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INFLAMMASOME-MEDIATED INFLAMMATION AND FIBROSIS – IT IS MORE THAN JUST THE IL-1 β

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Significant advances have been made over the past several years in understanding the importance of the intracellular proinflammatory structure termed the inflammasome in promoting inflammatory, autoimmune and metabolic diseases. Sterile inflammation is triggered when cells sense danger from exposure to damage-associated molecular patterns (DAMPs) released by dying cells rather than pathogen-associated molecular patterns (PAMPs) from microorganisms. Inflammation in the liver is regulated by Kupffer cell recognition of DAMPs and PAMPs, and the resulting activation of these resident macrophages leads to the recruitment of circulating monocytes and neutrophils that together comprise the hepatic innate immune response. DAMPs identified to induce sterile inflammation include extracellular ATP, high mobility group box protein 1 (HMGB1), heat shock protein 70, hyaluronic acid, nuclear DNA and uric acid. DAMPs are sensed by extracellular pattern recognition receptors (PRRs) which include nucleotide-binding oligomerization domain-like receptors and toll-like receptors (TLR). After DAMP stimulation two steps lead to inflammasome activation. First, transcription of cytokine and inflammasome component genes is increased. Then inflammasome assembly occurs that allows activation of caspase 1 which cleaves pro-interleukin (IL)-1 β into its active secreted form. Secreted IL-1 β regulates the recruitment and activation of other immune cells to amplify the inflammatory response.⁽¹⁾

Overactivation of the sterile inflammatory response has been implicated in the pathogenesis of a number of liver diseases including drug-induced liver injury, alcoholic and nonalcoholic steatohepatitis, and ischemia/reperfusion injury.⁽¹⁾ In the mouse model of acetaminophen hepatotoxicity, DAMPs including HMGB1, hyaluronic acid, DNA, keratin and heat shock protein 70 are released. With antibody neutralization of HMGB1, or in mice lacking TLR4 or TLR9 receptors, liver injury from acetaminophen is significantly reduced.⁽¹⁾ The PRRs TLR2, TLR4 and TLR9 have been shown to mediate steatosis and hepatitis in murine nonalcoholic fatty liver disease driven by inflammasome-produced IL-1 β .^(2,3) TLR4 and TLR9 receptor signaling also underlies mouse models of liver injury and inflammation from alcohol and ischemia/reperfusion.⁽¹⁾ Together these studies demonstrate a central role for the DAMP-PRR-inflammasome cascade in liver injury.

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The specific inflammasome NLRP3 (NOD, LRR and pyrin domain-containing 3) has emerged as an important mediator of sterile inflammation including in the liver. In murine liver injury components of the NLRP3 inflammasome pathway are up regulated and their deletion reduces liver disease.^(4,5) Wree *et al.*,⁽⁶⁾ previously described the ability of the NLRP3 inflammasome to generate spontaneous liver inflammation, hepatocyte death by pyroptosis, and fibrosis in studies employing transgenic mice with constitutive NLRP3 activation globally or conditionally in myeloid cells. In a continuation of this work in this issue of HEPATOLOGY they further investigated the mechanism by which NLRP3 drives hepatic inflammation and fibrosis.⁽⁷⁾ They hypothesized that NLRP3 inflammasome effects are mediated not only by inflammasome-produced IL-1 β , but also by the inflammasome-independent cytokines tumor necrosis factor (TNF) and IL-17 which have known roles in liver injury and fibrosis.

To examine this question, they crossed *Nlrp3*^{A350V} mice with constitutive myeloid cell NLRP3 activation with global TNF or IL-17 knockout mice. Inflammation in *Nlrp3*^{A350V} mice was associated with elevated IL-1 β , TNF, and IL-17 levels, and increased chemokine (C-X-C motif) ligand 1 and 2 resulting in hepatic neutrophil translocation. The knockout mice had increased numbers of hepatic macrophages with a proinflammatory phenotype. Increased hepatic stellate cell (HSC) activation and hepatic fibrosis were also found in *Nlrp3*^{A350V} mice. All of these NLRP3-induced effects were completely ameliorated by knockout of TNF, indicating that TNF is a critical effector molecule for NLRP3. Knockout of IL-17 reduced some of the changes caused by NLRP3 inflammasome activation but had little effect on the development of inflammation and fibrosis as compared to TNF. With high-dose lipopolysaccharide administration, liver inflammation was amplified in *Nlrp3*^{A350V} mice as reflected by increased neutrophil and macrophage infiltration. Inflammation was abrogated in *Nlrp3*^{A350V} mice with the TNF but not IL-17 knockout. These findings delineate a new mechanism by which TNF acts as a central mediator of liver disease.⁽⁸⁾

The study establishes TNF as the principal cytokine responsible for the recruitment of proinflammatory cells and activation of HSCs after macrophage NLRP3 activation (Fig. 1). This role of TNF could be due in part to enhanced IL-1 β signaling as the NLRP3-TNF double knockout mice had reduced IL-1 β levels indicating a positive feedback of TNF on IL-1 β . TNF can induce hepatocyte apoptosis and necrosis which serve as a stimulus for HSC activation, and liver fibrosis often correlates with the extent of hepatocyte death. Alternatively DAMPs can directly cause HSC activation. In experimental liver fibrosis, hepatic expression of the NLRP1, NLRP3 and absent in melanoma 2 inflammasomes is increased in HSCs as well as in Kupffer cells, suggesting that HSC inflammasome activation may be functionally important.⁽⁹⁾ IL-1 β and TNF may also cause liver fibrosis by increasing HSC survival.⁽¹⁰⁾ Thus, TNF may promote inflammasome-dependent liver fibrosis through multiple mechanisms.

The findings of this paper are important in light of the failure of anti-TNF therapy in human trials of alcoholic hepatitis due largely to increased mortality from infections. IL-1 β lacks the anti-microbial functions of TNF suggesting that IL-1 β might be a safer biological target than TNF in liver disease patients who are susceptible to infections. If TNF production is

secondary to IL-1 β , then there is a further rationale for the current efforts to test anti-IL-1 β therapy in human liver disease. However, against the possible efficacy of mono-therapy with an anti-IL-1 β agent is that the authors previously employed this strategy in *Nlrp3*^{A350V} mice and achieved a reduction in inflammation but not fibrosis.⁽⁶⁾ Therapeutics to specifically block TNF effects on injury and inflammation may therefore be unavoidable for anti-cytokine therapy to succeed in preventing fibrosis in some liver diseases.

Wree *et al.*,⁽⁶⁾ have shown previously that global NLRP3 activation produces a more severe phenotype than the myeloid specific activation of NLRP3 suggesting the importance of this inflammasome in cells other than macrophages. Further studies are needed to examine the role of inflammasomes and their downstream cytokines individually in hepatocytes, Kupffer cells and HSCs to further clarify the inter-relationship of these cells to the development of hepatic sterile inflammation and fibrosis. Specific cell targeted therapies can then be designed to dampen inflammation and fibrosis. These studies are important in light of the current lack of any hepatic anti-cytokine therapy despite the long-established role of proinflammatory cytokines in liver disease.

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Abbreviations

DAMP	damage-associated molecular pattern
HSC	hepatic stellate cell
HMGB1	high mobility group box protein 1
IL	interleukin
NLRP3	NOD, LRR and pyrin domain-containing 3
PRR	pattern recognition receptor
TLR	toll-like receptor
TNF	tumor necrosis factor

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FIG. 1.

Effects of the NLRP3 inflammasome on liver injury and fibrosis. DAMPs produced by dying hepatocytes are sensed by PRRs on Kupffer cells leading to the assembly and activation of the NLRP3 inflammasome and increased pro-IL-1 β production. Caspase 1 is activated and cleaves pro-IL-1 β into its active secreted form. IL-1 β can directly promote hepatocyte cell death, the recruitment of monocytes and neutrophils and hepatic stellate cell (HSC) survival and activation. IL-1 β also induces TNF synthesis and secretion from Kupffer cells which further increases IL-1 β production. TNF functions as the principal effector cytokine in mediating the effects of inflammasome-dependent IL-1 β on liver injury, inflammation and fibrosis.