Reperfusion injury in the heart can occur either as a result of transient arterial occlusion (eg, due to vasospasm or thrombus formation with spontaneous lysis) or as an iatrogenic consequence of thrombolytic or angioplastic therapy. A basic research objective is to delineate pathogenesis in order to devise effective prevention and/or treatment strategies. This, of course, applies to most biomedical research, and one general approach to this and other conditions is a reductionist model in which the responses of isolated cell types are investigated in tissue culture. This experimental approach has the advantage of distinguishing primary responses to the stimulus from those that are secondary to the responses of other cell types within the organ. Not only the target cell but also the inciting stimulus can be well defined in cell culture. Arterial occlusion leads to ischemia, which involves deprivation of energy substrates (glucose and oxygen) and accumulation of toxic metabolites (H\(^+\) and K\(^+\)), as well as secondary alterations in endothelial and inflammatory cell function. In contrast, cultured cells (eg, cardiac myocytes) can be subjected to anoxia/hypoxia under highly controlled conditions. Furthermore, the expression of specific proteins can be experimentally manipulated in an attempt to establish molecular mechanisms. Two obvious limitations of tissue culture systems involve the analysis of responses that are not cell autonomous (ie, involve more than one cell type) and those that are cell-type or developmental-stage specific (eg, adult versus neonatal cardiomyocytes). Thus, the relevance of responses in tissue culture to organ function in vivo must be confirmed experimentally, eg, by the use of transgenic or knockout mouse models.

Webster et al\(^1\) have used cultured neonatal rat cardiac myocytes to explore the mechanisms of cell death triggered by hypoxia and reoxygenation. They recently reported that under their culture conditions these cells undergo apoptosis via a p53-independent pathway, in contrast to a previous study that implicated p53 in this process.\(^2\) They also used Langendorff-perfused hearts from wild-type and p53-knockout mice to provide compelling evidence that cell death induced by ischemia-reperfusion did not require p53 expression.\(^1\) To follow up this interesting but essentially negative result, the same group now reports that hypoxia-reoxygenation results in the induction of neutral sphingomyelinase activity, accumulation of ceramide, and increased c-Jun N-terminal kinase (JNK) activity.\(^3\) This cascade can be inhibited by pretreatment of cells with antioxidants, suggesting that it is triggered by reactive oxygen species (ROS) generated during reoxygenation. Ceramide is a signaling molecule, generated from sphingomyelin by the action of acidic and neutral sphingomyelinases (reviewed in Reference 4), that has been linked to the JNK and apoptotic pathways in a number of cell types (reviewed in Reference 5). However, additional studies are required to determine whether ceramide-induced JNK activation is necessary and sufficient to trigger apoptosis in cardiac myocytes. The results reported by Hernandez et al\(^3\) in this issue of Circulation Research are in agreement with a previous study linking both oxygen/glucose deprivation in cardiac myocytes and ischemia/reperfusion in the intact heart with increased ceramide production and apoptosis.\(^6\) Reoxygenation-induced ceramide production is a specific response of cardiac myocytes, as exposure of cardiac fibroblasts to ceramide induced JNK activity whereas hypoxia-reoxygenation did not.\(^3\)

These results provide a basic molecular framework for understanding one apoptotic pathway that is induced by reperfusion (Figure 1), but multiple questions remain to be answered by future studies: (1) Which ROS (superoxide, hydrogen peroxide, hydroxyl radical, peroxynitrite, or others) generated by reperfusion are responsible for the induction of neutral sphingomyelinase activity? (2) What is the mechanism by which these ROS are generated? Several sources of oxygen free radicals have been identified including xanthine oxidase, the mitochondrial electron transport chain, and NADPH oxidase. (3) What is the mechanism by which ROS induce neutral sphingomyelinase activity? (4) What is the mechanism by which ceramide induces JNK activity? (5) What is the mechanism by which JNK activity triggers apoptosis in cardiac myocytes? (6) What other apoptotic pathways are induced by reoxygenation or other aspects of reperfusion?

Diacylglycerol generated by phosphatidylcholine-specific phospholipase C was shown to induce ceramide production by acidic sphingomyelinase in lymphoid cells treated with tumor necrosis factor (TNF).\(^7\) The generation of diacylglycerol by phospholipase C has been implicated in mechanisms of ischemic preconditioning (reviewed in Reference 8), suggesting that this enzyme may be involved in reperfusion-induced ceramide production. Exposure of cardiac myocytes and other cell types to doxorubicin also induces oxygen free
radical generation that leads to ceramide production, JNK activation, and apoptosis, which is mediated by acidic sphingomyelinases and can be inhibited by pretreatment with L-carnitine.9,10 Thus, elucidation of pathways of ceramide generation may lead to therapeutic strategies for preventing myocardial apoptosis in several clinical contexts.

Myocardial cell death resulting from reperfusion in vivo is not entirely cell autonomous, as alterations in endothelial and inflammatory cell function are also important pathogenetic factors. Reperfusion results in the production of chemotactic cytokines by vascular endothelial cells that result in the binding of polymorphonuclear leukocytes (PMNs) (via cell surface adhesion molecules) and their subsequent migration through the vessel into surrounding tissue. To investigate these processes, Kokura et al.,11 in a study also reported in this issue of Circulation Research, cocultured human umbilical vein endothelial cells (HUVECs) with PMNs and T lymphocytes isolated from peripheral blood. Their studies suggest that a complex interplay between these three cell types occurs after anoxia-reoxygenation in which hydrogen peroxide produced by HUVECs stimulates T cells to secrete TNF-α, which induces the expression by HUVECs of the cell surface molecule E-selectin, a mediator of PMN adhesion (Figure 2).

As in the study by Hernandez et al.,3 the production of ROS after reoxygenation appears to trigger the cascade described by Kokura et al.,11 but in the latter system the effects of ROS are not limited to the cell type within which they are produced. In both cases, however, many of the details regarding the mechanisms and direct effects of ROS production are yet to be delineated. Transgenic mice that overexpress copper-zinc superoxide dismutase manifest dramatically reduced myocardial reperfusion injury and reperfusion-induced superoxide generation (as demonstrated by electron paramagnetic resonance spin trapping) in the hearts of nontransgenic mice.12 These results are consistent with the hypothesis that superoxide production triggers the pathological processes described in these two reports. Finally, it is not clear how ischemic preconditioning blocks the pathophysiological processes described in these two reports.3,11 Recent experiments have demonstrated that delayed (late phase or second window) preconditioning does not occur in Nos2 knockout mice, which lack expression of inducible nitric oxide synthase.13 It will be of great interest to determine whether nitric oxide directly or indirectly affects either oxygen radical generation or a subsequent step in the pathways that are described in these two reports.

References
Cellular and Molecular Dissection of Reperfusion Injury: ROS Within and Without
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