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Cardiac Progenitor Cells:

The Revolution Continues

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In the last few years we have witnessed one of most extraordinary revolutions in cardiovascular medicine, namely, an explosion of basic and clinical studies that support the notion that the diseased heart can be repaired by administration of stem cells, resulting in formation of functional new myocytes and vessels. Although the mechanism by which cell therapy improves cardiac function and anatomy remains uncertain, translation of basic findings to the clinical setting is proceeding at a feverish pace.¹ A multitude of small, mostly nonrandomized clinical studies have reported improvement in cardiac perfusion and function after therapy with various cell types in patients with acute myocardial infarction or chronic ischemic cardiomyopathy.¹ Larger, randomized, double-blinded studies will be reported soon; if they confirm the salubrious effects of cell-based therapies observed in the initial trials, our management of acute myocardial infarction and heart failure will change dramatically.

One of the most important and unresolved issues in this scenario is the identity of the ideal cell for myocardial reconstitution. Although most of the clinical studies reported to date have used bone marrow– or skeletal muscle–derived cells, a host of other cells are being investigated in the experimental laboratory. Among these, resident cardiac stem cells (CSCs), discovered by Anversa's group in 2003,² hold great promise. In their initial report,² Beltrami et al identified a lin[−]/c-kit⁺ population of primitive cells that can be clonally expanded, differentiates into cardiac myocytes, smooth muscle cells, and endothelial cells in vitro, and is able to reconstitute infarcted myocardium in vivo. It is of translational interest that these cells can effect cardiac repair when delivered via the intravascular route.³ CSCs have also been shown to be present in the human heart, where they give rise to new myocytes in patients with aortic stenosis⁴ and ischemic cardiomyopathy.⁵ Because CSCs are normal components of the adult heart and appear to be responsible for the physiologic and pathologic turnover of cardiac myocytes and non-myocytes, they may be particularly suitable for reconstituting dead myocardium.

In addition to CSCs, over the past 2 years several other populations of cardiac primitive cells have been described that are able to differentiate into cardiomyocytes or regenerate infarcted myocardium or both. In 2003, Oh et al⁶ identified Sca-1⁺ cardiac progenitors that expressed

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CD31 but not c-kit or other markers of hematopoietic or endothelial progenitors. When injected intravenously into a mouse with myocardial infarction, these cells were able to generate cardiomyocytes, both with and without cell fusion. These cells differ from those described by Beltrami et al² in that they consistently express Sca-1 but not c-kit; they also differ from the cells described by Pfister et al⁷ (see below) in that they express CD31. In 2004, Messina et al⁸ reported the formation of cardiospheres from human and murine hearts that expressed Sca-1, c-kit, KDR/flk-1, and CD31. These cardiospheres were clonogenic in vitro and repaired infarcted mouse hearts in vivo. A detailed characterization of these proliferating cardiospheres grown in vitro from human endocardial biopsies revealed that they are positive for c-kit, MDR1, connexin 43, and the cardiac transcription factor Nkx2-5.⁹ Also in 2004, Martin et al¹⁰ reported the identification of an Abcg2-expressing cardiac SP cell population from embryonic as well as adult mouse hearts that are capable of proliferation and differentiation into a cardiomyocytic phenotype. Interestingly, these CSPs were negative for CD31.¹⁰ In 2005, Pfeister et al⁷ described a pool of cardiac primitive cells that were identified by their ability to efflux the Hoechst 33342 dye (a property similar to that of cells identified in other organs and termed side-population [SP] cells; this property is attributable to expression of ATP-binding cassette transporters). These cardiac SP cells (CSPs) were found to be Sca-1+/CD31-, to differentiate into cardiomyocytes in vitro, and to possess the properties of stem cells (self-renewal and clonogenicity).⁷

In 2005, Laugwitz et al¹¹ reported yet another primitive cell population in the heart that expressed the transcription factor isl1 but did not extrude Hoechst 33342 and did not express c-kit. These cells exhibited a cardiomyocytic phenotype when cocultured with neonatal cardiomyocytes in vitro and showed electrical as well as contractile properties reminiscent of neonatal cardiomyocytes.¹¹ Despite these findings, however, the isl1-positive cells described by Laugwitz et al cannot be regarded as cardiac stem cells until they are shown to be multipotent. Furthermore, the cells studied by Laugwitz et al were isolated from neonatal hearts; it is unknown whether cells with the same properties can be isolated from the adult heart as well (a critical issue from the standpoint of therapeutic application). In fact, isl1-positive cells have not been described in the adult left ventricle, with the possible exception of the outflow tract. Moreover, isl1-positive cells have not been shown to be able repair the infarcted heart or to regenerate cardiac tissue in vivo. In view of these considerations, the conceptual and therapeutic significance of the observations of Laugwitz et al¹¹ are unclear.

At present, the relationship among the various cardiac progenitor cells outlined above is unclear, and this is an important area for future research. Nevertheless, it is remarkable that many different laboratories have arrived at conceptually similar conclusions in such a short time frame. The discovery of resident cardiac progenitors represents a momentous milestone in cardiac biology, for it refutes the long-held view of the heart as a terminally differentiated organ, and instead supports a new paradigm in which the heart is a self-renewing organ that undergoes a continuous turnover. The slowness of this turnover is probably the reason it has not been recognized before; the number of progenitor and cycling cells in the heart is so low that it is very arduous to detect them unless one wishes to embark on a painstaking analysis. The cardiac progenitor cell revolution has shown that the heart is not different from other organs, in which repositories of stem/progenitor cells have been reported for many years.¹²

Although the existence of cardiac resident progenitors is becoming accepted in the scientific community, the source of these cells and their kinetics remain largely unknown. In the current issue of *Circulation Research*, Mouquet and colleagues¹³ shed light on this very problem by investigating the kinetics of CSPs under physiologic conditions and after myocardial infarction. Using enhanced green fluorescent protein chimeric mice, they found that, under normal conditions, the CSP pool in the heart was maintained without any significant contribution from bone marrow cells. However, after myocardial infarction,

CSPs were acutely depleted and then were reconstituted to baseline levels both via proliferation of resident CSPs and by homing of CD45+ bone marrow cells. After homing to the infarcted heart, the bone marrow-derived cells underwent a phenotypic conversion evidenced by a loss of the CD45 antigen.¹³

These observations are important because they are the first evidence of a possible extracardiac origin of cardiac progenitors. As is the case for all new observations, the findings of Mouquet et al raise a number of questions that will need to be addressed in future studies. For example, why does the bone marrow contribute to replenishment of CSPs after acute injury but not under normal conditions? Could this reflect the expression of ischemia-induced chemoattractants that are essential for the homing of circulating bone marrow cells to the heart? Does the bone marrow contribute to maintaining the pool of CSPs in the chronic phase of myocardial infarction and in other cardiomyopathies? What are the specific signals that are responsible for the homing and phenotypic conversion of bone marrow cells into CSPs? Does the ability of the bone marrow to replenish CSPs become impaired with aging? With regard to the last point, recent work from Anversa's laboratory^{14,15} indicates that senescence of CSCs associated with age results in cardiac contractile dysfunction, suggesting that CSCs cannot be replenished, at least effectively and in sufficient numbers, from extracardiac reservoirs. Because the CSPs studied by Mouquet et al^{7,13} may be phenotypically distinct from the c-kit+ CSCs described by Beltrami et al,² findings obtained in one cell type may not be applicable to the other. Alternatively, the findings of CSP replenishment by bone marrow cells¹³ may reflect an acute release of chemoattractants during myocardial infarction that is absent in the slow process of aging. Longer follow-up of the chimeric mice used by Mouquet et al¹³ may reveal whether cardiac CSP levels are sustained throughout life or decline with age.

Another important question pertains to the nature of the bone marrow cells that home to the heart and give rise to CSPs. Although SP cells are typically identified not on the basis of antigen expression per se but by their ability to extrude the Hoechst 33342 dye, SP cells in the bone marrow¹⁶ as well as peripheral blood¹⁷ are predominantly CD31+. Thus, the cells that homed to the myocardium after infarction in the Mouquet study¹³ may either have come from the smaller subfraction of bone marrow SP cells that are CD31- or have lost the CD31 antigen after engraftment. However, the loss of CD31 may potentially be associated with a lack of the vasculogenic differentiation potential that a bona fide CSC ought to possess. Whether these CSPs are capable of generating vessels in the heart remains to be determined. In addition to the CD45+ cells that Mouquet et al found to home to the heart, the bone marrow has also been reported to harbor CD45- cells that are committed to cardiac differentiation and are released into the peripheral blood after infarction.¹⁸ The relationship, if any, between CD45+ precursors of CSPs and CD45- cardiac-committed cells is unknown at present. Nevertheless, these considerations emphasize the potential importance of the bone marrow in cardiac homeostasis.

The observations of Mouquet et al have significant clinical implications. Earlier work from these authors⁷ has shown that CSPs grow colony-forming units in vitro, differentiate into functional cardiomyocytes, and express connexin 43. If CSPs are also identified in humans, they may be harvested from the heart or the bone marrow or both and expanded to obtain numbers of cells sufficient for therapeutic use. The finding that the cardiac pool of CSPs can be quickly replenished by bone marrow cells (within 7 days) suggests that it may even be possible to harvest CSP precursors from the peripheral blood, thereby obviating the need to perform a cardiac biopsy or cardiac surgery to obtain cardiac progenitor cells. However, two important issues need to be addressed before clinical exploitation of CSPs becomes feasible. First, the ability of CSPs to improve left ventricular function and structure after

transplantation into infarcted myocardium must be documented. Second, the phenotypic identity of the bone marrow stem cells that give rise to CSPs needs to be ascertained.

In conclusion, Mouquet et al¹³ have made an important contribution to the field of cardiac biology and regeneration by elucidating the kinetics of CSPs under physiologic and pathologic conditions. Because CSPs are phenotypically distinct from other primitive resident cells previously described in the heart, the present findings may not be applicable to all cardiac progenitors. Nevertheless, the fact that the bone marrow is the source of at least one type of primitive cells in the postnatal heart is novel and significant, both conceptually and therapeutically. Future studies should aim at unraveling the kinetics of the other cardiac primitive cells described to date and at elucidating whether these cells are truly distinct populations or represent different stages in the evolution of a common cell type. If it turns out that bone marrow-derived cells (either the precursors of CSPs described by Mouquet et al¹³ or the cardiac-committed cells described by Kucia et al¹³) play a significant role in cardiac turnover under physiological and pathological conditions, this would fundamentally change not only our treatment of heart disease but also our understanding of the bone marrow: far from being merely the site of hematopoiesis, this organ would be regarded as the fountain of youth that rejuvenates other tissues by releasing progenitors capable of homing and differentiating into various tissue types.

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