The report of Andrassy and colleagues in the current issue of Circulation adds to the growing body of evidence that the receptor for advanced glycation end products (RAGE) and its ligands, particularly high mobility group box-1 (HMGB1), are central mediators of ischemia/reperfusion (I/R) injury in the heart. A major cause of injury, especially in the reperfusion phase, is the influx of inflammatory cells into the stressed heart. Andrassy and colleagues showed that infiltrating leukocytes express proinflammatory HMGB1 and that HMGB1 plays fundamental roles in injury responses in the I/R heart. In homeostasis, HMGB1 is largely a nuclear protein. In stress conditions, HMGB1 may be released from injured cells, particularly on necrosis. The chief receptor for HMGB1, RAGE, is expressed in multiple cell types in the I/R heart, such as inflammatory cells, and also in cardiomyocytes and vascular cells (endothelial cells and smooth muscle cells). Although HMGB1 may interact with distinct receptors beyond RAGE (for example, toll receptors), the current study of Andrassy and colleagues reveals major roles for RAGE in transducing the effects of HMGB1 in the heart, as administration of recombinant HMGB1 or antagonists of this molecule had significant proinjury effects in the wild-type mouse heart, but no additive effects were noted in mice devoid of RAGE. The biology of RAGE, however, is complex and extends beyond HMGB1 in the injured heart.

RAGE is a Multiligand Receptor: Impact on Myocardial Infarction

In addition to HMGB1, other ligands of RAGE likely play pivotal roles in the response to ischemia or I/R in the heart. For example, rapid generation of pre–advanced glycation end products (AGE) species (methylglyoxal) and AGEs in transient occlusion/reperfusion of the LAD coronary artery was ligated with the left anterior descending (LAD) coronary artery was ligated without reperfusion. Pathogenic effects of S100b on apoptosis, hypertrophy, and cardiac function were demonstrated in the S100b-expressing transgenic mice. However, in the S100b null mice, these pathological effects were greatly reduced. Taken together, these considerations suggest that each of these RAGE ligand families may contribute to I/R injury in the heart. Experiments with the soluble ligand-binding decy of RAGE, soluble or sRAGE, supported that binding up RAGE ligands and blocking their access to the cell surface receptor imparted cardioprotection. In this context, a question that arises is, if multiple ligands of RAGE are potentially involved in I/R injury in the heart, then why did HMGB1 blockade alone exert such a potent effect in the studies of Andrassy and colleagues? Previous findings indicated that the ligands of the receptor cross compete with each other in ligand-binding assays, and recent insights suggest that perhaps multimeric higher-order structures of these ligands may indeed be the key species interacting with the RAGE extra-cellular domain. Thus, once the central ligand-binding domain(s) of the receptor are occupied in stress settings, blockade of RAGE, or any of the ligands may be sufficient to prevent further binding and activation.

Of note, the RAGE ligand family also exerts injurious effects in the diabetic heart. In diabetic mouse hearts, copious formation of AGEs is in place at baseline and is augmented by I/R in the isolated perfused heart. A major finding in that work is that in mice devoid of RAGE, both basal and I/R-stimulated increases in AGE formation were greatly reduced. These data underscore that RAGE, an activator of NADPH oxidase, both transduces the effects of reactive oxygen species (ROS) and contributes to AGE generation, likely via ROS, to stimulate a vicious cycle of cell stress. A salient feature of AGE-RAGE interaction in I/R was its impact on energy metabolism.
cantly higher after I/R in RAGE-null, RAGE-signaling mutant, and sRAGE-treated wild-type mice versus controls, suggesting a link between AGE-RAGE and glycolytic metabolism, likely via ROS-mediated effects on GAPDH. Studies addressing metabolic consequences of non-AGE ligands interaction with RAGE will reveal if blockade of such interactions will improve metabolic viability of the ischemic heart.

**HMGB1-RAGE: Signaling to Harm or Healing in the I/R Heart?**

The work of Andrassy and colleagues reveals that RAGE deletion in vivo and in vitro (macrophages) was associated with decreased phosphorylation of extracellular signal-regulated, c-Jun N-terminal mitogen-activated protein kinases. In distinct studies, RAGE-deficient or sRAGE-treated wild-type mouse hearts after I/R of the LAD coronary artery revealed suppression of c-Jun N-terminal kinase and signal transducer and activator of transcription 5 activation, and in RAGE-null hearts, upregulation of signal transducer and activator of transcription 3 activity. In the latter studies, these signaling pathway effects were associated with decreased release of cytochrome c and apoptosis in the ventricle tissue. Thus, in addition to inflammatory events, RAGE signaling likely contributes to proapoptotic mechanisms in the heart after I/R.

Collectively, these findings suggesting that blockade of ligand-RAGE interaction or genetic deletion of RAGE impart cardioprotection stand in contrast to those illustrating that local administration of HMGB1 to the infarcted murine heart facilitated regeneration, at least in part by stimulating proliferation and differentiation of local cardiac c-kit+ cells. Limana and coauthors showed that RAGE was expressed in this population of cells, both basally and after infarction. To address these apparently discrepant results, we must first examine the study conditions. Andrassy and colleagues tested higher doses of HMGB1 (10 µg/mouse) delivered through intraperitoneal administration of HMGB1 may exert its effects via different receptors or different cell types. Recently, cell-specific effects relevant to the heart have been further probed in vitro. Tzeng and colleagues showed that incubation of adult feline cardiomyocytes with HMGB1 (100 ng/mL) caused negative inotropic effects by impairing sarcomere shortening via decreasing calcium availability through modulation of membrane calcium influx. Notably, Andrassy and colleagues showed that incubation of adult feline cardiomyocytes with HMGB1 (10 µg/mL) had no effect on neonatal ventricular cardiomyocytes, with respect to activation of mitogen-activated protein kinases or upregulation of proinflammatory cytokines. Thus, significant differences between the 2 studies (adult versus neonatal feline or murine cardiomyocytes, respectively; dose of HMGB1; and experimental end points) render conclusions about the overall beneficial or injurious effects of HMGB1 on cardiomyocytes premature at this time. Intriguingly, this concept of dose-dependent effects of RAGE ligands has parallels in the biology of S100b. In cultured neuron-like cells, Huttenen and colleagues showed that nanomolar concentrations of S100b induced trophic survival benefits (increased expression of Bcl-2). In contrast, micromolar concentrations of S100b were toxic to these cells and induced apoptosis in an oxidative stress-dependent manner.

What about other cell types within the heart? Rossini and colleagues suggested that HMGB1 exerts its proregenerative effects on fibroblasts. These authors showed that incubation of RAGE-expressing human cardiac fibroblasts with HMGB1 (especially at doses of 100 ng/mL or less) upregulated a host of proinflammatory species, such as vascular endothelial growth factor, Interferon-γ, Interleukins-10, -1β, -4 and -9, and tumor necrosis factor α. Indeed, conditioned media from these cells stimulated the migration and proliferation of c-kit+ cardiac stem cells in a manner significantly more profound than that exerted by direct incubation of these stem cells with HMGB1. Thus, paracrine effects of HMGB1 stimulated by cardiac fibroblasts may override potentially negative effects on cardiomyocytes. However, it remains to be determined if local administration of HMGB1 to the injured heart would remain protective in ischemia and reperfusion injury after LAD infarction. In reperfusion, the ability of HMGB1 protein to survive and counteract the explosive release of prooxidative and proinflammatory factors into the heart during that period must be probed.

**HMGB1: Insights into Cellular Fate from In Vitro Analyses**

Recent in vitro studies testing HMGB1 have also provided possible insights into the disparate findings of injury versus regeneration. Although Limana and colleagues did not test specifically if RAGE (or perhaps toll-receptor) blockade prevented the salutary effects of HMGB1 in the infarcted heart, they showed that c-kit+ cells expressed RAGE. Further, others have shown that cardiomyocytes express this receptor, especially after infarction. It is plausible that local administration of HMGB1 may exert its effects via different receptors or different cell types. Recently, cell-specific effects relevant to the heart have been further probed in vitro. Tzeng and colleagues showed that incubation of adult feline cardiomyocytes with HMGB1 (100 ng/mL) caused negative inotropic effects by impairing sarcomere shortening via decreasing calcium availability through modulation of membrane calcium influx. Notably, Andrassy and colleagues showed that incubation of adult feline cardiomyocytes with HMGB1 (10 µg/mL) had no effect on neonatal ventricular cardiomyocytes, with respect to activation of mitogen-activated protein kinases or upregulation of proinflammatory cytokines. Thus, significant differences between the 2 studies (adult versus neonatal feline or murine cardiomyocytes, respectively; dose of HMGB1; and experimental end points) render conclusions about the overall beneficial or injurious effects of HMGB1 on cardiomyocytes premature at this time. Intriguingly, this concept of dose-dependent effects of RAGE ligands has parallels in the biology of S100b. In cultured neuron-like cells, Huttenen and colleagues showed that nanomolar concentrations of S100b induced trophic survival benefits (increased expression of Bcl-2). In contrast, micromolar concentrations of S100b were toxic to these cells and induced apoptosis in an oxidative stress-dependent manner.

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**The Multiple Sides of RAGE-Dependent Inflammation: Harnessing Primal Responses to Heal the Heart**

These considerations suggest that HMGB1/RAGE-dependent inflammation is a double-edged sword. What is the
evidence? In addition to the present study,\textsuperscript{1} 2 additional reports have suggested that in allo-immune responses in murine cardiac transplantation, HMGB1-mediated inflammation was highly detrimental to the allograft. Allotransplantation resulted in marked upregulation of both RAGE and HMGB1 (especially in inflammatory cells) in the allograft; blockade of ligand-RAGE interaction with sRAGE or direct blockade of HMGB1 (with RA-box) both increased allograft survival and suppressed the destructive allo-inflammatory response.\textsuperscript{17–18}

The RAGE sword, however, clearly has a softer edge, because RAGE-dependent inflammatory mechanisms may exert beneficial responses. For example, RAGE is essential for effective T-lymphocyte priming reactions in vivo and in vitro.\textsuperscript{13} Further, in acute crush of the sciatic nerve in mice, administration of sRAGE, or F(ab')\textsubscript{2} fragments of anti-RAGE, anti-S100/calgranulin or anti-aminophetin (ie, anti-HMGB1) immunoglobulin G significantly suppressed the regenerative response, as measured by functional and pathological end points.\textsuperscript{10} Deeper probing revealed that impairment of RAGE signaling in vivo in either, especially both axonal and macrophage cellular components in the injured sciatic nerve greatly suppressed regeneration.\textsuperscript{20} Thus, multiple interacting RAGE-expressing cells bathed in RAGE ligands may contribute to these injury responses. In such complex environments, autocrine, paracrine, or combined effects of RAGE ligands in distinct cell types may signal to yield either injury or repair.

In conclusion, lessons learned from studies in which RAGE ligands or RAGE itself have been antagonized, or RAGE/RAGE signaling has been modulated in transgenic mice, reveal that settings exist in which RAGE ligands such as HMGB1 may exert salutary or detrimental effects. Exciting research efforts underway at this time, focused on harnessing the healing powers of RAGE, hold promise for tipping the balance of the primal attack responses of RAGE to its regeneration potential from the nervous system to the heart. Learning how to capture and prevail over the damaging primal effects of RAGE may lead us to new ways to transform RAGE into redemption.

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