Video Article

Mouse Model of Alloimmune-induced Vascular Rejection and Transplant Arteriosclerosis

Winnie Enns[1], Anna von Rossum[1], Jonathan Choy[1]

[1]Department of Molecular Biology and Biochemistry, Simon Fraser University

Correspondence to: Jonathan Choy at jonathan.choy@sfu.ca

URL: http://www.jove.com/video/52800
DOI: doi:10.3791/52800

Keywords: Medicine, Issue 99, Transplantation, Vascular rejection, Transplant arteriosclerosis, Artery, Aorta

Date Published: 5/17/2015

Citation: Enns, W., von Rossum, A., Choy, J. Mouse Model of Alloimmune-induced Vascular Rejection and Transplant Arteriosclerosis. *J. Vis. Exp.* (99), e52800, doi:10.3791/52800 (2015).

Abstract

Vascular rejection that leads to transplant arteriosclerosis (TA) is the leading representation of chronic heart transplant failure. In TA, the immune system of the recipient causes damage of the arterial wall and dysfunction of endothelial cells and smooth muscle cells. This triggers a pathological repair response that is characterized by intimal thickening and luminal occlusion. Understanding the mechanisms by which the immune system causes vasculature rejection and TA may inform the development of novel ways to manage graft failure. Here, we describe a mouse aortic interposition model that can be used to study the pathogenic mechanisms of vascular rejection and TA. The model involves grafting of an aortic segment from a donor animal into an allogeneic recipient. Rejection of the artery segment involves alloimmune reactions and results in arterial changes that resemble vascular rejection. The basic technical approach we describe can be used with different mouse strains and targeted interventions to answer specific questions related to vascular rejection and TA.

Video Link

The video component of this article can be found at http://www.jove.com/video/52800/

Introduction

Over the past 30+ years, advances in immunosuppressive drugs have diminished graft rejection due to acute rejection but chronic rejection remains a main challenge. The main manifestation of chronic heart transplant rejection is transplant arteriosclerosis (TA) [1,2]. This condition is characterized by intimal hyperplasia and vasomotor dysfunction of allograft arteries and develops as a result of immunological targeting of endothelial and smooth muscle cells by the recipient immune system. The specific targeting of the graft vasculature due to recognition of foreign peptide-major histocompatibility complex (MHC) is highlighted by the development of TA exclusively in graft arteries while sparing host vessels [3]. In keeping with this is the observation that TA does not occur experimentally when the recipient is genetically identical to the donor or when the recipient lacks T and B cells [4]. Immune-mediated vascular injury and dysfunction causes the development of intimal thickening and fibrosis, as well as the aberrant accumulation of lipids and ECM proteins, in TA [5]. Intimal thickening tends to be concentric throughout the entire arterial tree [4-6]. Graft loss and death usually occur as a result of progressive ischemia resulting from luminal occlusion of allograft arteries [4].

In 1991, Mennander *et al.* [7] pioneered an aortic interposition model in rats to model TA. Several groups have subsequently adapted this procedure for use in mice. In this model, allograft aortic segments develop lesions that have features comparable to TA observed in clinical transplants. This includes intimal thickening characterized by the accumulation of smooth muscle-like cells and recipient leukocytes [7]. Over the past 2 decades this model has been used to generate important insight into the mechanisms of vascular injury, rejection and TA. It can be used to examine questions related to immune and vascular responses during arterial pathology. The choice of antigen mismatch impacts the ability to appropriately address these questions.

Transplantation across complete MHC barriers permits a comprehensive evaluation of immune responses that are known to be involved in organ transplant rejection. This includes direct CD4 and CD8 T cell recognition and targeting of foreign peptide-MHC presented by graft-derived cells, indirect CD4 (and possibly CD8) T cell recognition and targeting of graft-derived alloantigens presented by recipient antigen presenting cells, and antibody-mediated recognition of alloantigens on vascular cell surfaces [8]. However, the vascular response to injury in complete MHC-mismatched experiments may be different than that observed clinically. Johnson *et al.* [9] showed that, in aortic interposition grafts transplanted across a complete MHC mismatch barrier, most of the neointimal cells are of recipient origin and not of donor origin. This is different than that observed in human transplants where most intimal smooth muscle cells are of donor origin [9,10]. To account for this limitation, alternate experimental models that involve grafting across minor histocompatibility antigen mismatches have been developed that trigger vascular responses that more closely resemble those observed in clinical transplantation [11]. While these alternate models allow for important conclusions to be made regarding the vascular responses that drive the development of TA, the immunological processes that cause vascular rejection in minor histocompatibility antigen mismatched grafts do not completely re-capitulate those which occur in the clinical setting. For instance, minor histocompatibility antigens are recognized poorly by graft reactive antibodies [12]. Given the above considerations, it is important to consider the pathological question being examined when choosing the type of antigen mismatch used in an aortic interposition model. Here we describe a

detailed protocol for murine aortic interposition grafting. We describe interposition grafting between complete MHC-mismatched mice but the same protocol is used for grafting across other antigen mismatched mouse strains.

Protocol

All the protocols in this study were reviewed and approved by the Simon Fraser University animal care ethics committee. Use Balb/cYJ ($H2^d$) donor mice and C57Bl/6 ($H2^b$) recipient mice to examine allogeneic reactions. Mice are used for experiments between the ages of 8 to 12 weeks. Use either female or male mice. Syngeneic controls consist of aortic segments from C57Bl/6 donors into C57Bl/6 recipients.

1. Donor and Recipient Preparation

Note: Both the donor and recipient are anesthetized and prepared before the surgery to minimize ischemia of the graft. Injectable anesthetics are used in the protocol to prevent obstruction of the animal by equipment needed for the delivery of inhaled anesthetics. However, if desired, inhaled anesthetic is an appropriate alternative. From the initial injection of the anesthetics, the entire procedure takes approximately 90 min to complete. Ischemic time of the graft is less than 30 min.

1. Anesthetize the mouse with intraperitoneal injections of ketamine (100 mg/kg; 10 mg/ml) and xylazine (10 mg/kg; 1 mg/ml). The mouse will be sedated within 10 to 15 min. Assess the depth of anesthesia by pinching the fatty part of the animal foot pad. Administer 1/3 of the original dose of the anesthetic cocktail, as needed, until the animal does not exhibit withdrawal reflex. Monitor the respiratory rate closely after every cocktail is administered. During surgery, assess the level of anesthesia every 15 min by pinching the anterior abdominal wall with a pair of forceps. The mice should remain deeply anaesthetized for 60 to 90 min.
2. Lubricate the mouse's eyes with an ophthalmic ointment to prevent dryness while under anesthesia.
3. Shave hair as close to the skin as possible on the abdominal ventral region from the mid-thorax to the pubis. Be particularly careful not to nick any skin. Do not use depilatory cream because it may be absorbed into the skin and could be inflammatory.
4. Place the animal in supine position on a towel above the heating table or pad.
5. Clean the surgical site with a preliminary scrub with 2% chlorohexidine. Prepare a large working area to maximize the surgical field. Start scrubbing at the center of the surgical site and move to the outside in a linear or circular manner. Dispose of the gauze. Repeat this procedure at least 5 more times. Apply an alcohol prep pad to the same area. Once the alcohol is dry, prepare the clean area with Betadine solution and drape with sterile gauze.

2. End-to-end Anastomosis Procedure

1. Donor Operation
    1. Maintain aseptic technique throughout the operation. Clean all countertop and surgical table surfaces with 0.5% accelerated hydrogen peroxide solution prior to use. Wrap and autoclave all surgical instruments, gauzes, drapes and gowns prior to use. Verify sterility of the instruments with a steam sterilizer indicator strip placed in each pack. Sterile surgical gloves are used and disposed of between surgeries. For multiple surgeries, sterilize the surgical instruments between uses with the hot glass bead sterilizer.
    2. Place the donor mouse in a supine position on a clean, thin Plexiglas board wrapped with sterile drape under the operating microscope at 8-30X magnification.
    3. After ensuring adequate surgical anesthesia as outlined in section 1.1, proceed with surgery.
    4. Using sterile scissors, make a single midline lower longitudinal abdominal incision, from the pubis to the xyphoid process.
    5. Using a small retractor, open the abdominal walls to expose the cavity.
    6. Using sterile cotton tipped applicators, gently retract the intestines superiorly to the animal's left and cover with gauze moistened with saline solution. Move the reproductive organs inferiorly and locate the infrarenal aorta and the inferior vena cava (IVC). Moisten the exposed tissues periodically with saline solution.
    7. Using the medical No. 5 forceps, separate the aorta from the IVC, from the level of the left renal artery to the bifurcation. Use 10-0 polyamide monofilament sutures to ligate the small branches near the aorta.
    8. Once the donor's aorta has been separated from the IVC, saturate the vessel with saline, cover the exposed cavity with moistened gauze and set the donor aside onto a sterile area. Check the status of the donor (respiratory and cardiovascular function, and depth of anesthesia) every 15 min. Begin operating on the recipient, isolating the aorta as described below (Steps 2.2.1 to 2.2.7).
    9. Once the recipient aorta has been separated from the IVC and set aside, return the donor under the microscope. Cross clamp (proximal and distal to the segment of interest) the donor aorta, approximately 5 mm apart, with two 4 mm microvascular clamps.
    10. Using the Vannas-Tubingen microscissors, transect a small graft segment (3 to 4 mm in length) of the abdominal aorta.
    11. Using a 25 G 5/8 needle attached to a syringe, flush the excised aorta with heparinized (100 U/ml) saline solution. Ensure the tip of the needle does not come into contact with the vessel.
    12. Place aorta in heparinized (100 U/ml) saline solution on ice and set aside. While still under deep anesthesia, release the microvascular clamps. Euthanize the donor by exsanguination.
    13. Implant the donor vessel within 30 min of excision. Although it is possible to use one donor vessel for multiple recipients by excising a larger length of aorta, keep the ischemic time to less than 30 min.

2. Recipient Operation
    1. Maintain aseptic technique throughout the operation, as in donor operation.
    2. Place the recipient mouse in a supine position on a thin Plexiglas board wrapped with sterile drape under the operating microscope at 8-30X magnification.
    3. After ensuring adequate anesthesia, proceed with surgery when the animal does not exhibit withdrawal reflex.
    4. Using sterile scissors, make a single midline lower longitudinal abdominal incision, from the pubis to the xyphoid process.
    5. Using a small retractor, open the abdominal walls to expose the cavity.

6. Using sterile cotton tipped applicators, gently retract the intestines superiorly to the animal's left and cover with gauze moistened with saline solution. Move the reproductive organs inferiorly and locate the infrarenal aorta and the IVC. Moisten the exposed tissues periodically with saline solution.
7. Separate the aorta from the IVC, from the level of the left renal artery to the bifurcation. If necessary, use 10-0 polyamide monofilament sutures to ligate the small branches near the aorta.
8. Cross clamp (proximal and distal to the segment of interest) the aorta, approximately 5 mm apart, with two 4 mm microvascular clamps.
9. Using the Vannas-Tubingen microscissors, make a single horizontal aortotomy and resect a small segment (no more than 0.5 mm) of the abdominal aorta to accommodate the donor aortic graft.
10. Flush the excised aorta with heparinized (100 U/ml) saline solution. The donor aortic graft should be of appropriate length to connect the recipient's transected aortic ends.
11. Place the donor aortic graft in the orthotopic position and anastomose the donor's graft end to the recipient's end, matching the respective graft anatomical orientation with that of the recipient.
12. Gently grasp the tunica externa of the vessel and evert it slightly using the medical No.5 forceps. Using the forceps, drive the needle attached to the 10-0 polyamide monofilament suture through the full thickness of the vessel wall in order to secure the donor aortic graft to the recipient's resected vessel. Take care to ensure that the vessel opening is not closed off due to inadvertent stitching of the back wall of the vessel.
13. For continuous stitches, place stay sutures at 9 o'clock in both the upper and lower ends of the graft. Starting at the upper end of the graft from 3 o'clock, anastomose the resected ends with 2 running sutures and secure the suture to the stay suture.
    1. Flip the graft over and continue the running suture to dorsal part of the vessel, meeting the origin stay suture. Secure without applying much pressure on the vessel. Repeat the suturing for the lower anastomosis.
    Note: The interrupted sutures start the same way as the continuous suture with the exception that the vessel is anastomosed with three separated stitches between the stay sutures. Anastomosis time is usually 20 min.
14. Once the anastomosis is complete, release the distal microvascular clamp to allow retrograde blood to flow and check for leakage of the anastomosed sites. If there is a leakage, immediately place a stitch to close the defective site. If there is no bleeding at the sites, then release the proximal clamp.
15. Examine the transplant and check that there is no blood obstruction in the graft, and the proximal and distal portion of the recipient's vessel. Vigorous pulse pattern in both the donor's and recipient's vessel is a primary indication that the blood is flowing freely. Using the pair of forceps, gently grasp one end of the stay sutures and slightly evert the vessel to inspect the back wall of the vessel.
    Note: There should be no puckering of the vessel wall at both ends of the anastomosed sites. Poor blood flow after removal of the clamps is a sign of thrombosis.
16. Using cotton tipped applicators, return the intestines into the abdominal cavity.
17. With the Castroviejo needle holder and Graefe forceps, close the abdominal wall with 5-0 polypropylene sutures using continuous stitching. Close the skin layer with the same sutures using subcuticular closure.
18. Administer Torbugesic (1 mg/kg) i.m. immediately upon completion of the transplant.
19. Give immediately, in the order, Atipamezole (1 mg/kg), ketoprofen (5 mg/kg) and warmed Lactated Ringer's solution subcutaneously.
20. Immediately after surgery, place mice in a heated cage under a water blanket overnight (12 hr). During the anesthetic-recovery period, place the animals alone in a clean, dry unobstructed area. Line the cage (autoclaved) with clean paper towels and adjust the temperature of the water blanket to approximately 20 to 22 °C. Provide dry and wet kibbles ad libitum on the cage floor.
    Note: An important component of post–surgical care is the observation of the animal and appropriate intervention, as required, during recovery from anesthesia and surgery. The necessary intensity of monitoring will vary with the animal and might be greater during the immediate anesthetic recovery period as compared to later in postoperative recovery.
21. Continually monitor animals having undergone anesthesia monitored until they recover completely. The animal must be able to maintain unassisted sternal recumbency and it must appear calm and free of pain before it can be left unattended.
22. Give Buprenorphine (0.1 mg/kg BID) and ketoprofen (5 mg/kg SID) for a period of three days, both subcutaneously.
23. Monitor cardiovascular and respiratory function, body temperature, and postoperative pain or discomfort during recovery from anesthesia for a minimum of three days. Additional care may be required, such as administration of analgesics and other drugs, and parenteral fluids to minimize dehydration and electrolyte loss.
24. Assess the success of the transplant surgery by observing the motor function of the hind limbs. Complete success of the transplant involves unobstructed blood flow to the hind limb and tail, which should be immediate upon recovery of the animal, and complete recovery with no paralysis of the hind limbs on the second day

3. Tissue Collection

Note: Pre-determined end points range from 3-60 days depending on the type of analysis desired and the nature of the antigen mismatch. Generally, intimal thickening is robust at day 30 after transplantation across complete MHC mismatched mouse strains.

1. Anesthetize the mouse with intraperitoneal injections of ketamine (100 mg/kg; 10 mg/ml) and xylazine (10 mg/kg; 1 mg/ml). Assess the depth of anesthesia by pinching the fatty part of the animal foot pad. Proceed with surgery when the animal does not exhibit withdrawal reflex.
2. Clean the surgical site with water and ending with 70% alcohol.
3. Place the animal in supine position on a tray lined with absorbent pad.
4. Open the abdominal cavity with a large midline longitudinal incision. Place a self-retaining retractor to expose the cavity, the IVC and the abdominal aorta.
5. Gently separate the transplanted donor aortic segment from the neighboring IVC using a pair of medical No. 5 forceps. Be particularly careful not to strip the adventitia of the transplanted vessel.
6. Expose the thoracic cavity by cutting through the ribs along both sides of the thoracic spine all the way to the thoracic inlet. Reflect the anterior chest wall superiorly to expose the pericardium and secure the ribcage with a pair of hemostat.

7. Once the heart is exposed, insert a 25 G 5/8 needle (attached to a syringe) into the left ventricle and flush with 0.1 ml of heparinized (100 U/ml) saline solution.
8. Using a pair of scissors, remove the right atrium to allow the blood, heparinized saline, and fixative to leave the body during perfusion. Perfuse the animal with 5 ml of 4% paraformaldehyde or until the fluid runs clear. Remove the heart to ensure euthanasia.
9. Excise transplanted vessel segment and immerse in 4% paraformaldehyde for no more than one hour. Set the transplanted vessel in OCT (optimal cutting temperature) matrix and flash freeze. Section the vessel under a cryostat set at 8 um thickness. Stain sections with Haematoxylin & Eosin and/or Verhoeff-van Gieson (van Gieson) elastic stain.

4. Morphological Analysis of Grafts

Note: TA is characterized by intimal thickening [13]. In this model, intimal thickening and a resultant reduction in the size of the lumen are reflective of the severity of immune-mediated vascular injury. Syngraft controls are used to determine the baseline characteristics of vessels in the absence of allogeneic immune responses. These controls also permit evaluation of arterial damage that occurs as a result of the surgical procedure. There should be no intimal thickening in syngrafts.

1. To examine intimal thickening and luminal occlusion, measure the area within the endothelial cell layer, internal elastic lamina (IEL) and external elastic lamina (EEL) on elastic van Gieson stained artery segments with an image analysis software. The parameters below can be used to determine arterial changes reflective of TA.
    1. Measure the absolute intimal area: area within the internal elastic lamina - the area within the endothelial cell layer. This provides an absolute measurement of intimal expansion, which is the hallmark of TA.
    2. Measure the absolute medial area: area within the external elastic lamina - area within the internal elastic lamina. This provides an absolute measurement of medial degradation or growth, which can occasionally occur as a result of immune-mediated changes to this region of the vessel wall.
    3. Measure the total vessel area as the area within the external elastic lamina. This provides a measurement of vessel remodelling which involves expansion or constriction of artery as a result of immunological damage.
    4. Measure the intima/Media: (area within the internal elastic lamina – area within the endothelial cell layer) / (area within the external elastic lamina – area within the internal elastic lamina). This provides a relative measure of intimal expansion and normalizes for differences in vessel size.
    5. Measure the % Luminal Narrowing: [(area within the internal elastic lamina – area within the endothelial cell layer) / area with the internal elastic lamina)]*100. This provides a measure of the extent of luminal occlusion, which results from a combination of intimal expansion and remodeling (inward or outward) of the vessel wall.

Representative Results

In this model, the abdominal aorta from a Balb/cYJ mouse is interposed into the infrarenal aorta of a C57Bl/6 recipient. This permits a comprehensive evaluation of alloimmune responses that target allograft arteries. Immune-mediated vascular injury in this model initiates vascular reparative responses that culminate in intimal thickening, luminal narrowing and recruitment of immune cells (**Figures 1** and **2**). These criteria then serve as a read-out for the severity of alloimmune responses, vascular rejection, and TA. Success of the procedure can be assessed by the development of robust intimal thickening of allograft artery segments and the absence of intimal thickening in syngraft controls. Clinically relevant immunosuppression may be used with this model to more closely resemble clinical transplantation. This model may also be used with transgenic animals to study the effect of specific proteins/pathways in alloimmune responses as we have done [14].

Allograft aortic segments develop intimal thickening

The amount of immune-mediated allograft vascular damage was examined by quantifying intimal thickening and luminal narrowing in grafted aortic segments at day 30 post-transplantation. Allograft artery segments develop significant intimal thickening and luminal occlusion and no changes are observed in syngraft control artery segments (**Figure 1**).

Accumulation of leukocytes in allograft aortic segments.

The accumulation of leukocytes in allograft arteries was examined by quantifying the number of CD4 T cells, CD8 T cells, and macrophages (Mac-3)[15] by immunohistochemistry. Mac-3 was used to stain for macrophages in this study although other markers, such as F4/80, can also be used [16]. CD4 T cells, CD8 T cells and macrophages were detected in the intima of allograft arteries (**Figure 2**)

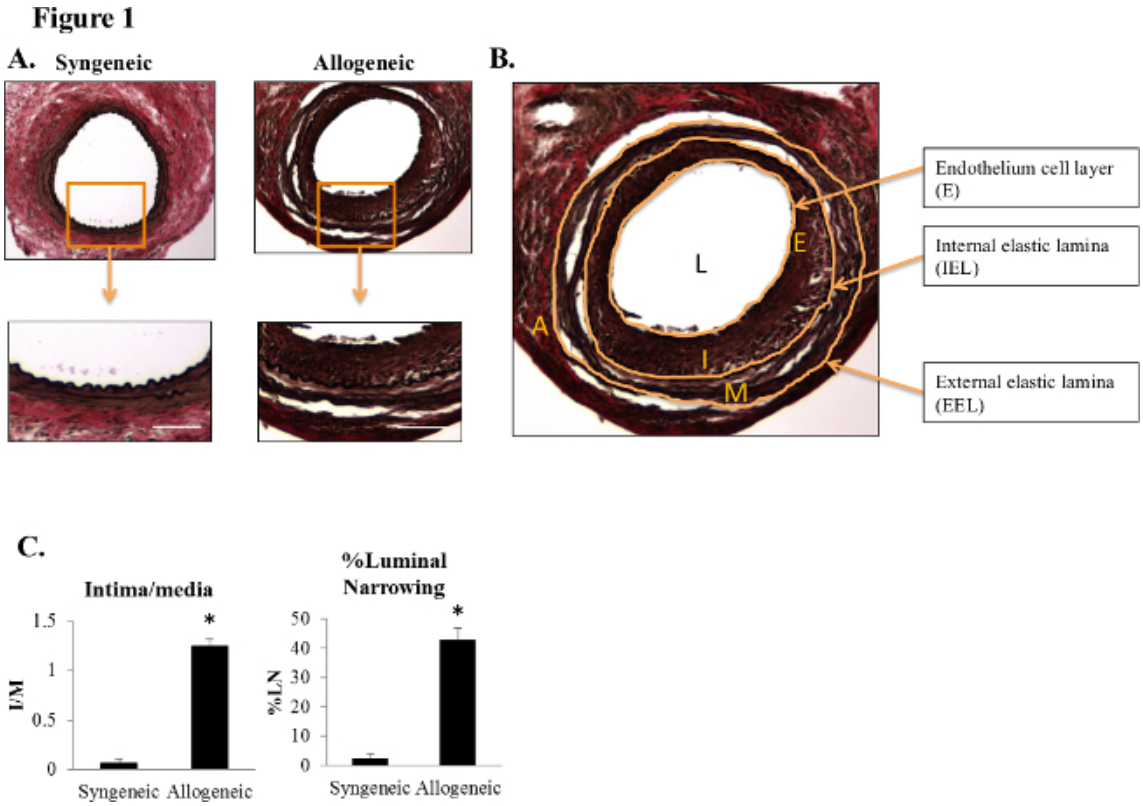

Figure 1: Histological analysis of grafted arteries. (A) Abdominal aorta segments from syngeneic and allogeneic mice were interposed into the resected abdominal aortas of C57Bl/6 mice. The grafted arteries were harvested at day 30 post-transplant. Representative photomicrographs of elastic van Giesen stained arteries are shown. Scale bar = 0.1 mm = 100 µm. **(B)** Diagram depicting how the different layers of the vessel are measured, L: lumen, E: endothelial layer, I: intima, M: media, A: adventitia. **(C)** Quantification of intima/media ratio and % luminal narrowing from 30 day syngeneic (n = 3) and allogeneic (n = 6) transplants, *P <0.05. Please click here to view a larger version of this figure.

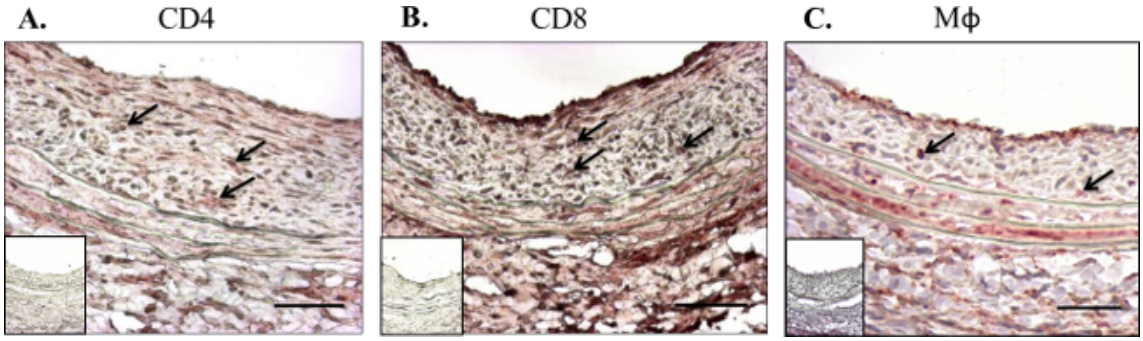

Figure 2: Immune cell accumulation in allograft arteries. Representative photomicrographs of allograft aortic segments immunohistochemically stained for **(A)** CD4, **(B)** CD8, and **(C)** Mac-3 and counterstained with hematoxylin are shown. Scale bar = 0.1 mm = 100 µm. Please click here to view a larger version of this figure.

Discussion

We have described a protocol for aortic interposition grafting in mice that is useful for studying immune-mediated vascular rejection and TA. This model can be used to investigate the causes of TA as well as the development of novel therapeutic strategies. It has been used in the past to establish an essential role of adaptive immunity, cytotoxic T cell responses, cytokine-mediated CD4 T cell effector responses, and antibody-mediated graft damage in TA [14,17-21]. Artery transplantation in mice is difficult due to the obvious small size of the animal; however, with practice and due diligence, successful surgeries can be accomplished. Success depends on the patency of the vessel. This involves ensuring that the graft is not damaged by improperly handling the vessel with forceps, constriction of the vessel lumen, suturing the back wall of the vessel, and repetitive suturing of the same site. Leakage of the vessel is also problematic and requires care to ensure that the number and position of the stitches are divided evenly around the vessel wall. It is also essential that graft ischemia is minimized to less than 30 minutes. With this, a success rate of greater than 95% can be attained.

The aorta is the largest artery in the mouse so this procedure is the simplest microsurgical approach for investigating vascular rejection in this model animal. Also, the aortic segment is grafted in a physiologically relevant location, experiences normal blood flow, and intimal thickening

develops rapidly. The other main model used for the assessment of vascular rejection and TA is heterotopic heart transplantation, which has the advantage of examining the development of TA in coronary arteries and in the context of heart transplantation that is the most relevant clinical scenario for TA. However, heterotopic heart transplants are placed in a non-physiological location within the body (usually anastamosed to the vena cava and aorta in the abdomen) and the heart does not pump blood through the ventricles due to the retrograde nature of the blood supply into the transplanted heart. As such, the nature of blood flow through the coronary tree may be different from what is normally experienced by the coronary vasculature [22,23]. Also, strategies to overcome acute rejection of the heart must be incorporated in order to evaluate arterial changes. In both models, it is important to carefully choose the type of antigen mismatch utilized in order to be able to appropriately address specific questions related to vascular rejection and TA.

In summary, aortic interposition grafting is a powerful technique for investigating immune-mediated arterial damage and TA. It can be routinely mastered with practice and diligence on the part of the researcher. Once mastered, this procedure can be modified for the interposition grafting of other arterial segments, such as the carotid artery, that may enable the examination of additional scientific questions. Also, the use of this model can be extended beyond the study of transplantation. Interposition grafting of arteries can be used to introduce arterial segments from modified (*e.g.* transgenic or lipid-fed) mice into non-modified counterparts, or vice versa, to isolate the biological effects of molecules to artery wall cells versus non-arterial wall cells [24,25].

Disclosures

None.

Acknowledgements

This work was supported by grants from the Canadian Institutes of Health Research and Heart and Stroke Foundation of BC & Yukon (JCC).

References

1. Billingham, M. E. Graft coronary disease: the lesions and the patients. *Transplant Proc.* **21**, 3665-3666 (1989).
2. Foegh, M. L. Chronic rejection--graft arteriosclerosis. *Transplant Proc.* **22**, 119-122 (1990).
3. Libby, P., Pober, J. S. Chronic rejection. *Immunity.* **14**, 387-397 (2001).
4. Tellides, G., Pober, J. S. Interferon-gamma axis in graft arteriosclerosis. *Circulation research.* **100**, 622-632 (2007).
5. Johnson, D. E., Gao, S. Z., Schroeder, J. S., DeCampli, W. M., Billingham, M. E. The spectrum of coronary artery pathologic findings in human cardiac allografts. *The Journal of heart transplantation.* **8**, 349-359 (1989).
6. Gao, S. Z., Alderman, E. L., Schroeder, J. S., Silverman, J. F., Hunt, S. A. Accelerated coronary vascular disease in the heart transplant patient: coronary arteriographic findings. *Journal of the American College of Cardiology.* **12**, 334-340 (1988).
7. Mennander, A., *et al.* Chronic rejection in rat aortic allografts. An experimental model for transplant arteriosclerosis. *Arterioscler Thromb.* **11**, 671-680 (1991).
8. Choy, J. C. Granzymes and perforin in solid organ transplant rejection. *Cell Death Differ.* **17**, 567-576 (2010).
9. Johnson, P., Carpenter, M., Hirsch, G., Lee, T. Recipient cells form the intimal proliferative lesion in the rat aortic model of allograft arteriosclerosis. *Am J Transplant.* **2**, 207-214 (2002).
10. Minami, E., Laflamme, M. A., Saffitz, J. E., Murry, C. E. Extracardiac progenitor cells repopulate most major cell types in the transplanted human heart. *Circulation.* **112**, 2951-2958 (2005).
11. Yu, L., *et al.* AIP1 prevents graft arteriosclerosis by inhibiting interferon-gamma-dependent smooth muscle cell proliferation and intimal expansion. *Circ Res.* **109**, 418-427 (2011).
12. Miller, C., DeWitt, C. W. Cellular and humoral responses to major and minor histocompatibility antigens. *Transplant Proc.* **5**, 303-305 (1973).
13. Tsutsui, H., *et al.* Lumen loss in transplant coronary artery disease is a biphasic process involving early intimal thickening and late constrictive remodeling: results from a 5-year serial intravascular ultrasound study. *Circulation.* **104**, 653-657 (2001).
14. Rossum, A., Enns, W., Shi, P., MacEwan, G. E., Choy, J. C. Bim regulates allogeneic immune responses and transplant arteriosclerosis through effects on T cell activation and death. *Arterioscler Thromb Vasc Biol.* **34**, 1290-1297 (2014).
15. Ho, M. K., Springer, T. A. Tissue distribution, structural characterization, and biosynthesis of Mac-3, a macrophage surface glycoprotein exhibiting molecular weight heterogeneity. *J Biol Chem.* **258**, 636-642 (1983).
16. Inoue, T., Plieth, D., Venkov, C. D., Xu, C., Neilson, E. G. Antibodies against macrophages that overlap in specificity with fibroblasts. *Kidney international.* **67**, 2488-2493 (2005).
17. Shi, C., *et al.* Immunologic basis of transplant-associated arteriosclerosis. *Proc Natl Acad Sci U S A.* **93**, 4051-4056 (1996).
18. Skaro, A. I., *et al.* CD8+ T cells mediate aortic allograft vasculopathy by direct killing and an interferon-gamma-dependent indirect pathway. *Cardiovasc Res.* **65**, 283-291 (2005).
19. Tellides, G., *et al.* Interferon-gamma elicits arteriosclerosis in the absence of leukocytes. *Nature.* **403**, 207-211 (2000).
20. Wang, Y., *et al.* Interferon-gamma induces human vascular smooth muscle cell proliferation and intimal expansion by phosphatidylinositol 3-kinase dependent mammalian target of rapamycin raptor complex 1 activation. *Circ Res.* **101**, 560-569 (2007).
21. Soulez, M., *et al.* The perlecan fragment LG3 is a novel regulator of obliterative remodeling associated with allograft vascular rejection. *Circ Res.* **110**, 94-104 (2012).
22. Choy, J. C., Kerjner, A., Wong, B. W., McManus, B. M., Granville, D. J. Perforin mediates endothelial cell death and resultant transplant vascular disease in cardiac allografts. *Am J Pathol.* **165**, 127-133 (2004).
23. Choy, J. C., *et al.* Granzyme B induces endothelial cell apoptosis and contributes to the development of transplant vascular disease. *Am J Transplant.* **5**, 494-499 (2005).
24. Reis, E. D., *et al.* Dramatic remodeling of advanced atherosclerotic plaques of the apolipoprotein E-deficient mouse in a novel transplantation model. *Journal of vascular surgery.* **34**, 541-547 (2001).

25. Potteaux, S., *et al.* Suppressed monocyte recruitment drives macrophage removal from atherosclerotic plaques of Apoe-/- mice during disease regression. *J Clin Invest.* **121**, 2025-2036 (2011).