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EDITORIAL

Inflammasome-*more* Injury in Cholestasis

Cholestatic liver diseases refer to a diverse group of disorders that disrupt bile flow. They occur secondary to diminished bile acid secretion by hepatocytes, or impaired transport through the biliary system. The resulting accumulation of bile acids has previously been shown to initiate liver injury by inducing expression of inflammatory genes and cytokines in hepatocytes.[1,2] However, it is unclear what mediates the downstream inflammatory response. In this issue of *Cellular and Molecular Gastroenterology and Hepatology*, Cai et al[3] demonstrate that the inflammasome, a danger-sensing cytosolic multiprotein complex, is involved in the pathogenesis of cholestatic liver injury.

The inflammasome classically consists of an upstream sensor protein of the NOD-like receptor family, an adaptor protein, and the downstream effector protease caspase-1.[4] Upon stimulation by a variety of microbial or sterile stressors, inflammasome assembly leads to autocatalytic cleavage of caspase-1, which in turn processes proinflammatory cytokines pro-interleukin 1β (pro-IL1β)and pro-IL18 into their mature and bioactive forms. The inflammasome has already been implicated in a number of noncholestatic liver diseases, but its role in mediating liver injury in the setting of cholestasis was unclear.

Cai et al now convincingly show that the inflammasome is indeed involved in the pathogenesis of cholestatic liver injury. They first demonstrate its activation in patients with cholangiopathies. Namely, they found significantly increased hepatic expression of key inflammasome pathway components (NLPR3, IL1β, CASPASE-1) in patients with primary biliary cholangitis and primary sclerosing cholangitis as compared with healthy control subjects.

To functionally investigate the role of the inflammasome in cholestatic liver injury, Cai et al induced obstructive cholestasis by performing bile duct ligation (BDL) in *Casp1*$^{-/-}$ mice, which are deficient in inflammasome activation. While similar elevation of serum bile acids was observed in wild-type and *Casp1*$^{-/-}$ mice upon BDL as compared with animals undergoing sham surgery, liver injury was attenuated in the *Casp1*-deficient mice, as evidenced by a lower rise in plasma alanine aminotransferase, alkaline phosphatase, and IL1β levels. Paradoxically, this reduction in liver injury was associated with an increase in hepatic fibrosis, which was accompanied by higher expression of collagen type 1 alpha 1 and tumor necrosis factor α in the *Casp1*$^{-/-}$ BLD mice as compared to the wild-type BLD group.

Cai et al next examined whether loss of the inflammasome changed the hepatic immune cell composition, and whether the alteration might explain the different levels of liver injury in *Casp1*$^{-/-}$ vs wild-type mice. They found an increase in the macrophage marker F4/80 in *Casp1*$^{-/-}$ BDL mice, with further immunophenotyping indicating that the macrophages were predominately of the anti-inflammatory M2 type. Collectively, the results suggest that inflammasome activation in the setting of cholestasis promotes hepatocyte injury and the release of IL1β, and that it suppresses anti-inflammatory responses by macrophages.

Last, Cai et al examined whether the inflammasome is directly activated by bile acids using hepatocytes and macrophages from wild-type and *Casp1*$^{-/-}$ mice. They found that endogenous conjugated bile acid species (taurocholic acid and glycocholic acid) at pathophysiologically relevant concentrations did not activate the inflammasome in either hepatocytes or macrophages. As expected, there was increased chemokine production, but bile acid exposure did not lead to caspase-1 cleavage or increased pro-IL1β production, indicators of inflammasome activation. The absence of direct activation of the inflammasome by bile acids contradicts previous reports, possibly because of the use of hydrophobic bile acid species at high levels in the prior studies.[5,6] Cai et al speculate that instead of direct bile acid toxicity, the "danger signal" triggering inflammasome activation might be a factor that is released from hepatocytes undergoing necrosis in the setting of bile acid accumulation.

Overall, this interesting study shows that inflammasome activation plays a role in promoting cholestatic liver injury by suppressing the anti-inflammatory M2 macrophage population. The relative increase in IL4 expression, an anti-inflammatory cytokine produced by M2 macrophages, has been shown to stimulate the profibrotic cytokine transforming growth factor β1. This could potentially explain why fibrosis was increased in *Casp1*-deficient mice after BDL. What remains to be determined is, what exactly is the source of inflammasome activation? Cai et al speculate that it is the dying hepatocytes that release damage-associated molecular patterns that go on to activate the inflammasome in macrophages. Future studies will be needed to test this hypothesis and to further explore the provocative finding regarding the potential role of the inflammasome in blocking liver fibrosis.

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Conflicts of interest
The authors disclose no conflicts.

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