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REVIEW

Toll-like receptors in alcoholic liver disease, non-alcoholic steatohepatitis and carcinogenesis

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Abstract

Activation of innate immune systems including Toll-like receptor (TLR) signaling is a key in chronic liver disease. Recent studies suggest that gut microflora-derived bacterial products (i.e. lipopolysaccharide [LPS], bacterial DNA) and endogenous substances (i.e. high-mobility group protein B1 [HMGB1], free fatty acids) released from damaged cells activate hepatic TLRs that contribute to the development of alcoholic (ASH) and non-alcoholic steatohepatitis (NASH) and liver fibrosis. The crucial role of TLR4, a receptor for LPS, has been implicated in the development of ASH, NASH, liver fibrosis, and hepatocellular carcinoma. However, the role of other TLRs, such as TLR2 and TLR9 in chronic liver disease remains less clear. In this review, we will discuss the role of TLR2, 4, and 9 in Kupffer cells and hepatic stellate cells in the development of ASH, NASH, and hepatocarcinogenesis.

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Key words

intestinal microflora, liver fibrosis, LPS, MyD88, TLR4.

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Nonstandard abbreviations used

ALD, alcoholic liver disease; ASH, alcoholic steatohepatitis; BM, bone marrow; CDAA, choline deficient amino acid defined; DAMP, damage-associated molecular pattern; DEN, diethylnitrosamine; HCC, hepatocellular carcinoma; HSC, hepatic stellate cells; JNK, c-Jun N terminal kinase; MyD, myeloid differentiation factor; NAFLD, non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis; PAMP, pathogen-associated molecular pattern; TAK1 Δ HEP, hepatocyte-specific TAK1 deleted; TBK1, TANK-binding kinase 1; TLR, Toll-like receptor; XBP-1, X-box binding protein-1.

Introduction

Toll-like receptors (TLRs) are a family of pattern-recognition receptors that play a critical role in the activation of innate immune system by recognizing pathogen-associated molecular patterns (PAMPs).^{1,2} Endogenous components derived from dying host cells, termed damage-associated molecular patterns (DAMPs), can also activate TLRs.^{3,4} To date, more than 10 members of the TLR family have been identified in mammals. TLRs are type I transmembrane proteins characterized by an extracellular leucine-rich domain and a cytoplasmic tail that is responsible for ligand recognition.^{1,2} After binding to corresponding ligands, TLRs transduce signals via myeloid differentiation factor (MyD) 88, a common signal adaptor molecule shared by IL-1 receptor and all members of TLRs except for TLR3.^{1,2} This cascade leads to

the activation of NF- κ B and results in the production of various proinflammatory cytokines, including TNF- α and IL-6. The MyD88-dependent pathway activates p38 and c-Jun N terminal kinase (JNK) as well. In contrast, TLR3 and TLR4 utilize MyD88-independent, TIR domain-containing adaptor-inducing interferon- β (TRIF)-dependent pathway. Subsequently, TRIF associates with TNF receptor-associated factor (TRAF 3 and TRAF 6) to activate TANK-binding kinase 1 (TBK1) and IkappaB kinase i (IKKi), which results in the activation of transcription factor IRF-3 (interferon regulatory factor-3) and induction of IFN- β .^{1,2} Accumulating evidence has demonstrated that TLRs play important roles in the pathophysiology of a variety of liver diseases,⁵ which may attribute to wide expression of TLRs on all types of liver cells, including hepatocytes,^{5,6} Kupffer cells,^{5,7} sinusoidal endothelial cells,⁸ hepatic stellate cells (HSCs),⁹⁻¹¹ biliary epithelial cells,⁵ as

well as immune cells such as liver dendritic cells.⁸ In this review, we summarize the recent findings regarding the role of TLRs in alcoholic liver disease (ALD), non-alcoholic fatty liver disease (NAFLD) and hepatocellular carcinoma (HCC).

Toll-like receptors 4 and alcoholic liver disease

It has been acknowledged for many years that chronic alcohol abuse causes hepatic steatosis, alcoholic hepatitis, and ultimately cirrhosis. The pathogenesis of ALD involves complex interaction between the direct effects of alcohol and its metabolite, acetaldehyde, in various cell types in the liver.^{12,13} Activation of Kupffer cells via TLR4 signaling is crucial in the pathogenesis of alcohol-induced liver injury. Since the disruption of intestinal barrier by ethanol generally increases permeability for macromolecular substances,¹⁴ Lipopolysaccharide (LPS) levels in systemic and portal blood are significantly increased in patients and animals with chronic alcohol consumption.^{15–17} Mice deficient in TLR4, CD14, and LPS binding protein (LBP) are resistant to alcohol-induced liver injury.^{16,18,19} Moreover, gut sterilization with antibiotics decreased plasma LPS levels, liver steatosis, inflammation, and injury in mice on chronic ethanol abuse.²⁰ Thus, it is conceivable that translocated LPS from the gut microflora activates hepatic TLR4 signaling in alcoholic liver disease. Although LPS alone fails to mimic the pathology of alcoholic steatohepatitis, ethanol administration increases the sensitivity to LPS-induced hepatocyte injury and cytokine production in the animal model.^{21,22} Previous studies emphasized the pathophysiological importance of TLR4 on Kupffer cells in alcoholic liver disease, however, lacked to demonstrate the role of TLR4 on HSCs.^{16,20} A recently published study investigated the relative contribution of TLR4 expressed on Kupffer cells and HSCs in ALD.²³ In addition to the previously established concept, it demonstrated that TLR4 signaling is important in both bone marrow (BM)-derived cells including Kupffer cells, and endogenous liver cells including HSCs for alcohol-induced hepatocyte injury, steatosis, inflammation, and fibrogenesis (Figure 1).²³ Moreover, activation of TLR4 signaling in HSCs is more important than TLR4 in Kupffer cells for HSC activation. In HSCs, activated TLR4 signaling downregulates transforming growth factor-beta (TGF- β) pseudoreceptor BAMBI, resulting in enhancement of TGF- β signaling.¹¹ BMP and activin membrane-bound inhibitor (BAMBI) downregulation is dependent on MyD88, but not TRIF.¹¹ Interestingly, the TLR4-TRIF-IRF3 dependent pathway is more important than the TLR4-MyD88 dependent pathway to develop alcoholic steatohepatitis, and the responsible cell types for the TLR4-TRIF-IRF3 pathway are BM-derived cells including Kupffer cells.^{12,24}

In addition to LPS, other bacterial products can be translocated into the portal vein in individuals with chronic alcohol consumption. In particular, bacterial DNA was found in serum and ascites of patients with advanced liver cirrhosis that lead to increases of cytokine production in peritoneal macrophages.^{25,26} Bacterial DNA, which is recognized by TLR9, sensitizes the liver to injury induced by LPS via upregulation of TLR4, MD-2, and induction Th1-type immune response in the liver.²⁷ Therefore, it is highly anticipated that TLR9 signaling will influence pathogenesis of alcohol liver disease. However, it has yet to be fully elucidated.

Expression of TLR1, 2, 6, 7, and 8 was elevated in wild-type mice that received the Lieber-DeCarli chronic alcohol-feeding model. The treatment with alcohol resulted in sensitization to liver inflammation and damage by TLR1, 2, 4, 6, 7, 8, and 9 ligands due to increased expression of TNF- α .²⁸ However, some investigations found deficiency in TLR2 had no protective effect on alcohol-induced liver injury in a chronic ethanol-feeding mouse model.²⁴

Taken together, it is clear that alcohol consumption leads to the activation of innate immunity via TLRs signaling. Recent studies demonstrated that TLR4 signaling contributes to the dissection of molecular mechanism in ALD, indicating the indispensable role of both Kupffer cells and HSCs in mediating the effect of gut-derived endotoxin in ALD and suggesting the role of other TLRs in modulation of alcohol-induced liver injury.

Toll-like receptors in NASH

Non-alcoholic steatohepatitis (NASH) is hepatic manifestation of metabolic syndrome. NASH is characterized by steatosis, inflammation, and progressive fibrosis that ultimately lead to the end stage of liver disease.^{29,30} Recent evidence suggests that overgrowth of intestinal bacteria and increased intestinal permeability are associated with the development of NASH.^{31,32} In chronic liver disease, including NASH, intestinal permeability is increased due to bacterial overgrowth or altered composition of bacterial microflora.³³ Systemic inflammation related to NASH also injures epithelial tight junctions,³¹ resulting in deregulation of intestinal barrier functions. Indeed, plasma levels of LPS were elevated in patients with chronic liver diseases, including NASH.³⁴ These findings suggest that hepatic immune cells might be exposed to high levels of TLR ligands derived from gut bacterial products, which might trigger liver injury in NASH. In fact, several reports demonstrated the importance of TLR4 and intestine-derived LPS in the animal model of NASH.^{35,36} Interestingly, pathological effect of TLR4 in Kupffer cells is achieved by inducing reactive oxygen species (ROS)-dependent activation of X-box binding protein-1 (XBP-1).³⁷ Moreover, other bacterial products such as bacterial DNA, a ligand for TLR9, was detected in the blood of the murine NASH model developed by 22 weeks of choline-deficient amino acid-defined (CDAA) diet feeding.³⁸ This evidence suggests that activation of TLR9 signaling plays an important role in the development of NASH. In CDAA diet-induced NASH, translocated bacterial DNA binds to TLR9 on Kupffer cells to produce IL-1 β , which in turn stimulates hepatocytes for lipid accumulation and cell death (Figure 1). Concurrently, IL-1 β activates HSCs to induce liver fibrosis.³⁸ Besides TLR4 and TLR9, TLR2 also plays a crucial role in the progression of NASH. In CDAA-diet induced NASH, TLR2 mediates liver inflammation and fibrosis, and the indispensable cell type expressing functional TLR2 is Kupffer cells. However, induction of hepatic steatosis is independent of TLR2 signaling (Figure 1).³⁹

Several plausible theories have been proposed to explain the ability of free fatty acids (FFAs) to activate TLR signals. Non-pathogenic substances, such as FFAs, and denatured host DNA may activate TLR2, TLR4, and TLR9.^{10,40–42} For instance, palmitic acid and oleic acid act through TLR4 on macrophages and 293 cells.⁴¹ Palmitic acid and stearic acid, potential TLR4 ligands, are abundant in dietary fat, and high levels of circulating FFAs have

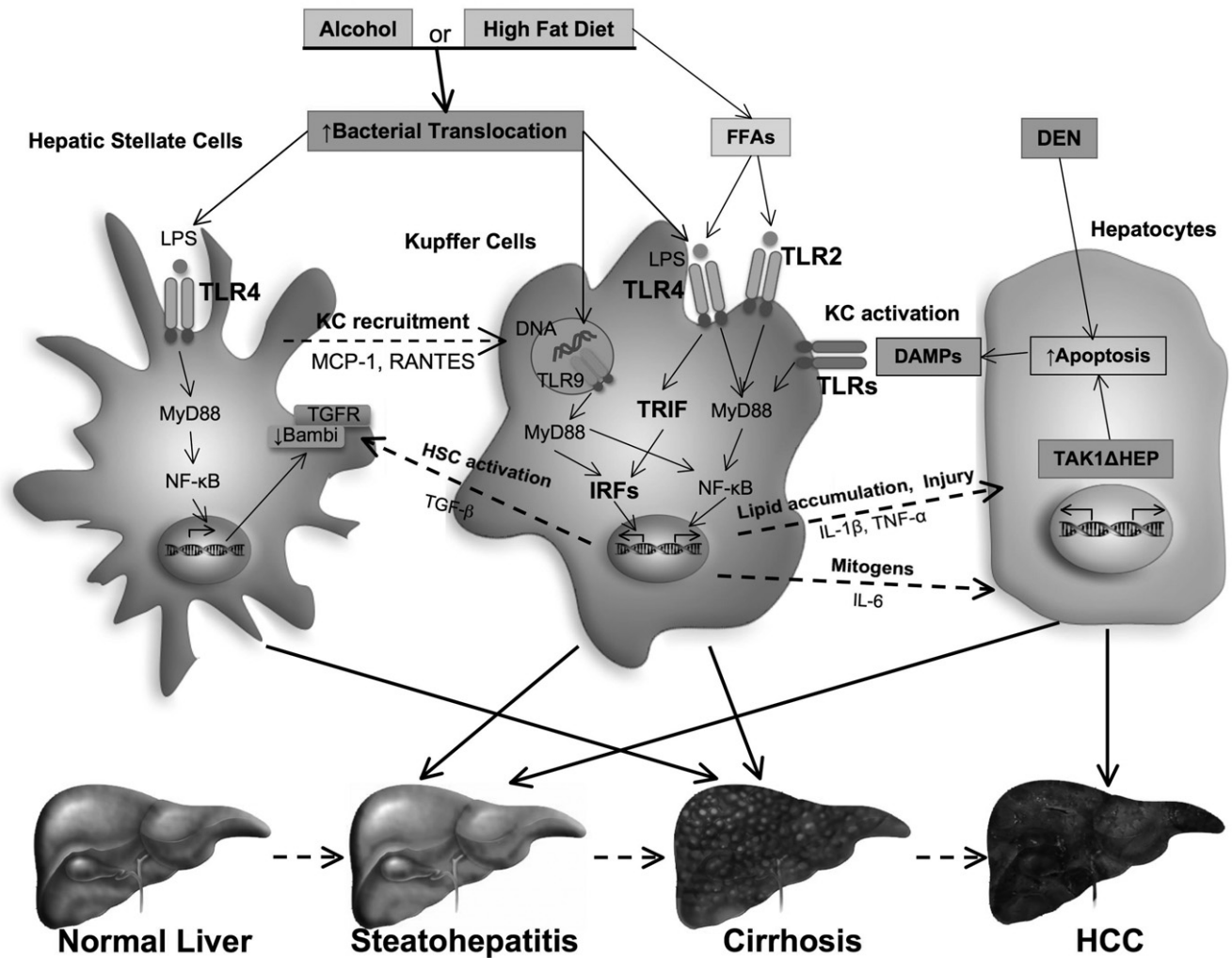


Figure 1 Role of Toll-like receptors (TLRs) in alcoholic liver disease, non-alcoholic fatty liver disease, hepatocellular carcinoma (ALD, NAFLD, and HCC).

Following alcohol consumption or excessive high-fat diet intake, bacterial translocation occurs due to the overgrowth of intestinal bacteria or disruption of intestinal barrier functions. Translocated intestine-derived pathogen-associated molecular patterns (PAMPs; lipopolysaccharide [LPS] and DNA) activate TLR signaling cascades in multiple hepatic cell types that regulate the inflammatory response. TLR2, TLR4 and TLR9 activation on Kupffer cells (KCs) induces production of various cytokines such as transforming growth factor-beta (TGF-β), IL-1β and TNFα, that subsequently induce hepatic stellate cell (HSC) activation and hepatocyte lipid accumulation and apoptosis. TLR4 activation on HSCs has also been shown to promote recruitment of KCs and directly augments fibrogenic response through downregulation of Bambi. Downstream signaling events include the MyD88-dependent NF-κB activation and TRIF-dependent IRF activation. Additionally, dead hepatocytes cause activation of KCs via TLRs and induce production of inflammatory cytokines and mitogen such as IL-6, which promote the HCC development. Consequently, hepatic TLRs play a pivotal role in the four sequential hallmarks of liver disease: steatosis, steatohepatitis, fibrosis, and HCC.

been observed in patients with NAFLD.⁴³ In addition, it is intriguing that the lipid component of LPS is sufficient to trigger TLR4 signaling. In particular, a medium-chain fatty acid component of LPS, lauric acid, has been shown to initiate TLR4 signaling in a macrophage cell line.^{44,45} These data suggest a strong relevance between TLR4 and lipid components. However, several reports have shown that FFAs do not directly stimulate TLR4 signaling.^{46,47} Since hepatocytes undergo apoptosis and necrosis in NASH, liver cells may constantly be exposed to denatured host DNA. However, it is not clear whether host DNA is a functional

ligand for TLR9. Indeed, the unmethylated CpG-motif is uncommon in mammalian DNAs.⁴⁸ Although some FFAs and denatured host DNA are attractive candidates for TLR ligands, further investigations are needed to determine whether these substances are capable of activating TLRs in NAFLD.

Recent studies have focused on TLR signaling in Kupffer cells that mediates the progression of simple steatosis to NASH. Other resident liver cells and BM-derived immune cells also produce various mediators modulating the severity of NAFLD in response to TLR ligands. Thus, understanding of cell type-specific TLR

signaling will provide new insight into the therapeutic management of NAFLD.

Toll-like receptors and carcinogenesis

Approximately 80% of HCC are preceded by chronic liver inflammation, fibrosis, and cirrhosis.⁴⁹ Under the pathologic conditions, the liver may be exposed to various TLR ligands via the portal vein, leading to an uncontrolled activation of innate immunity that may result in inflammatory liver diseases.⁵ Many factors are capable of activating TLRs in the liver. Among them, fibrosis, hepatitis B and C infection, alcoholic liver disease, and NASH are important etiologies for HCC. Therefore, it seems clear that TLRs play a role in the inflammation-associated liver cancer development. The chemical carcinogen, diethylnitrosamine (DEN), induces hepatocyte death, compensatory proliferation, and eventually HCC development in mice, closely resembling human HCC with poor prognosis.^{50,51} Mice deficient in TLR4 and MyD88, but not TLR2, have marked decreases in the incidence, size, and number of chemical-induced liver cancer, indicating a strong contribution of TLR signaling to hepatocarcinogenesis.^{5,52} Recently, two reports demonstrated that gut microbiota and TLR4 play a role in HCC promotion, but not in HCC initiation, mediating increased proliferation, production of proinflammatory cytokines (TNF- α , IL-6), expression of the hepatomitogen epiregulin, and prevention of apoptosis. Interestingly, gut sterilization, germfree status or TLR4 inactivation significantly reduced the development of HCC.^{53,54} Clinical and epidemiological evidence implicates long-term alcohol consumption in accelerating hepatitis C virus (HCV)-mediated tumorigenesis. HCV NS5A transgenic mice with long-term alcohol feeding develop typical tumors associated with NS5A mediated TLR4 overexpression in hepatocytes.⁵⁵ Recently, we reported that hepatocyte-specific TAK1 deleted (TAK1 Δ HEP) mice generated by intercrossing TAK1-floxed mice with Alb-Cre mice showed spontaneous HCC development.⁵⁶ In TAK1 Δ HEP mice, additional deletion of MyD88, TLR4 or TLR9 signaling provides a resistance for HCC development (Seki, unpublished data; Figure 1).

To understand liver tumorigenesis, it is very important to analyze which cell types are involved in the process. Kupffer cells may be the major cells expressing TLRs in the liver. Kupffer cells are liver tissue macrophages and express most of the major TLRs. In contrast, hepatocytes, the liver parenchymal cells, only show weak TLR2 and TLR4 expression and less response against their ligands.⁵⁷ TLR2 expression in hepatocytes is upregulated by LPS, TNF, and others, which suggest that hepatocytes become more sensitive in the inflammatory condition.⁵⁸ It is assumed that dying hepatocytes following DEN may activate myeloid cells such as Kupffer cells via TLRs and induce proinflammatory cytokines and hepatomitogens, such as IL-6, which enhance the development of HCC.^{54,59} However, Schwabe and colleagues argued that TLR4 on resident liver cells, but not BM-derived cells, is required for promotion of HCC.⁵³

In conclusion, there is clear evidence that TLRs and MyD88 signaling is associated with hepatic inflammation and hepatomitogen expression, which appear to be essential for hepatocarcinogenesis. These observations suggest that better understanding of TLR signaling pathways in the liver will help to clarify the mechanisms of liver tumorigenesis and provide new therapeutic targets for HCC.

Conflict of interest

The authors do not have any conflict of interest.

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