-aorta model. Rejection was confirmed at this time by laparotomy and histological examination. Rats bearing rejected grafts were sacrificed under terminal anesthesia by exsanguination. Prior to the collection of tissues, the

gross morphology of the transplanted heart and thoracic aorta was assessed for the cluster grafts. The transplanted aortic segment was palpated and compared to the nature of the recipient's own aorta. Furthermore, the transplanted aorta was transected to assess the presence of any gross luminal changes and the nature of arterial blood flow across the divided aorta. Additionally, this segment was slit open longitudinally to look for adherent thrombi or occlusion of its lumen. Finally, the heart was divided transversely in order to assess clinical changes in a manner comparable to that of the aorta.

Histology

The excised tissues were fixed in formol saline. A transverse block was taken through both ventricles and processed routinely through paraffin wax. Hematoxylin and eosin-stained sections were examined by an observer unaware of the treatment given. Features assessed included myocardial damage, interstitial inflammation and vascular changes. These were scored on a semiquantitative basis on the degree of changes or the approximate area of myocardium involved in the section: 0 = none; 1 = very mild or 0 %–4 %; 2 = mild or 5 %–24 %; 3 = moderate or 25 %–49 % and 4 = severe or > 50 %. A score of 4 for irreversible damage would indicate that at least half of the myocardium showed either myocyte loss from earlier damage and/or acute infarction and, therefore, incompatible with graft function. Myocardial damage associated with a diffuse lymphoid inflammatory infiltrate was considered to be possibly reversible and equivalent to acute rejection. Vascular changes were difficult to compare as intramural and epicardial vessels were often not present in the sections examined, owing to the nature of the time point at which specimens were collected in the study (completed rejection correlating with no graft heart beat).

The donor aorta was slit longitudinally and transverse sections were processed as for hearts. The aortic tissue was assessed histologically for intimal fibrosis, inflammatory cells and their position, adventitial and adjacent fibrosis, and vascularity. A semiquantitative scoring system was used to grade the changes in the aorta as follows: 0 = none; 1 + trace; 2 + mild; 3 + moderate and 4 + severe. The sum of the scores for each aorta was compared for the statistical analysis.

Statistical analysis

The histological score data and survival data for the different groups were analyzed using Kruskal-Wallis and Mann-Whitney tests. A P value below 0.05 was considered significant.

Results

Technical

The donor operation (donor ischemic time was counted starting from the moment of skin incision) lasted twice as long for the cluster graft procurement as for procurement of the isolated heart (Table 2). This difference is reflected in the overall slightly longer total donor ischemic time for the cluster grafts (45 min versus 34 min). Revascularization of the grafted en bloc unit was the endpoint for donor ischemic time. There were no technical failures for either of the two models tested in this series of experiments in the short or longer term.

Table 2 Technical results[a]

		Isolated heart	Cluster heart-aorta
(A)	Donor operation[b]	7	15
	Recipient preparation	7	7
	Transplantation	20	23
	Total graft ischemic time	34	45
(B)	Technical mortality	None	None

[a] Based on a single surgeon completing both donor and recipient procedures in series
[b] Times indicated are maximum limits and reported in minutes

Graft survival for the two models (Table 1)

The survival data for the recipients under the immunosuppressive treatment arm are shown here purely for the purposes of demonstrating and comparing the survival using the two models of transplantation under various immunosuppression protocols; they are not intended for the purposes of comparing the relative efficacy of one pharmacologic agent to another using graft survival as an endpoint. There was no difference in graft survival comparing untreated allografts in the isolated and cluster transplant models ($P > 0.05$). This trend was also present when matching groups were compared from the isolated and cluster series in the immunosuppression arm of the study ($P > 0.05$).

Postoperative findings: macroscopic

While the heart in the cluster grafts demonstrated acute rejection in untreated allografts, the accompanying thoracic aorta was grossly normal to sight and touch in comparison to the recipient's aorta. Division of the transplanted aorta resulted in brisk bleeding. Examination of the luminal side of this segment did not show any macroscopic evidence of adherent thrombi or vessel wall destruction as noted in its cardiac counterpart of the cluster grafts. The aortic anastomotic area appeared normal. Accompanying these signs was a shortened length of transplanted aorta. In the allotransplanted recipients under short-term immunosuppression, a similar gross morphological picture was seen at rejection. A normal cardiac and aortic transplant was seen in syngeneic recipients sacrificed at 5 months post-transplantation.

Histological results

Cardiac grafts

Rejection was documented in every heart graft (treated or control) originating from the isolated or cluster heart series. These rejected grafts were characterized

by a marked, diffuse lymphocytic infiltrate located throughout the interstitium, epicardium and perivascular spaces. Varying degrees of myocyte damage and fibrosis were observed but these predominantly indicated the presence of extensive and irreversible myocardial damage. Intramural and epicardial vessels, when identified, demonstrated changes including cellular infiltrates, thrombosis or intimal proliferation in untreated allograft recipients. In contrast, syngeneic hearts, belonging to either the isolated or cluster transplant groups, showed viable myocardium without epicardial or intramural vascular changes up to 100 days post-transplantation.

Aorta

Syngeneic recipients achieved a median score of 2 (range 0–3) at 5 months postoperatively. Statistically, this was not significantly different from the score obtained for normal aortas of age- and sex-matched control cohorts (0–3). However, untreated allografted recipients achieved a median score of 9 (range 2–13) 5 days postoperatively, significantly different from that of syngeneic and normal controls ($P < 0.05$). Taking into account the type of immunosuppressant used in this study (cyclosporin, FK 506 and leflunomide) and the schedules adopted (prevention of rejection under short and longer term treatments), the following results were obtained for aortas originating from the cluster grafts:

1. Untreated allograft aortas had higher scores than normal or syngeneic controls ($P < 0.05$).
2. The cardiac component of the cluster allografts under immunosuppression rejected within 2 weeks of discontinuing immunosuppression, whereas the aortic histology was surprisingly free of parallel changes, both in quantity and quality. From these data on the aortic component of the cluster graft it is evident that cardiac allograft survival can be extended sufficiently to allow for expression of aortic changes due to rejection, although the latter were minor in comparison to those seen in the heart graft. Owing to the nature of the endpoint in this study (cessation of heart beat correlating with graft rejection), the coronary vessels were found to be obliterated. Furthermore, the effects of a rejecting cardiac allograft on aortic rejection can be appreciated in this setting; there was a distinct asynchrony present between changes in the heart as a whole versus changes in the aorta. Statistically, the aortic scores obtained under these conditions were not found to be significantly different from their untreated allografted controls ($P > 0.05$).
3. The aortic score data did not reveal either a statistically significant dose-dependent effect for a given immunosuppressant (10-day course of Lef, 5 mg, vs Lef, 10 mg, vs Lef 20 mg; $P > 0.05$) or a time-dependent trend suggestive of higher scores associated with longer periods under a given immunosuppressant (10 days vs 30 days; $P < 0.05$).

Discussion

Cryopreserved human aortic allografts have been used to treat various forms of human primary aortic vessel disease [13, 26] or to correct infectious complications of primary procedures resulting from the use of prosthetic conduits [2, 24]. Postoperatively, calcific degenerative changes were commonly encountered in such grafts, the implicating factor being ascribed to immunological influences. These degenerative changes have been largely confirmed in a number of experimental models of large-vessel transplantation that have emerged over the years [10]. The general theme behind the investigations that followed the application of these models focused on the effects of cold preservation on the immunogenicity and endothelial cell viability of such grafts [7, 10, 17, 18]. These studies suggested that these grafts were especially prone to rejection because their recipients did not receive immunosuppression. What is also notable is that little mention is made of the aortic grafts at autopsy.

There is a close analogy between the latter and the situation in human cardiac transplantation. At autopsy or at regrafting for chronic rejection, the condition of the aorta is not described in great detail. Two possible inferences can be drawn; either the changes in the transplanted aorta are unremarkable for rejection in both quantity and severity, or the transplanted aorta, unlike the heart, is not subjected to the same detailed histological and immunohistochemical investigations. Shibata et al. [25] maintain that the main branches of the coronary system have been the focus of investigative attention in studies addressing transplant arteriosclerosis and that less attention has been devoted to the study of the same process in the more distal coronary branches. Shibata et al. further state that this distinction has gained importance in heart transplantation because it has been suggested that whereas the process is primarily immune-mediated in the former type of vessels, immunological damage assumed a minor role in the more distal myocardial vessels [25]. Insofar as the pathogenesis of transplant arteriosclerosis remains incompletely understood, appropriate and specific therapeutic interventions to prevent or reverse these changes will remain largely empirical and continuing research efforts in this field will find justification.

A survey of the transplant literature revealed that Boecyx et al. [5] alluded to the use of an extended aortic segment accompanying a cardiac graft in the context

of examining the role of portal venous drainage in rat cardiac allotransplantation. The purpose of using a longer aortic segment was to overcome the technical hurdle of draining the cardiac graft via the pulmonary artery into the recipient's portal vein. In another report, Goss et al. [11] investigated tolerance in the absence of immunosuppression in rat heart allografts. Goss et al. also used a longer aortic segment with the transplanted heart; no mention was made as to the choice of such a graft combination.

This study describes the techniques of the cluster model of heart plus entire thoracic aorta transplantation. The cluster model was proposed with a view to (a) studying graft vessel disease in larger vessels using the aorta as part of the cluster, (b) additionally studying the aortic component of the cardiac allografts and (c) developing a more comprehensive and efficient model that allows investigation of cardiac and aortic rejection simultaneously but separately. The principles of procurement and subsequent grafting of the cluster model are quite similar to those of the isolated heart model of transplantation. However, the performance of this technique warrants further knowledge of the potential pitfalls that can diminish the success of the model. The most important points of concern relate to insufficient cardiac venting, the use of excessive strain on the thoracic aorta during procurement, air embolism due to trapped air in the aortic segment, and torsion of the transplanted aortic segment due to malposition. We have adapted the procurement procedure of the aortic segment so as to isolate and excise this vessel in the cold phase that follows cardioplegia. Such a technique obviates the need for excessive forces on this vessel during its dissection. Unless the aorta is harvested without physical forces damaging its wall, such factors will themselves result in thrombosis or vascular intimal proliferation under immunosuppression and, in all likelihood, confound the effect of independent variables in an experimental protocol. The development of intimal proliferation in rat carotid arteries using balloon catheter injury confirms the importance of physical stresses on a vessel [12].

The structure of the aorta is strikingly different from the intramural and epicardial vessels. Whereas there is more elastic lamina in the aorta, the coronary vessels contain more muscle. This anatomic fact raises the issue of the applicability of data originating from the isolated aorta transplant model, which is used to understand the process of chronic rejection in the myocardial vessels. Since both types of vessels (aorta and myocardial) are targets of the process, and because the clinical outcome along a temporal sequence is more dramatic (i.e. infarction) for smaller size vessels, the cluster model allows for a broader investigation of the different size vessels. Graft vessel disease is routinely studied with ease by examining the aorta in an isolated transplant model. The cluster model continues to provide that avenue of investigation. Our observations of the early development of intimal proliferation in myocardial vessels of either isolated or cluster hearts under immunosuppressive therapy for rejection agree with those of earlier groups [20, 21]. The occurrence of this phenomenon in a relatively short period of time post-transplantation in a non-histocompatible untreated host, where cardiac rejection normally occurs within 5 days of transplantation, points to the strong possibility that immunosuppression can create a state of relatively weak allogeneic incompatibility favoring the process of vascular intimal proliferation. These observations confirm the fact that cardiac allograft survival can be prolonged significantly so as to observe simultaneous changes in graft aorta and coronary vessels. In our study, however, such changes were found to be mild compared to those detected in the accompanying grafted hearts. Although these findings are quite common in weakly allogeneic donor/recipient combinations (e.g. DA × Wistar and F344 × Lew) selected for the provision of prolonged heart allograft survival without the cover of immunosuppression, the more clinically relevant model, as reflected in our DA × PVG and DA × Lew combinations, highlights the non-exclusivity of the process in relation to time or the degree of donor/recipient disparity. It furthermore brings to the fore the clinical importance of these early changes due to acute rejection, changing immunosuppressive needs and rescue efforts that commonly plague most solid organ clinical transplants within 3 months of grafting.

Our study clearly shows that graft survival in both untreated and immunosuppressed recipients is not statistically different for the two models compared. There was no occasion in the cluster graft series where the aorta was rejected earlier than the accompanying heart. On the contrary, we have demonstrated the relative sparing of the aorta versus in intramural and epicardial vessels in terms of rejection. These data underscore the argument that under favorable circumstances (optimal immunosuppression and the duration of immunosuppressive treatment) it is possible to examine the two processes (cardiac and aortic rejection) simultaneously but separately. A distinct limitation in only examining the aorta of the cluster graft as a window into the events occurring in the intramyocardial vessels is that by virtue of the larger diameter of this vessel, the changes seen in it will not accurately reflect and correlate with changes in the smaller diameter myocardial vessels on a temporal basis, especially if the endpoint employed is myocardial infarction. A different limitation of the cluster model, however, is that whereas aortas can be hypothermically stored in University of Wisconsin solution for as long as 24 h and subsequently transplanted in experimental preservation protocols, hearts may not provide the same experimental latitude to the researcher.

In conclusion, the results of this comparative study demonstrate that the model is technically feasible and extremely reproducible with a high success rate. The data also show that it was possible to apply the cluster model to study experimental allograft rejection and obtain comparable survival data in comparison to the isolated heart model. Additionally, the cluster model was proposed with a view to gathering data on a range of vessel sizes in one animal. The observations from this study lead us further to believe that this model is a significant improvement over the isolated heart and isolated aorta models of transplantation that can be applied successfully in experimental studies evaluating novel immunosuppressants for acute and chronic rejection. The success of the cluster model in strongly allogeneic transplant combination lends credence to its potential application to strain combinations with a lesser degree of histoincompatibility, where longer term observations can be made. Under such conditions the cluster model would allow for the study of cardiac and aortic rejection concurrently, but independently, in one recipient.

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