Myocardial neovascularization by bone marrow angioblasts results in cardiomyocyte regeneration


Departments of Surgery and Medicine, Columbia University, New York, New York 10032

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Schuster, M. D., A. A. Kocher, T. Seki, T. P. Martens, G. Xiang, S. Homma, and S. Itescu. Myocardial neovascularization by bone marrow angioblasts results in cardiomyocyte regeneration. Am J Physiol Heart Circ Physiol 287: H525–H532, 2004; 10.1152/ajpheart.00058.2004.—The primary cardiac response to ischemic insult is cardiomyocyte hypertrophy, which initiates a genetic program culminating in apoptotic myocyte loss, progressive collagen replacement, and heart failure, a process termed cardiac remodeling. Although a few cardiomyocytes at the peri-infarct region can proliferate and regenerate after injury, no approaches are known to effectively induce endogenous cardiomyocytes to enter the cell cycle. We recently isolated, in human adult bone marrow, endothelial progenitor cells, or angioblasts, that migrate to ischemic myocardium, where they induce neovascularization and prevent myocardial remodeling. Here we show that increasing the number of angioblasts trafficking to the infarct zone results in dose-dependent neovascularization with development of progressively larger-sized capillaries. This results in sustained improvement in cardiac function by mechanisms involving protection against apoptosis and, strikingly, induction of proliferation/regeneration of endogenous cardiomyocytes. Our results suggest that agents that increase myocardial homing of bone marrow angioblasts could effectively induce endogenous cardiomyocytes to enter the cell cycle and improve functional cardiac recovery.

stem cells; myocardial remodeling; myocardial infarction

AN INTEGRAL COMPONENT of the healing process after a myocardial infarction is compensatory hypertrophy of viable cardiomyocytes at the peri-infarct rim to increase pump function in response to the loss of infarcted tissue (9, 24). However, cardiomyocyte hypertrophy initiates a genetic program that culminates in apoptotic loss of the cardiomyocytes, expansion of the initial infarct area, progressive collagen replacement, and heart failure (1, 5, 19, 20), a process termed cardiac remodeling. Pharmacological means to prevent cardiomyocyte hypertrophy remain the mainstay of postinfarct therapy to prevent remodeling and heart failure. Recent observations have suggested that a second compensatory response of viable cardiomyocytes at the peri-infarct region is to proliferate and regenerate after injury (3, 7). Although cardiomyocyte regeneration can be accomplished by providing exogenous precursors, for example, from the bone marrow (17), no approaches are known to effectively induce endogenous cardiomyocytes to enter the cell cycle.

We recently put forward the hypothesis that hypertrophied cardiomyocytes undergo apoptosis, because the endogenous capillary network cannot provide the compensatory increase in perfusion required for cell survival (12). Vascular network formation is the result of a complex process that begins in the prenatal period with induction of vasculogenesis by hemangio blasts, cells derived from the human ventral aorta that give rise to endothelial and hematopoietic elements (4, 6, 10, 13). Postnatal vasculogenesis occurs via pathways dependent on elements in the adult bone marrow and has been described in various animal models (2, 8, 15, 23, 25). In previous studies, we showed that human adult bone marrow contains cells with phenotypic and functional characteristics of embryonic angioblasts that are capable of homing to ischemic myocardium and inducing myocardial neovascularization (17). This process subsequently results in reduced cardiomyocyte apoptosis, prevention of adverse remodeling after acute infarction, and functional cardiac recovery (12).

The extent to which inductive cues from this angioblast population might additionally result in cycling and regeneration of endogenous cardiomyocytes has not been previously studied. In this study, we examined whether there was a dose-dependent relation between angioblast migration to the ischemic heart and subsequent myocardial neovascularization. Our results indicate that myocardial neovascularization results in regeneration and cell cycling of endogenous cardiomyocytes and suggest that agents that increase myocardial homing of bone marrow angioblasts could effectively induce endogenous cardiomyocytes to enter the cell cycle and improve functional cardiac recovery.

MATERIALS AND METHODS

Purification and characterization of cytokine-mobilized human CD34+ and CD34− cells. Single-donor leukopheresis products were obtained from humans treated with recombinant granulocyte colony-stimulating factor (G-CSF, 10 μg/kg sc; Amgen) daily for 4 days. Donors were healthy individuals undergoing standard institutional procedures of bone marrow mobilization, harvesting, and isolation for allogeneic stem cell transplants. Mononuclear cells were separated by Ficoll-Hypaque, and highly purified CD34+ cells (>98% positive) were obtained using magnetic beads coated with anti-CD34 MAb (Miltenyi Biotech). Purified CD34 cells were stained with fluorescein-conjugated MAbs against CD34 and CD117 (Becton-Dickinson), AC133 (Miltenyi Biotech), CD54 (Immunotech), CD62E (BioSource), VEGFR-2, Tie-2, von Willebrand factor, endothelial nitric oxide synthase, and CXCR4 (all obtained from Santa Cruz Biotechnology) and analyzed by four-parameter fluorescence using FACScan.

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(Becton-Dickinson). Cells positively selected for CD34 expression were also stained with phycoerythrin-conjugated anti-CD117 MAb (Becton-Dickinson) and sorted for bright and dim fluorescence using a Facstar Plus (Becton-Dickinson) and a phycoerythrin filter. Intra-cellular staining for GATA-2 was performed by permeabilizing 1 × 10^6 cells from each of the brightly and dimly fluorescing cell populations with a Pharmingen Cytofix/Cytoperm kit by incubation for 30 min on ice with 10 μl of fluorochrome-conjugated MAb against CD117 and CD34 surface antigens (Becton-Dickinson). After resuspension in 250 μl of Cytofix/Cytoperform solution for 20 min at 4°C, cells were incubated with a fluorochrome-labeled MAb against GATA-2 (Santa Cruz Biotechnology) or IgG control for 30 min at 4°C and analyzed by three-parameter flow cytometry. On the basis of criteria of simultaneous CD117 bright fluorescence and intracellular GATA-2 expression, ~6–12% of the CD34^+ population were angioblasts.

Animals, surgical procedures, injection of human cells, and quantitation of cellular migration into tissues. Rowett (rmu/rmu) athymic nude rats (Harlan Sprague Dawley, Indianapolis, IN) were used in studies approved by the Columbia University Institute for Animal Care and Use Committee. After anesthesia, a left thoracotomy was performed, the pericardium was opened, and the left anterior descend-}

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ligation. Each group consisted of 6–10 rats. We excluded all animals where the initial infarct was not large enough to cause reduction in ejection fraction by ≥50% within the first 48 h relative to normal animals. At that point, all animals included in the study were random-}

ized, and the surgeons and other technical staff were fully blinded to the experimental conditions. Histological and functional studies were performed at 2 and 15 wk. 

111In labeling of bone marrow-derived CD34^+ and CD34^− progenitors. G-CSF-mobilized cells were immunoselected for CD34^+ expression and resuspended in medium containing 20 μCi of 111In 8-oxyquinoline (oxine) per 10^6 cells. After the cells were washed, 2 × 10^6 111In 8-oxyquinoline (oxine)-labeled CD34^+ cells were infused intravenously into the nude rats 24 h after myocardial infarction or into noninfarcted animals. After 24 h, the animals were killed and the organs were harvested. 111In counts in each tissue were measured using a gamma spectrometer and calibrated as counts per minute per gram of tissue.

Histology and measurement of infarct size. After excision at 2 and 15 wk, left ventricles from each experimental animal were sliced at 10–15 transverse sections from apex to base. Representative sections were fixed in formalin and stained for routine histology (hematoxylin and eosin) to determine cellularity of the myocardium, expressed as cell number per high-power field (HPF, ×600). Masson’s trichrome stain, which labels collagen blue and myocardium red, was used to evaluate collagen content on a semiquantitative scale (0 to 3+) as follows: light blue (1+), light blue and patches of dark blue (2+), and dark blue (3+) staining. This enabled us to measure the size of the myocardial scar using a digital image analyzer. The lengths of the infarcted surfaces, involving epicardial and endocardial regions, were measured with a planimeter digital image analyzer and expressed as a percentage of the total ventricular circumference. Final infarct size was calculated as the average of all slices from each heart. All studies were performed by a blinded pathologist. Infarct size was expressed as percentage of total left ventricular area. Final infarct size was calculated as the average of all slices from each heart. Evaluation of capillary density. To quantitate capillary density and species origin of the capillaries, additional sections were stained freshly with MAb directed against rat or human CD31 (Serotec and Research Diagnostics, respectively), factor VIII (Dako, Carpinteria, CA), and rat or human major histocompatibility complex (MHC) class I (Accurate Chemicals). Arterioles were differentiated from large capillaries by the presence of a smooth muscle layer, identified by staining sections with a monoclonal antibody against muscle-specific desmin (Dako). Staining was performed by immunoperoxidase technique using an avidin-biotin blocking kit, a rat-absorbed biotinylated anti-mouse IgG, and a peroxidase conjugate (all obtained from Vector Laboratories, Burlingame, CA). Capillary density was determined at 2 wk after infarction from sections labeled with anti-CD31 MAb, confirmed with anti-factor VIII MAb, and compared with the capillary density of the unimpaired myocardium. Values are expressed as the number of CD31^+ capillaries per HPF (×400).

Measurement of myocyte apoptosis by DNA end labeling of paraffin tissue sections. For in situ detection of apoptosis at the single-cell level, we used the TdT-mediated dUTP nick end-labeling (TUNEL) method (Boehringer Mannheim, Mannheim, Germany). Rat myocardial tissue sections were obtained from LAD-ligated rats at 2 wk after injection of saline or CD34^+ human cells and from healthy rats as negative controls. Briefly, tissues were deparaffinized with xylene and rehydrated with graded dilutions of ethanol and two washes in phosphate-buffered saline (PBS). The tissue sections were then digested with proteinase K (10 μg/ml in Tris·HCl) for 30 min at 37°C. The slides were washed three times in PBS and incubated with 50 μl of the TdT reaction mixture (Boehringer Mannheim) overnight at 37°C in a humid atmosphere for 60 min at 37°C. For negative controls, TdT was eliminated from the reaction mixture. After three washes in PBS, the sections were incubated for 30 min with an antibody specific for fluorescein-conjugated alkaline phosphatase (Boehringer Mannheim). The TUNEL stain was visualized with a substrate system in which nuclei with DNA fragmentation stained blue (5-bromo-4-chloro-3-indolylphosphate-p-toluidine-nitro blue tetrazolium substrate system; Dako). The reaction was terminated after 3 min of exposure with PBS. To determine the proportion of blue-staining apoptotic nuclei within myocytes, tissue was counterstained with a monoclonal antibody specific for desmin. Endogenous peroxidase was blocked with a 3% hydrogen peroxidase solution in PBS for 15 min and then washed with 20% goat serum solution. An antitroponin I antibody (Accurate Chemicals) was incubated overnight (1:200) at 40°C. After they were washed three times, the sections were treated with an anti-rabbit IgG and then with a biotin-conjugated secondary antibody for 30 min (Sigma, St. Louis, MO). An avidin-biotin complex (Vector Laboratories) was added for an additional 30 min, and the myocytes were visualized brown after 5 min of exposure in 3,3′-diaminobenzidine solution (Sigma). The peri-infarct region or border zone was defined as the region of myocardium extending 0.5–1 mm from the infarcted tissue or infarct scar (20). Apoptotic myocytes at the peri-infarct region were quantified by an observer blinded to the experimental conditions along the entire length of the defined peri-infarct region. Three levels separated by 100 μm were evaluated for each condition, with four sections at ×20 magnification analyzed by an ocular grid at a given level. Each section contained ≥250 cells and approximated 1 mm^2 of tissue, and results are expressed as the mean for all sections. Stained cells at the edges of the tissue were not counted. Results are expressed as the mean number of apoptotic myocytes per square millimeter at each site examined.

Quantitation of cardiomyocyte proliferation. Cardiomyocyte DNA synthesis and cell cycling were determined by dual staining of rat myocardial tissue sections obtained from LAD-ligated rats at 2 wk after injection of saline or CD34^+ human cells and from healthy rats as negative controls for cardiomyocyte-specific troponin I and human- or rat-specific Ki-67. Briefly, paraaffin-embedded sections were micro-}

waved in a 0.1 M EDTA buffer and stained with a polyclonal rabbit antibody with specificity against rat, but not human, Ki-67 (18) at 1:3,000 (Promega; gift of Giorgio Cattoretti, Columbia University) or mouse monoclonal antibody recognizing human and rat Ki-67 (MB-1) at 1:300 dilution (Dako) and incubated overnight at 4°C. After they were washed, the sections were incubated with a species-
specific secondary antibody conjugated with alkaline phosphatase at 1:200 dilution (Vector Laboratories) for 30 min, and positively staining nuclei were visualized as blue with a 5-bromo-4-chloro-3-indolyl phosphate-p-toluidine-nitro blue tetrazolium substrate kit (Dako). Sections were then incubated overnight at 4°C with a monoclonal antibody against cardiomyocyte-specific troponin I (Accurate Chemicals), and positively staining cells were visualized as brown through the avidin-biotin system described above. Cardiomyocytes

Fig. 1. Degree of neovascularization and level of protection against cardiomyocyte apoptosis are dependent on absolute angioblast numbers in ischemic heart. A and B: 1.7-fold higher numbers of medium (3–6 contiguous endothelial lining cells) and 3.3-fold higher numbers of large (>6 contiguous endothelial lining cells) capillaries in the group receiving 2 × 10^6 CD34^+ human cells than in other groups (P < 0.01). Values are means ± SE of 3 separate experiments. C and D: significantly reduced numbers of apoptotic myocytes at the peri-infarct region in the group receiving 2 × 10^6 CD34^+ human cells (C) and greater mean diameter of viable myocytes at this site (D) than in the other groups (P < 0.01). Values are means ± SE of 3 separate experiments. *P < 0.05; **P < 0.05.
progressing through the cell cycle in the infarct zone, peri-infarct region, and area distal to the infarct were calculated as the proportion of troponin I-positive cells per HPF coexpressing Ki-67. For confocal microscopy, fluorescein isothiocyanate-conjugated rabbit anti-mouse IgG was used as secondary antibody to detect Ki-67 in nuclei. A Cy5-conjugated mouse MAb against α-sarcocinotype actin (clone 5C5; Sigma) was used to detect cardiomyocytes, and propidium iodide was used to identify all nuclei. In separate experiments, animals receiving saline or CD34+ cells after LAD ligation were given bromodeoxyuridine (BrdU) ad libitum in their drinking water daily. After the animals were killed, paraffin-embedded tissue was incubated with a mouse anti-BrdU antibody (Roche Molecular Biochemicals) and then with a biotinylated rabbit anti-mouse IgG antibody (Jackson ImmunoResearch) diluted 1:3,000 with D-PBS. The biotin was detected by using an avidin-biotin complex kit (Vector Laboratories), as described above.

Analyses of myocardial function. Echocardiographic studies were performed using a high-frequency linear array transducer (SONOS 5500, Hewlett Packard, Andover, MA). Two-dimensional images were obtained at midapical and apical levels. End-diastolic and end-systolic left ventricular volumes (EDV and ESV) were obtained by a biplane area-length method, and percent left ventricular ejection fraction (LVEF) was calculated as follows: [(EDV − ESV)/EDV] × 100.

RESULTS

Degree of neovascularization and level of protection against cardiomyocyte apoptosis are dependent on absolute angioblast numbers in the ischemic heart. We first examined the relation between the number of angioblasts injected and the resultant myocardial neovascularization. At 2 days after LAD ligation, animals were intravenously injected with saline or 10⁴, 10⁵, or 2 × 10⁶ G-CSF-mobilized human CD34+ bone marrow cells. The migratory patterns of human CD34+ cells injected into LAD-ligated nude mice were measured by 111I labeling of CD34+ cells. We were able to determine that, after LAD ligation, 23% of the intravenously injected human cells were present in the heart 24 h after injection. In contrast, only 7% of injected CD34+ cells homed to the lung, 15.6% to the liver, and 27.5% to the spleen. This confirms selective trafficking to the heart after LAD ligation. Induction of neovascularization at 2 wk was measured by quantitative analysis of medium- and large-sized capillaries, defined, respectively, as having three to six or more than six contiguous endothelial lining cells. Mean lumen diameter was 0.020 ± 0.002 and 0.053 ± 0.004 mm for medium- and large-sized capillaries, respectively (P < 0.001). The group receiving 2 × 10⁶ CD34+ cells demonstrated 1.7-fold more medium-sized capillaries and 3.3-fold more large-sized capillaries than the other two groups (Fig. 1, A and B; both P < 0.01). Moreover, the group receiving 2 × 10⁶ CD34+ cells demonstrated significantly reduced numbers of apoptotic myocytes at the peri-infarct region and greater mean diameter of viable myocytes at this site than the other groups (Fig. 1, C and D; both P < 0.001). Together, these data indicate that the degree of myocardial neovascularization and the subsequent protection of hypertrophic cardiomyocytes against apoptosis depend on the number of angioblasts in the ischemic myocardium.

Myocardial neovascularization is accompanied by sustained regeneration of endogenous cardiomyocytes. Although myocyte hypertrophy and increase in nuclear ploidy have generally been considered the primary mammalian cardiac responses to ischemia, damage, and overload (9, 24), recent observations have suggested that human cardiomyocytes have the capacity to proliferate and regenerate in response to injury (3, 7). Therefore, we investigated whether induction of neovascularization could also result in cardiomyocyte proliferation and/or regeneration. At 2 wk after LAD ligation, rats receiving 2 × 10⁶ CD34+ human cells demonstrated numerous “fingers” of cardiomyocytes of rat origin, as determined by expression of rat MHC class I molecules extending from the peri-infarct region into the infarct zone. Similar extensions were seen less frequently in animals receiving 10⁶ CD34+ human cells and very rarely in the other groups. The islands of cardiomyocytes at the peri-infarct rim in animals receiving 2 × 10⁶ CD34+ human cells contained a high frequency of rat myocytes with DNA activity, as determined by dual staining with a MAb reactive against cardiomyocyte-specific troponin I and a rabbit polyclonal antiserum with specificity for rat, but not human, Ki-67 (Fig. 2A) (22). Triple immunofluorescence using confocal microscopy confirmed the presence of cycling rat cardio-myocytes and demonstrated a speckled pattern of Ki-67 reactivity within cycling nuclei (Fig. 2B). In contrast, in animals receiving saline, there was a high frequency of cells with fibroblast morphology and reactivity with rat Ki-67, but not troponin I, within the infarct zone. The majority of cells staining positive for Ki-67 and troponin I were not in the process of cytokinesis, as defined morphologically. However, we did, on occasion, observe rare cardiomyocytes undergoing cytokinesis, and only in the setting of angioblast-induced neovascularization.

The number of cardiomyocytes progressing through the cell cycle was 40-fold higher at the peri-infarct region of rats receiving 2 × 10⁶ human CD34+ cells than at sites distal to the infarct, where myocyte DNA activity was not different from that in sham-operated rats. The number of cycling cardiomyocytes was 20-fold higher at the peri-infarct rim of animals receiving 2 × 10⁶ human CD34+ cells than in noninfarcted hearts (1.19 ± 0.2% vs. 0.06 ± 0.03%, P < 0.01) and 3.5-fold

Fig. 2. Peri-infarct neovascularization is accompanied by cell cycling and regeneration of endogenous cardiomyocytes. A: section from infarct of representative animal receiving 2 × 10⁶ CD34+ human cells showing “finger” of cardiomyocytes of rat origin, as determined by expression of rat major histocompatibility complex (MHC) class I molecules, extending from the peri-infarct region into the infarct zone. These cellular islands contain a high frequency of myocytes staining positively for cardiac-specific troponin I and rat-specific Ki-67 (arrows). Sections from infarcts of representative animals receiving saline do not show the same frequency of dual-staining myocytes. B: confocal microscopy of section of peri-infarct tissue from representative animal receiving human angioblasts demonstrates cardiomyocytes with nuclei (labeled blue by Cy5) that concomitantly expressed Ki-67 antigen (labeled green by FITC-conjugated secondary antibody) and with cytoplasm that concomitantly stained for troponin I (labeled red by propidium iodide-conjugated MAb). Arrow, cell-cycling cardiomyocyte with nucleus staining positive for Ki-67. C: significantly higher index of cell-cycling cardiomyocytes at the peri-infarct region in animals receiving 2 × 10⁶ CD34+ than in saline controls or sham-operated animals (both P < 0.01). No difference between the groups is seen at sites distal to the infarct. LAD, left anterior descending coronary artery. D and E: cardiomyocyte cell cycling as defined by Ki-67 or bromodeoxyuridine (BrdU) positivity was induced with increasing concentrations of CD34+ cells.
higher than in the same region in LAD-ligated controls receiving saline (1.19 ± 0.2% vs. 0.34 ± 0.1%, P < 0.01; Fig. 2C). Induction of cell cycling correlated with increasing concentrations of CD34+ cells (Fig. 2D), suggesting a dependency on a minimum number of cells necessary to overcome the threshold preventing progression of cardiomyocyte cell cycling. To better analyze differences in cell cycling throughout the experimental period, dual staining was performed for troponin I and BrdU in animals that had received BrdU daily in their drinking water from the time of surgery to death 2 wk later. Results were strikingly similar to those obtained with Ki-67 staining, and differences between the groups were even more evident, with 24-fold more cycling cardiomyocytes at the peri-infarct rim in the group receiving $2 \times 10^6$ human CD34+ cells than in
noninfarcted hearts and 5-fold more than in LAD-ligated controls receiving saline (both \( P < 0.01 \)).

Enhanced cardiomyocyte survival and regeneration result in reduced fibrosis and improvement in cardiac function. We next examined the effect of increasing the number of human angioblasts trafficking to ischemic myocardium on long-term myocardial function, defined as the degree of improvement in LVEF and reduction in left ventricular end-systolic area at 15 wk after intravenous injection (Fig. 3, A and B). Only modest improvement in these parameters was observed in the group receiving \( 10^4 \) or \( 10^6 \) human CD34+ cells compared with rats receiving saline. In contrast, rats receiving \( 2 \times 10^6 \) human CD34+ cells had a mean recovery in LVEF of 34 ± 4% and a mean reduction in left ventricular end-systolic area of 37 ± 6% (both \( P < 0.001 \)). Quantitation of the ratio of fibrous tissue to myocytes at 15 wk demonstrated significantly reduced propor-

![Fig. 3. Enhanced cardiomyocyte survival and regeneration result in reduced fibrosis and improvement in cardiac function. A and B: relation between the number of CD34+ human cells injected intravenously (10^4, 10^6, and 2 \times 10^6) and improvement in myocardial function at 15 wk, defined as mean left ventricular ejection fraction and mean left ventricular area at end systole. No significant improvement in these parameters was observed in groups receiving 10^4 or 10^6 human CD34+ cells compared with rats receiving saline alone. Rats receiving 2 \times 10^6 CD34+ human cells demonstrated significant recovery in left ventricular ejection fraction and reduction in left ventricular end-systolic area (both \( P < 0.001 \)). C: at 15 wk the mean proportion of scar per normal left ventricular myocardium in rats receiving 2 \times 10^6 human CD34+ cells was significantly reduced compared with rats in other groups (\( P < 0.01 \)). Values are means ± SE of 3 separate experiments. D: sections of rat hearts stained with Mason’s trichrome at 15 wk after LAD ligation demonstrate greater loss of anterior wall mass, collagen deposition (blue), and septal hypertrophy in rats receiving 10^4 CD34+ human cells than in rats receiving 2 \times 10^6 CD34+ human cells.]}
tions of scar per normal left ventricular myocardium in the group receiving $2 \times 10^6$ human CD34$^+$ cells compared with each of the other groups ($P < 0.01; \text{Fig. } 3C$). The overall effects of medium- and large-sized capillaries combining to protect against myocyte apoptosis and induce myocyte proliferation/regeneration are shown dramatically in Fig. 3D, where, in contrast to controls, injection with $2 \times 10^6$ human CD34$^+$ cells resulted in almost complete salvage of the anterior myocardium, normal septal size, and minimal collagen deposition.

**DISCUSSION**

Our data show that the degree of bone marrow-dependent neovascularization induced in the ischemic myocardium is directly related to the numbers of CD34$^+$ angioblasts homing to the ischemic site. As we previously showed (12), the newly developed vessels are of human and rat origin, indicating vasculogenesis from direct vascular incorporation and angiogenesis due to paracrine effects of secreted proangiogenic factors (11). Increasing the numbers of mobilized angioblasts in the ischemic myocardium results in development of capillaries of intermediate and large lumen size and contributes to improved heart function by two complementary mechanisms: 1) protection of at-risk, but otherwise viable, myocytes against apoptosis and 2) induction of myocyte proliferation/regeneration. Together, the results indicate that strategies to increase myocardial homing of human bone marrow-derived angioblasts may augment neovascularization of ischemic myocardial tissue and functional cardiac recovery after acute infarction.

The most striking finding in this study is that, in parallel with growth of larger-sized capillaries accompanying injection of high concentrations of human angioblasts, ischemic rat hearts developed prominent islands of regenerating myocytes at the peri-infarct region, a site recently reported to have an intrinsic capacity for self-renewal after ischemia (3, 7). In animals receiving human angioblasts, rat cardiomyocytes at the peri-infarct region demonstrated a 39-fold increase in mitotic activity compared with sites distal from the infarct and a 4.4-fold higher mitotic activity than in saline-treated animals. The regenerative response identified here bears striking similarity to the spontaneous myocardial regeneration seen in MRL mice after cryogenic injury to the heart (14). This mouse strain demonstrates prominent spontaneous neovascularization and wound repair after myocardial or other injury (14) and has mitotic indexes approaching 20% at the site of cardiomyocyte regeneration.

Efficient delivery of nutrients and growth factors to rat cardiomyocytes by the neovascularure would provide a unifying mechanism to account for the effects on cardiomyocyte apoptosis and cardiomyocyte cycling/regeneration. Protection of hypertrophied cells against apoptosis only requires sufficient extracellular concentrations of glucose necessary to sustain glycolysis (26). In contrast, cell cycle initiation and cellular proliferation require insulin- and Akt-dependent glucose transport and phosphorylation events (21). Because Akt phosphorylation has been shown to be critical for survival of mesenchymal stem cell-derived cardiomyocytes and subsequent functional cardiac recovery (16), this may also be an important pathway involved in cycling of endogenous cardiomyocytes. Irrespective of the precise underlying mechanisms, our study demonstrates that, by inducing processes of antiapoptosis and preregeneration of endogenous cardiomyocyte tissue, significant long-term improvement in cardiac function and salvage of myocardial mass can be achieved. Strategies to optimize the number of angioblasts homing to the ischemic heart may directly impact clinical protocols using bone marrow in patients with myocardial infarction.

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