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• MECHANISMS OF INJURY CONTROL AFTER LIVER ISCHEMIA-REPERFUSION

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Trauma is the leading cause of death in the United States for those between the ages of 1 and 45. The majority of trauma patients' deaths (75%) occur as a result of their initial injuries within the first 24 hours. The remaining 25-40,000 patients die within two days to several weeks after injury as a result of multiple organ failure. Death from cardiac, pulmonary or renal failure may be averted in many patients by aggressive support of these organs. However, the development of hepatic failure is associated with a virtually uniform mortality without liver transplantation. As with trauma, hepatic failure after elective liver resection or liver transplantation carries a high morbidity and mortality.

for global immunosuppression, as evidenced by their failure to express major histocompatibility complexes. These subjects fall victim to multiple organ failure when they fail to recognize a "second hit" challenge due to inhibition of their inflammatory response to injury. Thus, uncontrolled pro-inflammatory or anti-inflammatory states result in poor clinical outcomes.

Therapeutic strategies to improve outcomes after IR have been aimed at disrupting single components of a widely redundant inflammatory cascade. To date, however, laboratory successes have not translated to clinically relevant therapies. Given that many patients present for treatment after the pro-inflammatory phase of injury is well underway, a more recent approach

We hypothesize that negative regulators of cytokine and chemokine signaling play a pivotal role in the evolution of hepatic IR injury, and that it is the lack of appropriate negative regulation which leads to the uncontrolled inflammation associated with multiple organ failure.

Organ failure is often preceded by inadequate tissue and cellular perfusion which produces an ischemic phase of injury and culminates in a gradual loss of cellular homeostasis. Reperfusion has been postulated to initiate a complex chain of "second-phase" events that leads to a neutrophil-mediated injury. The risk of multiple organ failure (MOF) with its attendant high mortality has been postulated to significantly increase when pro-inflammatory cytokine and chemokine expression is sustained and unchecked, as might be expected with serial inflammatory stimuli. At the opposite extreme of the systemic inflammatory response spectrum is the less well defined anti-inflammatory response syndrome (CARS). Animals and patients subjected to prolonged periods of hemorrhagic shock or simulated post-operative infection show evidence

focuses 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, extravascular inflammation, and subsequent organ failure. Further, identification and potential exploitation of natural inflammatory control mechanisms offers a new avenue for clinical management of a spectrum of ischemia-reperfusion injuries.

Hepatic Ischemia-Reperfusion: Evolution of an Injury

The liver is particularly vulnerable to the effects of ischemia-reperfusion (IR) because of its "open architecture". The early phase of IR injury reflects the direct effects of local toxic reactive oxygen products that injure

tissue and mediate neutrophil activation and chemoattraction. The hypoxia/re-oxygenation of ischemia-reperfusion also initiates the recruitment of leukocytes to the specific site of injury through a spectrum of cytokines and chemotactic proteins expressed by Kupffer cells and hepatocytes. Activated PMNs alter microcirculatory perfusion through adhesion to endothelium, and adherent PMNs diapedese from the sinusoid, through the fenestrated endothelium, into the parenchyma where release of additional reactive oxygen products compounds the local injury. This PMN-mediated phase of hepatic IR occurs relatively late in reperfusion, with neutrophil infiltration dominating between 8 and 24 hr of reperfusion. This neutrophil-mediated phase of liver injury either continues to progress, culminating in necrosis and liver failure, or resolves, with resumption of normal liver function and a return to homeostasis and health.



FIGURE 1

The events which determine whether injury progresses to irretrievable necrosis or resolves are poorly understood. Based on the hypothesis that uncontrolled inflammation is largely responsible for the morbidity and mortality associated with ischemia-reperfusion injury (IR), the vast majority of investigations addressing mechanisms of IR have focused on understanding and modulating the pro-inflammatory phase of injury. Laboratory studies have advocated inhibition of reactive oxygen products, neutralization of inflammatory mediators with cytokine specific antibodies or soluble receptors, or blockade of neutrophil adhesion mechanisms to control the acute inflammation associated with IR.

Translation into clinically relevant therapies, however, has been disappointing, primarily because therapeutic strategies have been aimed at disrupting single components of a widely redundant inflammatory cascade. Given the critical role that cytokines and chemokines play in both early and late events after ischemia-reperfusion, an alternative approach to control of inflammation is to examine and potentially

exploit the natural mechanisms that regulate and inhibit cytokine and chemokine production.

JAK-STAT Cell Signaling and Suppressors of Cytokine Signaling (SOCS proteins) in Inflammation

The spectrum of cytokines that contribute to inflammation and its resolution utilize common cell signaling pathways to mediate their effects. A key pathway involves the Janus family of tyrosine kinases (JAK-Tyk) and the signal transducers and activators of 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 target gene transcription.

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. In addition to effecting cytokine signaling, STAT-mediated cell signaling 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 TNF α , IFN γ , IL-1, IL-6, IL-10 and Growth Hormone (GH). These mediators are, in turn, controlled, at least in part, by SOCS proteins.

The pattern and time-course of SOCS mRNA observed following cytokine stimulation appears to be both stimulus and tissue dependent. For example, although constitutively expressed in thymus and spleen, the level of SOCS-1 mRNA are very low in un-stimulated liver cells. Injection of IL-6 or IFN γ results in dose dependent increased levels of SOCS-1, SOCS-2, SOCS-3 and CIS RNA. Both SOCS-1 and SOCS-3 mRNA are detectable in liver within 20 min after IL-6 injection in mice. SOCS-1 mRNA levels return to baseline within 4 hr while SOCS-3 mRNA is sustained for up to 8 hr. SOCS-2 and CIS mRNA remains elevated for 24 h.

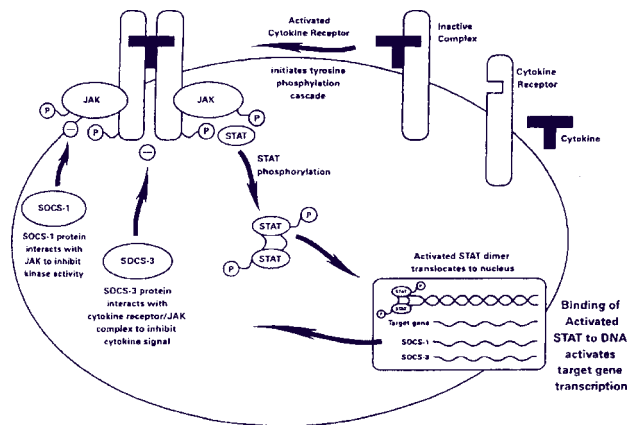


FIGURE 2

The importance of SOCS proteins to liver injury is apparent from studies in SOCS-1^{-/-} mice. These mice exhibit stunted growth and die before weaning with fatty degeneration of the liver and monocytic infiltration of several organs. In addition, the thymus of SOCS-1^{-/-} mice is markedly reduced in size and there is progressive loss of maturing B-lymphocytes in bone marrow, spleen, and peripheral blood. Animals lacking SOCS-1 may be rescued by injection of antibodies to IFN γ . Mice lacking both SOCS-1 and IFN γ however are viable and healthy. These data suggest that it is the loss of balance between the pro-inflammatory IFN γ and its negative control mechanism that results acute fulminant liver injury. Interestingly, SOCS-1 appears to be primarily important to limiting the duration of response to cytokines, rather than the magnitude of the response. There is also evidence that SOCS-3 is up-regulated by IL-10 via a STAT-independent mechanism, implying that additional cytokine-activated transcription factors are involved with SOCS transcription.

Current Laboratory Focus

Most laboratory models of acute hepatic IR utilize a fixed period of ischemic injury, applying the variables of reperfusion time and treatment regimens to delineate and understand the cascade of inflammatory events that follow ischemia. Clinically, the response to hepatic IR injury varies with the injury itself, and the balance between pro-inflammatory mediators and negative control mechanisms likely determines whether tissue heals or proceeds to cellular apoptosis and/or necrosis. We hypothesize that negative regulators of cytokine and chemokine signaling play a pivotal role in the evolution of hepatic IR injury, and that it is the lack of appropriate negative regulation which leads to the uncontrolled inflammation associated with multiple organ failure. To

this end, our current work is focusing on determining the role of JAK/STAT signaling and SOCS-mediated negative regulation of inflammation on the evolution of liver injury severity. We have developed a mouse model of lobar IR with a spectrum of ischemic injury and are testing our hypothesis by applying measurements of SOCS gene and protein expression and immunohistochemical techniques to longitudinal time course studies of injury severity in normal mice, mice treated with interferon gamma (IFN γ), and mice conditionally deficient for SOCS-1 or SOCS-3.

Early or sustained induction of SOCS proteins would potentially significantly alter the evolution of increasingly severe injury. If true, exploitation of these natural inflammatory control mechanisms offers a potential new avenue for clinical management of IR injuries. We have previously shown that IFN γ is protective in a model of liver IR when given in a dose known to restore clinical immunocompetence. High dose IFN γ pre-treatment of normal, immunocompetent rabbits blunts progression of liver IR injury, as evidenced by decreased glutamate pyruvate transaminase concentrations, while lower dose IFN γ pre-treatment or saline control is associated with a significantly increased cellular injury 24 hr after liver IR. Histologic injury, characterized by midzonal and centrilobular necrosis, does not progress beyond the first phase of neutrophil-independent, oxygen free radical mediated injury when animals are pre-treated with high doses of IFN γ . Late neutrophil infiltration is virtually eliminated. Our data have since been corroborated by other investigators utilizing high dose IFN γ in a rat model of liver IR. They further showed amelioration of the associated secondary lung injury. Proinflammatory cytokine and chemokine expression in both liver and lung is markedly attenuated by high dose IFN γ pre-treatment. Although the mechanism(s) by which IFN γ influences the progression of acute inflammation after IR has not been elucidated, given that IFN γ is a potent inducer of SOCS-1, early or sustained expression of SOCS-1 may account for these observations.

Summary of Significance

We hypothesize that the relative balance between acute inflammatory mediators and negative regulating cell signaling events determines whether an ischemia-reperfusion injury progresses or resolves. Expanding our understanding of normal mechanisms of inflammation control will potentially open new therapeutic strategies to the management of acute injury.

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