REVIEW ARTICLE

Toll-like receptors and liver disease

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Abstract

Toll-like receptors (TLRs) are pattern recognition receptors that play an important role in host defence by recognizing pathogen-associated molecular patterns (PAMP). Recent studies indicate that TLR signalling plays an important role in progression of chronic liver diseases. Ongoing clinical trials suggest that therapeutic manipulation of TLR pathways may offer novel means of reversing chronic liver diseases. Upon activation by their respective ligands, TLRs initiate an intracellular pro-inflammatory/anti-inflammatory signalling cascade via recruitment of various adaptor proteins. TLR associated signalling pathways are tightly regulated to keep a check on inappropriate production of pro-inflammatory cytokines and interferons thereby preventing various autoimmune and inflammatory processes. Herein, we review the current state of knowledge of hepatic distribution, signalling pathways and therapeutic modulation of TLRs in chronic liver diseases.
In an environment filled with limitless toxic chemicals and microorganisms, host survival requires a highly efficacious and dynamic immune system. Broadly, two separate yet interlinked immune systems, innate and adaptive immunity, coexist. Innate immunity encompasses responses that are universal, rapid, highly conserved and present from birth and generally form the first line of defense, whereas adaptive immunity responses are highly specific and develop more slowly following exposure to toxins or pathogens. Various classes of pathogens (e.g. bacteria, fungi, virus and parasites) have specific highly conserved structures called Pathogen-associated molecular patterns (PAMP). PAMP are relatively invariant and microbes cannot mutate them to avoid host defence mechanisms. Host recognition of PAMP involves specific protein receptors collectively known as the pattern recognition receptors (PRR) (1, 2). Toll-like receptors (TLRs) constitute the most important class of PRR (3).

**TLR Structure**

TLRs have a single trans-membrane domain along with an extracellular leucine-rich repeat domain and an intracellular cytoplasmic domain (TIR) (Fig. 1). The latter domain shares homology to interleukin 1 receptor (4). In total, 13 TLRs are known to be present in mammals, but only TLRs 1–10 are expressed and functional in humans. Expression of distinct TLRs with highly variable extracellular domain plays a pivotal role in recognizing different PAMP, thereby diversifying the spectrum of the body’s innate immune response.

**Distribution and ligands of TLR**

TLRs are widely distributed throughout the body and are expressed by endothelial, epithelial, mesenchymal and various immune cells (Table 1) (5). Despite being widely distributed their expression is highly regulated and specific, such that monocytes and macrophages express all TLRs except TLR3, whereas B and T lymphocytes express TLRs 3, 7, 9, 10 and TLRs 1, 2, 4, 5, 9 respectively (6). In immune cells, TLRs 1, 2, 4, 5, 6 and 10 are located within the plasma membrane, whereas TLRs 3, 7, 8 and 9 are intracellularly linked to endosomal membranes (7). This distinctive compartmentalization helps to prevent redundant activation of TLR signalling, thereby allowing a secure control over production of various inflammatory mediators like cytokines and interferons (8). In lieu of distinct pathogenic exposure, organ specific expression of various TLRs has also been noted i.e. hepatic expression, detailed below, differs from lung expression (9).

Ligands for TLRs include the PAMP and the death-associated molecular proteins (DAMP: endogenous ligands both intracellular and extracellular, which are released in response to cell necrosis or tissue injury respectively). PAMP include bacterial lipoprotein, lipopolysaccharide and nucleic acid structure. DAMP include but are not limited to heat shock proteins (10), IgG chromatin complex (11), versican (12), high mobility group protein B1 (13) and heparin sulphate (14).

Heterodimers of TLR1/2 recognize triacyl lipopeptides and Gram-positive bacteria, whereas TLR2/6 heterodimers bind diacyl lipopeptides. TLRs 4 and 5 recognize Gram-negative bacteria, DAMP and monomeric flagellin. Furthermore, TLRs 7 and 8 are activated by ssRNA in contrast to TLRs 3 and 9, which respond to dsRNA and unmethylated CpG motifs in bacterial DNA. Among human TLRs, TLR10 is the only family member without a definite known ligand, function and localization. Phylogenetic analysis reveals that TLR10 is closely related to TLR1 and TLR6, both of which mediate immune responses in cooperation with TLR2 (15). Chimeric receptor studies containing the extracellular recognition domain of TLR10 and the intracellular signalling domain of TLR1 revealed that TLR10 senses triacylated lipopeptides and a wide vari-

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**Fig. 1.** TLR structure: TLRs are type 1 transmembrane proteins that have distinct extracellular domains but homologous intracellular domains. Although distinct, the extracellular domains typically have leucine-rich repeat domains. The intracellular domain (TIR) shares homology to the interleukin 1 receptor and has potential binding sites for intracellular signalling molecules. Certain TLRs (e.g. TLR1/2 and TLR2/6) may form heterodimers with each other, which affects ligand binding.
ety of other microbial-derived agonists shared by TLR1 (16). TLR10 is also highly expressed in B cells, suggesting that it may play a critical role in B cell function (17).

**TLR Signalling**

On binding to their respective ligands, TLRs initiate an immune response both in infectious and non-infectious states. Apart from TLRs prime role in activation of an innate immune response, they also play a vital role in maturation of antigen-presenting cells (APC) (18), which may activate the adaptive immunity.

Upon formation of TLR-ligand complexes, an intracellular pro-inflammatory/anti-inflammatory signalling cascade is stimulated via recruitment of various adaptor proteins. The adaptor proteins include: the Myeloid differentiation factor 88 (MyD88), TIR domain-containing adaptor protein (TIRAP), TIR domain-containing adaptor protein inducing interferon-β (TRIF) and TRIF-related adaptor molecule (TRAM). These adaptor molecules play an important role in multiple signalling pathways, thereby enabling the TLR–ligand complex to activate multiple pathways leading to expression of pro-inflammatory/anti-inflammatory cytokines and interferons. TLR signalling cascade progression may be MyD88 dependent or MyD88 independent. All TLRs except TLR3 utilize the MyD88-dependent pathway, whereas TLR4 signalling is unique in its ability to progress via both pathways (Fig. 2).

**MyD88 dependent pathway**

TLRs 1/2, 2/6, 4, 5, 7, 8 and 9 are known to function via a MyD88-dependent pathway (1). Upon binding with their ligands, TLRs 1/2, 2/6 and 4 utilize adaptor protein TIRAP to indirectly activate the MyD88-dependent pathway. On activation, MyD88 further recruits IRAK-1 and 4, TRAF-6 and TAK-1 (3). Activation of TAK-1 increases expression of transcriptional factors IKK-β and α, NF-kβ and also induces the MAP kinase pathway thereby producing another transcriptional factor, AP-1. NF-kβ and AP-1 induce transcription of various pro-inflammatory cytokine mRNA, e.g. IL-1, TNF-α.
Activation of TLRs 5, 7, 8 and 9 also causes production of transcription factors NF-κB and AP-1, but does not require TIRAP to activate MyD88. TLRs 7 and 9 recruit IRAK-1, 4 and TRAF-6 that phosphorylates transcriptional regulator IRF-7, which induces expression of type 1 interferon mRNA.

Co-immunoprecipitation experiments indicate that similar to TLR2/1, TLR2/10 complexes utilize the MyD88-dependent pathway (15). However, the cytoplasmic domain of TLR10 neither alone nor along with TLR2 is able to activate typical TLR-induced signalling pathways (15). Therefore, human TLR10 cooperates with TLR2 in the sensing of microbes and fungi, but possesses a signalling function that is still enigmatic.

MyD88-independent pathway

On stimulation by its ligand, TLR3 directly binds TRIF as an adaptor protein. Although TLR4 also recruits TRIF, it utilizes TRAM as a mediator to recruit TRIF (1, 3). TRIF further recruits TRAF3, TBK-1 and IKK, which in turn phosphorylates transcription factor IRF-3, thereby inducing IFN-β mRNA expression. TRIF also associates with RIP-1, which, via the TRAF-6 intermediate stimulates the expression of NF-κB and AP-1 (18), culminating in increased production of various pro-inflammatory cytokines.

Regulation of TLR Signalling

Certain organs (e.g. liver and gastrointestinal tract) are constantly exposed to microbes, both pathogenic and non-pathogenic, commensal organisms; therefore, TLR-associated signalling pathways must be tightly regulated to keep a check on inappropriate production of pro-inflammatory cytokines and interferons. Any breach in these regulatory mechanisms may predispose to various autoimmune and chronic inflammatory diseases. The TLR signalling cascade is regulated at multiple levels, including receptor, adaptor protein, as well as at a nuclear level.

Triad domain-containing protein 3 (TRIAD3A) (19), Radio protective 105 (RP105) (20), soluble TLR2 (sTLR2) and sTLR4 (21), and Single Ig IL1 receptor-related molecule (SIGGR) (22) regulate the TLR signalling at the receptor level. MyD88s (23), Src homology 2 (SH2) domain-containing protein tyrosine phosphatase-1 (SHP-1) (24), Peptidyl-prolyl cis/trans isomerase (PIN1) (25), Suppressor of cytokine signalling (SOCS-1) (26–28), Cylindromatosis (CYLD) (29) and IRAK-M (30) play an important role in signalling regulation by modulating various intracellular signalling and adaptor proteins. TRAIL-R (31) and Bcl-3 (32) control the production of cytokines and interferon by adjusting production, activity and nuclear translocation of various TLR-activated transcription factors. This multi-tiered regulation permits a finely balanced immune response and highlights the importance of TLR signalling in innate immune responses.

Hepatic expression of TLRs

Seventy five to 80 per cent of the liver’s blood supply comes from the portal vein, which drains the gastrointestinal tract. In spite of a constant inflow of gut-derived microbes to the liver, liver TLRs are not constantly activated. This high threshold for activation of liver TLRs has been referred to as tolerance. Tolerance is associated with low level expression of TLRs and signalling molecules such as MD2 and MyD88 (33) and up-regulation of IRAK-M (30).

The liver plays diverse roles in the body and to carry out such diverse functions, a variety of cell types are necessary. Broadly, liver cells can be divided into parenchymal (hepatocytes) and non-parenchymal cells, a mixed group of cell types. Each cell type has a distinct function and a highly specific distribution of TLR (Fig. 3).

Hepatocytes

Hepatocytes, parenchymal liver cells, constitute 60 per cent of liver cells and carry out most of the synthetic and metabolic functions of the liver. Using RT–PCR, it has been shown that hepatocytes contain transcripts of TLRs (1–9) and are responsive to multiple PAMP (33–35). Hepatocytes are known to uptake and eliminate LPS from the portal circulation and excrete it into bile (36). The uptake of LPS by hepatocytes in vivo occurs via a CD14-TLR4-MD2-dependent mechanism, and is mediated by β2-integrin-induced activated p38 MAPK (37).

Non-parenchymal cells (NPC)

Kupffer cells

Kupffer cells are resident macrophages in the liver that reside in the sinusoidal space and phagocytose circulating particles and microbes. They express TLRs 2, 3, 4 and 9 (38, 39), although their threshold for activation is higher than other immune cells given their milieu (40). CD14, a lipopolysaccharide-binding protein, has been implicated in LPS-induced activation of TLR4 on Kupffer cells (41). Upon activation, Kupffer cells produce a myriad of pro-inflammatory mediators such as IL-6, IL-12, IL-18 and TNF-α, as well as an anti-inflammatory mediator IL -10 (42).

Hepatic stellate cells

Hepatic stellate cells are resident hepatic fibroblasts localized to the peri-sinusoidal space of Disse. HSC play a central role in development of liver fibrosis and may also play a role in regulating immune responses. HSC
are known to express TLRs 2, 4 and 9 (43–45). HSC stimulation with PRR-specific ligands causes activation of the MAP kinase as well as NF-κB pathways followed by secretion of TGF-β, IL-6, IL-8 and various chemokines such as (MCP-1, MIP-2 and ICAM-1). In turn, TGF-β, a potent stimulator of collagen deposition paves the pathway towards fibrosis/cirrhosis. Chimeric C3H/HeJ mice with a TLR4 mutation limited to HSC have decreased hepatic fibrosis in response to LPS, supporting a key role for HSC in linking hepatic inflammation and fibrosis (46). A similar effect was not noted in chimeric C3H/HeJ mice with a TLR4 mutation in Kupffer cells.

**Biliary epithelial cells**

Biliary epithelial cells form the intrahepatic biliary ducts and mainly express TLRs 2, 3, 4 and 5 (47). They are frequently exposed to various gut-derived microbes ascending the biliary tree from the duodenum. Under non-pathogenic conditions, increased expression of IRAK-M plays a vital role in preventing unwanted activation of the TLR signalling cascade, whereas under inflammatory conditions, up-regulation of BEC-associated TLRs was associated with IFN-γ and TNF-α exposure (48). Pro-inflammatory chemokines released by BEC play an important role in biliary pathogenic responses.

**Sinusoidal endothelial cells**

Sinusoidal endothelial cells line the liver sinusoids and express TLR4 and 9 (49, 50), but the importance of the TLR signalling in SEC in inflammatory responses is not clear. SEC may have an important role as antigen-presenting cells (APC) (51).

**Bone marrow-derived immune cells**

Various bone marrow-derived immune cells including B cells, T cells, NK cells and dendritic cells circulate through the liver and migrate into the liver parenchyma. Natural Killer (NK) cells express transcripts of TLR (1–10) (52–54). Ligands for TLRs 2 and 3 directly stimulate the NK cells, whereas ligands for TLRs 7, 8 and 9 stimulate them indirectly (55). Upon activation, NK cells produce IFN-γ and regulate liver injury as well as regeneration. Whether, TLRs on the other immune cells within the liver play an important role in hepatic

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**Fig. 3.** Diagrammatic representation of the liver and its various cell types along with their location and distinct TLR expression. Under non-inflammatory conditions, expression levels of TLRs are much reduced among non-immune cells within the liver. The hepatocyte is the parenchymal cell of the liver. Bile (yellow) is secreted into the cannicular space between hepatocyte couplets and eventually drains into ducts formed by biliary epithelial cells (BEC). Kupffer cells are resident hepatic macrophages that remain within the hepatic sinusoids formed by sinusoidal epithelial cells (SEC). Other immune cells, such as myeloid dendritic cells (MDCs), plasmacytoid dendritic cells (PDCs) and natural killer cells (NK cells), pass through the sinusoids. Stellate cells reside within the perisinusoidal space of Disse (PSD) between hepatocytes and SEC. The sinusoidal membrane is fenestrated, such that ligand molecules passing through the sinusoids may come into direct contact with stellate cells and hepatocytes.
inflammation is uncertain. B cells express TLRs 1, 7, 9 and 10 (51), but TLRs do not play a major role in antibody production (56). T cells express TLRs 1, 2, 4, 5 and 9 (6, 51). Although T cells demonstrate significant mRNA levels of TLRs 2 and 4, only activated T cells express surface TLRs 2 and 4. In recent studies, it has also been shown that TLR4 receptors on T cells do not respond to LPS (57). Reduced TLR4 expression by DC confers tolerance to LPS-induced activation; however, this tolerance can be overcome by conditions (e.g. chronic liver disease) that lead to above normal LPS levels (58).

TLRs in liver diseases

The Liver represents a major target of bacterial PAMP in many disease states. There is increasing evidence that TLRs play an important role in the pathogenesis and progression of many liver diseases including non-alcoholic fatty liver disease (NAFLD), alcoholic liver disease (ALD), viral hepatitis, autoimmune liver disease, hepatic fibrosis and liver cancer (18, 59–62). The extent of TLR involvement in each disease or condition is being actively investigated in anticipation of future therapeutic interventions.

Non-alcoholic fatty liver disease

Currently, NAFLD is the most common chronic liver disease in the USA (63). Its pathologic spectrum ranges from simple fatty liver (hepatic steatosis) to cirrhosis with intervening steatohepatitis. Many animal models have demonstrated increased serum PAMP levels in non-alcoholic steatohepatitis (NASH) (45). Recently, similar results have also been shown in NAFLD patients (64, 65). Alterations in intestinal flora and increased gut permeability have been held responsible for increased serum PAMP levels in NASH (66, 67). These PAMP are known to stimulate various TLRs, thereby inducing hepatic inflammation via production of various inflammatory mediators such as cytokines, chemokines and interferons. Depending upon the model investigated, animal models have suggested that TLRs 2, 4 and 9 may play role in NAFLD onset or progression. Methionine choline-deficient (MCD) diet has widely been used to study NAFLD/NASH model in mice. Wild-type (Wt) mice fed on MCD diet have increased evidence of steatohepatitis, endotoxaemia and increased TLR4 expression, whereas TLR4-mutant mice showed much less liver injury despite similar levels of endotoxaemia (45). Allina et al. have suggested that air particulate matter stimulation of TLR4 on Kupffer cells enhances progression of NAFLD in mice fed high-fat chow (68). Wt mice fed on choline-deficient, l-amino acid diet developed NASH characterized by steatosis, inflammation and fibrosis, whereas TLR9−/− mice showed significantly less steatohepatitis and fibrosis (69). The role of TLR2 in the pathogenesis of NASH/NAFLD is still not clear. Recently, murine mice models have demonstrated the importance of TLR2 in diet-induced obesity, insulin resistance and hepatic steatosis (70). In contrast, Szabo et al. have demonstrated a protective role of TLR2 in fatty liver disease (71). Further studies are needed to delineate the role of TLR2 in human NAFLD.

Alcoholic liver disease

As in NAFLD, not all alcoholics develop significant liver disease, indicating a second factor to be necessary for ALD progression (72). Progression of ALD may depend on Kupffer cell activation by gut-derived microbes. Multiple studies have demonstrated the role of alcohol in increasing gut permeability by disrupting tight junctions (73, 74), thereby increasing plasma LPS levels (73). A significant reduction in liver injury was noted upon gut flora alteration by antibiotic (75) and probiotics (76). The composition of gut flora and its ability to activate hepatic TLRs may be a key factor in progression of ALD. Under non-inflammatory conditions, Kupffer cells, known to play a critical role in pathogenesis and progression of ALD, secrete anti-inflammatory cytokines, e.g. IL-10, whereas post-LPS mediated TLR4-dependent stimulation, Kupffer cells produce large amounts of pro-inflammatory cytokines including TNF-α, IL-1, IL-6 and IL-8, chemokines and profibrogenic factors such as TGF-β, which mediate liver inflammation and stellate cell activation (60, 77, 78). In turn, the activated stellate cells induce liver fibrosis.

CD14 and lipopolysaccharide-binding protein (LBP) are important for LPS-induced activation of TLR4 on Kupffer cells. C3H/HeJ mice, which have mutated TLR4 genes showed no signs of liver injury on chronic alcohol exposure (79) and a similar reduction in ALD was seen in CD14-deficient mice (80) or when Kupffer cells were depleted with gadolinium chloride (7). Hritz et al. have also demonstrated TLR4-dependent downstream signaling cascade in alcoholic liver disease to proceed via the MyD88-independent pathway (81). This was further confirmed by reduced injury in TLR4-deficient but not MyD88-deficient mice.

In a study by Gustot et al. using the Lieber-DeCarli chronic alcohol fed mouse model, increased expression of TLR1, 2, 6, 7, 8 and 9 was observed in addition to TLR4 (82). Upon exposure to their respective ligands, increased TNF-alpha mRNA expression was seen in the livers of EtOH-fed mice compared with control mice, thereby demonstrating a link among TLRs, alcohol and inflammation.
**Hepatitis B**

Hepatitis B is a worldwide health problem and is expected to affect one-third of the global population. In the USA, there are an estimated 1.25 million hepatitis B-infected individuals (83) with an increased risk of developing cirrhosis, hepatic decompensation and hepatocellular carcinoma (HCC). Interpersonal variations in outcomes of HBV infection and treatment are dependent on host susceptibility to virus and up-regulation of antiviral immune response (84).

HepG2 cells, a hepatoma cell line able to replicate hepatitis B virus (HBV), are often used for HBV research as an in vitro model. Total RNA was extracted from HepG2 cells and TLR expression was studied; TLRs 2, 3, 4, 5, 6, 7 and 9 mRNA were highly expressed, whereas TLRs 1, 8 and 10 showed no or very weak expression (85). Activation of TLRs 2, 3, 4, 7 and 9 by their respective ligands induced an IFN response in HepG2 cell and inhibited HBV DNA replication and RNA transcription (85). In another in vitro study, transfection of HBV-positive cell lines with TLR adaptor molecules led to increased TLR activity, thereby significantly reducing levels of HBV DNA and mRNA (86). In HBV transgenic mice, viral replication was almost completely abolished in a non-cytopathic and a non-inflammatory way by the administration of 10 μg of TLRs 3, 4, 5, 7 and 9 ligands (87). Also, inhibition of replication was associated with intrahepatic increase in IFN-α/β. The antiviral effect of TLR ligands was annulled in the IFN-α/β receptor knock-out HBV transgenic mice (88).

Recently, studies in HBeAg-positive chronic hepatitis B (CHB)-infected subjects have shown significant down-regulation of TLR2 on peripheral blood monocytes, hepatocytes and Kupffer cells in their livers (88). In contrast, HBeAg-negative CHB patients showed up-regulation of TLR2 and cytokine expression. Therefore, HBeAg-induced down-regulation of TLR2 via precore protein may be responsible for rapid progression of disease in HBeAg-positive patients (88). In conclusion, TLR activation on both parenchymal (84) and non-parenchymal liver cells (87) may repress HBV replication.

**Hepatitis C**

Hepatitis C (Hep C) is the most common cause of liver transplantation in the USA. The ability of hepatitis C virus (HCV) to dampen the immune system, thereby maintaining an innate environment congenial to its persistence, has been revealed (59, 89). Treatment with IFN-γ attempts to re-invigorate antiviral immune responses within the liver.

Increased mRNA levels for TLRs 2, 6, 7, 8, 9 and 10 were shown in both monocytes and T cells of Hepatitis C-infected individuals (90, 91). Up-regulation of TLRs 2 and 4 protein expression in peripheral blood monocytes (PBMC) has also been shown via flow cytometric analysis in chronic Hep C-infected individuals (91). Upon exposure to TLR 2 and 4 ligands, PBMC from chronic Hepatitis C-infected individuals exhibited a greater increase in cytokine production than PBMC from healthy subjects (91). In contrast, suppressed expression of TLRs 3 and 7 in cells transfected with the entire HCV genome and hepatoma cells with HCV expression, respectively, has been shown (92, 93). Earlier studies had demonstrated HCV-induced down-regulation of TLR3–TRIF–TBK1–IRF3 and TLR–MyD88 pathway. NS5A/4A was shown to inhibit IRF3 phosphorylation and activation, block TLR3-mediated signalling pathways and proteolyse TRIF (94–96). Also, NS5A inhibited cytokine production via interaction with MyD88 through the IFN sensitivity-determining region (ISDR) and thereby inhibiting IRAK 1 recruitment (89). Thus, HCV-induced inhibition of TLR signalling pathways may play a vital role in its chronicity. In a clinical study by Horseman et al., once-daily treatment with intravenous isatibarine (800 mg), a selective TLR7 agonist, for 7 days caused a significant reduction in viral load compared with untreated subjects. Furthermore, the treatment was well tolerated and showed a low frequency of mild-to-moderate side effects (97). Kawai et al. have also demonstrated the ability of a TLR7 agonist to decrease HCV RNA in infected subjects (98).

Recently, specific polymorphisms in TLR genes have been associated with more advanced Hepatitis C infection (99,100). These polymorphisms possibly contribute to the variable rate of disease progression and treatment responses in Hepatitis C-infected individuals. More functional studies and longitudinal studies are needed to delineate the role of these polymorphisms.

**Primary biliary cirrhosis**

Primary biliary cirrhosis is a slowly progressive, cholestatic autoimmune liver disease characterized by T cell-mediated destruction of small and medium-sized bile ducts within the liver. The biopsy findings in those with PBC show clustering of both autoreactive T cells and NK cells around small bile ducts (101). A potential role of TLR4 ligand-stimulated NK cells in destruction of BEC in the presence of TLR3 ligand-stimulated monocytes has been shown in PBC patients (102). Also, biliary LPS levels are increased in PBC patients and BECs from those with PBC showed highly elevated TLR4 expression as compared with controls (103). The latter innate immunity responses may be critical for PBC disease progression.

Other studies suggest that overall innate immunity may be impaired in those with PBC. Patients with PBC have increased incidence of recurrent urinary tract infections; furthermore, various studies have demonstrated significant association between urinary or vaginal infections and endotoxaemia in bile ducts as a result of elevated DAMP and PAMP levels, thereby suggesting that impaired innate immunity may play a role in the
pathogenesis and progression of PBC (104). However, stimulation of TLRs 2, 3, 4, 5 and 9 on peripheral blood mononuclear cells (PBMC) significantly increased levels of pro-inflammatory cytokines production in those with PBC compared with healthy controls (105).

A polyclonal increase in IgM levels is characteristic of PBC. RT–PCR studies have shown significantly increased mRNA levels of type-1 IFN and TLR3 in portal tract and liver parenchyma in early-stage PBC (106). In vitro stimulation of PBMC from PBC patients by TLR9 ligand has shown to increase production of IgM via induction of TLR9 receptors on B cells, thereby demonstrating the role innate immune system might play in regulation of adaptive immune responses (107).

**Primary sclerosing cholangitis**

Primary sclerosing cholangitis is a chronic cholestatic liver disease characterized by progressive periductal fibrosis around both intra and extrahepatic bile ducts (108). The mechanism for this increased periductal fibrosis remains unclear. In comparison with controls, sera of a significantly higher percentage of those with PSC had anti-BEC IgG antibodies (109). Recently, stimulation of BECs with PSC IgG, but not control IgG, has shown to induce expression of TLRs 4 and 9 on BECs (110). Upon stimulation with LPS and CpG DNA, TLR-expressing BECs produced high levels of interleukin-1ß, interleukin-8, interferon-γ, TNF-α, GM-CSF and TGF-β (110). TGF-β strongly activated HSC to produce collagen. Thus, PRR induced BEC production of cytokines and interferon may play an important pathogenic role in PSC.

**Autoimmune hepatitis**

Autoimmune hepatitis is characterized by immune-mediated destruction of hepatocytes and high titres of IgG and antinuclear autoantibodies. A variety of drugs and toxins are known to trigger autoimmune hepatitis, but typically the trigger is unknown. A few case reports have suggested that autoimmune hepatitis may be induced by a viral infection. In a mouse model, Lymphocytic Choriomeningitis Virus has been shown to induce AIH via activation of TLR3 on antigen-presenting cells (APC) (111). Activated APC secrete type-1 IFN and TNF-α/β. These pro-inflammatory mediators further activate Kupffer cells, hepatocytes and endothelial cells to produce a chemo-attractant CXCL-9, which in turn attracts CD8+ T cells to perpetuate the onset of AIH (111). Additional studies are needed in individuals with AIH to evaluate the importance of TLR3 activation.

**Fibrosis and cirrhosis**

A common response to any chronic liver injury is fibrosis. Upon continued liver insults, progression of fibrosis to cirrhosis may occur. HSC have been shown to play a central role in pathogenesis of fibrosis and cirrhosis. HSC, upon activation, transform into myofibroblasts and deposit type I collagen, thereby playing a vital role in pathophysiology of fibrosis and cirrhosis.

HSC can be activated either via pro-inflammatory cytokines, growth factors secreted by TLR4-LPS pathway stimulated Kupffer cells, or directly via LPS-TLR4-dependent stellate cell stimulation. However, quiescent stellate cells are somehow resistant to activation owing to high-level expression of BAMBI (bone morphogenetic protein and activin membrane-bound inhibitor) that inhibit TGF-β receptor signalling (112), whereas upon TLR4 activation, BAMBI expression is down-regulated leading to unrestricted activation of TGF-β-dependent signalling. A significant association between TLR4 mutant liver cells and reduction in liver fibrosis was demonstrated in bile duct ligated (BDL) mice (113). Furthermore, TLR4 SNPs (T399I and/or D299G) have been shown to confer reduced LPS responsiveness in cultured human or mouse HSCs. The SNPs reduced NF-κB activation and pro-inflammatory cytokine expression in HSCs and attenuated progression of fibrosis to cirrhosis (114).

Other TLRs may also regulate liver fibrosis. In experimental hepatic fibrosis models, reduced HSC activation and collagen deposition were also seen in TLR9 knockout mice (115). Deficiency of TLR3-induced NK cell-dependent apoptosis of HSC has been held responsible for progression of alcohol-induced liver fibrosis (116). Although TLR2-induced activation of quiescent HSC is very minimal, TNF-α, IL-1β treatment up-regulates TLR2 expression, thereby priming HSCs to increase NF-κB activation and IL-8 production in response to TLR2 ligands (117).

**Hepatocellular carcinoma**

Worldwide, liver cancer is the fifth most common cancer and the third most common cause of cancer mortality (118). HCC is also the most prevalent primary liver cancer. The presence of chronic hepatic inflammation and fibrosis, which are regulated by TLR activation as discussed above, in cirrhotic livers is known to promote HCC formation in approximately 10% of those with cirrhosis. TLR-induced, NF-κB pathway-dependent, cytokine and growth factor production by inflammatory cells has been suggested to modulate onset and progression of cancer in general (119).

Diethyl nitrosamine (DEN) is a well-known chemical carcinogen that induces HCC in mice. Upon treatment with DEN, mice deficient in TLR4 and MyD88 had a significant decline in incidence, size and number of liver tumours (59, 77). Various clinical and animal studies have suggested that alcohol and HCV act synergistically in progression of liver disease and increase risk for liver cancer. Recently, in a mice study, this collusion was shown to proceed via TLR4 with involvement of Nanog,
a TLR4 downstream gene (120). Dapito et al. (121) recently demonstrated that activation of the LPS–TLR4 pathway provided proliferative and anti-apoptotic signals to non-marrow-derived resident liver cells, thereby promoting HCC progression. Moreover, LPS reduction by gut sterilization or TLR4 inactivation reduced HCC by 80%, therefore providing a strong rationale for targeting the intestinal microbiota and TLR4 for the primary or secondary prevention of HCC. Hence, TLRs may be an important link between chronic liver inflammation and HCC formation. Further studies are needed to better understand this link.

Future therapy

In contrast to other organs, the liver is a forgiving organ with great regenerative powers. Morbidity and mortality from chronic liver disease nearly always requires the development of cirrhosis. Fortunately, progression of chronic liver disease to cirrhosis typically takes decades. Thus, interventions that even mildly inhibit the progression of liver disease might have a big impact on morbidity and mortality from liver disease. As discussed in this review, inhibition of hepatic TLR signalling, particularly TLR4 or TLR7 may have a direct impact on liver disease progression.

Not surprisingly, pathogens (HBV and HCV) have developed strategies to suppress or evade the hepatic TLR system to facilitate chronicity of infection. Therefore, therapeutic manipulation of the hepatic TLR system is of prime importance for the development of novel treatments for the management and cure of chronic inflammatory liver diseases. Various animal models (122) and currently running phase III clinical trials (123) involving use of small molecule agents, such as Lipid A and TAK-42, a TLR-4 antagonist, have shown some benefit in management of septic patients. Also, some oral medications (e.g. simvastatin, hydrochloroquine) already in use for other indications have been found to decrease TLR expression or activity (124). Such agents may prove broadly beneficial in the management of various chronic liver diseases. As TLRs are ubiquitously expressed, another beneficial goal would be to target anti-TLR therapy to the liver. First pass metabolism may limit non-hepatic effects of oral agents. For example, budesonide has proven quite useful in the treatment of autoimmune hepatitis and avoids systemic effects of prednisone therapy.

In the absence of cirrhosis, chronic liver disease is largely asymptomatic and not a major cause of morbidity or mortality. Because of inherently slow disease progression, clinical trials with large enrolments and clearly defined endpoints may be needed to confirm the benefits of TLR-based therapies (TBT) in preventing progression of chronic liver disease to cirrhosis and HCC. Alternatively, TBT can be tested as adjuvant therapy for those enrolled in trials of direct-acting agents (DAA) against HCV or in trials of chemotherapeutic agents against HCC. The promise definitely exists for ground breaking TBT to treat chronic liver disease in the near future.

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