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A Novel Protective Role for FXR against Inflammasome Activation and Endotoxemia

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During conditions of impaired bile flow (cholestasis), increased serum bile acids (BAs) are prognostic markers of sepsis. In this issue, Hao et al. (2017) show that the BA receptor FXR binds NLRP3 inflammasome in macrophages and inhibits activation of inflammasome components, thus reducing endotoxemia in cholestasis.

Sepsis is a life-threatening condition caused by a dysregulated host response to infection. The liver is an essential organ that acts as a guardian, modifier, and target of sepsis (Strnad et al., 2017). Any condition of impaired hepatic BA flow is defined as cholestasis, which is a common co-morbidity of sepsis; therefore, measurement of plasmatic BA level is routinely used as a sepsis prognostic marker. BAs are synthesized from cholesterol in the liver, stored in the gallbladder, and post-prandially released into the intestine in order to facilitate the absorption of dietary lipids and liposoluble vitamins. These amphipathic molecules travel along the intestine and once in the distal ileum 95% of them are actively absorbed and return to the liver via the portal vein in the so-called enterohepatic circulation. BAs are crucial metabolic players and important signaling molecules (Thomas et al., 2008); thus, the physiologic orchestration of BA enterohepatic circulation is essential.

The nuclear Farnesoid X receptor (FXR) is the master regulator of BA homeostasis, governing their synthesis, transport, and metabolism (Modica et al., 2010). BA circulation is tightly controlled by a number of membrane transport systems. The organic anion transporting polypeptides (Oatps) and the Na^+ taurocholate cotransport protein (Ntcp) mediate hepatic BA uptake at the basolateral membrane of hepatocytes. After conjugation, BAs are secreted into the bile via the canalicular membrane ATP-binding cassette (ABC) transporters, mainly the bile salt export pump (Bsep) and the multidrug related protein 2 (Mrp2). On the contrary, the basolateral membrane ABC transporters, such as Mrp3 and Mrp4, and the organic anion transporters α and β (OSTα/β) drive an alternative route to drain the BAs from the liver to the systemic circulation in case of obstruction to bile flow. Alterations of the equilibrium of these transport systems may lead to a cholestatic disorder.

Inflammasomes are multimeric protein complexes involved in the host defense protecting activity against invading pathogens and in physiological aberrations such as cancer and auto-inflammatory, metabolic, and neurodegenerative diseases. Inflammasomes induce the inflammatory process by triggering the maturation of proinflammatory cytokines, such as interleukin-1β (IL-1β), to engage innate immune defense response (Man and Kanneganti, 2015; Schroder and Tschopp, 2010). In macrophages, the activation of the NLRP3 inflammasome, a well-characterized inflammasome implicated in sepsis and infectious diseases, requires two signals. The first signal, known as priming, is characterized by a transcriptional upregulation of Nlrp3 and IL-1β induced by pathogen-derived factors, particularly the bacterial lipopolysaccharide (LPS). Subsequently, host-derived danger-associated molecular patterns (DAMPs), mainly ATP, mediate the second signal activating NLRP3 to trigger the inflammasome assembly and IL-1β release (Man and Kanneganti, 2015).

In the present study, Gonzalez and coworkers (Hao et al., 2017) add new insights into BA functions and explain how they trigger an inflammatory response by acting as DAMPs activating the NLRP3 inflammasome. Using a non-targeted metabolomic approach, the authors observed that high concentrations of BAs act as early biomarkers of LPS-induced endotoxemia in mice. FXR mRNA and protein levels were found to be significantly reduced in murine LPS-induced endotoxemia. Moreover, after LPS treatment, a decreased expression of *Ntcp* and *Bsep* accompanied by a concomitant increase of *Ostβ* was observed, showing a BA spill over into the blood systemic circulation, increased serum BAs levels, and their subsequent uptake into macrophages. The authors demonstrated that in activated macrophages, but not monocytes, BAs are able to stimulate both signal 1 and 2 of NLRP3 inflammasome activation, in synergy with LPS and ATP, respectively, in a calcium influx-dependent manner (Figure 1). The discovery that BA could modulate NLRP3 activating DAMPs in macrophages confirms that in vivo BAs drive metabolic derangements exacerbating inflammatory responses in combination with PAMPs and host-derived DAMPs.

Although inflammasomes fine tune the physiological host defense response, their excessive activation is involved in the development of autoinflammatory and metabolic diseases (Man and Kanneganti, 2015). Gonzalez and coworkers (Hao et al., 2017) identified the BA receptor FXR as a negative regulator of the NLRP3 inflammasome. *Fxr* null mice were shown to be more sensitive, while FXR-overexpressing mice were more resistant, to LPS-induced endotoxemia, with higher levels of mature IL-1β and

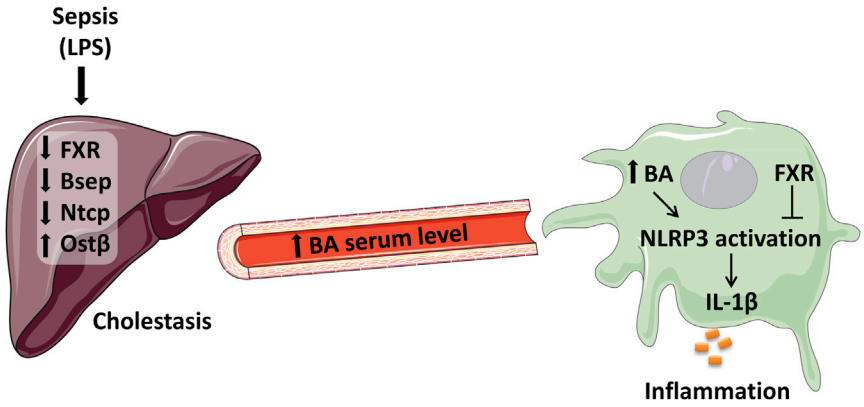

Figure 1. Proposed Link between Cholestasis and Poor Prognostic Outcome of Sepsis
Decreased expression of FXR, Bsep, and Ntcp and increased expression of Ostβ during sepsis lead to bile acid (BA) spill over into the blood, which increased serum BAs levels and their subsequent uptake into macrophages. BAs activate NLRP3 inflammasome in the macrophages promoting the release of proinflammatory cytokines such as IL-1β. On the contrary, FXR has a direct negative regulatory effect on NLRP3 activation.

activated caspase-1 in their macrophages. Interestingly, treatment of wild-type mice with the FXR agonists GW4064 or obeticholic acid (OCA) was found to be inefficient to improve survival and decrease caspase-1/IL-1β activation, while overexpression of FXR per se did. This scenario strongly suggests a ligand-independent physical interaction between FXR and NLRP3 inflammasome components preventing their assembly and thereby repressing NLRP3 activation. In addition, the bone marrow of wild-type (WT) and whole-body *Fxr* null ($Fxr^{-/-}$) mice were transplanted into WT mice. $Fxr^{-/-}$-WT mice showed increased IL-1β plasma levels after LPS treatment, further confirming the direct negative regulatory effect of macrophage FXR on Nlrp3 activation (Figure 1).

The work by Gonzalez and colleagues (Hao et al., 2017) provides a mechanistic understanding of the association between cholestasis and poor prognosis in septic patients. While the physiological role of BA and FXR in macrophages remains to be established, BAs and FXR play a pivotal role in the regulation of NLRP3 inflammasome and therefore in the outcome of sepsis. Indeed, BAs promote calcium influx-dependent NLRP3 activation, while FXR negatively regulates the inflammasome activation probably via direct physical interaction with its components, avoiding NLRP3 inflammasome assembly. Given the dual FXR ability to control inflammasome, adjuvant FXR therapeutic exploitation could be suggested in sepsis and cholestasis. A major goal during cholestasis is to reduce levels of cytotoxic pro-inflammatory BAs. This could be achieved via intestinal FXR activation with subsequent induction of the enterokine FGF19 that inhibits hepatic BA synthesis (Inagaki et al., 2005; Modica et al., 2012; Degirolamo et al., 2016). The present study highlights the importance of FXR activation in macrophages with complementary inhibition of the NLRP3 inflammasome and subsequent reduction of sepsis and endotoxemia, which are traveler fellows of cholestasis.

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