A Model of Sequential Heart and Composite Tissue Allotransplant in Rats

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Background: Some of the 600,000 patients with solid organ allotransplants need reconstruction with a composite tissue allotransplant, such as the hand, abdominal wall, or face. The aim of this study was to develop a rat model for assessing the effects of a secondary composite tissue allotransplant on a primary heart allotransplant.

Methods: Hearts of Wistar Kyoto rats were harvested and transplanted heterotopically to the neck of recipient Fisher 344 rats. The anastomoses were performed between the donor brachiocephalic artery and the recipient left common carotid artery, and between the donor pulmonary artery and the recipient external jugular vein. Recipients received cyclosporine A for 10 days only. Heart rate was assessed noninvasively. The sequential composite tissue allotransplant consisted of a 3 × 3-cm abdominal musculocutaneous flap harvested from Lewis rats and transplanted to the abdomen of the heart allotransplant recipients. The abdominal flap vessels were connected to the femoral vessels. No further immunosuppression was administered following the composite tissue allotransplant. Ten days after composite tissue allotransplantation, rejection of the heart and abdominal flap was assessed histologically.

Results: The rat survival rate of the two-stage transplant surgery was 80 percent. The transplanted heart rate decreased from 150 ± 22 beats per minute immediately after transplant to 83 ± 12 beats per minute on day 20 (10 days after stopping immunosuppression).

Conclusions: This sequential allotransplant model is technically demanding. It will facilitate investigation of the effects of a secondary composite tissue allotransplant following primary solid organ transplantation and could be useful in developing future immunotherapeutic strategies. (*Plast. Reconstr. Surg.* 126: 80, 2010.)

Does a sequential allotransplant adversely affect a primary transplanted organ? As abdominal wall, hand, and face transplants become more common, this question is important for those patients who have a previous critical organ transplant. The purpose of this study was to develop a rat model of a secondary composite tissue allotransplant following a primary solid organ allotransplant.

Heterotopic heart transplantation was initially described by Mann in dogs and further improved

by anastomosing the donor aorta and pulmonary artery to appropriate recipient vessels.¹⁻⁵ Transplantation of multiple tissues derived from ectoderm and mesoderm is known as “composite tissue allotransplantation,” as originally described by

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Peacock.⁶ Composite tissue allotransplants includes body structures, such as the hand, abdominal wall, face, and other tissues. This is in contrast to solid organ transplantation involving a relatively uniform structure (e.g., heart, lung, and kidney). Composite tissue allotransplantation for reconstruction is being performed with increasing frequency. Worldwide, approximately 60 composite tissue allotransplant procedures have been performed through 2008, including 30 unilateral or bilateral hand transplantations, four partial face transplantations, six knee joints, and several other allograft transplantations, such as larynx and nerve.⁷⁻⁹

The performance of composite tissue allotransplantation has been limited by the required lifelong immunosuppression, with its resulting toxicity and limited tissue function due to chronic rejection and fibrosis.⁷⁻⁹ Of the 600,000 patients in the United States who have undergone solid organ transplantation and are already on immunosuppression therapy, some suffer from tissue loss due to impaired wound healing, trauma, burns, and resection of malignancies that are common in patients on long-term immunosuppression. Although these patients may benefit from a composite tissue allotransplant, there is concern that the sequential composite tissue allotransplant could adversely affect the immune function and rejection of the transplanted solid organ. Therefore, the objective of this study was to develop a model for assessing the effects of a composite tissue allograft on a heart allotransplant in the rat.

METHODS

Heterotopic Heart Allotransplantation Model

All protocols were approved by the Institutional Animal Care and Use Committee before the initiation of any studies. Surgery was performed as described by Chen in 1991,^{10,11} with several modifications. Ten donor male Wistar Kyoto rats (150 to 200 g) were anesthetized with isoflurane (2% in oxygen) and ventilated via a tracheotomy. Great care was taken to maintain rectal temperature at 36°C to 39°C. Through an abdominal midline incision, the peritoneal cavity was opened, and the inferior vena cava was exposed. Heparin (200 IU in 2 ml of saline) was injected into the inferior vena cava. A bilateral thoracotomy was then performed and the anterior chest wall reflected. The thoracic aorta and the inferior vena cava were transected above the diaphragm. The aorta was mobilized from the surrounding tissues, and the heart, lung, and thymus were harvested en bloc. The heart was cooled for 15 seconds to 4°C in

Ringer's solution to arrest the beating heart and to slow metabolism. The main bronchus and the thymus were removed. The pulmonary artery branch going to the left lung was separated and ligated. The right pulmonary artery was transected as distally as possible and used as an outflow tract. Approximately 3 mm of the brachiocephalic artery from the aorta was preserved as an inflow tract. The other vessels of the heart were ligated.

Ten recipient male F344 rats (400 to 500 g; Charles River Laboratories, Wilmington, Mass.) were similarly anesthetized, positioned supine, and ventilated via a tracheotomy. A cervical midline incision was made ventrally to expose the left external jugular vein and common carotid artery. After undermining of the skin of the left neck, the left submandibular gland and sternocleidomastoid muscle were excised to create a space for the heart transplant. The proximal common carotid artery and external jugular vein were clamped proximally and ligated distally using a 4-0 suture. A modified sleeve technique¹² was performed for the anastomoses between the donor brachiocephalic artery and recipient common carotid artery using 8-0 nylon sutures (Fig. 1). Next, the donor pulmonary artery and recipient external jugular vein were connected end to end using interrupted 9-0 nylon sutures. After the clamps were released, the donor heart filled with blood immediately and began fibrillating. Within approximately 1 minute after reperfusion, the donor heart spontaneously began to contract with normal rhythm. The incision was closed after thorough hemostasis, and a 4-mm length of one of the cutaneous sutures was left to accentuate the motion of the underlying transplanted heart. The heart rate of the heterotopic allotransplanted hearts was easily measured noninvasively via direct observation and video recorded. For native hearts, heart rate was measured using a veterinary pulse oximeter (Mediad Palco, Torrance, Calif.) and confirmed grossly by chest wall motion. Beating of the native heart was faster and easily distinguished from the transplanted heart beat. Heart allotransplant survival time was defined as the period from reperfusion to the cessation of the donor heart beat. The operation was considered a technical failure if the donor heart stopped beating within 3 days.

Immunosuppression Protocols

Because the purpose of the study was to assess rejection severity and not to prevent rejection as would be done clinically, we chose an immunosuppression protocol that would produce predictable

Fig. 1. Surgical schema of heterotopic heart allotransplant procedure and isotopic composite tissue allotransplant (BA, brachiocephalic artery; EA, epigastric artery; EV, epigastric vein; CCA, common carotid artery; FA, femoral artery; FV, femoral vein; PA, pulmonary artery; EJV, external jugular vein).

rejection. All heart allotransplant recipients received cyclosporine A (5 mg/kg intraperitoneally) (Sandimmun Neoral; Novartis Pharma, Basel, Switzerland) every other day for 10 days after the heart transplant. As detailed below, all immunosuppression was then stopped in all groups.

Sequential Composite Tissue Allotransplant Transplantation Procedure

On day 10 after heart transplantation, all rats were again anesthetized and had their abdomens shaved and prepared. The composite tissue allotransplant protocol was generally as described by Hewitt et al.,¹³ with modifications as recently described by Pradka et al.¹⁴ The femoral nerve was minimally exposed to reduce the potential for hypokinesia. A 3 × 3-cm abdominal flap was elevated, and the superficial epigastric vessels were transected, creating a free flap.

As a control for the trauma induced by the secondary surgery, an autograft group ($n = 5$) had the abdominal flap isotopically sutured in place after the vessels were anastomosed. In the composite tissue allotransplant group ($n = 5$), the abdominal flap was discarded, and five male Lewis rats (400 to 500 g; Charles River Laboratories) were anesthetized and had an identical 3 × 3-cm abdominal flap harvested on the superficial epigastric

artery and vein. The harvested flap was transplanted to the recipient F344 rat that had previously received the heart allotransplant. Anastomoses between the epigastric artery and vein were performed, and care was taken to avoid injury to the femoral nerve (Fig. 1). After the abdominal flap surgery, no additional immunosuppression therapy was administered. All rats were observed and recorded daily. On day 20, all rats were again anesthetized. The allotransplanted and native hearts and the allotransplanted and native abdominal walls were all harvested en bloc and immersion fixed in 10% neutral buffered formalin for 24 hours, followed by immersion in phosphate-buffered saline. Paraffin-embedded specimens were sectioned and stained with hematoxylin and eosin. In summary, the timeline of the study was as follows:

- Day 0: Perform heart allotransplant ectopically and begin immunosuppression; record heart rates.
- Day 10: Perform either abdominal autologous composite tissue island flap isotopically or abdominal composite tissue allotransplant. Stop immunosuppression in all rats.
- Day 13: Assess perfusion of abdominal flap using fluorescein.
- Day 20: Harvest abdominal flaps and heart.

All data are presented as mean $\pm$ SEM.

RESULTS

Animal Survival Rate

The survival rate in this study was 80 percent. One rat died possibly from a compressed trachea after the heart allotransplantation. Another rat died from pulmonary insufficiency following a prolonged anesthesia time during the sequential composite tissue allotransplant operation.

Heart Rate and Rejection

The average total ischemia time of the transplanted heart was 52 ± 3 minutes, spent almost entirely at 4°C . The ischemia time spent above 4°C was 15 ± 5 seconds. The heart rate of transplanted hearts was easily observed noninvasively. (See Video, Supplemental Digital Content 1, which demonstrates postoperative noninvasive observation of allotransplant heart rate, <http://links.lww.com/PRS/A165>; the end of the suture amplifies the motion of the beating heart. Abdominal composite tissue allotransplant is also depicted.) With immunosuppression, after transplantation, the heart rate averaged 150 ± 22 beats per minute (mean $\pm$ SEM). The native heart rate in the same rats averaged 176 ± 17 beats per minute. Slowing of the allotransplanted heart rate usually began 4 to 5 days after stopping immunosuppression, which is similar to previously published reports⁵ and averaged 83 ± 12 beats per minute at 20 days after transplant.

Composite Tissue Allotransplant Rejection

The average total ischemia time of both composite tissue allotransplant and autograft flaps was 64 ± 5 minutes at 4°C plus approximately 10 seconds above 4°C . The technical success of each composite tissue allotransplant was confirmed on day 3 by the color and turgor of each flap, along with fluorescein perfusion followed by gross Wood's lamp observation (Fig. 2). Using gross clinical observation, the rejection of the composite tissue allotransplant was indicated by redness that first appeared at the lateral margins on day 6 after transplant (Fig. 2). Rejection continued to progress to obvious firmness (day 9), followed eventually by frank necrosis (Fig. 2).

Histopathology

Native myocardium and skin had unremarkable morphology and cellularity. The allotransplanted heart showed typical signs of acute rejection, including both perivascular and diffuse

myocardial lymphocytic infiltration (Fig. 3). The abdominal flap autograft histopathology appeared normal (Fig. 4). The allotransplanted abdominal flap had typical perivascular and diffuse lymphocytic infiltration (Fig. 4).

DISCUSSION

From the first successful human kidney allotransplant performed by Merrill et al. in 1954¹⁵ to the first partial face allotransplant by Devauchelle et al. in 2005,¹⁶ both solid organ allotransplantation and composite tissue allotransplants have been used to restore form or function. Of the 600,000 patients in the United States who have undergone solid organ transplantation, an increasing number of them could benefit from sequential composite tissue allotransplantation to reconstruct complex defects. It is believed that composite tissue derived from both the ectoderm and mesoderm has high tissue antigenicity. The effect, however, that a sequential composite tissue allotransplant may have on immune function, particularly in a recipient with a primary solid organ allotransplant, is not well known. The objective of our study was to develop a model for assessing the effects of a composite tissue allotransplant on a previous heart allotransplant.

The most popular heterotopic heart transplantation model uses the peritoneal cavity as the recipient site.¹⁷ In that model, the recipient vessels are the abdominal aorta and inferior vena cava, which are not far from the common iliac vessels. This would have created a high risk of thrombosis if the composite tissue allotransplant vessels were connected to the iliac vessels or femoral vessels. To reduce this risk, we transplanted the heart from a small donor rat (150 to 200 g) to the neck of a larger recipient (400 to 500 g).

During the study, one rat died, apparently from a compressed trachea due to a hematoma caused by a leaking arterial anastomosis. To minimize arterial leaks, we began to use a "sleeve" technique modified from Neren's method.¹² Technical points that were important to maximize success in this heterotopic heart transplantation model included a short amount of time above 4°C while ischemic, a large subcutaneous space, maintenance of normal body temperature, and meticulous hemostasis. For the sequential composite tissue allotransplant technique, minimizing surgical trauma and anesthesia time was important for success.

We recognize that ceasing immunotherapy following composite tissue allotransplantation

Fig. 2. A 3 × 3-cm abdominal flap after allotransplantation. (Above, left) Brightfield photograph on day 3 after composite tissue allotransplantation. (Above, right) Wood's lamp illumination 15 minutes after sodium fluorescein injection on day 3. (Center, left) Brightfield photograph on day 6. (Center, right) Brightfield photograph on day 9. (Below) Brightfield photograph on day 12.

allowed rejection to develop unchecked. If effective immunotherapy had been continued, we expect that no rejection would have occurred. This model, however, was developed to assess alteration of rejection by the secondary allotransplant. Thus, reliability of the rate and severity of the rejection was important to establish. Other immunotherapeutic strategies and other combinations of large and small mismatched

strains of rats could be worthwhile investigations with this surgical model.

CONCLUSIONS

This surgical technique is technically demanding but reliable. The risk and technical requirements of this model are higher than those of the separate individual transplants.

Fig. 3. Heart histopathology (hematoxylin and eosin stained) on day 20. (Left) Native heart. (Right) Allotransplanted heart with obvious lymphocytic infiltration.

Fig. 4. Composite tissue histopathology (hematoxylin and eosin stained) on day 20 (10 days after composite tissue allotransplantation). (Left) Autografted skin. (Right) Allotransplanted skin with typical acute rejection consisting of obvious perivascular lymphocytic infiltration.

Maintaining survival and function of transplanted tissues in this model is more difficult due to the need for sequential operations. A major advantage of this model is that transplanted heart function can be assessed and recorded easily by direct clinical observation non-invasively in the recipient's neck. This study presents a unique model that can be used to investigate the effects of a sequential composite tissue allotransplant on an allotransplanted solid organ and may facilitate development of future immunotherapy strategies for composite tissue allotransplantation following solid organ allotransplantation.

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