Hepatoprotection via the IL-6/Stat3 pathway

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Stat3 is a vital transcription factor that is activated downstream of the gp130 receptor, primarily via IL-6 signaling in adult liver. A new study (see the related article beginning on page 989) demonstrates that Stat3 provides hepatoprotection against Fas-mediated apoptotic liver damage by two mechanisms: direct inactivation of caspases and reduction of reactive oxygen species.

IL-6 is a critical proregenerative factor and acute-phase inducer in the liver that also confers resistance to liver injury by hepatic toxins, ischemia, and Fas (Figure 1). Its effects are mediated almost exclusively on hepatocytes within the liver. Although the source of IL-6 within the liver has not been unequivocally established, studies with bone marrow transplantation provide evidence that hepatic Kupffer cells (liver macrophages) are responsible for production of IL-6 in response to lipopolysaccharide or TNF (13). Secreted IL-6 acts on neighboring hepatocytes in a paracrine fashion to stimulate liver regeneration and repair. IL-6-bound to the soluble IL-6 receptor signals via gp130 and Janus kinase-1 (JAK-1), leading to activation of the Stat3 transcription factor and the MAPK signal transduction cascade. IL-6–/– livers induce little Stat3 in response to IL-6 activation during liver regeneration after partial hepatectomy, hepatic injury, or acute-phase liver injury and allow predictions about potential therapeutic interventions that could prove beneficial in a variety of liver insults.

The IL-6 signal transduction pathway in liver injury and regeneration

IL-6 acts on neighboring hepatocytes in a paracrine fashion to stimulate liver regeneration and repair. IL-6–/– livers induce little Stat3 in response to IL-6 activation during liver regeneration after partial hepatectomy, hepatic injury, or acute-phase liver injury and allow predictions about potential therapeutic interventions that could prove beneficial in a variety of liver insults.

Figure 1

Model for IL-6/Stat3 signaling pathway. IL-6 binds to its soluble receptor, sIL-6r, which binds to the gp130 receptor, resulting in the activation of Janus kinase (JAK). This leads to activation of the MAPK pathway and activation of Stat3 by tyrosine (Y) phosphorylation. Dimerized Stat3 is able to translocate into the nucleus and activate gene transcription. In the liver, this process promotes liver regeneration, the acute-phase response, and hepatoprotection against Fas and toxic damage. P, phosphate.

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Nonstandard abbreviations used: reactive oxygen species (ROS); signal transducer and activator of transcription-3 (Stat3).
induction, suggesting that Stat3 may mediate many of the effects of IL-6 (1, 7–10). Conditional Stat3 knockout mice have been used to show that Stat3 is an important component of the IL-6 response during liver regeneration and the acute-phase response, but Stat3 does not account for all of the effects of IL-6, particularly those that are mediated by MAPK activation (14). Up to 40% of the immediate-early genes induced during liver regeneration are regulated at least in part by IL-6, and a significant subset of these are also regulated by Stat3.

**Modulation of Stat3 levels in liver cells points to its critical role in hepatocyte survival**

Stat3 is a vital, ubiquitously expressed protein that is activated by a number of ligands in addition to IL-6 (15, 16). It has important roles in mitogenesis and antiapoptosis. Stat3 has been shown to be involved in the transcriptional upregulation of many genes, not only acting by direct DNA binding, but acting in some cases as a coactivator of transcription factors such as activator protein-1 and hepatocyte nuclear factor-1 (17). Stat3 knockout results in early embryonic lethality, but conditional knockouts provide useful tools to examine the actions of Stat3 in specific tissues. In the study by Haga et al. (12), two animal models were used to examine the effects of Stat3 modulation in Fas-mediated liver injury: mice injected with adenoviruses expressing constitutively active Stat3 and other proteins; and mice with hepatocyte-specific Stat3 gene deletions. Adenoviruses injected intravenously normally homed to the liver, infecting more than 80% of hepatocytes and allowing for expression of encoded proteins. Haga et al. demonstrate that constitutively active Stat3 provided protection against Fas-mediated liver injury, and that Stat3 deficiency led to Fas sensitivity. The antiapoptotic proteins FLIP, Bcl-2, and Bcl-xL, which block caspase activation, are elevated in IL-6–treated livers (9). Haga and colleagues report here that these proteins were also elevated in Stat3-overexpressing livers, providing evidence that Stat3 mediates the major antiapoptotic effects of IL-6 (Figure 2). Whereas IL-6–mediated elevation of antiapoptotic proteins is largely posttranscriptional (9), mRNA for these proteins was elevated in the Stat3-overexpressing livers (12). This difference could be due to the massive overexpression of Stat3 and the fact that adenovirus infection confers a degree of transcriptional induction not seen in normal mice.

This mechanistic evaluation was taken to another level by the demonstration that not only anticaspase agents, but also the antioxidant N-acetyl cysteine, were able to provide some protection against the effect of Stat3 deficiency on Fas-mediated apoptosis (12). Having identified ROS as a component of Fas-mediated liver injury, Haga et al. identified an endogenous antioxidant, Ref-1, as a target of Stat3. Expression of Ref-1 provided hepatoprotection, strongly suggesting that Ref-1 is a critical component of Stat3-mediated hepatoprotection. Ref-1, a dual-function protein upregulated by increases in ROS, is an endonuclease in the basal excision repair pathway and a reducing agent that facilitates the DNA-binding properties of redox-sensitive transcription factors (18–21). Ref-1 is able to suppress ROS generation and hepatic apoptosis (Figure 2).

**Hepatoprotection by redox-dependent and -independent mechanisms**

These findings provide important insights into the hepatoprotective properties of IL-6 and its major anti-injury mediator Stat3 (12). Though not yet shown, it is expected that IL-6 induces Ref-1, as IL-6 is the major regulator of Stat3 activation in Fas- and toxin-mediated liver injury. Stat3 has not yet been shown to be hepatoprotective in toxic liver damage, but based on these findings, Stat3 is predicted to be hepatoprotective in liver injury. Toxin-mediated liver injury occurs largely through the generation of ROS and direct mitochondrial damage, leading to hepatic necrosis with a lesser degree of apoptosis (1). The level of oxidants may be so high that glutathione is depleted, thereby precluding the activation of caspases, a glutathione-dependent process. By inducing both antioxidant Ref-1 and caspase inhibitors such as Bcl-2, FLIP, and Bcl-xL, IL-6, Stat3, and similar cytokines are hepatoprotective in a broad spectrum of liver injuries mediated by Fas and liver toxins (22). Cytokines such as IL-6 also promote liver regeneration, another component of the hepatoprotective

![Figure 2: Proposed model for the actions of IL-6 and Stat3 that result in hepatoprotection against Fas activation. Interaction of FasL with its receptor activates the caspase cascade that is blocked by IL-6 and Stat3 through the upregulation of FLIP, Bcl-2, and Bcl-xL. Fas activation also generates an oxidative stimulus that is blocked by the upregulation of Ref-1. FADD, Fas-associated death domain; Rac1, RAS-related C3 botulinum toxin substrate 1; Apaf1, apoptotic protease activating factor; Bax, Bcl2-associated X protein; Bid, BH3-interacting domain death agonist.](image-url)
mechanism that restores liver mass after necrotic or apoptotic injury has occurred. The link between intracellular signals resulting in mitogenic and antiapoptotic effects of these agents remains to be completely dissected.

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Selectin and selectin ligand binding: a bittersweet attraction

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Inhibition of leukocyte migration into target organs has long been an attractive, though challenging, basis for anti-inflammatory strategies. However, to date, the manipulation of leukocyte rolling along blood ves
sels has not yielded successful new therapies. An important study (see the related article beginning on page 1008) may now open new avenues in this exciting field of anti-inflammatory therapies by introducing a putative inhibitor of poly-N-acetyllactosamine biosynthesis that affects selectin ligand activity and shows efficacy in a rodent skin inflammation model.

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flammatory compounds for the treatment of skin diseases.

Nonstandard abbreviations used: sialyl Lewis X (sLeX); cutaneous lymphocyte-associated antigen (CLA); β1,4-galactosyltransferase (GalT); α2,3-sialyltransferase (ST3GalV); α1,3-fucosyltransferase (FucT); P-selectin glycoprotein ligand-1 (PSGL-1); contact hypersensitivity (CHS); peracetylated-4-fluorinated-α-glucosamine (4-F-GlcNAc).

Tissue-specific localization of T cells is a requirement for immune surveil-
lance in the skin and in addition plays a pivotal role in the pathogen-
esis of numerous inflammatory skin disorders. Indeed, the evidence that T cells are crucial factors in mediating psoriasis, allergic contact der-
matitis, atopic dermatitis, and cuta-
aneous T cell lymphomas is so strong that these diseases are now consid-
ered as T cell–mediated dermatoses (1). Consequently, insight into mecha-
nisms of T cell recruitment to the skin (and other target organs) may lead to novel anti-inflammatory therapies, and the subject is there-
fore of particular interest.

Selectin and selectin ligand interactions mediate leukocyte rolling along the endothelium

The multistep cascade of T cell migra-
tion has been well described (Figure 1), and the molecular basis for T cell skin homing has been reviewed recently (2). The first steps of T cell localization to all tissues include leukocyte tethering and rolling along the vessel wall, which is mediated primarily by interactions between selectin and selectin ligand (3). A number of studies have demonstrat-
ed the pivotal role of E- and P-selectin for leukocyte rolling as well as their overlapping and mutually compensating functions (4). Therefore, it was not surprising that a neutralizing anti-
body solely against E-selectin was found to be without beneficial effects in a recent clinical trial (5). Probably for these reasons, the development of a potent, but E-selectin–specific low-
molecular weight antagonist called ESA-2 (6) was stopped. The lesson learned from these findings was that potent and clinically active selectin antagonists have to interfere with at least two of the three selectins (E, P, and L) in order to show in vivo efficac-
y. Some such antagonists have been reported recently (7).