Video Article

Murine Heterotopic Heart Transplant Technique

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Abstract

It is now over forty years since this technique was first reported by Corry, Wynn and Russell. Although it took some years for other labs to become proficient in and utilize this technique, it is now widely used by many laboratories around the world. A significant refinement to the original technique was developed and reported in 2001 by Niimi. Described here are the techniques that have evolved over more than a decade in the hands of three surgeons (Plenter, Grazia, Pietra) in our center. These techniques are now being passed on to a younger generation of surgeons and researchers.

Based largely on the Niimi experience, the procedures used have evolved in the fine details - details which we will endeavor to relate here in such a way that others may be able to use this very useful model. Like Niimi, we have found that a video aid to learning is a priceless resource for the beginner.

Video Link

The video component of this article can be found at https://www.jove.com/video/51511/

Introduction

In an era when it is possible to perform kidney, lung, liver and pancreas transplants in mice, the cornerstone of basic organ transplant and immunology research since 1973[1-4] remains the heterotopic heart transplant model in the mouse. In the intervening years several papers have been published detailing improvements/refinements[5,6] to this procedure.

As a model of solid organ primarily vascularized transplantation this procedure is second to none. Once mastered this procedure lends itself to research into allogeneic rejection responses[7], the development of chronic vasculopathies[8] and the mechanisms of ischemia reperfusion injury[9].

The keys to successfully learning this procedure are just like any other surgery, patience on the part of the instructor and the trainee and attention to detail. At the beginning of the process the new surgeon will find that they will spend many hours on each transplant. As experience is gained, surgical times, and therefore ischemia, will drastically reduce. Paying attention to the details of every step will sooner or later lead to success.

While the instructor can do their best to pass on, and to anticipate, all of the possible pit falls that may be encountered during these surgeries, the “creative” trainee will likely find some of their own!

The basics of the procedure are as follows. The donor ascending aortic arch is end-to-side anastomosed to the recipient abdominal aorta and the donor pulmonary artery is end-to-side anastomosed to the recipient abdominal inferior vena cava (IVC). Blood flows from the recipient aorta in retrograde fashion through the donor aorta to the coronary arteries. Once the blood has flowed through the coronary system it drains into the right atrium via the coronary sinus, is pumped into the right ventricle and then via the pulmonary artery into the recipient IVC. In this way the coronary system is supplied arterial blood and sinus rhythm returns to the graft within 1-2 min of reperfusion. Since the left chambers of the heart are essentially pressure under-loaded the left ventricular free wall will atrophy over time.

Protocol

All animals were housed under pathogen-free conditions at the University of Colorado Barbara Davis Center Animal Facility under IACUC approval and cared for according to NIH guidelines.

Depth of anesthesia is judged by toe pinch initially and by observance of respiration rate once the procedure has begun.

1. Donor Heart Harvest

1. Anesthetize the donor mouse by injecting pentobarbital (60 mg/kg IP).
2. Immobilize the mouse by 4-way restraints and clip the fur. Wipe the skin with alcohol.
3. After dissecting away the skin, open the abdominal cavity by making a transverse incision just inferior to the diaphragm.
4. Cut the diaphragm posterior to costal insertion and lift the anterior chest wall anteriorly and superiorly exposing the heart.
5. Extend a full thickness cut up the posterior lateral thoracic cavity on the left and right sides of the chest.
6. Isolate the inferior vena cava (IVC) and place a loose 5-0 silk suture around the IVC adjacent to its insertion into the right atrium.
7. Inject 1.0 cc of 4 °C heparinized saline (200 u/cc) into the IVC, then ligate the vessel with the 5-0 silk suture and divide.
8. Isolate the right superior vena cava (rSVC) in similar fashion and ligate with 5-0 silk suture and divide.
9. Gently roll the heart towards the animals' right side and isolate the left superior vena cava (lSVC), ligate with a 5-0 silk suture and divide exposing the left pulmonary artery.
10. Secure the heart under a wet gauze while the thymus is blunt dissected away from the main pulmonary artery (PA) and the left and right PA branches and ascending aortic arch.
11. Blunt dissect the aortic arch free of the surrounding tissues. Micro-scissors are used to divide the aorta proximal to the right brachial-cephalic artery; *i.e.* there should be no branches between the heart and the division point of the aorta.
12. This section of the aortic arch forms the arterial cuff for the implant process.
13. Reflect the aortic cuff inferiorly to expose the PA trunk and the left and right branches of the PA.
14. Blunt dissect the left and right PA branches away from the surrounding tissues as far from the heart as possible. This allows for easy dissection of the PA trunk from surrounding tissues.
15. Divide the PA trunk as distally as possible, just proximal to its bifurcation. This section of the pulmonary artery forms the venous cuff for the implant process.
16. Place a 5-0 silk suture around the base of the heart and tie. The heart is then cut free at the base, and placed in 4 °C saline. Total harvest time is approx. 10-15 min.

2. Heart Implant Technique

1. Anesthetize the recipient mice with pentobarbital (60 mg/kg IP initial dose, 25 mg/kg IP supplemental dose if required).
2. Clip the fur and immobilize the mouse by 4-way restraints and prep the skin with povidone-iodine and drape in a sterile fashion.
3. Make a 2 cm midline vertical abdominal incision and enter the abdominal cavity.
4. Retract the bowel superiorly and externalized on to the chest. Keep wrapped in sterile moist gauze (sterile saline) throughout the case.
5. Isolate the abdominal aorta and inferior vena cava (IVC) below the renal vessels and place 4-0 cotton ties around the aorta and IVC superior then inferior to the anastomosis site.
6. Identify any lumbar vessels within the field and ligate with 10-0 nylon suture.
7. Knot the cotton ties, first the inferior followed by the superior. In this way some blood is retained in the aorta making the aortotomy easier.
8. Form the aortotomy with a 30 G needle to enter the lumen of the aorta. Extend the incision with fine micro scissors to a length of approximately 2 mm. This incision is made in a straight line along the longitudinal axis of the vessel.
9. Make an end to side anastomosis of the donor aorta to the recipient aorta in the following fashion. Place a 10-0 nylon suture stay stitch in the donor aorta and to the inferior angle of the incision in the recipient aorta and tie. Place a second 10-0 nylon opposite the first in the donor aorta and the superior corner of the incision in the abdominal aorta and tie.
10. Make a running suture line from superior to inferior in the lateral wall of the aorta and tie against the previously placed stay stitch. Be sure to bring the intimas (inner vessel surface) together as you stitch. Then suture the medial side in a running fashion and tie. The first and last stitch on each side should be placed as close to the stay stitches as possible. Then aim to have 3 evenly spaced stitches between these two making a total of 5 stitches.
11. Make an end to side anastomosis of the donor pulmonary artery to the recipient IVC in the following fashion. Puncture the IVC with a 30 G needle and extend the incision for approx. 2 mm with fine micro scissors. This incision is made in a straight line along the longitudinal axis of the vessel.
12. Tie the donor pulmonary artery to the inferior corner of the incision in the IVC with 10-0 nylon. Place a second 10-0 nylon opposite the first in the donor artery and the superior corner of the incision in the IVC and tie.
13. Make a running suture line between the pulmonary artery and the IVC and tie. The first and last stitch on each side should be placed as close to the stay stitches as possible. Then aim to have 5 evenly spaced stitches between these two making a total of 7 stitches.
14. Release the distal 4-0 cotton tie re-establishing venous flow.
15. Once hemostasis of the venous anastomosis has been observed the proximal 4-0 cotton tie is gradually loosened and the arterial anastomosis observed for hemostasis. When both anastomoses are considered secure, remove the cotton ties from the mouse.
16. Return the bowel to the abdomen. The abdominal wall is closed in two layers using 5-0 Silk suture in a running fashion.
17. Administer a 1.0 ml bolus of sterile, warm normal saline into the abdomen as fluid resuscitation upon closing, and 0.8 ml of normal saline is injected subcutaneously post-operatively. No other supportive measures are required during the surgery. Recover the animal on a warming blanket. Total implant time is approx. 90-120 min for beginners, 45-60 min with experience. Administer buprenorphine analgesia, 0.05 mg/kg, SC, 0.1-0.2 ml at the beginning of the procedure and every 6-12 hr for 72 hr post-op.

3. Graft assessment

1. Assess graft function daily by trans-abdominal palpation.
2. Hold the mouse as though it were to be given an intraperitoneal injection.
3. Gently press the tip of a forefinger against the abdominal wall and ascertain the beating strength and regularity of the graft.
4. Give palpation quality a score from 4 (normal amplitude and frequency) to 0 (non-beating rejected graft).

IMPORTANT NOTES:

All instruments are sterilized, sterile gloves are worn throughout the procedure and a sterile field is maintained. The donor and recipient surgeries are performed with the use of an operating microscope. Ensure that the anastomoses are “clean”. That is, that the back walls are not caught when placing stitches. This will cause a significant constriction to flow that will more than likely result in a failed graft and in extreme cases to hind-limb paralysis. It is also vitally important that full thickness passes including the vascular adventitia and the intima of the suture needle are achieved. Evertion of the edges also ensures that there is intima-to-intima contact, which aids in sealing and healing of the anastomoses. Another vitally important factor is ensuring that the tension of the anastomotic suture lines is also optimal. Too loose and there will be irreversible leaking, too tight and stricture to flow will result. If on the arterial side this will result in poor perfusion of the graft, if on the venous side a congested heart will result.

Representative Results

The utilization of this surgical technique opens the way for either simple graft survival/rejection studies, or quite complex experimental protocols. In the study briefly described in the figure below, we sought to define the involvement, if any, of Fas and/or perforin as mechanisms of CD4 T cell mediated cardiac rejection. This was made possible by the extraordinary array of mouse strains that are available today. Results demonstrate that the direct rejection of cardiac allografts by CD4 effector T cells requires the alternative contribution of graft Fas expression and T cell perforin expression. To our knowledge, this is the first demonstration that cytolytic activity by CD4 T cells can play an obligate role for primary acute allograft rejection *in vivo*.

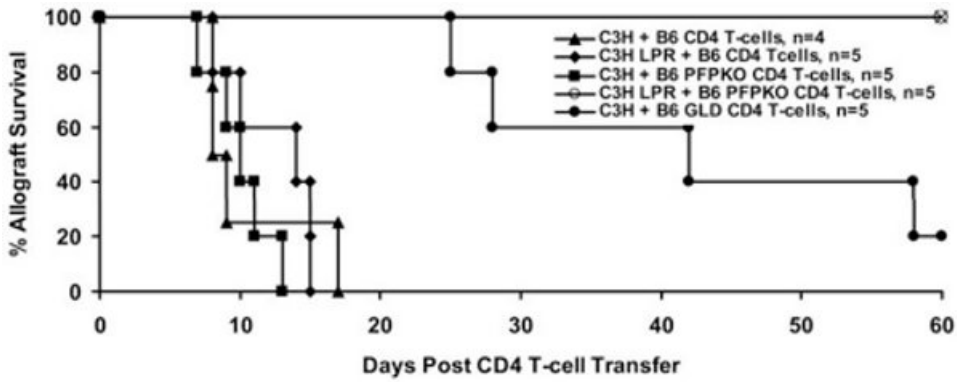

Figure 1. Perforin and Fas Represent Obligate and Parallel Pathways of CD4 T cell-Mediated Cardiac Rejection B6, B6 PFPKO (perforin knock-out), and B6*gld* (Fas-ligand deficient) CD4 T cells were utilized to reconstitute B6*rag*$^{-/-}$ recipients of C3H wild type or Fas-deficient C3H/*lpr* cardiac allografts. Removal of Fas alone (♦, p=NS vs. control Wt C3H + B6 CD4 T cells) from the donor hearts or removal or perforin alone from the CD4 T cells (■, p=NS vs. control Wt C3H + B6 CD4 T cells) did not abrogate rejection. Interestingly, the removal of FasL from effector CD4 T cells did delay rejection significantly (●, $p < 0.02$ vs. Wt C3H + B6 CD4 T cells, $p < 0.01$ vs. Wt C3H + B6 PFPKO CD4 T cells, and $p< 0.01$ vs. C3H/*lpr* + B6 CD4 T cells). However, most allografts were still rejected (4 of 5). Significantly, the simultaneous removal of both donor Fas and CD4 T cell perforin completely abrogated rejection (○, $p < 0.002$ vs. control Wt C3H + B6 CD4 T cells, Wt C3H + B6 PFPKO CD4 T cells, and C3H/*lpr* + B6 CD4 T cells). This abrogation was significantly more robust than the individual removal of CD4 T cell FasL (○, $p < 0.003$ vs. control Wt C3H + B6*gld* CD4 T cells). From Grazia *et al*[10]. Reprinted with permission.

Discussion

This surgical technique is not easy to master, but once mastered is a powerful research tool. The researcher/surgeon is rewarded by consistency of technique and by attention to detail. Patience during the learning phase is key. As published by Niimi*3*, with the aid of a video based learning tool it takes an average of 11 attempts to achieve the first successful procedure and 78 attempts to achieve a 90% success rate. Videos have become an important teaching tool in surgery[11,12].

Troubleshooting

Bleeding from the anastomoses may occur and this is likely due to either lack of correct tension in the sutures, or too few sutures. While a clotting inducing agent such as Gelfoam can be useful for reducing leaks, we recommend that the surgeon should rely on good technique. Congested non-beating heart is most commonly due to anastomoses that are too tight, particularly on the venous side. A non-beating, non-perfused graft is commonly caused by an air bubble that has traveled into one of the coronary arteries. It is important to maintain a damp-to-wet field to avoid the entry of bubbles into the vessels.

Limitations of the Technique

This technique is not suitable if a researcher wants to investigate effects on a fully functioning heart. That would require an orthotopic transplant technique, which to date has proved impossible to perform.

Significance with Respect to Existing Methods

If one wishes to study the effects on a fully vascularized, solid organ transplant in the mouse, then the heart model is probably the simplest to master. Mouse models of lung, kidney and liver transplantation do exist, but are much harder to learn and perfect.

Critical steps Within the Protocol

It is vitally important that full thickness passes including the vascular adventitia and the intima of the suture needle are achieved. Evertion of the edges also ensures that there is intima-to-intima contact, which aids in sealing and healing of the anastomoses. Another vitally important factor is ensuring that the tension of the anastomotic suture lines is also optimal. Too loose and there will be irreversible leaking, too tight and stricture to flow will result. If on the arterial side this will result in poor perfusion of the graft, if on the venous side a congested heart will result.

Above all, repetition, consistency of procedure and continual attention to detail will yield great results and fundable and publishable data.

Disclosures

The authors have nothing to disclose.

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References

1. Corry, R. J., Winn, H. J., Russell, P. S. Heart transplantation in congenic strains of mice. *Transplant Proc.* **5** (1), 733. (1973).
2. Corry, R. J., Winn, H. J., Russell, P. S. Primarily vascularized allografts of hearts in mice. The role of H-2D, H-2K, and non-H-2 antigens in rejection. *Transplantation.* **16** (4), 343. (1973).
3. Niimi, M. The technique for heterotopic cardiac transplantation in mice: experience of 3,000 operations by one surgeon. *J Heart Lung Transplant.* **20** (10), 1123. (2001).
4. Plenter, R. J., Zamora, M. R., Grazia, T. J. Four decades of vascularized heterotopic cardiac transplantation in the mouse. *J Invest Surg.* **26** (4), 223. (2013).
5. Su, S. *et al.* Modified suture technique in a mouse heart transplant model. *Asian J Surg.* **34** (2), 86. (2011).
6. Mao, M. *et al.* A novel and knotless technique for heterotopic cardiac transplantation in mice. *J Heart Lung Transplant.* **28** (10), 1102. (2009).
7. Csencsits, K. L., Bishop, D.K. Contrasting alloreactive CD4+ and CD8+ T cells: there's more to it than MHC restriction. *Am J Transplant.* **3** (2), 107. (2003).
8. Hasegawa, T., Visovatti, S. H., Hyman, M. C., Hayasaki, T., Pinsky, D. J. Heterotopic vascularized murine cardiac transplantation to study graft arteriopathy. *Nat Protoc.* **2** (3), 471. (2007).
9. Linfert, D., Chowdhry, T., Rabb, H. Lymphocytes and ischemia-reperfusion injury. *Transplant Rev.* (Orlando). **23** (1), 1. (2009).
10. Grazia, T.J. *et al.* Acute cardiac allograft rejection by directly cytotoxic CD4 T cells: parallel requirements for Fas and perforin. *Transplantation.* **89** (1), 33. (2010).
11. Liu, F., Kang, S. M. Heterotopic heart transplantation in mice. *J Vis Exp.* (6), 238. (2007).
12. Plenter, R. J. Heterotopic heart transplantation in mice. *European Society for Surgical Research.* 45th Annual Congress. (2010).