Smooth Muscle Cells, But Not Myocytes, of Host Origin in Transplanted Human Hearts

Ruchira Glaser, MD; Min Min Lu, MD; Navneet Narula, MD; Jonathan A. Epstein, MD

Background—There is increasing evidence to support a role for stem cells in the regeneration and repair of the human cardiovascular system. However, significant controversy still remains about the extent of chimerism present in blood vessels and myocytes of transplanted human hearts.

Methods and Results—We investigated the contribution of infiltrating host cells to human cardiac allografts by evaluating the origin of vascular smooth muscle cells and cardiac myocytes in hearts after orthotopic cardiac transplantation. Smooth muscle cells were identified in pathological human coronary artery specimens with antibodies against smooth muscle α-actin. DNA in situ hybridization for the human Y chromosome was then performed on the same samples to identify cells of male origin. Both myocytes and vascular smooth muscle cells were examined for the presence of the Y chromosome in sex-mismatched specimens. In positive control samples, 34.7% of nuclei contained a detectable Y chromosome; in sex-mismatched samples, 2.6% of the smooth muscle cells examined were of host origin. The Y chromosome in myocyte nuclei in male positive controls was detected; however, despite examination of >6000 myocyte nuclei in sex-mismatched specimens, we were unable to detect any nuclei with the clear presence of the Y chromosome.

Conclusions—Vascular smooth muscle cells of infiltrating host cell origin can be found in human cardiac allografts. However, unlike prior reports, we found no evidence that chimerism is present in cardiac myocytes. (Circulation. 2002; 106:17-19.)

Key Words: transplantation ■ muscle, smooth ■ myocytes

Recent observations suggest that circulating stem cells differentiate into, among other cells, vascular smooth muscle cells. Both animal and human experiments have implicated remote progenitor cells in both the pathogenesis of vasculopathy and myocyte regeneration.1–4 For instance, coronary artery neointimal smooth muscle cells expressed LacZ after ROSA26 (LacZ) mice received wild-type cardiac allografts, identifying significant host origin of the neointima.5 Similarly, human smooth muscle cells of host origin were identified in the vascular lesions of renal allografts6 and in the normal vessels of cardiac allografts.7 Finally, a significant population of cardiac myocytes of host origin has been identified.7 Nevertheless, there remains significant controversy about chimerism in the human heart, with conflicting data recently presented.7,8

Methods

Cardiac Specimens
Pathological autopsy specimens of 6 human male recipients of female cardiac allografts constituted the experimental group; specimens from 2 male recipients of male cardiac allografts and 1 female recipient of a female cardiac allograft were used as positive and negative controls, respectively. Sections of 5-μm thickness were obtained and included cardiac myocardium and both small and large coronary arteries. Samples were obtained according to protocols approved by the institutional review board at the University of Pennsylvania.

Immunohistochemistry and DNA
In Situ Hybridization
Smooth muscle cells were identified by means of immunohistochemistry with antibodies against smooth muscle α-actin. DNA in situ hybridization for the human Y chromosome was subsequently performed with CEP Y satellite III (Vysis), a Y chromosome repeat marker that is labeled with fluorescein, on the same samples to identify cells of male origin. Finally, cell nuclei were counterstained with Hoechst nuclear dye.

Analysis
Cells expressing smooth muscle α-actin with nuclei containing an identifiable Y chromosome were identified as vascular smooth

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muscle cells of male origin. An independent pathologist blinded to the experimental results scored the specimens for degree of transplant arteriosclerosis and cardiac rejection.

**Results**

In positive control male samples, 34.7% of nuclei (694 of 2000 nuclei) contained a detectable Y chromosome. The fact that some male nuclei did not have detectable Y chromosome staining was most likely related to the thickness of the sections (Figure, a). Consistent with the high specificity of the assay, no fluorescence signal indicative of Y chromosome was detected in female negative control samples.

Smooth muscle cells containing a Y chromosome were identified in the neointimal lesions of the experimental sex-mismatched specimens, providing definitive evidence of coronary smooth muscle cells of host origin within the allograft. The majority of smooth muscle cells of host origin were identified in medium and small arteries. On average, 2.6% (154 of 6000 nuclei counted) of smooth muscle cells examined were of host origin (Figure, b and c). There was significant variation in the percentage of host smooth muscle cells detected in allograft samples, ranging from 0.8% to 5.6% of smooth muscle cell nuclei examined. Given the sensitivity of our assay, we estimate that, at most, 16% of smooth muscle cells within the regions of the cardiac allografts that we examined were of host origin.

The clinical and pathological characteristics of the patients are summarized in the Table. The patients had varying lengths of time after transplantation, as well as varying degrees of rejection vasculopathy. We found no clear correlation between the degree of cardiac rejection, transplant vasculopathy, or time since transplantation and the number of host neointimal cells. Vessels with advanced neointimal smooth muscle proliferation did not have markedly increased (or decreased) numbers of host cells. We did not identify evidence of host cell-mediated angiogenesis because we did not find any vessels in which all or most cells were of host origin.

Finally, we were able to detect the Y chromosome in myocyte nuclei in male positive controls (Figure, d). However, although we examined >6000 myocyte nuclei in sex-mismatched specimens, we were unable to detect any nuclei with clear evidence for the presence of the Y chromosome. We did detect other, unspecified Y chromosome–positive cells within the specimens that we examined; these probably represented lymphocytes, monocytes, and/or fibroblasts, but we did not characterize these cells further.

**Discussion**

Our data demonstrate the presence of infiltrating host cells in the vascular smooth muscle cells of cardiac allografts. The degree to which host cell infiltration occurs seems to be somewhat variable, although generally rare. Although there did not seem to be a relationship between the degree of transplant arteriopathy or degree of cardiac rejection and the degree of chimerism in the vasculature, the number of patients examined was too small to draw clear conclusions about whether such a correlation exists.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Patient Age, y</th>
<th>Reason for Transplantation</th>
<th>Pathology</th>
<th>Time Between Transplantation and Autopsy</th>
<th>Host Origin Smooth Muscle Cells Detected, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>64</td>
<td>Idiopathic dilated cardiomyopathy</td>
<td>No acute cellular rejection; moderate to severe graft coronary atherosclerosis</td>
<td>10 y</td>
<td>3.1</td>
</tr>
<tr>
<td>2</td>
<td>29</td>
<td>Idiopathic dilated cardiomyopathy</td>
<td>Mild acute cellular rejection; mild graft coronary atherosclerosis</td>
<td>3.2 y</td>
<td>5.6</td>
</tr>
<tr>
<td>3</td>
<td>54</td>
<td>Ischemic cardiomyopathy</td>
<td>Moderate acute cellular rejection; moderate graft coronary atherosclerosis</td>
<td>3.7 y</td>
<td>0.8</td>
</tr>
<tr>
<td>4</td>
<td>26</td>
<td>Idiopathic dilated cardiomyopathy</td>
<td>Moderate acute cellular rejection; moderate graft coronary atherosclerosis</td>
<td>6 mo</td>
<td>3.4</td>
</tr>
<tr>
<td>5</td>
<td>67</td>
<td>Idiopathic dilated cardiomyopathy</td>
<td>No acute cellular rejection; mild graft coronary atherosclerosis</td>
<td>6.6 y</td>
<td>1.2</td>
</tr>
<tr>
<td>6</td>
<td>45</td>
<td>Idiopathic dilated cardiomyopathy</td>
<td>Moderate acute cellular rejection; severe graft coronary atherosclerosis</td>
<td>10.1 y</td>
<td>1.4</td>
</tr>
</tbody>
</table>
These results differ in several respects from those of Quaini et al. They reported that arterioles within cardiac allografts contained up to 60% smooth muscle cells of host origin. We did not identify any vessels with this degree of chimerism. They also identified up to 15% of myocyte nuclei that were of host origin, whereas we identified none. It is worth noting that Quaini et al found the highest levels of chimerism in relatively recent transplants (<28 days), and none of our patients fell into this category. Because we identified the Y chromosome in arterial vessels of experimental and control specimens, in addition to male (control) myocytes, it is unlikely that our inability to detect host-derived myocytes in experimental samples was due to a technical deficiency. It is possible that we detected smooth muscle cell chimerism but did not detect cardiac myocytes of host origin because smooth muscle proliferation and response to injury is prevalent after cardiac transplantation.

Cardiac transplantation is a life-saving therapy in many patients with end-stage heart disease. However, transplant vasculopathy remains the major cause of late graft failure and subsequent mortality. It has been hypothesized that neointimal proliferation in arteriosclerotic lesions is the result of inflammatory mediator–driven local recruitment of cells from the media to the intima. Our data reveal the presence of smooth muscle cells of host origin in the neointima of human coronary arteries. It remains to be determined if this mechanism can contribute to the neointimal proliferation of transplant arteriosclerosis. It is also possible that this process contributes to other prevalent cardiac vasculopathies, including atherosclerosis and postangioplasty and in-stent restenosis. Additional studies will be required to determine the degree to which infiltrating cells contribute to cardiac smooth muscle and myocyte chimerism and to identify the factors that modulate this process.

References
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