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- Cellular and Molecular Mechanisms of Myocardial Ischemia-Reperfusion Injury



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Cardiovascular disease is the leading cause of death in the United States. Although there are a variety of therapeutic options for patients with cardiac disease, heart surgery is a mainstay of treatment for patients with advanced acquired or complex congenital heart disorders. Despite advances in the techniques of heart surgery, ischemic cardiac injury results in considerable morbidity and mortality. To date, the therapy for acute ischemia of the heart has been largely directed towards re-establishing perfusion of ischemic myocardial, or towards the coagulation system to prevent thrombosis. These therapies have arguably reached an efficacious limit.

Our research focuses on understanding how the myocardium responds to ischemia at the molecular, cellular and physiological levels. The goal of our research is to translate an understanding of the molecular mechanisms of ischemic cell signaling into applications for clinical practice.

Ischemia-reperfusion Injury: Paradoxically, restoration of blood flow to oxygen-deprived tissue, the mainstay of therapy for ischemia, often causes further myocardial damage (termed "ischemia-reperfusion [I/R] injury"). I/R injury contributes significantly to morbidity and mortality in surgical patients, and is the principal pathogenetic event in stroke, complications of peripheral vascular disease, hemorrhagic shock, and early transplant graft dysfunction. The reperfusion of oxygen-deprived tissue can cause further myocardial injury by inciting a deleterious inflammatory reaction in and around the reperfused tissue. Because restoration of oxygen delivery to ischemic tissue is critical to survival, a substantial amount of research in the last decade has focused on treating or preventing this detrimental consequence of reperfusion. In our laboratory, we examine the molecular mechanisms of regional I/R injury that often complicate cardiothoracic surgical procedures.

Toll-like receptors: Increased expression of Toll-like receptors (TLRs) has been noted in biopsy samples of patients with severe congestive heart failure, suggesting that TLRs may serve a function apart from their classic role in recognizing microbial antigens. TLRs have been identified on cardiac myocytes, but the function of these receptors of innate immunity in the heart is unknown. We believe that TLRs expressed on cardiac myocytes are activated by reperfusion of ischemic myocardium. We postulate that TLR4 activation during ischemia and reperfusion leads to the activation of mitogen-activated protein kinase (MAPK) signaling pathways and specific transcription factors. These DNA-binding proteins can promote the transcription of genes encoding proteins that cause cardiac apoptosis, or that initiate an acute inflammatory process in the myocardium surrounding an infarction.

Research in our laboratory has identified the involvement of innate immunity receptors in the mechanism of ischemic injury. We have examined mice that are genetically engineered to lack Toll-like receptor 4 (TLR4). Compared to wild-type mice, TLR4-null mice develop a significantly smaller infarct after myocardial I/R injury — illustrating that this innate immune signaling pathway plays a role in the pathogenesis of I/R injury.

TLRs can signal through an adaptor protein called MyD88. MyD88-null mice also develop smaller myocardial infarct after I/R injury, indicating that I/R activates a TLR4- and MyD88-dependent signaling event that results in myocardial damage.

In addition, TLR4 is known to signal through MAP kinases. We have pharmacologically inhibited the activity of the MAP kinase p38, resulting in reduced infarct size after ischemia and reperfusion, compared to mice treated with vehicle alone. Thus,

we are able to apply what we are discovering about the basic science of myocardial I/R injury to potential clinical development.

Ischemic preconditioning: Ischemic preconditioning (IPC) of the myocardium is a phenomenon whereby *brief* repetitive periods of transient ischemia and reperfusion substantially protects the heart against subsequent *prolonged* ischemia. Adaptation of the heart to ischemia following IPC is a biphasic phenomenon. There is an early phase of protection that develops within minutes from the initial ischemic insult and lasts 2–3 hours, and a late (or delayed) phase that is acquired 24 hours later and lasts 3–4 days. The enhanced resistance to infarction and myocardial stunning afforded by IPC and the lasting nature of the response has generated considerable interest in this phenomenon as a potential therapeutic adjunct in the treatment of ischemic heart disease in humans.

The mechanism by which IPC exerts this cardioprotection remains unclear. The classic ligand for TLR4 is LPS (lipopolysaccharide; endotoxin), an integral component of the outer membrane of gram-negative bacteria. Transient activation of TLR4

matory response even in the absence of infection. Heat shock proteins (HSPs) are highly conserved molecules that participate in protein folding and assembly, as well as the translocation of proteins between cellular compartments following cellular stress. Interestingly, HSP60 and HSP70 have been identified as potential ligands for TLR4. In the heart, HSP70 is the primary stress protein responsive to oxidative stress. Increased expression of HSPs in the myocardium increases resistance to ischemia. Our laboratory has shown evidence that IPC is mediated, in part, by the expression of two inducible members of this family, HSP 70.1 and HSP 70.3. Thus, heat shock proteins are potential mediators of the late phase of IPC, and may work through Toll-like receptors.

The balance: Our studies indicate that TLR4 has a detrimental role in prolonged ischemia, but is necessary for the protective effect observed in brief episodes of ischemia. We hypothesize that IPC causes a shift in TLR4-mediated signaling, away from a MyD88-dependant pathway (leading to cellular death), and toward a MyD88-independent pathway, leading to the modulation of NFκB activation, ultimately

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by LPS in the heart confers functional protection from subsequent I/R injury, indicating that LPS treatment can substitute for ischemia in myocardial preconditioning. We have observed that when TLR4-null mice are treated with ischemic preconditioning, the myocardial infarction size remains large compared to the protection seen in wild-type mice, indicating that TLR4 is necessary for early ischemic preconditioning of the heart. However, MyD88-null mice are responsive to IPC, suggesting that the TLR4 signaling involved in myocardial protections does not require MyD88. Research is ongoing in our laboratory to further elucidate the role of Toll-like receptors in preconditioning.

There is also increasing evidence that endogenous ligands can stimulate TLRs, triggering an immune or inflammatory response. Signals from damaged or stressed cells may initiate an inflam-

mately resulting in cellular survival (Figure 1). The regulation of this proposed shift from TLR4-mediated cell death to TLR4-mediated cell survival raises intriguing possibilities for therapeutic intervention, and is an active area of research in our laboratory.

Ischemia reperfusion injury and ischemic preconditioning are critically important in cardiac surgery. Both cyto-destructive (infarction) and cyto-protective (IPC) molecular pathways can be activated following an ischemic event. Our goal is to understand these cellular events so that therapy can be developed to protect against myocardial damage.

Experimental techniques: We utilize cultured cells (cell lines and primary cell isolates) to examine molecular mechanisms that are involved in the response to I/R injury. These studies allow us to examine specific questions about the effects of hypoxia and reoxygenation on molecular pathways

in precisely controlled conditions. In addition, cell culture gives us the capability to move DNA sequences into cells in a controlled fashion to deduce cellular mechanisms of activation based on the over-expression of specific proteins. Finally, by employing differential array and DNA microchip technology, we can identify and characterize novel protein kinases or transcription factors that, in concert with NF-κB, regulate the cellular response to hypoxia and reoxygenation.

We pair these *in vitro* studies with *in vivo* mouse models of myocardial I/R injury and IPC, in which ischemia is induced in mouse hearts by transient occlusion of the left anterior descending coronary artery. Following reperfusion we determine the size of the infarction to quantify the magnitude of cardiac I/R damage. Although these mouse models are technically challenging, they allow for the use of transgenic and gene knockout strains to examine the effects of specific genotypic changes on myocardial I/R injury.

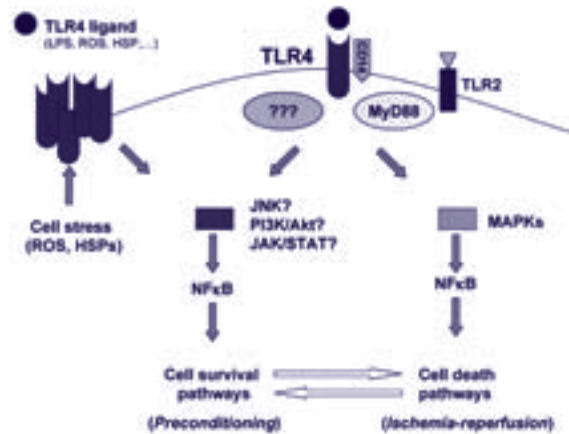


FIGURE 1: TLR4, and possibly other TLRs, are activated by oxidative stress during myocardial I/R injury, either by binding a putative endogenous ligand (e.g., HSPs) that circulates in response to myocardial I/R injury or because of physical alterations by oxygen radical species that cause TLR4 activation in the absence of ligand. Receptor dimerization leads to signal transduction via a MyD88-dependent or -independent pathway, resulting in transcription factor activation (e.g., NFκB). NFκB translocates to the nucleus to promote the transcription of genes encoding either cell survival proteins (following IPC) or cell death proteins (following I/R). Thus, ischemia-reperfusion can initiate selective myocardial signaling pathways that result in either myocardial damage or myocardial protection, depending on the nature of the stimulus.

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