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- Hepatic Ischemia-Reperfusion Injury: The Search for Control
- JAK/STAT Cell Signaling Pathway and Suppressors of Cytokine Signaling (SOCS Proteins)
- SOCS Proteins, Cytokine Control and the Response to Hepatic IR Injury



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Hepatic Ischemia-Reperfusion Injury: The Search for Control

The liver is particularly vulnerable to the effects of local ischemia followed by reperfusion (IR) during resection of hepatic tumors, surgical management of direct liver trauma and organ transplantation. Ischemia initiates a complex chain of events that is augmented during reperfusion and is characterized by early expression of inflammatory cytokines and chemokines, with subsequent neutrophil activation and infiltration. The resulting injury has the potential to evolve to liver failure. Experimental therapeutic strategies to improve outcomes after IR have been aimed

major role in determining whether injury resolves or progresses to irretrievable damage and organ failure. Understanding these relationships is central to effective clinical modulation of ischemia-reperfusion injury once it is underway and potentially offers an exploitable new avenue for clinical control of ischemia-reperfusion injuries.

Ironically, cytokines generally accepted as pro-inflammatory (and thus potentially harmful in nature), have also been shown to confer protection under clinically relevant conditions. For example, we have previously shown that IFN γ , long accepted as a primer of macrophages and T-cell immunity, is protective in a model of liver IR when given in a dose known to restore

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at disrupting individual components of this highly redundant inflammatory cascade, treating either prior to the onset of ischemia or at the time of reperfusion. To date, however, laboratory successes have not translated to clinically relevant therapies, at least in part because the pro-inflammatory phase of injury is well underway by the time patients present for treatment.

We have chosen an alternative approach to understanding the control of IR injury, focusing on signaling events that mediate the body's management of an acute inflammatory response rather than means of preventing inflammation from the outset. Given their critical role in the evolution of ischemia-reperfusion injury, it is likely that events that precede, trigger and regulate inflammatory cytokines and chemokines play a

immunocompetence. High dose IFN γ pre-treatment of normal, immunocompetent rabbits blunts progression of liver IR injury, as evidenced by decreased glutamate pyruvate transaminase (GPT) 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 centrolobular 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 treatment. Similarly, interleukin-6 (IL-6) is generally categorized as a pro-inflammatory cytokine but has been shown to be protective in liver IR. TNF α and IL-6 are also known to play a critical role early in liver regeneration following partial hepatectomy, serving to regulate the priming phase of regenerative repair.

JAK/STAT Cell Signaling Pathway and Suppressors of Cytokine Signaling (SOCS Proteins)

An effective response to injury requires balance between active inflammation and mediator regulation. In fact, the spectrum of cytokines that contribute to inflammation and its resolution utilize common cell signaling pathways to mediate their effects. One such key pathway involves the Janus family of tyrosine kinases (JAK-Tyk) and the signal transducers and activators of

(I-7, CIS) that regulate cytokine-triggered JAK/STAT signal transduction through direct negative feedback inhibition at key junctures within the pathway. In this way, the effects of major inflammatory mediators are held in check. Chemokines important to neutrophil trafficking also signal through JAK/STAT (STAT-5) and have been shown to be regulated, at least in part, by SOCS. In addition to their direct negative regulation of JAK/STAT, SOCS1 and SOCS3 have also been proposed as major inhibitors of the inflammatory processes mediated by various mitogen-activated protein kinase signaling (MAPK) mechanisms. Several cytokines important to IR, including TNF α , IL-1, IL-6 and Toll-like receptors, utilize these cell signaling pathways. Thus SOCS proteins may make important contributions to the regulation of inflammatory mediators outside a direct negative feedback loop. Because the induction of SOCS genes by one cytokine potentially modifies the duration and intensity of numerous cytokine signals, they are ideally situated to participate in cytokine signaling crosstalk, contributing to the overall regulation and control of the complex and redundant response that is the hallmark of an inflammatory response to injury.

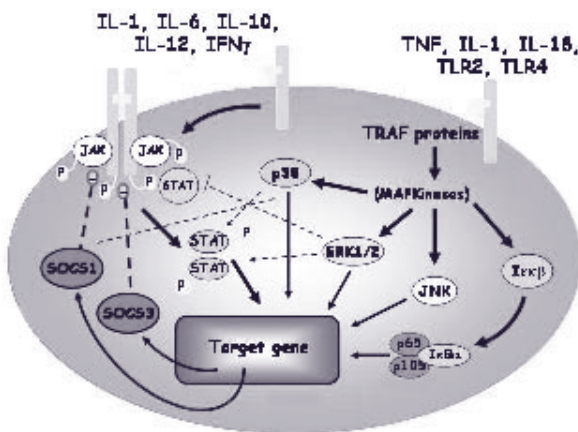


FIGURE 1: Cell Signaling Pathways of Inflammation

transcription proteins (STATs), which are initiated when cytokines such as IL-1, IL-6, IL-12 and IFN γ bind to their receptor and the receptor's cytoplasmic tail is phosphorylated. 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 STATs 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 inflammatory mediator gene transcription.

Among the gene targets of JAK/STAT-inducible early genes are a family of at least eight proteins, designated *Suppressors of Cytokine Signaling* or *SOCS proteins*

SOCS Proteins, Cytokine Control and the Response to Hepatic IR Injury

The potential importance of SOCS proteins to both acute and chronic liver injury is apparent from studies in transgenic mice. SOCS1 $^{-/-}$ 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 SOCS1 $^{-/-}$ mice is markedly reduced in size and there is progressive loss of maturing B-lymphocytes in bone marrow, spleen, and peripheral blood. Animals lacking SOCS1 may be rescued by injection of antibodies to IFN γ , implying that an uncontrolled pro-inflammatory response mediated by IFN γ contributes to this phenotype. Mice lacking both SOCS1 and IFN γ however are viable and healthy. In vitro studies of SOCS1 overexpression by IFN γ offer evidence that functionally, SOCS1 appears to be primarily important to limiting the duration of response to cytokines, rather than the magnitude of the response. This is supported by experiments confirming that IL-6 induces normal STAT activation in SOCS1 deficient cells while IFN γ stimulation results in prolonged STAT-1 expression.

Just as IFN γ is a potent inducer of SOCS1, IL-6 is a potent inducer of SOCS3 and over-expression studies suggest that SOCS3 is a pleiotropic negative regulator of

cytokines. Like SOCS1, deletion of the SOCS3 gene (SOCS3 $-/-$ mice) is a lethal defect, but comparative studies in conditional knock-out mice indicate that SOCS1 and SOCS3 each function in a remarkably specific manner. SOCS3 deficiency prolongs activation of STAT1 and STAT3 after IL-6 stimulation but activation of STAT1 after stimulation with IFN γ is normal. Although similar studies in mice with conditional-deletion of the SOCS1 gene are not completed, SOCS3 and SOCS1 appear to have reciprocal functions in IL-6 and IFN γ regulation and not only attenuate cytokine-specific intra-cellular signaling but also help to coordinate the biological responses by specific cytokines. Microarray analysis shows that IL-6 induces a pattern of gene expression in SOCS3 conditionally-deficient livers that mimics the pattern induced by IFN γ . Thus, both proteins may contribute to regulation of IFN γ and IL-6 signaling. While the role of SOCS1 and SOCS3 is to ensure the appropriate duration of cytokine signaling, like many body systems there appears to be redundancy between SOCS1 and SOCS3. Although not functionally interchangeable, these cytokine regulators represent overlapping potential mechanisms of cytokine control for a spectrum of disease.

In all, these data support the concept that it is the loss of balance between pro-inflammatory and negative control mechanism that tips the scales between acute fulminant liver injury and recovery. We hypothesize that SOCS proteins are at the fulcrum of the response to IR, such that, depending on the timing and intensity of a pro-inflammatory stimulus, the relative expression of SOCS proteins determines whether injury progresses or resolves. Thus the expression of SOCS proteins may represent an exploitable means of clinical injury control.

Our current work utilizes a murine model of hepatic IR to examine the role of SOCS proteins as critical modulators and gatekeepers of the phenotypic response to ischemia-reperfusion injury. In this model, mice undergo partial hepatic ischemia, retaining continuous perfusion to three small segments of the liver. As a first step, we have characterized compared injuries (histology, cytokine expression) and the pattern of SOCS expression in both previously ischemic and continuously perfused liver segments across a range of liver IR severity (20, 45, or 90 minutes of ischemia followed by variable periods of reperfusion). Table I summarizes these data. We have shown that SOCS3 appears to be induced as an early injury response gene, while SOCS1 expression is reserved as a second control mechanism, induced as an injury becomes increasingly severe.

GPT (4 hours after reperfusion)	+		++		+++	
	Isch	Perf	Isch	Perf	Isch	Perf
Neutrophils	–	–	+	–	++	–
Necrosis	–	–	+	–	++	–
SOCS3	++	++	++	++	++	++
SOCS1	–	–	+	+	++	++

TABLE 1: Summary of Increasing Injury Severity Effects
Mild Moderate Severe

Work to fully characterize the relationships between cytokine and chemokine expression, their cell signaling mechanisms and SOCS expression are ongoing. However, the severity of injury appears to be critical not only to the induction of pro-inflammatory mediators, but the timed expression, intensity and duration of potential injury control mechanisms. Interestingly, these effects are not limited to directly injured tissue. Continuously perfused liver invokes similar SOCS responses as tissue subjected to Ischemic injury after a broad range of IR injury, likely due to the effects of circulating mediators. Confirmation of the central role of SOCS proteins to the control of IR injury, however, will require evidence that deletion of individual SOCS genes worsens injury and that early and/or sustained expression is protective.

To accomplish this, we are extending our murine IR model to transgenic mice with conditional deletion of SOCS genes. Given the perinatal lethality of complete gene deletion, our collaborators in Australia have bred mice with SOCS deletion that is confined only to liver. This will allow us to test our hypotheses as to the relative importance of SOCS1 and/or SOCS3 to the evolution of liver IR injury. In wild type mice, a mild IR injury resolves without consequence, moderate IR should produce a more severe but potentially recoverable injury and prolonged ischemia should progress to irrevocable injury. With conditional deletion of SOCS3, significant worsening of mild injury (mimicking the severity of injury observed with longer periods of ischemia) would place SOCS1 and/or SOCS3 at the center of the early response to injury. Functional cell signaling redundancy between SOCS1 and SOCS3 may shift “responsibility” for injury control from one suppressor of cytokine signaling to another with conditional deletion of a single gene.

Alternatively, if SOCS3 and SOCS1 are “additive” mechanisms of control, conditional deletion of SOCS1 will likely have a lesser overall effect on mild IR but

dramatically undermine the capacity for recovery with a more severe IR injury characterized by further loss of hepatocytes with sustained GPT, prolonged cytokine and chemokine expression and early hepatic failure. The altered inflammatory milieu will also affect the more normal residual tissue (perfused lobes) due to an increase in circulating mediators, offering insights into the indirect effects of IR on hepatic reserve. As further proof of SOCS central role in IR control, we are also undertaking studies in which mice undergoing hepatic IR are stimulated with IL-6 or IFN γ , determining if

overexpression of SOCS1 or SOCS3 is responsible for injury protection. In addition, we are addressing the interface between SOCS regulation and other inflammatory signaling pathways extending these studies to evaluate both local hepatic and secondary lung injury after hepatic IR.

In summary, our findings will not only further characterize the nature of injury control but support further study of SOCS proteins as novel potential therapies to improve outcomes after ischemia-reperfusion.

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