Identification of Myocardial and Vascular Precursor Cells in Human and Mouse Epicardium

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Abstract—During cardiac development, the epicardium is the source of multipotent mesenchymal cells, which give rise to endothelial and smooth muscle cells in coronary vessels and also, possibly, to cardiomyocytes. The aim of the present study was to determine whether stem cells are retained in the adult human and murine epicardium and to investigate the regenerative potential of these cells following acute myocardial infarction. We show that c-kit$^+$ and CD34$^+$ cells can indeed be detected in human fetal and adult epicardium and that they represent 2 distinct populations. Both subsets of cells were negative for CD45, a cell surface marker that identifies the hematopoietic cell lineage. Immunofluorescence revealed that freshly isolated c-kit$^+$ and CD34$^+$ cells expressed early and late cardiac transcription factors and could acquire an endothelial phenotype in vitro. In the murine model of myocardial infarction, there was an increase in the absolute number and proliferation of epicardial c-kit$^+$ cells 3 days after coronary ligation; at this time point, epicardial c-kit$^+$ cells were identified in the subepicardial space and expressed GATA4. Furthermore, 1 week after myocardial infarction, cells coexpressing c-kit$^+$, together with endothelial or smooth muscle cell markers, were identified in the wall of subepicardial blood vessels. In summary, the postnatal epicardium contains a cell population with stem cell characteristics that retains the ability to give rise to myocardial precursors and vascular cells. These cells may play a role in the regenerative response to cardiac damage. **(*Circ Res*. 2007;101:1255-1265.)**

Key Words: epicardium ■ infarction ■ stem cells ■ cardiovascular differentiation

Myocardial infarction (MI) in the mammalian heart is associated with an acute inflammatory response, leading to the replacement of injured cardiomyocytes with granulation tissue and scar.[1] Recently it has been shown that the heart is a dynamic organ in which spontaneous myocyte regeneration together with myocyte death are major determinants of cardiac homeostasis in physiologic and pathologic conditions.[2] Myocardial regeneration appears to be mediated by multipotent cardiac stem cells (CSCs), resident in the heart, that give rise to new myocytes and vascular structures. A variety of studies document the presence of CSCs in the mouse,[3–10] rat,[11] dog,[12] and human adult heart.[7,13]

The adult myocardium is enveloped by a layer of epithelial cells called epicardium that during embryogenesis, plays an important role in the formation of the coronary vasculature. The epicardium has an extracardiac origin: at approximately stage 18 in the avian heart and 10.5 days post coitum in the mouse, a cluster of cells derived from septum transversum in mammals and located close to the liver primordium in other vertebrates, populates the myocardial external surface of the heart.[14] Epicardial cells synthesize a dense layer of extracellular matrix that resides between them and the myocardium in the subepicardial space. A subset of these cells delaminate from the epicardium and migrate into the subepicardium, where they generate a population of epicardially derived cells (EPDCs) after a process known as epithelial-to-mesenchymal transition. EPDCs are pluripotent stem cells that have a considerable importance in cardiac development both by contributing to several cell lineages within the heart[15] and by secreting factors that modulate myocardial development.[16]

Recently EPDCs have been identified in mammal adult epicardium.[17,18] In vitro studies show that both human and mouse EPDCs may be induced to differentiate into smooth muscle cells[17,18] whereas only murine EPDCs can give rise to endothelial cells.[17] At present, it is unknown whether human and murine adult EPDCs play a role in heart repair after tissue damage. This question has already found an answer in the teleost Zebrafish.[19] Among vertebrates, Zebrafish has the

Original received February 16, 2007; revision received September 7, 2007; accepted October 10, 2007.

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Circulation Research **is available at http://circres.ahajournals.org** **DOI: 10.1161/CIRCRESAHA.107.150755**

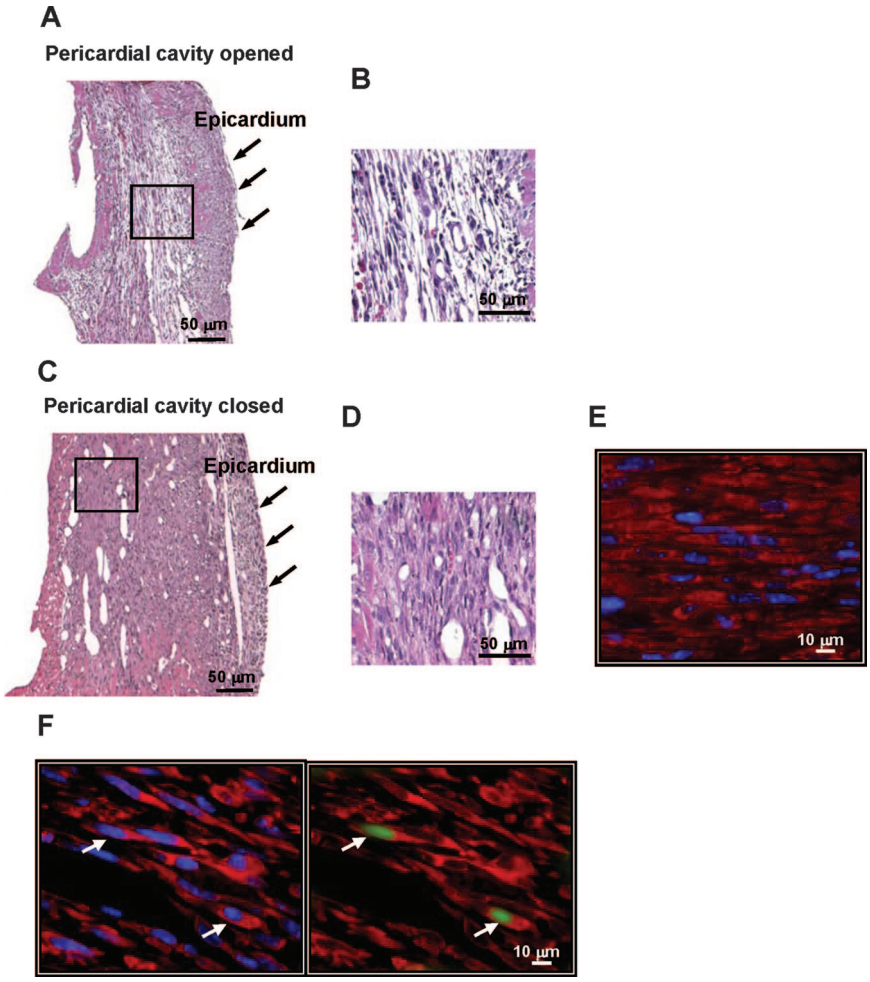

Figure 1. Pericardial sac integrity prevents myocardial tissue deterioration following infarction. Myocardial infarction was induced in mice by coronary artery ligation; the pericardial sac was left either open or closed. A, Hematoxylin/eosin staining of a 1-week infarcted heart in which the pericardial cavity was left open; the injury site is shown. B, High magnification of the inset shown in A. C, Hematoxylin/eosin staining of a 1-week infarcted heart in which the pericardial cavity was closed. The injury site exhibits a lower degree of fibrosis than in A and B. D, High magnification of the inset shown in C. E, Newly formed cardiac cells expressing α-sarcomeric actin are present in the infarcted heart with intact pericardial cavity. F, α-Sarcomeric actin-positive cells (left image) are proliferating cells, as indicated by Ki67 staining (right image). Red fluorescence indicates α-sarcomeric actin; green fluorescence, Ki67; blue fluorescence, Hoechst staining of nuclei.

ability to completely regenerate the heart following ventricular apex resection; under these conditions, epicardial cells undergo epithelial-to-mesenchymal transformation, migrate into the wound, and participate to myocardial regeneration and blood vessel formation.[19]

In adult human hearts, the subepicardial space contains adipose tissue depots, mostly localized next to the larger blood vessels and to the atrioventricular sulcus. Several experimental results have demonstrated that adipose tissue from different compartments is a source of cells with the ability to differentiate into adipocytes, osteoblasts, chondrocytes, and myoblasts.[20] Recently, the ability of these cells to differentiate toward the endothelial lineage both in vivo and in vitro has been demonstrated.[21,22]

Based on these findings, the aim of the present study was to establish whether epicardial progenitor cells play a role in the physiologic process of myocardial repair. We show here that both human and mouse epicardial/subepicardial compartments include cells expressing stem cell antigens c-kit and CD34. Some cells either express early markers of cardiac differentiation or differentiate into endothelial cells in vitro. In the mouse, after myocardial infarction (MI), epicardial c-kit$^+$ cells participate to the reparative process by proliferating and differentiating into myocardial and vascular cells.

Materials and Methods

An expanded Materials and Methods section containing details regarding cell isolation, immunofluorescence and immunohistochemical studies, flow cytometry, RT-PCR, data collection, and statistics is available in the online data supplement at http://circres.ahajournals.org.

Animals and Surgical Procedures

MI was induced by coronary artery ligation in C57BL6 female mice at 8 weeks of age (20-g body weight) as described previously.[23]

Human Heart Samples

Human heart samples were obtained from archived autopsy material of fetal and adult hearts.

Evaluation of Myocardial Function

Myocardial functions were evaluated by echocardiographic and hemodynamic studies.[23]

Cell Isolation

Cardiac cells were isolated from C57BL6 female mice at 2 to 3 months of age, and myocytes were discarded as previously described.[11]

Results

Influence of the Pericardial Sac on Myocardial Structure and Function Following Infarction

We first investigated whether the pericardial sac played a role in ventricular remodeling acutely after infarction and whether

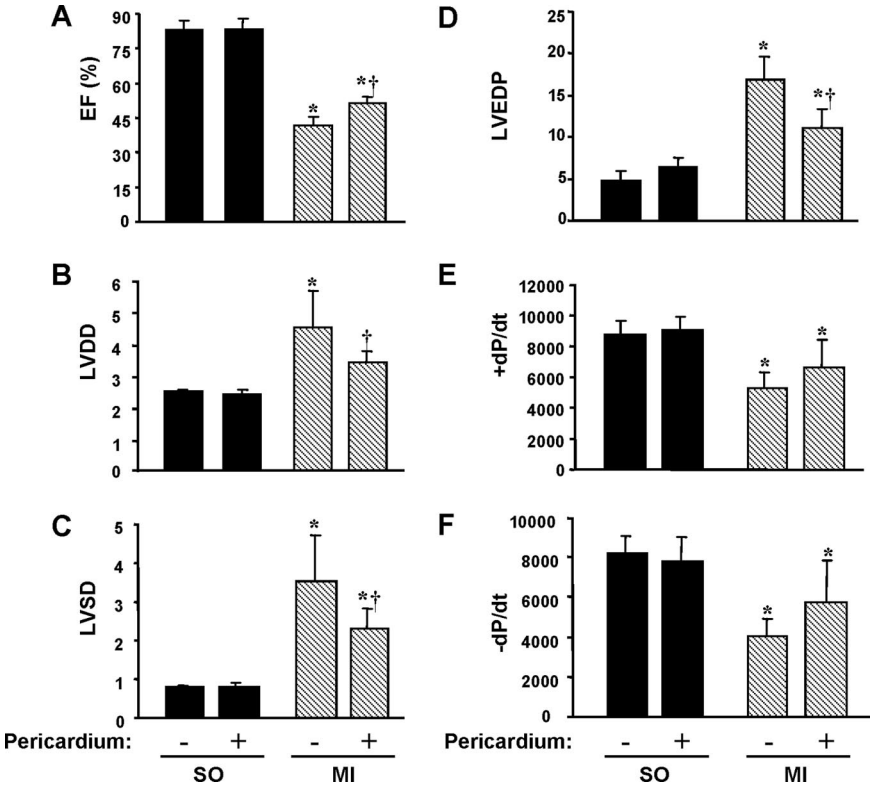

Figure 2. Pericardial sac integrity partially prevents myocardial function deterioration after infarction. A through F, Echocardiographic (A through C) and hemodynamic (D through F) assessments of cardiac function in sham-operated (SO) and infarcted (MI) murine hearts in the absence (-) and presence (+) of an intact pericardial sac. All studies were performed 1 week after surgery (n=6 for each of the four groups). †$P<0.01$ vs MI (-), *$P<0.01$ vs their respective sham-operated controls.

the epicardial cells did participate in the reparative process in the infarcted heart. To verify the first hypothesis, coronary artery ligation was performed in 2 groups of C57BL6 female mice. In the first group of animals, the pericardial cavity was opened just before ischemic injury (Figure 1A and 1B and supplemental Figure I), whereas in the second group, it was kept closed (Figure 1C and 1D and supplemental Figure I). All animals were euthanized 8 days later. Hematoxylin and eosin staining showed that when the integrity of the pericardial cavity was maintained, the scar displayed a better preservation compared with that formed when the pericardial cavity was opened (Figure 1A through 1D and supplemental Figure I). Moreover, in the presence of the intact pericardial cavity, foci of cardiac regeneration were detected in the infarcted region, as demonstrated by the presence of small cells expressing the cardiac marker α- sarcomeric actin in the cytoplasm (Figure 1E) and Ki67, a nuclear protein present in G_1, S, G_2, and early mitosis (Figure 1F).

Echocardiographic and hemodynamic measurements were performed 1 week after acute MI. In comparison with sham-operated mice, infarcted animals exhibited evidence of cardiac failure. However, infarcted mice in which the pericardium was kept closed, exhibited a better preservation of myocardial function and lower left ventricular (LV) diameters in comparison with mice in which the pericardial sac was left open (Figure 2A through 2F); in the former group, the ejection fraction was ≈24% higher (Figure 2A). Moreover, the dilation of the left ventricle was attenuated, as shown by a smaller increase in LV end-diastolic diameter and LV end-systolic diameter (Figure 2B and 2C and supplemental Table I). LV end-diastolic pressure was ≈33% lower (Figure 2D), whereas changes in both positive and negative dP/dt showed a trend suggesting less severe damage in the presence of a closed pericardial cavity (Figure 2E and 2F and supplemental Table I). In contrast, opening of the pericardial sac had no effect on myocardial function and LV diameters in sham-operated animals. Thus, the presence of an intact pericardial sac ameliorated LV function and remodeling of the heart acutely after infarction. To investigate whether the epicardial cells participated in the reparative process occurring after infarction, the epicardial cell layer was transduced with a lentiviral vector–expressing green fluorescent protein (LV-CMVGFP). Ten days after infection, analysis of GFP expression showed the transgene in mesothelial cells of the epicardium and of the pericardium, whereas no expression was found in myocardial cells (Figure 3A).[24] Three days after lentivirus injection, MI was induced and the animals were euthanized 7 and 21 days after injury (Figure 3B through 3D). At these time points, epicardial-derived GFP-expressing cells were detected within the infarcted wall of the left ventricle. Some cells expressed α-sarcomeric actin, indicating their differentiation into the myocardial lineage (Figure 3C and 3D).

Identification of c-Kit$^+$ and CD34$^+$ Cells in Human Fetal and Adult Epicardium

In these experiments, it was examined whether stem cells could be identified in the human fetal and adult epicardium. Stem cell antigens c-kit and CD34 were used for this analysis because they identify a subset of stem cells in several adult tissues.[11,22,25,26] In the fetal heart, the epicardium covers the outer edge of the heart. The external layer of flat mesothelial cells lies on a thin basal lamina, and underneath there is a layer of connective tissue, the subepicardial space, that

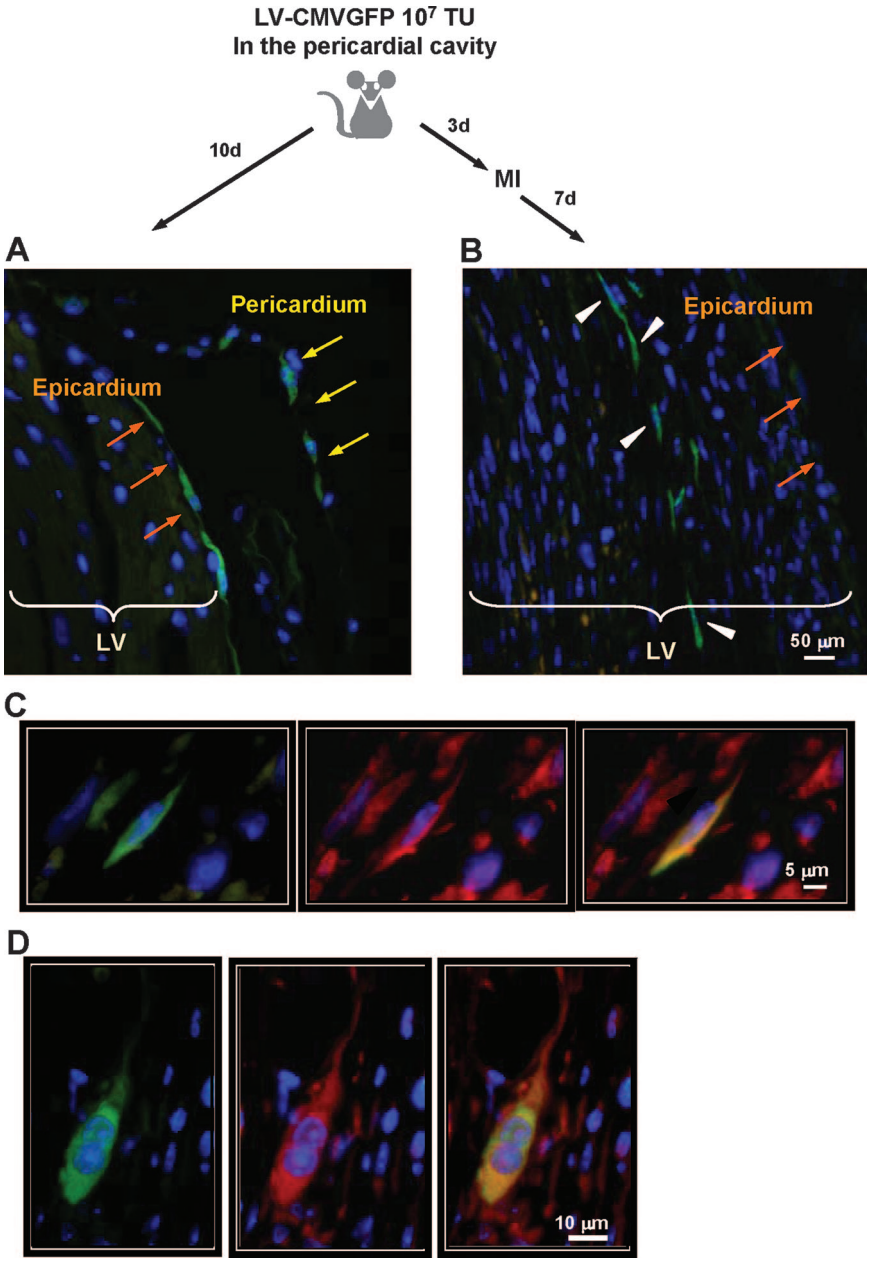

Figure 3. Epicardial cells migrate after MI. LV-CMVGFP (10^7 transducing units) was delivered in the pericardial sac, and MI was induced 3 days later. Noninfarcted (n=10) and infarcted (n=10) animals were euthanized 10 days after lentivirus injection. A, In noninfarcted hearts, GFP was detected both in the epicardial (orange arrows) and in the pericardial (yellow arrows) mesothelial cells but not in the myocardium. B, In infarcted hearts, epicardial GFP-expressing cells (arrowheads) were detected within the infarcted wall of the left ventricle (LV). C and D, Epicardial GFP-transduced cells expressed α-sarcomeric actin at days 7 (C) and 21 (D) after MI. Immunohistochemical analysis showing a GFP-positive cell in infarcted heart tissue (C and D, left images). The same cell expresses α-sarcomeric actin (C and D, middle images, red fluorescence). The merge of both images is shown (C and D, right images). Blue fluorescence indicates Hoechst staining of nuclei.

contains elastic fibers, as well as large vessels (Figure 4A). Importantly, coronary arteries originate in this region during cardiac development.[15] By immunohistochemistry, cells expressing c-kit (Figure 4B) and CD34 (Figure 4C) antigens were detected in the subepicardial region of human fetal hearts. These cells are distributed in the interstitial spaces of the subepicardial connective tissue. In the adult human myocardium, the subepicardial space is mostly occupied by adipose tissue (Figure 4D), which surrounds coronary arteries and veins. c-Kit$^+$ and CD34$^+$ cells were also identified in the subepicardial region of adult hearts, where they occupied the interstitial space between adipose cells (Figure 4E and 4F). Taken together, the results show that c-kit$^+$ and CD34$^+$ cells are present in human fetal and adult epicardium.

Flow Cytometric Analysis of c-Kit$^+$ and CD34$^+$ Human Epicardial Cells

Freshly isolated cell suspensions obtained from adult human epicardial biopsies were analyzed by flow cytometry to identify c-kit– and CD34-expressing cells. Epicardial c-kit$^+$ and CD34$^+$ cells appeared as 2 distinct populations and represented 0.8±0.5% and 8.6±2%, respectively, of the total cells isolated (Figure 5A and 5E). Both populations were negative for CD45, a cell surface marker expressed exclusively by the hematopoietic lineage (Figure 5B and 5C). CD34$^+$ cells were mostly negative for the endothelial/monocyte/macrophage marker CD31 (Figure 5D). Notably, CD34$^+$ cells share some phenotypic characteristics with EPDCs,[17,18] because they expressed the hyaluronate receptor (CD44), and a subset of these cells were also positive for the major T cell

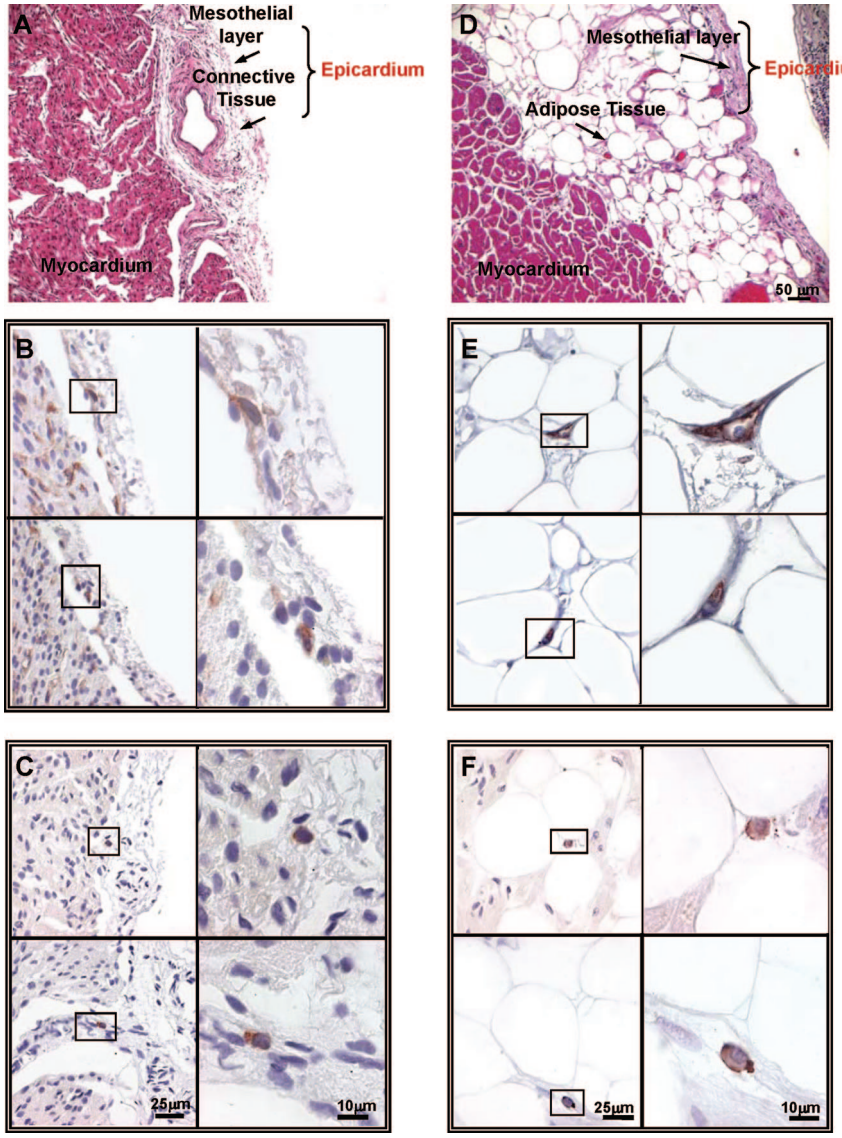

Figure 4. c-Kit– and CD34-expressing cells are present in human fetal and adult epicardium. A through F, Sections from fetal (A through C) and adult (D through F) hearts were stained for the following: hematoxylin and eosin to show epicardial structure (A and D); c-kit$^+$ cells (B and E); and CD34$^+$ cells (C and F). High magnification of insets is shown on the right of each image.

antigen (Thy-1; CD90) and endoglin (CD105) (supplemental Figure II).

Human Epicardial–Derived c-Kit$^+$ and CD34$^+$ Cells Are Potential Cardiac and Vascular Precursors

To assess whether c-kit$^+$ and CD34$^+$ cells present in the adult human epicardium were potential cardiogenic precursors, the expression of early cardiac markers was analyzed in the purified cell fractions (supplemental Figure III). Immunofluorescence analysis revealed that some c-kit$^+$ and CD34$^+$ cells expressed the early marker of cardiomyocyte differentiation Nkx2.5 and the cardiac transcription factor GATA4 (Figure 5F and 5G). Both populations also displayed the ability to acquire the endothelial phenotype, as shown by their capacity to uptake 1,1′-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine–labeled acetylated LDL (Ac-LDL-DiI) in 7-day primary cultures (Figure 5H).

c-Kit$^+$ Cells Resident in the Murine Epicardium Proliferate and Differentiate After MI

There are striking differences in the structure of the epicardium between humans and rodents (Figure 6A); in contrast to human epicardium, murine epicardium lacks adipose cells and consists of a monolayer of mesothelial cells on a thin layer of connective tissue formed by elastic fibers. By RT-PCR, the expression of c-kit, CD34, and the cardiac markers Nkx2.5 and GATA4 were identified in epicardial cells (Figure 6B). The expression levels of these markers were comparable to those detected in cells obtained from the heart after cardiomyocyte separation. Flow cytometric analysis of dissociated mesothelial cells revealed the presence of both c-kit– and CD34-expressing cells, which represented 1.4±0.7% and 0.48±0.14%, respectively, of the total epicardial epithelium (Figure 6C and 6G). Further characterization showed that, as in humans, c-kit$^+$ and CD34$^+$ cells lacked the hematopoietic lineage marker CD45 (Figure 6D and 6E).

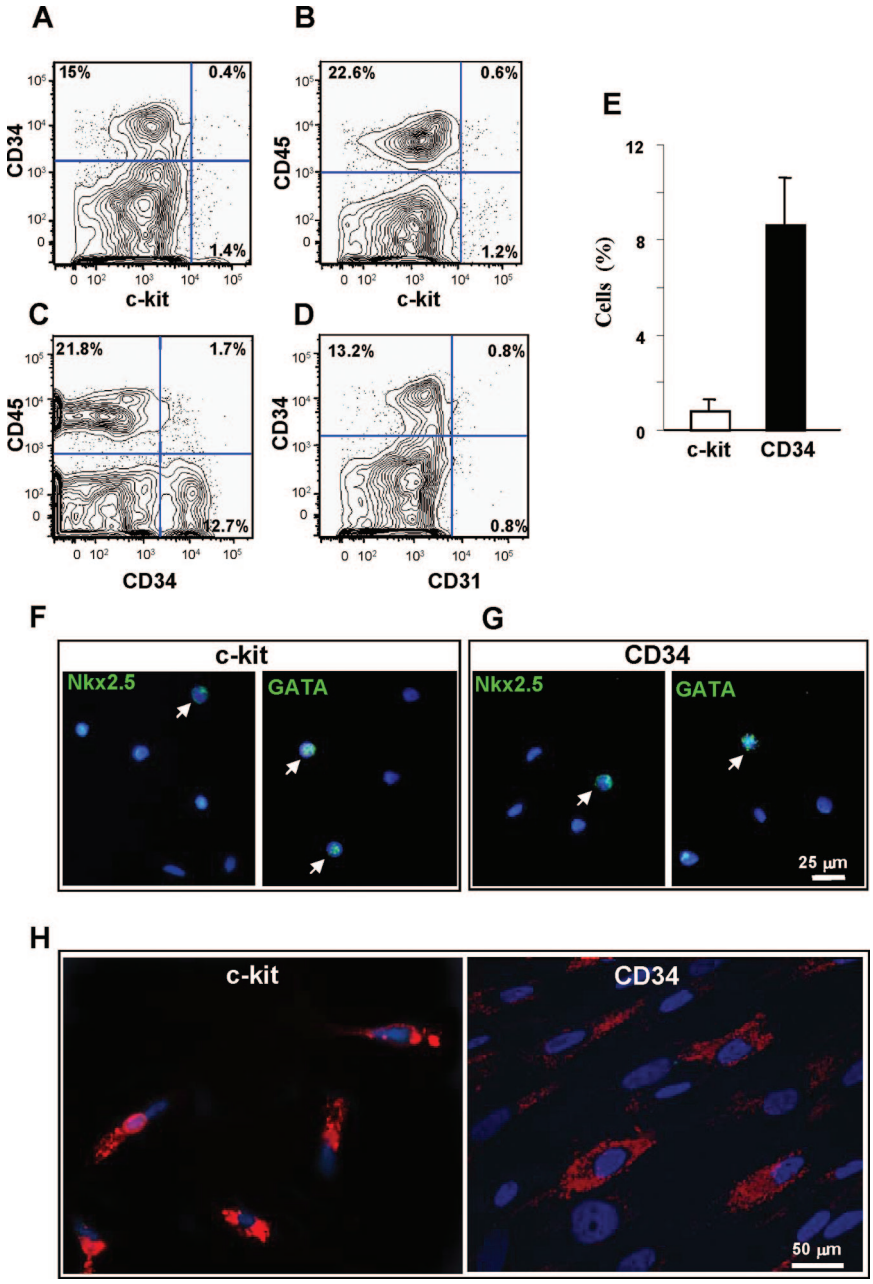

Figure 5. Characterization of human epicardial c-kit$^+$ and CD34$^+$ cells. A through E, Flow cytometric analysis of human epicardial cells. A, c-Kit$^+$ and CD34$^+$ are 2 distinct cell populations. Cells were stained with PC7-conjugated c-kit in combination with PC5-conjugated CD34. B and C, Epicardial c-kit$^+$ and CD34$^+$ cells were mostly negative for the hematopoietic marker CD45. Epicardial cells were stained either with PC7-conjugated c-kit (B) or PC5-conjugated CD34 (C), together with allophycocyanin-Cy7–conjugated CD45. D, CD34$^+$ cells did not express the endothelial antigen CD31. Cells were stained with PC5-conjugated CD34 and phycoerythrin-conjugated CD31. In the upper right corner of each quadrant, the cell percentage of 1 of 5 experiments is indicated. E, Bar graph of the percentages of human epicardial c-kit$^+$ and CD34$^+$ cells obtained by flow cytometry (n=5). F through H, Human epicardial c-kit$^+$ and CD34$^+$ cells express early cardiac markers and acquire endothelial phenotype in vitro. F and G, Magnetic cell-sorted c-kit$^+$ and CD34$^+$ cells were immunostained for Nkx2.5 and GATA4 (arrows). Green fluorescence indicates Nkx2.5 and GATA4; blue color, Hoechst to evidence nuclei. H, Magnetic cel-sorted c-kit$^+$ and CD34$^+$ cells were left in culture for 7 days and then incubated with Ac-LDL-DiI (red fluorescence) as an indicator of endothelial differentiation, followed by fixation and Hoechst nuclear staining. Ac-LDL-DiI uptake was detected in ≈10% of both c-kit$^+$ and CD34$^+$ cells.

Moreover, CD34$^+$ cells were negative for the endothelial marker CD31 (Figure 6F). Thus, c-kit$^+$ and CD34$^+$ cells are present in murine adult epicardium.

Cardiac stem cells (CSCs) have been recently identified in the mammalian heart as undifferentiated cells expressing variable combinations of stem cell markers c-kit, Sca1, and multidrug resistance (MDR)1.[7,11] These cells can give rise to all cardiac cell lineages after MI.[7,11] Approximately 60% of epicardial c-kit$^+$ cells were positive for MDR1, whereas only 6% expressed Sca1. Notably, both Sca1 and MDR1 were expressed at low levels in epicardial c-kit$^-$ cells (supplemental Figure IV). Considering the differentiation potential of CSCs and their immunophenotypic similarity to epicardial c-kit$^+$ cells, we investigated whether this latter population displayed properties comparable to those of CSCs, ie, whether epicardial cells increased in number and differentiated following MI.[13] To test this hypothesis, epicardial c-kit$^+$ cell proliferation and differentiation was assessed in the mouse heart at different time points after the induction of MI in the presence of an intact pericardial cavity. By immunohistochemistry, it was found that after infarction, the number of c-kit$^+$ cells increased in the epicardial compartment (Figure 7A through 7E). Three days after surgery, the absolute number of c-kit$^+$ cells (Figure 7F) as well as the fraction of c-kit$^+$ cells expressing Ki67 (Figure 7G) in the epicardium were higher in infarcted than in sham-operated animals. Notably, the increase of epicardial c-kit$^+$ cells was also detected in infarcted hearts with an open pericardial cavity; however, the absolute number was significantly lower compared with that found in presence of an intact pericardial cavity (Figure 7F). In the absence of infarction, the pericardial sac integrity did not affect c-kit$^+$ cell number (Figure 7F).

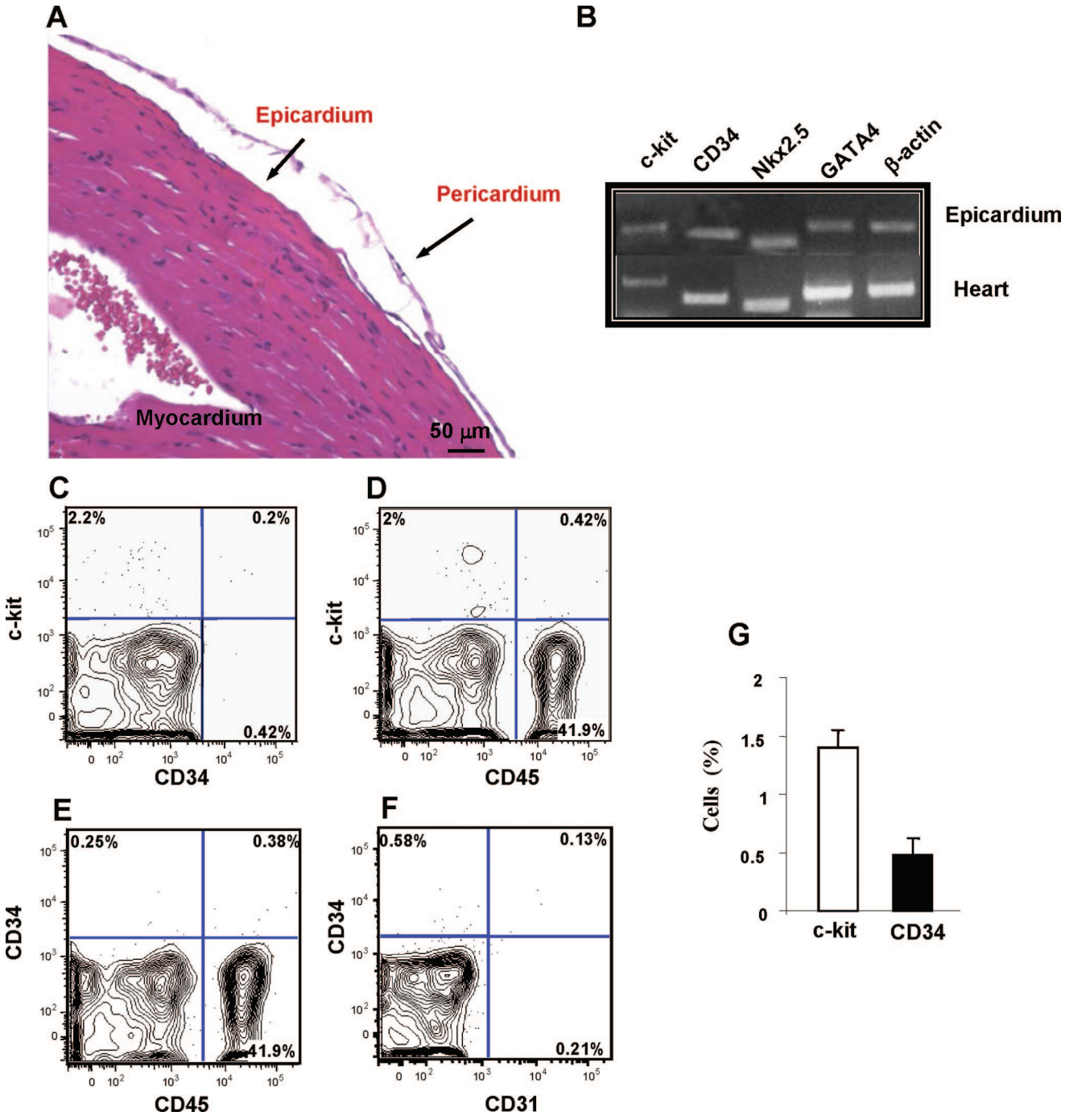

Figure 6. c-Kit$^+$ and CD34$^+$ cells are present in the murine adult epicardium. The murine epicardium consists of a monolayer of mesothelial cells on a thin layer of connective tissue. A, Hematoxylin/eosin staining of adult mouse heart showing the epicardial and pericardial layers. B, RT-PCR analysis of total mouse epicardial RNA showed the expression of cardiac markers in epicardial cells compared with cells obtained after perfusion of the heart and cardiomyocyte removal. C through G, Flow cytometric analysis of murine epicardial cells. C through E, c-Kit$^+$ and CD34$^+$ cells represent distinct populations mostly negative for the hematopoietic marker CD45. Epicardial cells were stained with either allophycocyanin-conjugated c-kit or phycoerythrin-conjugated CD34, together with PC5-conjugated CD45. F, CD34$^+$ cells did not express the endothelial antigen CD31. Cells were stained with phycoerythrin-conjugated CD34 and fluorescein isothiocyanate–conjugated CD31. G, Bar graph of the percentages of epicardial c-kit$^+$ and CD34$^+$ cells analyzed by flow cytometry (n=6).

Together, these results demonstrate that MI enhances epicardial c-kit$^+$ cell proliferation and that the presence of an intact pericardial cavity further increases their absolute number.

To verify whether following infarction, epicardial c-kit$^+$ cells differentiated toward myocardial, endothelial, and smooth muscle phenotypes, the expression of the cardiac transcription factor GATA4, the endothelial marker factor VIII, and the α-smooth muscle actin was assessed by immunohistochemistry. At day 3 after coronary ligation, some epicardial and subepicardial c-kit$^+$ cells (Figure 8A) also expressed GATA4 (Figure 8B). Quantification analysis revealed a 3-fold increase of c-kit$^+$/GATA4$^+$ cells in the

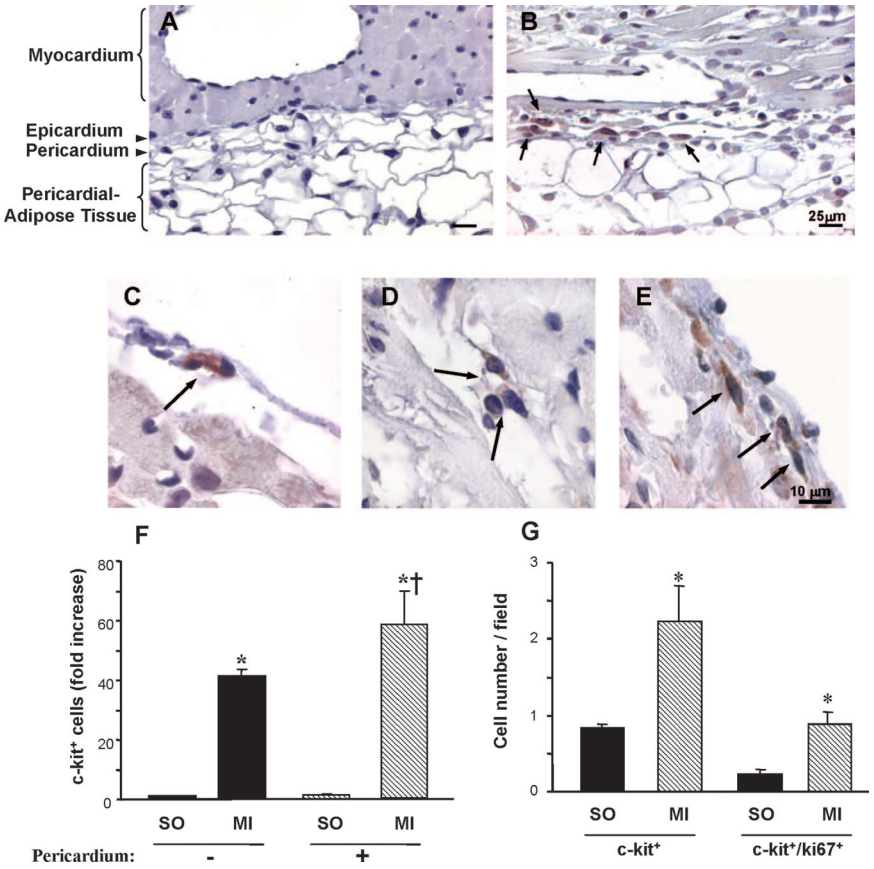

Figure 7. Epicardial c-kit$^+$ cells proliferate after MI. c-Kit$^+$ cell number increases in the epicardium after MI. A and B, Low-power view of c-kit$^+$ immunostaining in epicardial tissue of noninfarcted (A) and in 3-day infarcted hearts (B). In this example, c-kit$^+$ cells were not detected in the absence of infarction and appeared after infarction in the epicardial–subepicardial region. C through E, High-power magnification of c-kit$^+$ cells in the epicardial region 1 (C), 2 (D), and 3 (E) days after infarction. F, Quantification of c-kit$^+$ cells in the epicardium of sham-operated (SO) and 3-day infarcted hearts (MI) with the pericardium either open (+) or closed (−) (n=5/group). *$P<0.0001$ vs sham operated, †$P<0.02$ vs MI with pericardial sac open. G, Mean c-kit$^+$ and c-kit$^+$/Ki67$^+$ in epicardial cells isolated from sham-operated and MI hearts 3 days after coronary ligation (n=3/group). *$P<0.004$ vs sham operated.

infarcted compared with control hearts (Figure 8C). One week after MI, small blood vessels were found in the subepicardial space. Some of these vessels included c-kit$^+$ (Figure 8D and 8F) cells expressing either the endothelial marker factor VIII (Figure 8E) or smooth muscle actin (Figure 8G). Thus, MI induces epicardial c-kit$^+$ cell proliferation and their differentiation into a myocardial, endothelial, and smooth muscle phenotype.

Discussion

Recent studies have shown that the heart is not a terminally differentiated organ and that "resident" cardiac stem cells contribute to cardiac repair following injury,[5,23,27] as well as physiologic cardiac tissue homeostasis replacing dead cells during the lifespan.[13] c-Kit$^+$ CSCs have been found in niches, mostly localized in the atria and in the apex, as well as dispersed among myocardial cells throughout the heart.[28] The present study shows that c-kit$^+$ cells are also present in human and murine epicardium. Furthermore, in the mouse, these cells respond to MI as c-kit$^+$ cells within the myocardium; they proliferate, migrate toward the injury site, and exhibit evidence of differentiation toward the myocardial and vascular phenotype. The existence of progenitor cells in the epicardial compartment was supported by the following observations. First, in a mouse model of MI, we found a significant prevention of cardiac function impairment and LV remodeling in the presence of an intact pericardial cavity. These hearts were characterized by foci of cardiac regeneration in the infarcted region. The mechanical protection exerted on precursor cells resident in the epicardium by the pericardial compartment, as well as by the pericardial fluid, could explain such effects. Moreover, growth factors released in the pericardial fluid after acute MI[29–31] could play a role in the regenerative process modulating epicardial cell function. Second, epicardial mesothelial cells transduced with a lentiviral vector–expressing GFP were detected in the LV wall of mouse infarcted hearts at 1 and 3 weeks after injury and acquired a cardiac phenotype.

The presence of vascular progenitors in the epicardium has been widely demonstrated during cardiac development. In the embryo, the epicardium, which has an extracardiac origin, provides cells with vasculogenic potential, so called EPDCs, that form at least part of the major coronary vessels following an epithelial-to-mesenchymal transformation.[16,32] Only recently have EPDCs been described in adult human and mouse hearts.[17,18] Human EPDCs treated with transforming growth factor-β1 or bone morphogenetic protein-2 were able to differentiate into smooth muscle cells but failed to form endothelial cells.[18] Treatment of mouse epicardial explant cultures with thymosin-β4 showed extensive outgrowth of cells that differentiated into both endothelial and smooth muscle cells.[17] These data suggest that adult EPDCs may preserve the properties of their embryonic counterparts. Although it has been proposed that embryonic EPDCs could also give rise to cardiomyocytes,[33] at present, it has not been assessed whether adult EPDCs have a cardiogenic potential.[33–35]

Precursor cells able to give rise to cardiomyocytes, smooth muscle cells, and endothelial cells, have been identified in the

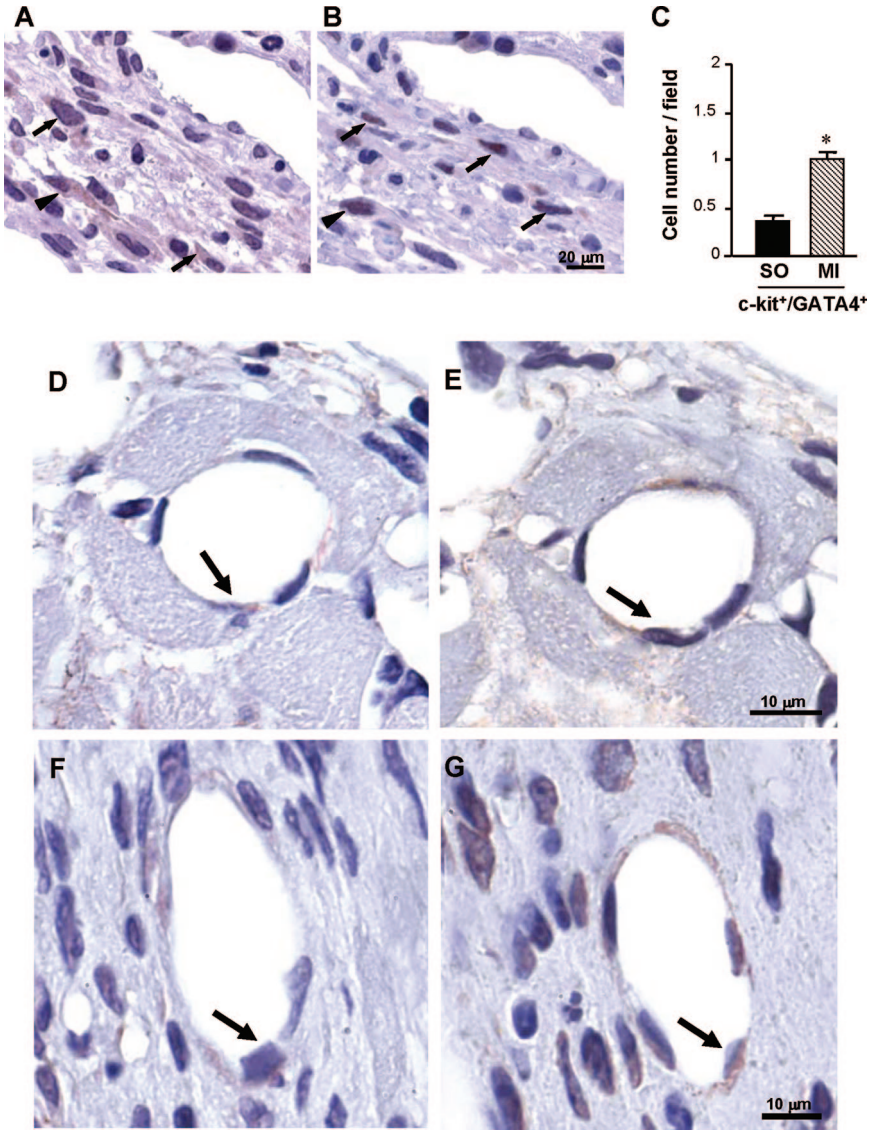

Figure 8. Epicardial c-kit$^+$ cells express cardiac and endothelial markers after MI. A and B, Immunohistochemical analysis on serial sections obtained from 3-day infarcted hearts showed c-kit$^+$–expressing (A) and GATA4-expressing (B) cells in the epicardium. Arrows in A and B indicate single-positive cells for c-kit$^+$ and GATA$^+$, respectively; arrowheads indicate double-positive cells. C, Bar graph indicating the mean c-kit$^+$ cells and c-kit$^+$/GATA4$^+$ cells detected in isolated epicardial cells (n=3). *$P<0.006$. D through G, At day 7 after infarction, c-kit$^+$ cells (D and F) were found in the wall of small subepicardial vessels, where they expressed von Willebrand factor (E) and α-smooth muscle actin (G).

rat,[11] mouse,[3–10] dog[12] and human[7,13] heart. Several stem cell-related antigens have been used to characterize these cells, and, among them, the stem cell antigen c-kit together with Sca1 and MDR1 identify a population of cardiac stem cells that may be induced to proliferate, differentiate, and participate in the reconstitution of damaged myocardium.[5,23] We demonstrated here that c-kit–expressing cells are present in mouse and human adult epicardium. These cells are localized in the mesothelial layer, which is the major constituent of murine epicardium, and also in the subepicardial compartment of human epicardium, which is characterized by the presence of adipose tissue. Although epicardial c-kit$^+$ cells express MDR1, at present, it remains to be investigated whether epicardial c-kit$^+$ and CSC c-kit$^+$ cells are the same population. In both mouse and human epicardial compartments, we also identified a population of CD34$^+$/c-kit$^-$ cells. The absence of CD31 in these cells demonstrates that they represent a population with vasculogenic and angiogenic potentials rather then mature endothelial cells. It is noteworthy that a similar cell subset was recently identified in the stromal vascular fraction of human adipose tissue as a population exhibiting endothelial cell progenitor characteristics.[22] Notably, CD34$^+$ cells were localized in the subepicardial space of human hearts, which is mostly occupied by adipose tissue. Although epicardial c-kit$^+$ and CD34$^+$ cells also have been identified in the peripheral blood, we excluded the possibility that the presence of such progenitors may be attributable to blood circulating into the epicardial fat tissue for the following reasons: first, they did not express the hematopoietic marker CD45; second, c-kit$^+$ and CD34$^+$ cells were highly represented in the human epicardium compared with the peripheral blood (c-kit$^+$, 0.5% versus 0.05%; CD34$^+$, 8.6% versus 0.09%, respectively).[36]

Cultured EPDCs isolated from human epicardial biopsies have a fibroblast-like shape and adhere to plastic dishes. The immunophenotypic characterization of in vitro–expanded EPDCs reveals that they express the mesenchymal markers CD44, CD90, and CD105 but are negative for the stem cell antigens CD34 and Sca1.[18] Moreover, they express the late cardiac marker GATA4.[18] In contrast to adult cultured EPDCs, freshly isolated human epicardial c-kit$^+$ and CD34$^+$

cells display a nonadherent phenotype and are positive not only for GATA4 but also for the early cardiac marker Nkx2.5. Furthermore, both epicardial populations exhibit the ability to acquire an endothelial phenotype in vitro, a property not demonstrated in human EPDCs. Interestingly, epicardial $CD34^+$ cells are positive for CD44, and a subpopulation of $CD34^+$ cells also expressed CD105 and CD90, suggesting that CD34 may identify progenitor cells able to give rise to cardiovascular cells and possibly to EPDCs.

After MI in mice, epicardial c-kit^+ cells enter the cell cycle and proliferate. At day 3 after injury, epicardial c-kit^+ cells expressing cardiac and vascular markers are detected in the epicardial and subepicardial region.

The importance of the epicardium in the reparative process after damage has been demonstrated recently in the zebrafish adult heart. Zebrafish is able to regenerate its heart completely, even after a cardiac mass loss as high as 20%.[37] Following surgical removal of the ventricular apex, new cardiomyocytes originate from undifferentiated progenitor cells and the epicardium plays a critical role during the entire process.[19] Specifically, a subpopulation of epicardial cells migrates into the wound and provides new vasculature to the regenerating tissue, whereas proliferating cardiomyocytes are detected in the subepicardial compartment.

In conclusion, we demonstrate here that the adult mammalian epicardium contains precursor cells able to give rise to cardiomyocytes and endothelial and smooth muscle cells. After infarction, epicardial precursor cells are activated and participate in the physiological process of myocardial repair. A better understanding of the mechanisms involved in adult epicardial precursor cell activation could help in the development of therapeutic strategies aimed at enhancing the regenerative capacity of these cells.

Acknowledgments

We thank C. Bertolami, A. Di Carlo, D. Avitabile, and E. De Falco for technical support; A. De Benedictis for expert advice; and G. Melillo for statistical analysis. We also thank R. Ricci, A. Alois, S. Leonardi Cattolica, R. Gallo, and P. Sordini for providing human biopsies.

Sources of Funding

This research was supported by a grant from the Italian Ministry of Health and European Union FP6 contract LSHD-CT-2004-5029288 (Application and Process Optimization of Stem Cell Products for Myocardial Repair [SC&CR]).

Disclosures

None.

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