Though quite encouraging, the results by Lenci et al are in contrast with the results of other studies that found that the majority of serologically HBsAg-negative liver-transplant recipients on prophylactic therapy have HBV-DNA or ccc-DNA detectable in liver and/or peripheral blood mononuclear cells.[4,5] The high prevalence of detectable virus in these sites (up to 23–90%) in previous studies has supported the use of indefinite HBV prophylaxis. In addition, the repeated use of HBV-DNA and ccc-DNA measurements in the liver as a guide to withdrawal of prophylaxis has its own limitations. Repeated liver biopsies are burdensome, are not free of complications, and assays for quantitation of intrahepatic HBV-DNA and ccc-DNA are not standardized. Sampling error and the variable accuracy of the assay may further add to the disparities in detection of intrahepatic HBV-DNA and ccc-DNA.

In spite of the limitations, study by Lenci et al[3] suggests that a special approach can be taken in a select group of patients at low risk of re-infection depending on the HBV-DNA at the time of transplantation. Those with undetectable HBV-DNA at transplant can be candidates for withdrawal of HBIg or possibly be candidates for withdrawal of all prophylactic drugs. Whereas those at a higher risk are treated by long-term combination low-dose HBIg plus nucleoside analogs. Though potent drugs with high genetic barrier are available to treat the recurrent HBV infection, the focus should still be on prevention of re-infection rather than on the treatment of recurrent infection.

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Nuclear Factor High-mobility Group Box1 Mediating the Activation of Toll-like Receptor 4 Signaling in Hepatocytes in the Early Stage of Non-alcoholic Fatty Liver Disease in Mice

Li L, Chen L, Hu L, et al. Nuclear factor high-mobility group box1 mediating the activation of toll-like receptor 4 signaling in hepatocytes in the early stage of nonalcoholic fatty liver disease in mice. *Hepatology* 2011, July 11.

Abstract

One of the challenges surrounding nonalcoholic fatty liver disease (NAFLD) is to discover the mechanisms that underlie the initiation of it. The aim of this study was to elucidate the effects of toll-like receptor 4 (TLR4) signaling in liver parenchymal cells during the early stage of NAFLD. Male TLR4-wildtype, TLR4-knockout, TLR2-knockout, MyD88-knockout, and TRIF-knockout mice were fed a normal diet or high-fat diet (HFD). Liver steatosis, alanine aminotransferase levels, nuclear translocation of nuclear factor kappa B (NF-κB) (P65), macrophage accumulation, and neutrophil infiltration were assessed. Using Kupffer cell depletion or bone-marrow transplantation, we examined the potential role of Kupffer cells and myeloid infiltrating cells during the initiation of NAFLD. Immunohistochemistry and western blotting were implemented to determine the release of high-mobility group box1 (HMGB1). The neutral antibody against HMGB1 was used to block the activity of free HMGB1. Here, we report that the activation of TLR4 signaling in hepatocytes, accompanied with the relocation of P65 in nucleus, was proven to play an important role during the initiation of NAFLD. Importantly, HMGB1 releasing from hepatocytes in response to free fatty acid (FFA) infusion was first reported as the key molecule for the TLR4/MyD88 activation and cytokines expression in vitro and in vivo. Treatment with neutralizing antibody to HMGB1 protects against FFA-induced tumor necrosis factor alpha and interleukin-6 production. *Conclusion:* Our study supports the notion that TLR4/MyD88 signaling in liver parenchymal cells plays a pivotal role during the early progression of HFD-induced NAFLD, in which free HMGB1 served as a positive component mediating TLR4 activation.

Comment

Pathogenesis of non-alcoholic fatty liver disease (NAFLD) is still ill understood. Insulin resistance, oxidative stress, endotoxemia, and gene polymorphism are thought to be involved in the pathogenesis of NAFLD. Gut-derived portal endotoxins contribute to the overactivation of Kupffer cell through toll-like receptors (TLRs) and therefore aggravate liver inflammation and cell injury which may lead

to the activation of hepatic stellate cells and induce liver fibrosis.[1] Among 13 TLRs identified in mammals, TLR2, TLR4, and TLR9 play a role in the development of NAFLD. In the innate immune system, TLRs are sensors that recognize bacterial and viral components such as lipopolysaccharide, bacterial DNA, and peptidoglycan. Recent data have demonstrated that the liver is exposed to a high load of TLR ligands due to bacterial overgrowth and increased intestinal permeability in NAFLD. Upon stimulation by these TLR ligands, hepatic immune cells produce various mediators that are involved in host defense.[1] These mediators in return alter the lipid metabolism, insulin signaling, and cell survival. Indeed, some TLR-deficient mice demonstrate lesser degrees of NAFLD even though TLR ligands are increased. Activation of the transcriptional factor nuclear factor kappa B (NF-κB), a downstream target for TLR-MyD88 signaling, is crucial for the inflammatory response in immune cells and is a key in the development of NAFLD.[2] TLR-MyD88 signaling pathway also activates JNK, a member of mitogen-activated protein kinases and JNK is important in the pathogenesis of NAFLD, because JNK activation plays a central role in the development of obesity and insulin resistance.[3]

The TLRs either detect exogenous ligands like detection of conserved pathogen-associated molecular patterns (PAMPs) or endogenous ligands like damage-associated molecular patterns (DAMPs) including the widely expressed nucleus protein high-mobility group box1 (HMGB1). Endogenous ligands including HMGB1 are released from damaged or stressed tissues which can also signal through TLRs. HMGB1, an early mediator of injury and inflammation, has been identified as the key factor linking TLR4 signaling and the development of hepatic and kidney ischemia/reperfusion injury.[4]

Even though the effects of adipose, stellate, and Kupffer cells have been well documented in vitro and in vivo, the elaborate role of hepatocytes in the progression of NAFLD remains unclear. In the article by Li et al, the authors have focussed on the role of hepatocytes in the pathogenesis of early NAFLD.[5] In addition, the authors tested the hypothesis that free fatty acid (FFA) related HMGB1 release mediates the activation of TLR4 signaling in hepatocytes and make the essential contribution to high-fat diet (HFD) induced early stage NAFLD. The authors found that palmitic acid could elevate the expression of HMGB1 in vitro and in vivo.[5] The relocation of HMGB1 intra- and intercellularly was induced by FFA/HFD administration by way of an unknown mechanism, which can in turn self-activate the TLR4 signaling on hepatocytes in an autocrine manner and other hepatocytes together with nonparenchymal cells in a paracrine manner. The accumulation of free HMGB1 in plasma may further enhance the inflammation and liver damage. In addition, stimulated TLR4 signaling in hepatocytes could promote lipid accumulation and hepatic steatosis, resulting in TLR4-dependent HMGB1 release and forming a positive feedback. In addition, they provided evidence for the release of the HMGB1 protein from the hepatocyte nucleus to the cytoplasm, intercellular space, and plasma of HFD-fed TLR4-WT mice as early as at 4 weeks. In contrast to the early release of HMGB1 protein, the prominent elevation of endotoxin in portal plasma was observed later than 8 weeks in vivo in response to HFD, suggesting that HMGB1 acts as a ligand for activation of TLR4 signaling in hepatocytes in the onset of NAFLD.

More studies are required to explore the roles of other TLRs in the development of NAFLD and the precise regulatory mechanism of TLRs-mediated HMGB1 release and activation and the possible therapeutic strategies for preventing the activation of TLR4 signaling in patients with NAFLD by way of blocking the activation of HMGB1 in the future.

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