Development of a Combined Heart and Carotid Artery Transplant Model to Investigate the Impact of Acute Rejection on Cardiac Allograft Vasculopathy

Behzad Soleimani, MD, MRCP, FRCS(C-Th),[a] Fumin Fu, MD,[b] Philip Lake, PhD,[b] and Victor C. Shi, MD[b]

Background: Cardiac allograft vasculopathy (CAV) is the leading cause of late allograft loss after heart transplantation. Although clinical studies are suggestive of an association between episodes of acute rejection and subsequent emergence of CAV, direct experimental evidence in support of a causal relationship is lacking.

Methods: We developed a new murine model of CAV in which a carotid artery and a heart graft are simultaneously transplanted into a single recipient. Transplants were performed across full or partial major histocompatibility complex (MHC) mismatched strain combinations. The heart grafts were either syngeneic with the carotid graft or from a third-party strain. Carotid arteries were harvested after 30 days and evaluated by morphometry and immunohistochemistry.

Results: In the fully mismatched combination, all heart grafts were rejected within 7 days, as determined by loss of pulsation. At 30 days, carotid allografts in the combined transplant group had significantly more intimal hyperplasia compared with isolated carotid allografts. The neointima consisted of abundant smooth muscle cells and leukocytes. Intimal hyperplasia was also significantly enhanced by acute rejection of the third-party donor heart. In the partial MHC mismatched combination, the heart graft survived indefinitely, and this was associated with diminished intimal hyperplasia in the cotransplanted carotid artery compared with the isolated carotid allograft.

Conclusion: We present direct experimental evidence that CAV is promoted by acute parenchymal rejection of the heart. This interaction between acute rejection and CAV is mediated by both allospecific and non-allospecific processes. Effective therapeutic strategy against CAV should therefore target non-allospecific mediators as well as prevent episodes of acute rejection. J Heart Lung Transplant 2008;27:450–6. Copyright © 2008 by the International Society for Heart and Lung Transplantation.

Cardiac allograft vasculopathy (CAV) is the leading cause of heart transplant failure after the first post-operative year.[1,2] The key pathologic process in CAV is the rapid development of intimal hyperplasia (IH). The development of IH is characterized by the accumulation of smooth muscle cells (SMCs) and extracellular matrix in a subendothelial location. This occurs together with infiltration of monocytes, T cells, fibroblasts, and dendritic cells.[3,4]

A number of experimental models have been developed to determine the cellular and molecular mediators of this complex process. Experimental heart transplants have long been recognized to develop CAV provided early acute rejection (AR) is prevented, generally by the use of immunosuppressants. Introduction of immunosuppressants can, however, increase the complexity of the models given that many of these agents have themselves been implicated in the pathogenesis of CAV.[5–7] In addition, immunosuppressants used experimentally generally have no clinical application and in some models result in permanent graft survival after a brief period of peri-operative treatment.[8] This is clearly not the case in clinical transplantation, and this discrepancy may have important implications when extrapolating results from these models to clinical CAV.

An alternative strategy is the use of partial major histocompatibility complex (MHC) or minor histocompatibility mismatched strain combinations. Because in clinical transplantation, donors and recipients are often mismatched in multiple major and minor histocompatibility loci, data generated by heart transplant models

From the [a]Department of Cardiac Surgery, National Heart and Lung Institute, Imperial College London, London, United Kingdom, and [b]Novartis Pharmaceuticals Corporation, East Hanover, New Jersey.

Submitted July 26, 2007; revised December 23, 2007; accepted January 12, 2008.

This work was funded by grants from the British Heart Foundation and Novartis Pharmaceutical Corporation.

Reprint requests: Victor C. Shi, Novartis Pharmaceuticals Corporation, One Health Plaza, East Hanover, NJ 07936-1080. Telephone: 862-778-8300. Fax: 973-781-8265. E-mail: victor.shi@novartis.com

 1053-2498/08/$–see front matter. doi:10.1016/j.healun.2008.01.015

Table 1. Donor and Recipient Strains in Experimental and Control Groups

Group	No.	Recipient strain	Carotid artery donor strain	Heart donor strain
I	6	C3H(H-2^k)	C57BL/10(H-2^b)	C57BL/10(H-2^b)
II	6	B10.BR(H-2^k)	B10.A(2R)(H-2^{h2})	B10.A(2R)(H-2^{h2})
III	6	C3H(H-2^k)	C57BL/10(H-2^b)	BALB/c(H-2^d)
Control I	6	C3H(H-2^k)	C57BL/10(H-2^b)	●●●
Control II	6	B10.BR(H-2^k)	B10.A(2R)(H-2^{h2})	●●●

using partial mismatch may only be applicable to a subset of patients with similar histoincompatibilities.

One further characteristic of these models is that in common with clinical CAV, the coronary artery lesions in experimental cardiac allografts are generally heterogeneous in location, distribution, and intensity,[9] making quantitative evaluation of IH severity in these models complex.[10] In the most common variant of experimental heart transplantation, in which the heart is grafted heterotopically, the left ventricle is completely offloaded and undergoes atrophy. This results in the formation of left ventricular thrombus, which can in turn serve as a source of cytokines such as transforming growth factor-β.[11] The impact of left ventricular atrophy and thrombus formation on development of IH in these models is unknown.

Arterial allograft models were developed as an alternative to whole organ grafts to allow mechanistic study of IH without the complications of an immune response to cardiac parenchymal tissue. In contrast with organ allograft models, the lesions seen in these models are concentric, uniform, and reproducible. In addition, most vessel transplant models require no immunosuppression because there is no acute destructive parenchymal rejection that would otherwise precede the emergence of IH. Absence of immunosuppression is, however, regarded by some authorities as a potential weakness of these models.[12]

To benefit from the advantages and avoid the shortcomings associated with the arterial and organ models, we developed a new model by cotransplanting a carotid artery and heart graft into a single recipient in the absence of immunosuppression. Advanced IH was reproducibly formed in the carotid artery graft within 30 days of transplantation. This model was then used to address the impact of acute rejection of the heart on the development of IH in the cotransplanted carotid artery.

MATERIALS AND METHODS

Animals and Experimental Groups

Inbred mice (28 to 32 g, 9-week-old males) were chosen for incompatibility in the H-2 region (Jackson Laboratory, Bar Harbor, ME). Three groups of combined transplants and 2 groups of isolated transplants were performed (n = 6 per group). In Group I, C57BL/10 (H-2^b) mice were used as donors of heart and carotid artery grafts and C3H (H-2^k) mice as recipients (full MHC mismatch). In Group II, B10.A(2R)(H-2^{h2}) mice were used as donors of heart and carotid artery grafts and B10.BR(H-2^k) mice as recipients (isolated class I MHC mismatch). In Group III, C3H(H-2^k) mice were recipients of a carotid artery graft from C57BL/10(H-2^b) mice and a heart graft from BALB/c(H-2^d) mice (third-party heart). Isolated carotid grafts were also performed for Groups I and II as controls. The experimental and control groups are summarized in Table 1. All animals received humane care in compliance with the *Principles of Laboratory Animal Care* formulated by the National Society for Medical Research and the *Guide for the Care and Use of Laboratory Animals* prepared by the Institute of Laboratory Animal Resources and published by the National Institutes of Health (NIH Publication No. 86-23, revised 1996).

Transplantation

The cotransplant model used in this study was generated by transplanting carotid artery and heart grafts simultaneously into 1 recipient. The 2 procedures were performed under the same anesthetic, but in sequence with the heart, followed by the carotid artery. All procedures were performed on anesthetized mice under a dissecting microscope (Leica, Solms, Germany).

Mouse carotid artery transplant model. This model has been described by us previously.[13] In the donor mouse, a mid-line incision was made extending from the mandible to the suprapubis; then, 2 ml of heparinized saline (50 U/ml) was injected through the inferior vena cava, and the aorta was divided. The carotid arteries that lie laterally to the trachea were removed and stored in saline at 4°C. A mid-line incision was then made from the mandible to the suprasternal notch of the recipient. The left internal carotid artery was dissected out, and proximal and distal microvascular clips (FE693K, Aesculap, Center Valley, PA) were applied. Two longitudinal arteriotomies (0.5 to 0.6 mm, 1 cm apart) were made on the carotid artery using the tip of a 30-gauge needle.

The graft was then transplanted as a loop by constructing 2 end-to-side anastomoses using 11-0 continuous nylon suture. Prominent pulsations were visible in both the transplanted loop and the native vessel after