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CELLULAR AND MOLECULAR GASTROENTEROLOGY AND HEPATOLOGY

POINT-COUNTERPOINT

Liver Steatosis is a Driving Factor of Inflammation

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Nonalcoholic fatty liver disease (NAFLD) is the most common liver disease associated with comorbidities, such as insulin resistance, cardiovascular, and metabolic diseases. A subset of patients with NAFLD progresses to nonalcoholic steatohepatitis (NASH), characterized by hepatocyte injury, lobular inflammation, and perisinusoidal fibrosis, which may progress to cirrhosis. A significant gap in understanding the causative factors and molecular mechanisms involved in liver injury and inflammation limits the progress in treating NASH. Accumulation of excess free fatty acids in hepatocytes as lipid droplets (called hepatic steatosis) is the stepping stone in the spectrum of NAFLD. Hepatic steatosis is mainly triggered by continuous low-fiber high-fat diets, impaired fatty acid metabolism, sustained adipose tissue lipolysis, and de novo lipogenesis.[1] Since the early 1970s, researchers have elegantly demonstrated that the accumulation of oxidizable lipids in hepatocytes increases lipid peroxidation and oxidative stress via peroxisomal β-oxidation. Conversely, the peroxidation of membrane lipids and their products directly contribute to hepatocyte injury by damaging various cellular organelles, such as the endoplasmic reticulum and mitochondria. This leads to activating multiple signaling pathways involved in apoptosis, necrosis, and pyroptosis (inflammasome), resulting in hepatocyte injury and inflammation.[2] Thus, hepatic lipotoxicity acts as the primary insult for initiating injury and inflammation in NASH pathogenesis.

The casual role of lipotoxicity in promoting inflammation in the context of NASH is unclear because of the coexistence of steatosis in combination with insulin resistance, adipokines, alteration in the immune system, and dysbiosis.[3] It is well acknowledged that intestinal microbial dysbiosis and dysregulated adipokine levels during NASH are closely associated with the severity of hepatic lipotoxicity.[4] However, in the setting up of NASH, they are considered secondary offenders. Supporting this concept, Zhang et al[5] demonstrated that lipotoxicity induced by a cholesterol diet sequentially promotes inflammation and hepatocyte injury, associated with gut microbiota dysbiosis. In contrast, decreasing cholesterol levels restored the gut microbiota and completely prevented the NASH development. Similarly, dysbiosis occurs in steatosis-prone leptin-deficient ($Lep^{ob/ob}$) mice, independent of dietary regimens, indicating that alterations in the host (lipid) metabolism is the primary event in the spectrum of liver diseases.[6] In addition, inhibiting the accumulation of saturated fatty acids in the hepatocytes through pharmacologic or genetic approaches improves insulin sensitivity and attenuates inflammation,[7] suggesting that hepatic lipotoxicity is sufficient to trigger the injury and inflammatory response in the liver.

How does lipotoxicity induce inflammation and injury in NASH? Hepatocytes enriched with mitochondria metabolizes fatty acids into acetyl-CoA via fatty acid β-oxidation. However, increased fatty acid delivery endorses lipotoxicity by generating excess reactive oxygen species, which causes mitochondrial damage. Under physiological conditions, mitophagy removes damaged mitochondria; however, lipotoxicity impairs mitophagy resulting in the accumulation of damaged mitochondria leading to hepatocellular injury (Figure 1).[8] The injured hepatocytes release danger signals, such as damage-associated molecular patterns (DAMPs), including mitochondrial DNA and high-mobility group box 1. Intracellular DAMPs are recognized by pattern recognition receptors, whereas extracellular DAMPs act through the receptors of advanced glycation end products and toll-like receptor 4 and 9 signaling. Activation of these receptors in hepatocytes and immune cells cooperatively triggers a sterile inflammation through diverse pathways, such as nuclear factor-κB, mitogen-activated protein kinase (p38 MAPK), (p42/44 MAPK), and c-Jun N-terminal kinase signaling cascades (Figure 1).[9,10] For instance, studies show that ablation of toll-like receptor 4 and 9 signaling in the hepatocytes or immune cells similarly attenuate high-fat-induced hepatic steatosis and inflammation, suggesting toll-like receptor signaling in parenchymal and nonparenchymal cells coordinates obesity-associated fatty liver disease.[11-13] Thus, lipotoxicity-mediated elevation in reactive oxygen species and mitochondrial damage sets a stage to trigger hepatic inflammation.

Lipotoxicity-mediated mitochondrial dysfunction and reactive oxygen species generation also activate nod-like receptor protein (NLRP3) inflammasome signaling by promoting NLRP3 oligomerization and inflammasome assembly. The NLRP3-inflammasome pathway acts as an adaptive mechanism to restore hepatocellular homeostasis during acute stress; however, its sustained activation from persistent injury promotes liver injury, pyroptosis, and fibrosis (Figure 1).[14-16] Studies revealed that NLRP3 signaling in parenchymal cells is crucial in NASH pathogenesis because global but not myeloid-specific activation of NLRP3 increases hepatocyte death and injury.[14,17] Mechanistically, NLRP3 activates caspase-1 in

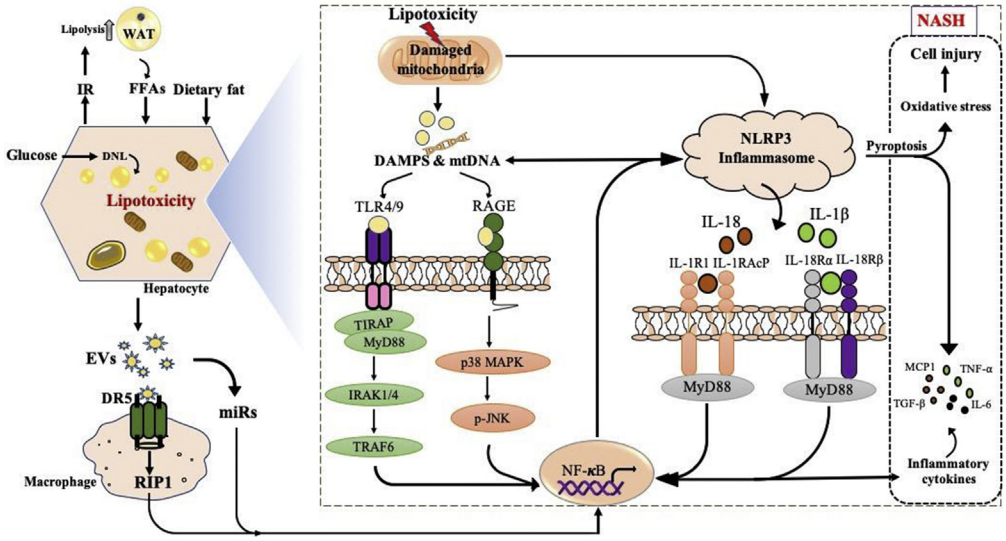

Figure 1. Lipotoxicity drives NASH. The schematic diagram representing the contribution of lipotoxicity in the pathogenies of NASH. The accumulation of toxic lipids in the hepatocytes caused by continuous supply of free fatty acids from adipocytes, diet, or de novo synthesis damages mitochondria resulting in the release of several mediators, such DAMPs and mtDNA. These mediators possess proinflammatory via TLR4/9 and RAGE, leading to the activation of MyD88, p38 MAPK, and NF-κB signaling. Furthermore, NF-κB and DAMPs can also activate the NLRP3 inflammasome, which induces IL-1β and IL-18 secretion. Signaling through IL-1R1 and IL-18Rα exacerbates inflammation and activates apoptotic and pyroptotic pathways. Moreover, EVs with cargo in the form of proteins and miRs released from the lipotoxic hepatocyte activates proinflammatory signaling in macrophages and other cell types in the liver. WAT, white adipose tissue; DNL, de novo lipogenesis; IR, insulin resistance; EVs, extracellular vesicles; DR5, death receptor 5; miR, micro RNA; RIP1, receptor-interacting serine/threonine-protein kinase 1 (RIPK1); DAMPs, damage associated molecular patterns; mtDNA, mitochondrial DNA; TLR, toll-like receptors; RAGE, receptor for advanced glycation end products; TIRAP, toll/IL-1 receptor domain-containing adaptor protein; MyD88, myeloid differentiation factor 88; IRAK, interleukin 1 receptor-associated kinase; TRAF, TNF receptor-associated factor; NF-κB, nuclear factor kappa binding protein; NLRP3, nod-like receptor protein; IL-18 and 1β, interleukin receptor; IL-1R1, IL-1 receptor; I-1RacP, IL-1 receptor accessory protein; IL-18Rα, IL-18 receptor α chain; IL-18Rβ, IL-18 receptor β chain; MCP1, macrophage chemoattract protein 1; TGFβ, transforming growth factor-β; TNFα, tumor necrosis factor-α.

hepatocytes, cleaving prointerleukin (IL)1β and pro-IL18β into their mature forms (IL-1β and IL-18). These proinflammatory cytokines induce hepatocyte pyroptosis via nuclear factor-κB activation.[18] Conflicting evidence also suggests that hepatocyte-derived saturated fatty acids, DAMPs, and stress molecules, such as uric acid and cholesterol crystals, activate the NLRP3 inflammasome in non-parenchymal cells leading to sterile inflammation and hepatocellular injury. For example, mitochondrial DNA released from the damaged hepatocytes activates the NLRP3 inflammasome in hepatic Kupffer cells leading to an inflammatory response.[19] Remarkably, various classes of lipids elicit a differential effect on inflammasome activation during NASH progression.[20] Therefore, understanding the lipid mediators of inflammasome activation may lead to novel therapeutic targets to treat NASH.

Recent studies show that hepatocytes communicate with neighboring cells via extracellular vehicles (EVs), composed of cargo in the form of proteins, lipids, and nucleic acids. Notably, the circulating levels of EVs are significantly elevated in human and mouse models of NASH.[21] It is also evident that the composition of the EVs cargo varies considerably between normal patients and patients with NASH, contributing to the worsening of liver injury and inflammation. Several mechanisms regulate EV release, including inositol-requiring enzyme 1α and death receptor 5. Studies show that lipids control the EV release and its cargo composition. For example, death receptor 5 proapoptotic signaling induced by the saturated fatty acids increases the release of EVs bearing tumor necrosis factor–related apoptosis-inducing ligand from the hepatocytes, which then activates the release of proinflammatory cytokines from the macrophages.[22] Similarly, EVs with integrin β1 as a cargo released from the lipotoxic hepatocytes mediates monocyte adhesion to liver sinusoidal endothelial cells, promoting hepatic inflammation.[23] Moreover, EVs barring the proinflammatory miR-1 released from the lipid-laden hepatocytes suppress KLF4 and activates nuclear factor-κB in the endothelial cells (Figure 1).[24] However, it remains unclear how a mere lipid overload regulates EV release and composition. EVs are the center of interest for future evaluation of novel therapeutic approaches in various diseases. Therefore, deciphering the mechanisms underlying lipid regulation of EV homeostasis will benefit in combating NASH.

Despite the remarkable progress in understanding the complex relationship between lipotoxicity and NASH progression, “the chicken or the egg paradox” still prevails on the causal

relationship between hepatic lipotoxicity and inflammation. A better understanding of the critical determinants of hepatic injury and inflammation driven by hepatic steatosis could help identify novel therapeutic targets for NASH.

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Conflicts of interest
The authors disclose no conflicts.

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