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## Cellular preservation therapy in acute myocardial infarction

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THE PAST DECADE has been characterized by unprecedented progress in cardiac cell biology and pathophysiology. The dogma of the heart being a terminally differentiated organ has been challenged by compelling evidence generated in several different laboratories around the world (6). These findings were received with a mixture of skepticism and enthusiasm, which has led to an exponential development in the field of cardiac regeneration. The relative ease of using autologous cells to promote cardiac regeneration paired with promising preclinical data has prompted multiple translational studies in patients with acute myocardial infarction (AMI) (5, 12). While the results of clinical studies with bone marrow-derived stem cells or mobilizing factors [such as granulocyte colony-stimulating factor (G-CSF)] produced only marginal positive benefits (5, 12), clinical observations have led to a reexploration of the original hypotheses generated in the laboratory (from bedside to bench). Some investigators now question the efficiency of cardiomyocyte regeneration and progenitor cell transdifferentiation (16). Furthermore, the paradigm shifted from a substantial role of cardiomyocyte regeneration to a combined role of cellular preservation and regeneration of resident cells mediated by paracrine mechanisms (9). Most important, the complexity of the events in infarct healing suggests that no single intervention may be sufficient to halt the course of post-AMI remodeling (Fig. 1).

**Ischemic necrosis.** Ischemic necrosis is the hallmark of acute myocardial infarction. A sudden obstruction to coronary flow leads to decreased tissue oxygen content, decreased ATP levels, tissue acidosis, and hypoglycemia, which trigger the activation of the mitochondrial apoptosis pathway (caspase-9 mediated) and alterations in cell membrane permeability leading to oncosis cell death. Cell membrane rupture releases cytosolic content into the surrounding tissue and stimulates further inflammation. The constellation of cell membrane rupture and tissue inflammation constitutes ischemic necrosis, which is the trigger for the process of infarct healing.

**Inflammation, granulation tissue, and scar formation.** Necrosis is a powerful chemotactic factor for inflammatory cells that infiltrate the damaged area in the attempt to remove debris and coordinate the reparative fibrosis. While inflammation is an essential step for infarct healing, a limitation of the inflammatory response may be a potential therapeutic intervention to limit unfavorable remodeling. Polymorphonucleates, monocytes, and lymphocyte infiltrate the infarct area at different times. This infiltrate is paired

with neoangiogenesis, hyperplasia of fibroblast, and increased collagen production and deposition. The inflammatory infiltrate disappears over time by means of cell apoptosis. Ultimate scar formation is characterized by a prevalence of connective tissues fibers and supporting cells. The fate of the infiltrate and the granulation tissue are major determinants for cardiac remodeling after AMI. For example, the inhibition of granulation tissue apoptosis is associated with the formation of a thicker scar, which ultimately reduces wall stress (10). However, the inflammatory infiltrate also stimulates the local production of inflammatory cytokines (i.e., interleukin-1) that further induce receptor-dependent caspase-8-mediated apoptosis in cardiomyocytes (4).

**Cardiomyocyte hypertrophy, apoptosis, autophagy, and degeneration.** Many cardiomyocytes are lost during the early course of AMI in the central area where tissue levels of oxygen are lowest. However, neither the region of the myocardium bordering this central area nor the remote myocardium is spared from damage. Increased wall stress leads to an increased metabolic demand of the surviving cardiomyocytes that leads to cell hypertrophy. This is more evident in the border zone in which there is a combination of hypertrophic stimuli (stretch, angiotensin, and norepinephrine) and inflammatory stimuli (interleukins and tumor necrosis factor- $\alpha$ ) paired with a suboptimal tissue perfusion. Cardiomyocytes in the border zone have been described as metabolically impaired (usually with anaerobic metabolism, electromechanical dissociation, and fetal gene switch) and morphologically abnormal (degenerated, with aberrant nuclei). These cells have multiple overlapping phenotypes, such as mitochondrial abnormalities, suggestive of oncosis; nuclear alterations and DNA fragmentation, suggestive of apoptosis; and cytoplasmic vacuolization, suggestive of autophagy (7–8, 11). While the presence of “degenerated” cardiomyocytes is generally accepted, many investigators debate the exact role of each cell death modality. This debate stems from inconsistencies between studies that vary in subjects studied, overlapping pathways and phenotypes, methods used, and interpretations given (1, 2). Nevertheless, the degree of cardiomyocyte degeneration correlates with clinical signs of adverse cardiac remodeling and predicts outcome independent of the markers used to detect cardiomyocyte degeneration (i.e., in situ end-labeling for DNA fragments, caspase-3 activation, annexin V staining for apoptosis, or ubiquitin staining for autophagy) (2–3, 8, 14). The most compelling evidence supporting a significant role for cardiomyocyte apoptosis derives from the elegant study by Wencker et al. (17) demonstrating mechanistically that cardiomyocyte apoptosis induced by caspase-8 overexpression is sufficient to cause dilated cardiomyopathy, heart failure, and death in the mouse.

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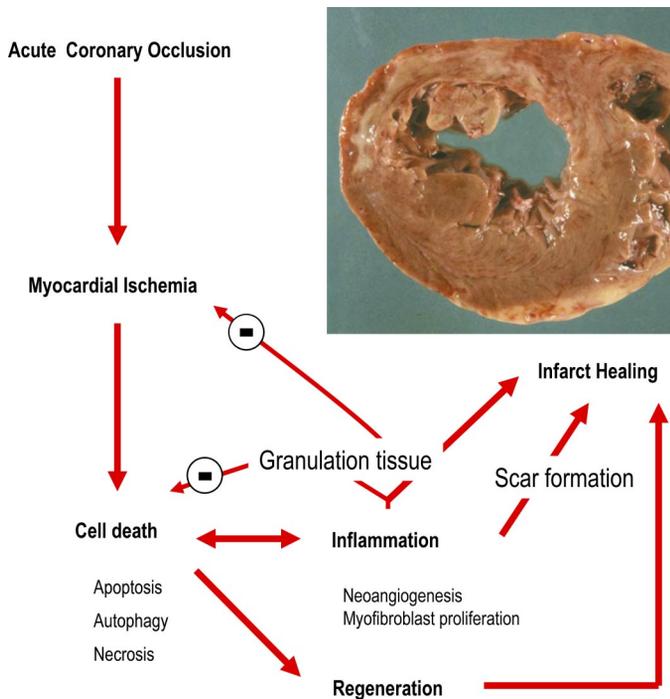


Fig. 1. The complexity of events leading to infarct healing after acute myocardial infarction, including cell death and regeneration, inflammation, and granulation tissue formation, is highlighted. A pathology image of adverse cardiac remodeling following acute myocardial remodeling (autopsy specimen) is shown.

**Cardiomyocyte regeneration and cardiac stem cells.** Ischemic necrosis triggers cardiac regeneration and cardiomyocyte mitosis (6). The rate of spontaneously occurring mitosis after AMI appears rather low (0.07% mitosis, and 0.7% Ki-67+ cardiomyocytes), yet suggests the potential for enhancing this process. A resident pool of c-kit+ cells in the heart has also been described. These cells express markers of lineage differentiation toward cardiomyocytes and are able to reconstitute cardiac tissue when injected in the animals with AMI (6).

**The balance between cell death and regeneration.** It appears obvious that the fate of the myocardium depends on the balance of several competing events. Whereas cell death of cardiomyocytes may be a detrimental factor, apoptosis of infiltrating leukocytes is an essential step of infarct healing. In contrast, if the regeneration of cardiomyocytes is of potential benefit, the regeneration of the nonmyocyte population can be potentially harmful. Moreover, if cardiomyocytes with replicative potential are homed to myocardial regions with increased cell death, increased inflammation, and/or increased wall stress, regeneration is unlikely to occur and further cell loss is expected.

**The current study: premises, results, and inconsistencies.** In their current study, Okada et al. (15) proposed a combined cardioprotective and antiapoptotic strategy to prevent heart failure. The authors focused on the effects of soluble Fas gene delivery on the granulation tissue in the infarct scar with or without the combined administration of G-CSF, a cytokine with pleiotropic effects. G-CSF-treated mice had smaller and thinner scars after AMI that suggested a cardioprotective effect (smaller scar) but an impairment in scar

formation (thinner scar), which may ultimately lead to a further increase in wall stress. Mice treated with sFas gene delivery, on the other hand, had shorter segmental scar length with increased wall thickness, suggesting a preservation of the ability to form scar. Most interestingly, the combined G-CSF and sFas group had the smallest scar area associated with greater scar thickness, suggesting that treatment with sFas gene delivery counterbalances the impairment in scar formation seen with G-CSF alone (15). In contrast with results from other groups, but consistent with prior findings from the same group, Okada et al. (15) find that the role of cardiomyocyte apoptosis or regeneration is negligible. Specifically, they report the rate of terminal deoxynucleotide transferase-mediated dUTP nick-end labeling (TUNEL)-positive cardiomyocytes to be ~0.02% and report no evidence of Ki-67 staining, which is in contrast with other reports (1, 3, 6, 8). The reason behind this discrepancy is not clear. In previous publications, these authors had criticized the use of TUNEL as a marker of apoptosis in cardiomyocytes by highlighting the high number of false-positive results at TUNEL (2). The current observation is reassuring in one sense, as false-positive results of TUNEL in cardiomyocytes after AMI appear to be limited (only 0.02% cells stain positive). Unfortunately, the authors make no further comments on cardiomyocyte structure or function. Interestingly, the same group of authors recently described the absence of TUNEL-positive cardiomyocytes but the presence of “degenerative nuclear changes” and “immunogold labeling indicating fragmented DNA in the nuclei” of many cardiomyocytes in the myocardium bordering the infarct (13). We hypothesize that these myocytes, with changes that the authors comments as “degenerative,” represent damaged cardiomyocytes that are functionally dead and may be referred to as “apoptotic.” This discrepancy highlights a lack of consensus regarding the definition of cell death in cardiomyocytes. We also wonder why such changes, “known to give false-positive TUNEL reactions” per the authors, do not result in TUNEL-positive staining in their samples. While we wait for further studies, the current study reconfirms the complexity of infarct healing and progression toward heart failure. With the preservation of the cellular component of the granulation tissue, the authors were able to reduce cardiac dilatation, reduce cardiac dysfunction, and increase survival following AMI without affecting the cardiomyocyte counterpart. These findings confirm once again that alterations of the healing process may favorably affect outcome.

**Cellular preservation therapy in heart failure.** In conclusion, infarct healing is characterized by complex and intervening events in which cell viability remains at jeopardy for weeks after the index event. The preservation of cell viability in cardiomyocytes bordering the infarct and in the granulation tissue within the infarct is essential to determine the fate of scar, the subsequent wall stress increase, and the strain on the noninfarcted myocardium. Moreover, the border zone and the healing scar constitute the scaffold for proliferation of resident stem cells and potential transplanted cells. Preserving cell viability constitutes the primary modality to preserve the ability to appropriately respond to additional injury.

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