Cardiac Chimerism as a Mechanism for Self-Repair
Does It Happen and If So to What Degree?

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Cardiac regeneration is an area that has gained considerable attention lately, especially with the finding that immature muscle cells and/or autologous stem cells can regenerate function in a previously injured heart.1,2,3 However, this fertile field remains unsettled enough that each piece of new data should be rigorously scrutinized. Toward that end, a controversy has arisen recently concerning the potential for cardiovascular self-repair evidenced by chimerism in an allografted human heart. Several investigators have allografted female hearts into human male recipients and examined the heart at the time of explantation for the presence of Y chromosome–positive cells either in the coronary vasculature or within cardiomyocytes.4–7 Conflicting results have been obtained. The report by Glaser et al4 in this issue of Circulation presents one side of the controversy, namely that regeneration is possible to a certain degree in the coronary vasculature but fails to occur within cardiomyocytes.4 In contrast, the recent report by Quaini et al5 claimed not only that vascular regeneration occurs, but also that repair of up to 30% of the donor myocardium takes place within 1 month of transplantation.

The notion of progenitor cell–based vascular repair arises naturally from our understanding of vascular biology, where endothelial and smooth muscle cell turnover and replacement are clinically accepted. In fact, the use of endothelial progenitor cells to repair damaged vasculature is an exciting new area of investigation.8 In contrast, the controversy over myocardial chimerism and repair is one that requires a paradigm shift from the accepted dogma of a myocardium that is incapable of self-repair to one in which host-derived stem cells can be recruited to, engraft within, and regenerate significant portions of human heart. If the data supporting this paradigm shift are accurate, they represent the newly found, extraordinary ability of the myocardium to regenerate — a development that could provide a basis for new types of therapy. Yet, as with any paradigm shift, the burden of proof is high, especially in an area with such dramatic therapeutic implications. It is imperative, therefore, to comment on both sides of the controversy, thus raising issues that must be addressed to convincingly resolve this debate.

At issue is the concept of cardiac chimerism and the degree to which it can occur. Glaser et al report the presence of infiltrating host cells comprising up to 5.6% of the vascular smooth muscle cells, but comprising none of the >6000 cardiomyocytes surveyed in the donor heart.4 These data are similar to the results obtained by other groups, including our own, that demonstrate very low levels of cardiomyocyte chimerism (∼0.02% to 1%) within an allografted human heart.5,6 In contrast, Quaini et al report that ≤30% of transplanted myocardium is regenerated within 4 to 28 days after allotransplantation by cardiac-derived stem cells originating from the recipient.5 These disparities in data could reflect either real differences in cardiac biology due to differences in the specimens examined or limitations in the criteria currently required to draw such conclusions. It is notable that the studies of Glaser,4 Hruban,6 and Laflamme7 are very consistent in terms of cardiomyocyte chimerism, or lack thereof, and do not likely reflect an inability to detect chimerism, because they used appropriate positive controls. In contrast, the data of Quaini et al stand alone. Again, this degree of difference and the implications for therapeutic potential suggest a high degree of scrutiny is needed for such a paradigm shift.

The degree of cardiac chimerism observed likely depends on several factors, including the methods used to identify “chimeric” cells, the limitations of those measurements, the timing of the observations, and the criteria by which chimerism is identified. Investigator differences in one or all of the above appear to account for the varied findings in the field. Yet, virtually all investigators agree that for chimerism to be claimed, it is imperative to show that the Y chromosome–positive nuclei detected in an allograft are unequivocally associated with either myocardial vessels or cardiomyocytes. Otherwise, it is possible to attribute some, if not all, of the Y chromosome–positive nuclei to alloimmune, inflammatory infiltration, and not cardiac regeneration. Incorrect attribution of Y chromosome–positive nuclei to cardiomyocytes could account for the differences in the data reported by Quaini et al versus the data of the other investigators. For example, although the fluorescent images presented by most investigators are aesthetically pleasing, they do not definitively demonstrate that the nuclei present are associated with the apparent surrounding cytoplasm. Most allografted hearts contain innumerable infiltrating recipient lymphocytes and macrophages, usually associated with allograft rejection, which are often intimately associated with donor cells. These
lymphocytes and macrophages can give the appearance of a recipient cell actually residing within the donor cell, particularly in fluorescence images. These false-positives can be especially common in the myocardium, because of the intertwining nature of fascicles and bundles of cardiac myocytes. Furthermore, as reported in a murine transgenic model, macrophages can develop networks of small metalloelastase-positive tunnels within the myocardium that can, on occasion, cross small vessels or cardiomyocytes. The nuclei of these inflammatory cells can be mistaken with those of cardiomyocytes.

Clearly, resolving this potential limitation in detection is crucial to solving the debate. One way to demonstrate that observed nuclei are truly associated with cardiocytes, and are not pseudonuclei, is by showing a concomitant stain with an antibody that recognizes a basement membrane component, such as laminin or collagen IV. This would show that there is no basement membrane around the “nucleus” and establish that it is not a pseudonucleus. As shown in the fluorescent and light micrographs presented, when examined under fluorescent conditions, pseudonuclei may appear to be present in allografted cardiomyocytes. If a large number of infiltrating lymphocytes is present, as is often seen during the early acute phase of rejection, this could falsely elevate the number of chimeric vascular or cardiac cells observed. This potential for incorrectly estimating the contribution of a nucleus either as positive or negative is further illustrated by the finding of Glaser et al in which only 34.7% of the positive control nuclei were detected as positive, presumably because of the thickness of the sections.

A point that cannot be ignored in the chimerism debate is the use of markers to identify chimeric cell differentiation. Positive immunohistochemical staining for a number of cardiac or vascular specific markers has been proposed as evidence of cardiac or vascular differentiation. These markers include smooth muscle α-actin, Mef2D, GATA-4, and β-cardiac myosin heavy chain. Yet, it has been shown that inflammatory cells can express several of these molecules. For example, T cells express Mef2D, GATA-4 is expressed in the spleen and in other blood cells, and even “cardiac-specific” molecules, such as β-cardiac myosin heavy chain, are expressed in peripheral blood cells. Therefore, the interpretation of these stains as proof of cardiac differentiation is debatable, particularly when there is an ongoing rejection process of the cardiac allograft. Thus, the host rejection response may be the source of Y chromosome–positive cells expressing these “cardiac-specific markers.”

A final point that must be considered is the timing of the measurements after transplantation. Quaini et al reported most active chimerism within 4 to 28 days after transplantation, with decreasing activity over 1 year. This time course is also the one expected for early acute allograft rejection that eventually resolves. The observed expression of c-Kit, MDR1, and Sca-1 gives credence to this conclusion in that, for example, c-Kit is induced in many types of repair and by inflammatory processes. Glaser et al measured chimerism from 3 to 10 years after transplantation, well after the time period for the resolution of potential acute rejection. The lack of chimeric nuclei seen in this period further raises the question of the origin of the Y chromosome–positive nuclei in previous studies.

It is reasonable for investigators in the field to accept that a limited number of observed Y-positive cells could be peripherally derived progenitor cells that are recruited to active areas of myocardial and vascular injury, and that this occurs to a very limited degree in the myocardium. In the absence of enhancing strategies, these progenitor cells appear unable to meet a long-term demand for organ repair, as evidenced by the inability of the vast majority of individuals to adequately repair their vasculature and heart over a lifetime of atherosclerosis and ischemic cardiomyopathy.

In conclusion, if the data supporting vascular and cardiac self-repair as reported by Quaini et al could be substantiated, the regenerative capacity of the myocardium could be enormous. However, the completion of this paradigm shift, from the dogma of nonregenerating myocardium to that of a self-repairing heart, requires unquestionable proof and a
reproduction of the data from a number of investigators. The reports by Glaser, Hruban, and Laflamme do not support such extraordinary regenerative capacity for the myocardium.\textsuperscript{3,6,7} Although we recognize the importance of challenging dogmas for the progress of science and the welfare of human kind, we also urge all involved in the field to rigorously evaluate the data.

References


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