

# Lorrie A. Langdale, M.D.

• Mechanisms of Injury Control after Hepatic Ischemia-Reperfusion



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## FUNDING

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Liver failure is often preceded by a period of inadequate tissue and cellular perfusion (ischemia). Hypoxia/re-oxia initiates early activation of Kupffer cells, producing a wave of reactive oxygen species (ROS) and proinflammatory cytokines, in particular  $TNF\alpha$ ,  $IL-1\beta$ , and  $IL-6$ , as well as chemokines. These cytokines, together with reactive oxygen species, act in a paracrine manner on adjacent hepatocytes and endothelial cells, resulting in direct cytotoxic effects. Hepatocytes (HC), in turn, amplify the Kupffer cell (KC) response by expressing and releasing mediators such as  $IL-6$  to further activate neighboring cells. Cytokines released into the circulation may also initiate secondary organ injury, setting the stage for multiple organ failure. Activated neutrophils are recruited by chemokines to the sites of primary and secondary injury. Working in concert with activated complement, these mediators exacerbate the initial injury through microcirculatory vasoconstriction and release of additional reactive oxygen products. This late phase may continue to progress, culminating in liver necrosis with attendant organ failure, or resolve with resumption of normal liver function and a return to homeostasis (Figure 1).

While the progression of injury following liver ischemia-reperfusion (IR) has been well described, the mechanisms of regulation contributing to injury control and ultimate resolution are less well understood. Therapeutic strategies to improve outcomes have been aimed at blocking individual components of this widely redundant inflammatory cascade prior to the onset of IR. To date, however, laboratory successes have not translated to clinically relevant therapies. Further, given that many patients present for treatment after

the pro-inflammatory phase of injury is well underway, a more realistic approach would focus on understanding the mechanisms of inflammation regulation and control. Understanding the mechanisms of cellular signaling that precede, trigger and control the inflammatory response to an injury could be key to effective clinical modulation of ischemia-reperfusion injury and its complications.

## Suppressor of Cytokine Signaling Control of Inflammation

A regulated response to injury requires both active inflammation, with the expression of pro-inflammatory cytokine and chemokine mediators and neutrophil activation and trafficking, and active inflammation control. The spectrum of pro- and anti-inflammatory cytokines that contribute to this dynamic process utilize common cell signaling pathways to mediate their effects. One key pathway involves the Janus family of tyrosine kinases (JAK-Tyk) and the signal transducers and activators of

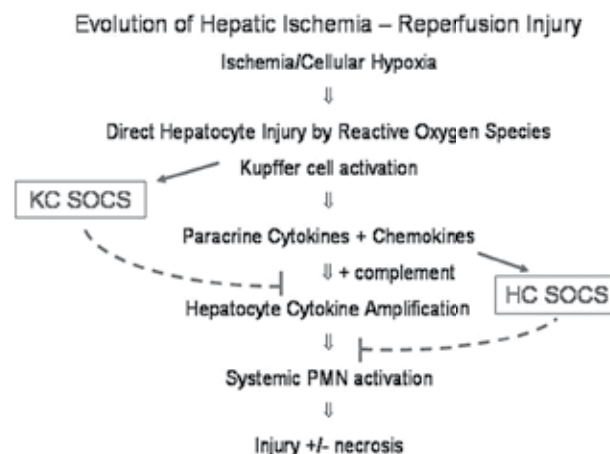


FIGURE 1: Evolution of Liver Injury and Resolution

transcription proteins (STATs). The JAK-STAT pathway requires cytokines to form a ligand-receptor complex that phosphorylates the cytoplasmic portion of the cytokine receptor. This receptor-associated Janus kinase (JAK) then forms a docking site for signal transducer and activator of transcription (STAT), and the resulting complex allows tyrosine phosphorylation of the STAT with formation of an activated dimer or tetramer. The STAT dimer/tetramer translocates to the nucleus and binds with a specific DNA sequence and/or other transcription factors to effect targeted gene transcription.

In addition to sustaining cytokine signaling, STAT-mediated cell signaling also induces the expression of suppressors of cytokine signaling (SOCS) proteins that serve as classic negative feedback mechanisms for cytokine expression. Numerous cytokines important to acute inflammation activate cells through JAK-STAT, including  $\text{TNF}\alpha$ ,  $\text{IFN}\gamma$ , IL-1, IL-6, IL-10 and erythropoietin. These mediators are, in turn, controlled, at least in part, by SOCS proteins.

Our current work focuses on determining the role of JAK/STAT signaling and SOCS-mediated negative regulation on the evolution of liver injury and resolution. We hypothesize that SOCS1 and SOCS3 are essential to the evolution and ultimate resolution of liver IR, cooperatively delimiting cytokine/chemokine-mediated primary and secondary injuries through negative regulatory cross-talk between cells as well as distinct intracellular signaling pathways. Using a murine model of hepatic IR, we are exploring the protective effects of SOCS-induction with erythropoietin as well as the injurious effects of SOCS1 or SOCS3 conditional deletion from hepatocytes on liver IR severity. We have shown that while SOCS3 expression is consistent across a broad range of IR injury from mild to severe, SOCS1 expression directly parallels the severity of ischemic injury. These data suggest that SOCS1 provides a second tier of cytokine regulation when SOCS3 alone is insufficient. Deletion of either protein from hepatocytes appears to be tolerated when injury is moderate, but loss of SOCS1 dramatically compounds injury severity when ischemia is prolonged. These data support the concept that while SOCS1 and SOCS3 share some functionality, they do not appear to be interchangeable.

## Erythropoietin — a Potential Injury Protection Strategy

Like many of the pro- and anti-inflammatory mediators important to hepatic IR, erythropoietin (rhEPO) also signals through JAK-STAT. Erythropoietin is a glycoprotein hormone vital to the differentiation of committed erythroid progenitor cells. Over the last decade, a variety of non-hematopoietic effects have been attributed to exogenous rhEPO, in particular, protection after ischemia in a variety of tissues, including brain, heart, kidney and, most recently, liver. This protection has been observed with pre- and post-injury treatment and has generally been ascribed to induction of anti-apoptotic genes. A less well explored function of rhEPO is its capacity to induce several suppressors of cytokine signaling (SOCS1, SOCS3 and Cis), primarily through STAT5 and STAT3.

We have shown that rhEPO primarily induces Cis-mediated regulation in erythroid progenitor cell lines. SOCS3 appears to be a dominant rhEPO-induced SOCS regulatory mechanism in normal liver. rhEPO induces both SOCS1 and SOCS3 early in reperfusion after severe IR. Like many of the cytokines important to IR, rhEPO is known to signal through STAT3 as well as STAT5. STAT5 is significantly blunted by rhEPO, while STAT3 signaling is sustained. This occurs in the absence of major pro-inflammatory mediators important to IR that signal predominantly through STAT3. SOCS3 is known to selectively regulate IL-6 associated STAT3 signaling but not that utilized by IL-10. Active negative regulation of pro-inflammatory mediators, coupled with sustained anti-inflammatory cytokine signaling mechanisms, would alter the balance of the response to severe IR and inhibit injury progression.

Our next phase of study will focus on the role of SOCS-mediated cytokine regulation in non-parenchymal liver cells, in particular Kupffer cells, utilizing mice with inducible deletion of SOCS1, SOCS3, or both regulatory genes in all liver cells. We hypothesize that rhEPO's direct effect is primarily on Kupffer cells, setting the stage for prompt regulation of the initial cytokine burst, without which the amplification of IR injury through targeted pro- and anti-inflammatory secondary gene responses in neighboring hepatocytes cannot proceed.

## Summary of Significance

Furthering our understanding of the cell signaling events that define and control the acute inflammatory responses to primary and secondary injury will foster the development of treatment strategies important to promoting injury progression, resolution and healing. Our long-term goal is to identify and potentially exploit the natural inflammatory control mechanisms as a novel avenue for clinical management of ischemia-reperfusion injuries.

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## RELATED PUBLICATIONS

1. Langdale LA, Wilson L, Jurkovich GJ, Liggitt HD. Effects of immunomodulation with interferon-gamma on hepatic ischemia-reperfusion injury. *Shock* 11:356-361, 1999.
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## OTHER CO-INVESTIGATORS

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