Toll Like Receptors in Chronic Viral Hepatitis – Friend and Foe

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1. Introduction

Chronic viral hepatitis caused by Hepatitis B virus (HBV), Hepatitis C virus (HCV) and Hepatitis D virus (HDV) infection is among the most frequent causes for liver related morbidity and mortality worldwide. In recent years, it has become clear that not only the adaptive but also the innate immune system is involved in the pathogenesis of these infections. The innate immune system represents the initial line of host defense against invading pathogens. Germline-encoded pathogen recognition receptors (PRR), that are able to recognize specific structures of microorganisms, are an important component of this system. Amongst these, Toll like receptors (TLR) are a family of PRR perceiving a wide range of microorganisms, including bacteria, fungi, protozoa and viruses. Hepatitis viruses have evolved evading strategies to subvert the innate immune system of the liver which is of relevance for understanding the mechanisms that lead to chronicity of these infections and to develop novel therapeutic approaches based on these findings. Thus, recent studies suggested that TLR-based therapies may represent a promising approach in the treatment in viral hepatitis. This chapter focuses on the role of local innate immunity of the liver in the pathogenesis of chronic viral hepatitis.

2. Innate immunity

It has been suggested that the innate immune system is of particular relevance in the early phase of viral and bacterial infections (Fearon and Locksley, 1996). PRR become activated immediately after exposure to infectious agents and activation of downstream signaling pathways leads to the expression of effector molecules that limit microbial replication (Biron, 1998; Epstein et al. 1996). PRR sense evolutionary highly conserved structures, so-called pathogen-associated molecular patterns (PAMPs). Within this process the TLR system is one of the main players (Medzhitov & Janeway, Jr., 2000; Medzhitov, 2001).

2.1 Toll like receptor system

Activation of the TLR system leads to the expression of pro-inflammatory (IL-6, IL-12, TNF-α,…) as well as anti-inflammatory cytokines (IL-10,….) by responsive cell types. TLR7, -8 and -9 additionally initiate Interferon-α (IFN-α) expression after binding of their specific ligands. Stimulation of TLR3 and -4 results in expression of IFN-β as well as immunoregulatory cytokines (Akira et al. 2006; Takeda & Akira, 2005).
TLRs are associated with cellular membranes and have a highly conserved cytosolic domain with similarity to the Interleukin-1 receptor and are therefore called Toll/IL-1 receptor (TIR). Binding of a specific pathogen-associated molecular pattern (PAMP) to these receptors leads to recruitment and activation of adapter molecules. All TLRs except TLR3 are able to activate the myeloid differentiation primary response gene 88 (MyD88). TLR2 and -4 integrate the TIR domain containing adapter protein (TIRAP) to active MyD88. MyD88-dependent signaling further involves Interleukin-1 receptor associated kinases 1 (IRAK1) and -4 (IRAK4) and Tumor necrosis factor (TNF) receptor associated factor 6 (TRAF6) to dissociate the Nuclear factor Kappa B (NFκB)-Inhibitor IκB. This is followed by translocation of NFκB into the nucleus and transcription of immunoregulatory genes (Takeda & Akira, 2005). MyD88 signaling additionally activates mitogen-activated protein kinases (MAPKs) which further activate AP-1 signaling resulting in cytokine expression. MyD88 signaling of endosomally located TLR7, -8 and -9 additionally promotes activation of Interferon regulatory factor 7 (IRF-7) which initiates the expression of IFN-α (Honda & Taniguchi, 2006).

TLR3 signaling is MyD88 independent. Activation of the TIR domain containing adaptor inducing IFN-β (TRIF) results in phosphorylation of IRF-3 followed by induction of IFN-β expression. TRIF additionally mediates activation of TRAF6 leading to translocation of NFκB as described before. TLR4 activates the toll-interleukin 1 receptor domain containing adaptor protein (TIRAP) to activate MyD88-dependent signaling as well as the TRIF-related adaptor molecule (TRAM) to enable TRIF dependent induction of IFN-β (Akira & Takeda, 2004; Honda & Taniguchi, 2006; Takeda and Akira, 2005) (Figure 1).

**Fig. 1. Toll like receptor signaling**
Activation of Toll like receptors leads to recruitment of the adaptor molecules MYD88, TIRAP, TRIF and TRAM. Downstream signaling involves TAK1, MAPKs, TRAF3, TBK1 and IKKs leading to nuclear translocation of transcriptions factors (AP-1, NFκB, IRF-3 or IRF-7) and subsequent transcription of inflammatory genes.
2.2 Local immune system of the liver
The liver represents an immunological organ in which blood from the gastrointestinal tract, enriched with nutrients and antigens, flows through sinusoids in close contact to antigen-presenting cells (APC) and lymphocytes (Figure 2). Physiological functions of the liver include protein synthesis and metabolism as well as removal of pathogens and antigens from the blood. This necessitates a locally regulated immune system. Efficient elimination of pathogenic microorganisms derived from the gastrointestinal tract must be accompanied by tolerance induction for a large number of harmless antigens to avoid unnecessary damage of hepatocytes that represent two third of the total liver cell population (Knolle & Gerken, 2000). The remaining cells consist of non-parenchymal liver cells (NPC) including Kupffer cells (KC), liver sinusoidal endothelial cells (LSEC), stellate cells, dendritic cells (DC) and intrahepatic lymphocytes.

It has been proposed that the liver is an organ of tolerance induction rather than induction of immunity. Therefore, the different types of APC may contribute in different ways to reach homeostasis of the local microenvironment (Racanelli & Rehermann, 2006). LSECs represent about 50% of the NPCs and form a fenestrated monolayer separating hepatocytes from the blood stream. LSECs are able to perform receptor-mediated endocytosis or phagocytosis with comparable efficacy as DCs. Processed peptides are loaded onto the major histocompatibility complex (MHC) class I and II molecules and are presented to passing lymphocytes. KCs represent approximately 20% of the NPC population of the liver, located in the hepatic sinusoids, where they are in close contact to the blood and passing lymphocytes. This exposed location enables Kupffer cells to take up antigens or debris from the blood stream and to induce inflammation or maintenance of tolerance (Sun et al. 2003).

2.3 The role of liver cells as part of the local innate immunity
TLR have recently been recognized to play an important role in the pathogenesis of chronic hepatitis. Activation of TLR signaling pathways results in an antiviral state of liver cells, thereby offering the possibility for the development of novel therapeutic strategies. Hepatocytes express TLRs and are able to respond to stimulation with TLR ligands. The expression of TLRs was demonstrated for primary human and murine hepatocytes as well as hepatoma cell lines including HepG2 and Huh7. The functionality of the TLR pathways in these cell systems was shown by analysis of cellular responses to various TLR ligands (Preiss et al. 2008; Thompson et al. 2009; Xia et al. 2008; Wu et al. 2007; Zhang et al. 2009; Broering et al. 2008).

Non-parenchymal liver cells; Kupffer cells; sinusoidal endothelial cells and hepatic stellate cells are important players to mount local innate and adaptive immune responses in the liver (Kimura et al. 2002; Knolle & Gerken, 2000). Studies regarding the diversification of TLR signaling pathways in NPC revealed that KC respond to all TLR ligands by producing TNF-α or IL-6. Only TLR3 and TLR4 activation leads to expression of IFN-β in KC. In addition, TLR1 and -8 ligands significantly upregulate MHC class II and costimulatory molecules. For TLR8-activated KC, high levels of T cell proliferation and IFN-γ production could be shown in mixed lymphocyte reactions (MLR). Similarly, LSEC respond to TLR3 ligands by producing IFN-β, to TLR1-4, -6 and TLR8-9 ligands by producing TNF-α, and to TLR3 and -4 ligands by producing IL-6. Interestingly, LSEC failed to stimulate allogeneic T cells in MLR despite significant upregulation of MHC class II and costimulatory molecules in response to TLR8 ligands (Wu et al. 2009). Taken together, NPC display a restricted TLR-
mediated activation profile when compared to ‘classical’ APCs which may also explain, at least in part, their tolerogenic function in the liver.

Fig. 2. Cell populations of the hepatic microenvironment
The hepatocytes are lined by the liver sinusoid endothelial cells (LSEC), preventing direct cell to cell contact to passing leucocytes, Kupffer cells (KC), dendritic cells (DC) or lymphocytes (T cells). Hepatic stellate cells (HSC) are located in the Space of Dissé between LSECs and hepatocytes.

3. Chronic viral hepatitis
Currently, five different human hepatitis viruses have been characterized: Hepatitis A virus (HAV), hepatitis B virus (HBV), hepatitis C virus (HCV), hepatitis D virus (HDV) and hepatitis E virus (HEV). HAV is a single-stranded, unenveloped RNA virus encoding for a single polyprotein. HBV is a virus with a partially double stranded DNA genome that replicates through RNA intermediates. HCV is a single-stranded RNA virus coding for a polyprotein, which is cleaved into a capsid protein, two envelope proteins and 6 non-structural proteins. HDV is a defective RNA virus, similar to viroids, that only encodes the capsid antigen (delta antigen) and is dependent upon the HBV coinfection, in particular the surface protein (HBsAg), for production of infectious virus particles. HEV is a labile RNA virus, unrelated to the other known hepatitis viruses. Chronic infections of hepatitis viruses, that are caused by HBV, HCV and HDV in immunocompetent patients, are frequently associated with progression of fibrosis and the development of liver cirrhosis and primary hepatocellular carcinoma (Hayashi & Zeldis, 1993; Perrault & Pecheur, 2009; Wedemeyer & Manns, 2010; Aggarwal & Naik, 2009).
It has been suggested that the interaction between hepatitis viruses and the innate as well as the adaptive immune system determines the outcome of these infections. Thus, studies regarding specific interactions between viral proteins and components of the immune system may introduce important information about the establishment of chronic infection. Here, we focus on the role of non-parenchymal liver cells and hepatocytes as part of the innate immune system of the liver and their relevance in the pathogenesis of viral hepatitis.

3.1 Hepatitis B virus
Hepatitis B virus is a hepatotropic non-cytopathic DNA virus which belongs to the Hepadnaviridae family. An estimated 400 million people worldwide suffer from chronic HBV infection, reaching higher prevalence in Asia and Africa. Patients with chronic infections mostly remain asymptomatic while 10-30% of these individuals develop liver cirrhosis and liver cancer. Although the mechanisms that are involved in viral clearance and persistence are still not fully clarified, it is evident that cell-mediated immune responses play an important role for viral clearance as patients with chronic HBV infection usually fail to develop adequate HBV-specific immune responses. (Bertoletti & Gehring, 2006). Pegylated interferon $\alpha$ (IFN-$\alpha$) and nucleos(t)ide analogues are used for therapy of chronic hepatitis B.

3.2 Hepatitis C virus
HCV, a member of the Flaviviridae family, hepaciviridae genus, is a global health care problem as more than 2% of the world’s population (170 million individuals) has been infected with the this hepatotropic virus (Alter, 2007). Infection with HCV leads to chronic hepatitis in 70-80% of the cases, thereby promoting serious hepatic disorders as liver cirrhosis and hepatocellular carcinoma (Di Bisceglie, 1997; Di Bisceglie, 1998). As a consequence, chronic HCV infection is a main indication for liver transplantation. Currently, standard of care is a combination therapy of pegIFN and ribavirin for patients infected with all 6 HCV genotypes (Manns et al. 2001), while addition of protease inhibitors have been licensed for the treatment of genotype 1 patients. Recently published clinical trials indicated that triple treatment with the protease inhibitors Telaprevir or Boceprevir in combination with pegIFN and ribavirin, compared to standard treatment with pegIFN and ribavirin alone, significantly improved sustained virologic response rates in naïve patients with HCV genotype 1 infection. In addition, these triple treatments resulted in significantly improved sustained response rates in patients with chronic HCV genotype 1 infection, who were nonresponders or relapsers to previous treatments (Bacon et al. 2011; Jacobson et al. 2011; Zeuzem et al. 2011; Poordad et al. 2011).

3.3 Hepatitis D virus
Hepatitis D virus (HDV) has primarily been identified as an additional antigen during HBV infection. It has been shown, that HDV only occurs in HBV infected patients, because it is using HBsAg as envelope protein, which is necessary for the cell entry of HDV (Rizzetto et al. 1977; Rizzetto et al. 1980). About 20 million HBV infected individuals are thought to be co-infected with HDV, occurring either as a superinfection of chronic HBV infection or a concomitant acute coinfection of HBV and HDV (Hadziyannis, 1997). The pathogenesis of HDV infection is only poorly understood. Whereas clinical observations identified that hepatitis D could be an immune-mediated disease process, specific clinical cases suggested that HDV induces cytopathic infections (Nakano et al. 2001). Chronic Hepatitis D is...
associated with a severe course of hepatitis, frequently leading to rapid fibrosis progression and the development of hepatocellular carcinoma (Wedemeyer & Manns, 2010).

4. Interplay of forces

The local induction of type I IFNs (IFN-α, -β) during the early phase of infection with HBV and HCV (Bigger et al. 2001; McClary et al. 2000) is crucially important as they are thought to limit HBV as well as HCV replication (Frese et al. 2001; Guidotti et al. 1994) while induction of IFN-γ during progression of viral infection may additionally inhibit viral replication (Frese et al. 2002; Guidotti et al. 1996).

4.1 Innate immunity against HBV

The role of the innate immune system during the early phase of HBV infection has been investigated in different experimental systems. Wieland et al. investigated the transcriptome of the liver in three chimpanzees during the course of acute HBV infection (Wieland et al. 2004). Their analysis focused on two diverse groups of cellular genes: those in the early phase are associated with the innate immune response, and those in the late phase are associated with the adaptive immune response that terminates infection. They demonstrated that this virus does not induce any genes during entry and expansion, leading the authors to suggest that HBV is a ‘stealth virus’ in the early phase of infection. By contrast, a large number of IFN-γ-regulated genes are expressed in the liver during viral clearance (Figure 3). This upregulation of IFN-γ-regulated genes in livers results from the adaptive T cell response as specific T-cells infiltrating the liver are major producers of IFN-γ (Wieland et al. 2004). Thus, HBV and HCV infections strongly differ in the early phase of infection, as HCV induces a strong IFN-α response in chimpanzees (Su et al. 2002).

There are data to suggest that HBV actively inhibits the induction of an early IFN response. Wu et al. showed a regulatory effect of TLR-activated KC and LSEC on the in vitro replication of HBV in a co-culture model utilizing HBV-Met cells (Pasquetto et al. 2002). TLR3- and TLR4-activated KC as well as TLR3-activated LSEC induced a MyD88-independent response inhibiting HBV replication. While HBV replicative intermediates were highly suppressed, viral mRNAs as well as secretion of HBsAg and HBeAg remained largely unchanged. The HBV suppressing effect mediated by TLR3 ligands was caused by IFN-β whereas TLR4-activated KC additionally induced undefined cytokines with antiviral activity (Wu et al. 2007).

Further studies included co-culture experiments with hepatocytes or NPC and HBV-Met cell supernatants, HBsAg, HBeAg as well as HBV virions resulting in abrogation of TLR-induced antiviral activity, correlating with decreased activation of IRF-3, NFκB and ERK1/2. In comparison to primary hepatocytes, HBV-infected HBV-Met cells did not induce antiviral cytokines upon TLR activation. TLR-induced expression of TNF-α and IL-6 was suppressed in the presence of high amounts of HBV. Accordingly, suppression of HBV replication by siRNA leads to activation or expression of pro-inflammatory transcription factors and cytokines (Wu et al. 2008). These data might explain why HBV does not induce a strong initial type I IFN response such as HCV and, therefore, behaves as a ‘stealth virus’ (Wieland et al. 2004).

Despite the fact that HBV does not induce an IFN response during the early phase of infection, it can be recognized by liver resident cells, thereby activating innate immune
responses without IFN induction. Hoesel et al. recently showed that HBV is recognized by hepatic NPC, mainly by Kupffer cells, upon infection of primary human liver cells in vitro. Within 3 hours, these cells release inflammatory cytokines including IL-1β, -6, -8 and TNF-α without inducing an IFN response. NFκB-dependent IL-6 secretion of activated KC is able to control HBV gene expression and replication in hepatocytes at the level of transcription. IL-6 leads to activation of MAPKs exogenous signal-regulated kinase (ERK) 1/2 and c-jun N-terminal kinase resulting in decreased expression of two transcription factors (hepatocyte nuclear factor (HNF) 1α and HNF 4α) that are essential for HBV gene expression and replication (Hoesel et al. 2009).

A recent publication studied the full replication cycle of hepatitis B virus (HBV) in primary hepatocyte cultures that were isolated from the northern treeshrew (Tupaia belangeri). The Tupaia model has been used to investigate the effect of cytokines on HBV infection. Stimulation of HBV infected primary Tupaia hepatocytes with recombinant Tupaia TNF-α led to viral suppression while covalently closed circular DNA and viral RNA were still detectable leading to the conclusion that TNF-α may also control HBV infection (Xu et al. 2011).

Consistently, Zhang et al. demonstrated that activation of cellular pathways by TLR ligands leads to inhibition of hepadnaviral replication (Zhang et al. 2009). Using the model of woodchuck hepatitis virus (WHV) infected primary hepatocytes (PWH), Poly I:C and LPS stimulation resulted in upregulation of cellular antiviral genes and TLRs. LPS stimulation led to a pronounced reduction of WHV replication intermediates without a significant IFN induction while Poly I:C transfection resulted in the production of IFN and a highly increased expression of antiviral genes in PWHs and slight inhibitory effect on WHV replication. LPS could activate NFκB, MAPK, and PI-3k/Akt pathways in PWHs. Furthermore, inhibitors of MAPK-ERK and PI-3k/Akt pathways, but not those of IFN signaling pathways, were able to block the antiviral effect of LPS. These results indicate that IFN-independent pathways which activated by LPS are able to down-regulate hepadnaviral replication in hepatocytes (Zhang et al. 2009).

A direct activation of cellular pathways through the expression of cellular adaptors involved in signaling has similar effects like the stimulation with TLR ligands. Guo et al. determined the effects of PRR-mediated innate immune response on HBV replication in hepatoma cell lines. Plasmids expressing TLR adaptors, MyD88, TRIF, or RIG-I/MDA5 adaptor or interferon promoter stimulator 1 (IPS-1) were transfected into cells and led to dramatic reduction of the levels of HBV mRNA and DNA in hepatoma cells. Analysis of involved signaling pathways revealed that activation of NFκB is required for all three adaptors to elicit antiviral response in both HepG2 and Huh7 cells while activation of IRF-3 is only essential for induction of antiviral response by IPS-1 in Huh7 cells (Guo et al. 2009).

Although recent publications consistently confirmed the antiviral role of TLR-mediated innate responses of hepatic cells, the antiviral mechanisms that are induced by activation of the TLR system are not fully understood. IFN-β has been identified as the major antiviral factor produced by NPCs in response to TLR3 and 4 ligands (Wu et al. 2007). Wieland et al. showed that IFN-β inhibits hepatitis B virus (HBV) replication by non-cytolytic mechanisms that either destabilize pregenomic (pg)RNA-containing capsids or prevent their assembly. Using a doxycycline (dox)-inducible HBV replication system, IFN-β pretreatment led to production of replication-competent pgRNA-containing capsids. The turnover rate of preformed HBV RNA-containing capsids is not changed in the presence of IFN-β or IFN-γ.
These conditions further inhibited pgRNA synthesis. Thus, type I and II IFNs prevent the formation of replication-competent HBV capsids (Wieland et al. 2005). IL-6 was shown to activate cellular signaling pathways in PHHs including the MAPK pathway and inhibit the HBV gene transcription (Hoesel et al. 2009). In PWHs infected with WHV, LPS activates the MAPK pathway and reduce the WHV replication, but unable to deplete WHV transcripts (Zhang et al. 2009). Future studies are necessary to clarify the mechanisms involved in TLR-mediated anti-HBV actions.

As TLR-mediated immune responses down regulate HBV replication, HBV developed mechanisms to counteract these antiviral functions. Hepatocytes and Kupffer cells isolated from liver biopsies of patients with chronic hepatitis B (CHB) showed significantly decreased expression of TLR2 on hepatocytes, KCs and peripheral monocytes in patients with HBeAg-positive CHB in comparison with HBeAg negative CHB and controls (steatosis patients). The level of TLR4 expression did not significantly differ between these groups. Hepatic cell lines harboring a recombinant baculovirus encoding HBV significantly reduced TNF-α expression as well as phospho-p38 kinase expression in the presence of HBeAg. Within the absence of HBeAg, HBV replication was associated with upregulation of the TLR2 pathway resulting in increased TNF-α expression (Visvanathan et al. 2007). Consistent with these findings, the TLR expression was significantly suppressed in liver tissue and PBMC of woodchucks chronically infected with WHV, assuming an important role of TLR2 during hepadnaviral infection and pathogenesis (Zhang et al. unpublished). HBV additionally blocked the gene expression of MyD88, an essential adaptor molecule in TLR-mediated innate immune responses. The terminal protein (TP) domain of the HBV polymerase was described to be responsible for this antagonistic activity. It is supposed that the HBV polymerase inhibits IFN-inducible MyD88 expression by blocking the nuclear translocation of STAT1 and therefore representing a general inhibitor of IFN signaling (Wu et al. 2007).

Fig. 3. Role of the innate immunity during pathogenesis of HBV
HBV infection only induces a weak type I IFN response. HBsAg or HBeAg inhibit endogenous expression of IFNs in the early phase of infection. Activated cells of the innate immune system may still produce type I IFNs, therefore limiting viral replication.

Isogawa et al. examined the ability of different TLRs to effect HBV replication in vivo. HBV transgenic mice have been injected with single doses of TLR2, -3, -4, -5, -7 and -9 ligands.
With the exception for TLR2, all of the ligands suppressed HBV replication in the liver in an IFN-α/γ-dependent manner (Isogawa et al. 2005). The potential of these TLR ligands to provoke the expression of antiviral cytokines at the site of HBV replication, leads to suggestion that TLR activation represents a powerful tool for novel therapeutic strategies in the treatment of chronic HBV infection.

4.2 Innate immunity against HCV

Standard treatment of HCV infection is the combination of pegylated type I IFN and ribavirin. Interferons are antiviral cytokines, produced after activation of the innate immune system during virus infection. Secretion of IFN leads neighbouring cells to switch to an ‘antiviral state’ and resists viral infection. Thus, an important role of the innate immune system is to limit viral replication and spread as well as to promote and orchestrate subsequent adaptive immune reactions. While the mechanisms that are utilized by the innate immune system to control HCV infection are not well defined, it is generally accepted that IFN signaling is likely to be involved. Viral proteins are able to potently activate the innate immune system. Nevertheless, HCV developed different mechanisms to evade cellular, antiviral and innate immune responses, reflecting the adaptation to its host.

Molecular patterns within the 5′ as well as the 3′ non-translated region (NTR) of the HCV genome have been identified as retinoic acid inducible gene I (RIG-I) activating PAMPs. RIG-I is a well characterized PRR sensing and affecting HCV replication. Binding of viral double stranded RNA to the cytosolic RNA helicase RIG-I recruits the mitochondria-associated CARDIF protein, which induces IKKe/TBK1 kinases-mediated IRF-3 phosphorylation and thereby induces IFN-β expression. It is supposed that HCV RNA initiates IFN-β secretion in a RIG-I-dependent manner through its 5′ and 3′ NTR secondary structure (Saito et al. 2007; McCormick et al. 2004; Sumpter, Jr. et al. 2005). It has been additionally demonstrated that TLR3 is able to detect HCV structures in cultured hepatoma cells, leading to activation of IRF-3 and expression of ISGs, which limit HCV replication. The HCV motif that triggers TLR3 signaling remains to be characterized (Wang et al. 2009). It is suggested that RIG-I and TLR3 represent independent signaling pathways that are involved in IRF-3- and NFκB-mediated antiviral state during HCV infection (Alexopoulou et al. 2001; Yoneyama et al. 2004) (Figure 4).

In defiance of this immune activation HCV is able to subvert the antiviral activity of the innate immune system. Recent data suggested that the viral NS3/4A serine protease of the Hepatitis C virus may enable its persistent infection. The NS3/4A protein causes specific proteolysis of the TRIF adaptor molecule downstream the TLR3 signaling. It has been demonstrated that the viral NS3/4A protease additionally leads to disruption of RIG-I signaling. Therefore the viral NS3/4A protease cleaves CARDIF and abrogates IKKe/TBK1-mediated IFN-β secretion (Li et al. 2005; Foy et al. 2005; Foy et al. 2003; Breiman et al. 2005; Vilasco et al. 2006).

Broering et al. and Wang et al. investigated the antiviral capacity of TLR-activated KC, LSEC and HSC against HCV. Despite the expression of all TLRs, murine KC and LSEC only suppressed HCV replication in a co-culture model after activation of TLR3 and -4 which were mediated by IFN-β only (Broering et al. 2008). Similar results were obtained for murine HSC. Here, IFN-β was responsible for the antiviral activity of TLR3-stimulated HSC, whereas additional cytokines of undefined nature seem to be involved in the TLR4-mediated antiviral effect. In case of human HSC, only TLR3 stimulation led to production of
antiviral cytokines. HCV suppression was related to the upregulation of ISGs and RIG-I in target cells (Wang et al. 2009).

![Diagram of HCV infection and innate immunity](image.png)

**Fig. 4. HCV triggers innate immunity**

Different structures of the hepatitis C virus can be detected by the innate immune system. This leads to cytokine secretion and induction of an antiviral state as well as inflammation processes. The virus developed evading strategies to subvert this antiviral state.

Recently established HCV cell culture models (Lindenbach et al. 2005; Wakita et al. 2005; Zhong et al. 2005) lead to the generation of infectious HCV particles. Zhang et al. generated cell culture derived HCV particles to study their immunomodulatory effects on freshly isolated PBMCs and pDCs obtained from healthy blood donors. While complete HCV particles were not able to induce cytokine production, purified HCV RNA led to immune stimulation, accompanied by enhanced TLR7 activation. It was suggested that the HCV RNA genome contains G/U-rich motifs which have immune-stimulatory capacity. It is hypothesized that HCV particles are digested by endosomal proteases, the uncoated viral genomes and therefore the G/U-rich motifs mediate TLR7 activation (Diebold et al. 2004; Jurk et al. 2002). Lee et al. additionally revealed an interaction between TLR7 and HCV, wherein TLR7 mediates interferon secretion as well as interferon-independent immune responses (Lee et al. 2006). Further analysis indicated a higher prevalence of single nucleotide polymorphisms (SNP) in TLR7 of patients chronically infected with HCV. These SNPs significantly correlates with the progression of liver fibrosis in patients with chronic HCV infection (Schott et al. 2007).

Another mechanism of HCV to evade the attack of the innate immune system targets TLR7. A significant decrease in TLR7 expression was shown in the presence of HCV in vitro and in vivo (Chang et al. 2010). It was proposed that HCV directly interferes with the transcriptional regulation of TLR7 mRNA. HCV replication level directly correlates with TLR7 expression, as reconstitution of TLR7 expression levels were achieved upon viral suppression. Despite this decrease in TLR7 mRNA in HCV-replicating cells, increased activation of IRF-7 was detected. This indicates that other PRR induce nuclear translocation of IRF-7 in HCV replicating cells (Chang et al. 2010).

Innate immunity is additionally activated by the HCV core protein leading to inflammation but failing to induce antiviral cytokines (Dolganiuic et al. 2004; Feldmann et al. 2006). Other studies have described that synthetic lipopeptide-complexes of the HCV core protein mediate the innate immune response through TLR2 and TLR4 (Duesberg et al. 2002).
addition, an increased expression of some TLRs as well as inflammatory cytokines was shown in PBMC of chronically infected HCV patients (Sato et al. 2007). Dolganiuc et al. identified pre-activated monocytes in patients with chronic hepatitis C. In this study, increased IFN-γ, endotoxin and HCV core protein seemed to modulate monocyte functions, resulting in the generation of MyD88/IRAK complexes, NFκB activation and increased expression of TNF-α. These findings lead the authors to suggest that LPS, HCV core protein and IFN-γ extend the activation of inflammatory monocytes/macrophages indicating a loss of TLR tolerance. These observations additionally lead to the assumption, that both host- as well as virus-derived factors influence macrophages to mediate persistent inflammation during chronic HCV infection (Dolganiuc et al. 2007).

4.2.1 Interferon response; friend or foe?

Elevated hepatic ISG expression in HCV infected chimpanzees as well as in patients was identified as a virus induced type I IFN response (Bigger et al. 2001; Bigger et al. 2004; Helbig et al. 2005). As already discussed HCV developed evading strategies to subvert the innate immune system. While infected cells are not able to sense the virus and subsequently secrete IFNs, it is supposed that the increased ISG expression during HCV infection is induced by an activated local innate immune system. Activation of the innate immune system, NPCs in particular, results in the expression and secretion of IFN-ǃ. Type I IFNs bind to their cell surface receptor leading to conformational changes, which activate the Janus kinase - Signal Transducers and Activators of Transcription (JAK–STAT) signaling pathways. Downstream phosphorylation of STAT-1 and STAT-2 recruits a third factor (IRF-9) forming the transcription-complex ISG factor-3 (ISGF-3). ISGF-3 translocates into the nucleus and interacts with IFN stimulated response elements (ISRE) in the promoter regions of ISGs. Expression of selected ISGs may result in HCV eradication in acute hepatitis C infection (Bigger et al. 2001). However, progression of persistent HCV infection can be established due to escape strategies against the immune system which may also be responsible for non-response to therapies that are based upon the administration of exogenous IFNs (Sato et al. 2007).

A subgroup of ISGs has been described to directly suppress HCV replication. Protein kinase R (PKR) for example, phosphorylates the alpha subunit of the eukaryotic initiation factor (eIF)-2 leading to suppression of translational processes (Gale, Jr. et al. 1999; Pflugheber et al. 2002). The RNA-specific adenosine deaminase 1 (ADAR1) binds to dsRNA resulting in destabilization of secondary structures (Taylor et al. 2005). The antiviral function of 2′-5′ oligoadenylate synthetases (2′-5′ OAS) is mediated by the activation of the latently expressed endoribonuclease RNaseL, which induce degradation of viral and cellular RNA (Silverman, 1994; Zhou et al. 1997). ISG56 is an IRF-3 responsive gene that blocks one of the eIF3 subunits resulting in inhibition of translation (Wang et al. 2003; Hui et al. 2003; Terenzi et al. 2005). Additional ISGs affecting HCV replication have been identified during IFN therapy including MxA, ISG 6–16 and Viperin. The mechanisms of action of these host factors are still unclear (Bigger et al. 2004; Helbig et al. 2005; Suzuki et al. 2004).

HCV evolved mechanisms to influence this type I IFN response induced by the JAK–STAT signaling. The HCV polyprotein has been described as a strong inhibitor of IFN-α-induced signaling, as it impairs ISGF3 DNA binding. This effect is mediated by an increase in STAT1–protein inhibitor of activated STAT1 (PIAS1) association, resulting in the decreased transcriptional activity of ISGF3 through hypomethylation of STAT1 (Blindenbacher et al.}
In addition, also HCV core protein effects JAK-STAT signaling. The core protein induces STAT1 degradation, inhibits STAT1 activation/phosphorylation and increases the induction of suppressor of cytokine signaling (SOCS) proteins (Bode et al. 2003; Lin et al. 2005; Lin et al. 2006). Furthermore, the core protein suppresses the binding capacity of ISGF3 to the ISRE, resulting in decreased expression of anti-HCV effective ISG (de Lucas et al. 2005). Moreover, HCV proteins directly inhibit the antiviral action of selected ISGs. The envelope protein E2 and the non-structural protein NS5A have been reported as antagonists of PKR, leading to the disruption of translation control by IFNs (Gale, Jr. et al. 1998; Taylor et al. 1999).

The local type I IFN response induced by HCV seems to be paradox, as these interferons can inhibit HCV replication. In addition, patients that are non-responders to IFN-based therapies have highly elevated expression levels of a subset of ISGs compared to patients who cleared the virus (Asselah et al. 2008; Chen et al. 2005). ISG15, an ubiquitin-like modifier, is one of these genes. ISG15 is conjugated to a subset of target proteins. The functional consequence of this ISGylation is still unclear. ISGylated proteins are not degraded by the proteasome as ubiquitinylated proteins are (Malakhov et al. 2002; Ritchie & Zhang, 2004). Recently published data indicate that ISGylation negatively modulates interferon signaling (Chua et al. 2009; Broering et al. 2010), in addition ISGylation and ISG15 itself directly promote HCV replication (Chen et al. 2010; Broering et al. 2010). These observations may explain why elevated expression of ISGs, and ISG15 in particular, during HCV infection is beneficial for the hepatitis C virus (Figure 5).

**4.3 Innate immunity against HDV**

There are only few studies on the interaction of HDV with host innate responses. Like many other viruses, HDV seems to have developed anti-IFN-α strategies as it negatively affects the activation of IFN-α signaling by interfering the JAK-STAT signal transduction pathway. An early publication from McNair et al. demonstrated that neither IFN-α nor IFN-γ was able to inhibit HDV gene expression and formation of genomic and antigenomic RNA in cell
lines stably transfected HDV genomes (McNair et al. 1994). However, such cell lines showed no defect in upregulation of ISGs like PKR, 2’-5’ OAS and IRF-1 or in induction of an antiviral status after IFN treatment. These cells were able to upregulate IFN-β after Poly I:C treatment. The presence of HDV RNA did not affect PKR function.

In contrast, Pugnale et al. showed in a transient transfection system that hepatoma cells replicating HDV have an impaired response to IFN-α (Pugnale et al. 2009). By unknown mechanisms, the phosphorylation of both STAT-1 and STAT-2 was greatly impaired, consequently, both factors did not relocate into the nucleus. In addition, the IFN-α stimulated tyrosine phosphorylation of IFN receptor-associated JAK kinase Tyk2 was also inhibited by HDV, without affecting either the tyrosine phosphorylation of Jak1 or the expression of type I IFN receptor subunits. Both studies showed consistently that IFNs are not able to suppress HDV. Pugnale et al. could detect the inhibition of IFN signaling in HDV replicating cells, likely due to the higher levels of viral RNA and proteins in the transient transfection system (Pugnale et al. 2009). Despite of the dsRNA nature of HDV genomic and antigenomic RNAs, there was no apparent activation of IFN pathways in the stably transfected cell lines, indicating that HDV RNA may be sequestered during replication (McNair et al. 1994).

Another report demonstrated that large HDV antigen (L-HDAg) may enhance the ISG MxA more than 3-fold. However, the upregulation of MxA by IFN-α is generally very strong and may reach levels of more than 100-fold compared to unstimulated controls. Thus, the ability of L-HDAg to activate ISG expression is rather low (Williams et al. 2009). Thus, HDV is a weak inducer of cellular IFN responses and is likely able to inhibit IFN signaling. In addition, it has also been reported that IFN-α inducible protein ADAR1 modulates HDV gene expression and genome replication by editing the HDV genome (Hartwig et al. 2004).

![Fig. 6. Role of the innate immunity during pathogenesis of HBV HDV coinfection](http://www.intechopen.com)

HBV as well as HDV only induce a weak type IFN response. HBsAg or HBeAg are supposed to inhibit endogenous expression of IFNs. Activated cells of the innate immune system might still produce type I IFNs. HDV is able to block JAK/STAT signaling, thereby subverting the IFN response. HDV additionally promotes NFkB activation and secretion of inflammatory cytokines.
NFκB activation is involved in many inflammatory processes and in cancer. The L-HDAg has been shown to mediate TNF-α-induced NFκB signaling, probably through the direct association with TRAF2, a protein implicating the early signaling events (Park et al. 2009). Several studies revealed a relationship between L-HDAg, S-HDAg, genomic RNA or antigenomic RNA and the proteome of the cell (Mota et al. 2009; Mota et al. 2008). Modified expression profile of proteins involving regulation of nucleic acid and protein metabolism, energy pathways, signal transduction, transport, apoptosis and cell growth were found. Host factors involved in HDV replication have been determined using a small inhibitory RNA (siRNA) screening. Cells stably transfected with the S-HDAg were treated with siRNA before HDV infection (Cao et al. 2009). It has been reported that a part of the genome, described as an RNA promoter, directly interacts with some cellular proteins (Beard et al. 1996) (Figure 6).

It could be demonstrated that two of these host factors, the eukaryotic translation elongation factor 1 alpha 1 (eEF1A1) and the glyceraldehyde-3-phosphate dehydrogenase (GAPDH), are involved in RNA processing and in the translation machinery. Of note, these genes are often considered and used as housekeeping genes (Sikora et al. 2009).

5. Coinfection of HBV and HCV

Clinical investigations on disease outcomes and progression in patients coinfected with HBV and HCV are diverse and contradictory. Viral interference or reciprocal replicative suppression of the two viruses probably occurs (Liaw et al. 1994; Zarski et al. 1998). Due to a missing model system for HBV/HCV coinfection in the past, virological and molecular aspects are poorly understood (Brass & Moradpour, 2009). Recent studies investigated the mechanisms of HBV and HCV coinfection by heterologous overexpression of viral proteins, leading to conflicting results. It has been demonstrated that the HCV core protein and NS5A negatively regulate HBV replication, whereas other studies could not confirm these findings (Pasquinelli et al. 1997; Chen et al. 2003; Schuttler et al. 2002).

It seems to be important to address the question whether direct interference occurs between the viruses in order to understand the disease progression. Recent studies have addressed this issue (Eyre et al. 2009; Bellecave et al. 2009). The human hepatoma cell line Huh-7 supports HBV replication and formation of HBV virions. Huh-7 cells also can be used to study the HCV life cycle, including viral entry, RNA replication and release of infectious particles. Using this cell culture system, these authors independently showed that HBV and HCV are able to replicate in the same cell, without showing interfering processes. HBV replicating cells can be infected with cell culture-derived HCV resulting in secretion of infectious HCV. In addition, the inhibition of one of these viruses did not influence the replication of the other. It is well known that HBV replication, for example, may be suppressed in the presence of replicative HCV infection while eradication of HCV may lead to replicative activity of HBV infection. This phenomenon may be explained by the fact that HCV may induce local IFN production by NPCs through activation of TLR3 which may lead to suppression of HBV replication (Broering et al. 2008; Wu et al. 2007) (Figure 7).

6. Conclusions

TLRs have been identified as key regulators of innate and adaptive immune responses in the liver as they play a critical role in the pathogenesis and progression of many liver diseases.
as well as in the regulation of tissue injury and wound healing processes. The local innate immune system represented by hepatocytes, liver sinusoidal endothelial cells, Kupffer cells and stellate cells, for example, is involved in the induction of systemic tolerance or inflammation and additionally cross-talks to the adaptive immune system. It has been suggested that the local innate immune system is of importance in the progression of HBV or HCV infection. PRRs, especially TLRs play a pivotal role in the pathogenesis of viral hepatitis due to their rapid signal transduction. It has been clearly demonstrated that TLRs participate in antiviral immunity.

Fig. 7. Role of the innate immunity during pathogenesis of viral hepatitis
HCV infection directly induces type IFNs, later viral proteins like NS3/4 inhibits endogenous expression of IFNs. Activated cells of the innate immune system might still produce type I IFNs resulting in increased ISG15 expression in infected cells and therefore promoting HCV replication. HBV likewise seems to inhibit endogenous IFN expression, additionally inhibiting TLR activation in the local immune system. This may explain way HCV but not HBV induce an initial type I IFN response during acute infection. In case of HBV/HCV co-infection, HCV-activated NPC may inhibit HBV replication by the production of type I IFNs.

As a consequence, various evading strategies have been evolved by hepatitis viruses (HBV, HCV and HDV) to counteract these antiviral activities:
The hepatic innate immune system is able to sense PAMP structures of these hepatotropic viruses, resulting in secretion of antiviral cytokines, in particular interferons, and therefore
limits viral replication. Conversely, the hepatitis viruses may counteract and subvert the innate immunity by evolutionary adaptation. The Hepatitis B virus for example developed a mechanism to block the expression of interferon sensitive genes, especially MyD88, an adaptor molecule involved in mostly all of the TLR signaling pathways. In addition HBV evades the TLR2 signaling, which results in limitation of viral replication, by directly decreasing its expression. These evasion strategies may explain the absence of an antiviral response during infection and the description as a ‘stealth virus’. Coinfection with Hepatitis D virus is additionally accompanied by further evasion of the IFN response.

In contrast to this Hepatitis C virus initially induces a strong type I IFN response. However, the virus is able to control this antiviral signaling by evasion strategies directly targeting the TLR3 or RIG-I pathway, which initiate IFN-β expression. In addition, HCV developed different mechanisms to block the JAK-STAT signaling, resulting in abrogation of the IFN response, in particular the expression of ISGs with antiviral functions. HCV paradoxically needs one of these response genes, ISG15, which promotes HCV replication and negatively regulates the IFN response.

These evolutionary developed adaptations of HBC, HCV and HDV to their host invert the benefits of the antiviral response, induced by the local innate immune system. The inadequate but consistent activation of the hepatic innate immunity results in tolerance induction and permits progression of viral persistence and chronic infections. Therefore, therapeutic manipulations of the hepatic TLR pathways are of high interest for the development of novel treatment strategies.

7. References


There are a lot of important issues related to viral hepatitis studies: molecular biology of viruses, laboratory diagnostics, epidemiology, treatment etc. However, there is a number of special textbooks and monographs on the subject. Considering this fact and rather fast progress in our understanding of the problem this book focuses on the important sections of the problem immune pathogenesis of parenterally transmitted viral hepatitis and some aspects of hepatitis diagnostics. Seven chapters were prepared by several groups of researchers to share information and results of studies with specialists working in the field and persons who are interested to learn about the viral hepatitis issue. The Nobel Prize Committee (the field of physiology and medicine, 2011) awarded Bruce A. Beutler and Jules A. Hoffmann for their discoveries concerning the activation of innate immunity whilst Ralph M. Steinman was awarded for his discovery of the dendritic cell and its role in adaptive immunity. We are proud to say that our book is in line with these discoveries, because 3 chapters cover the problems of innate and adaptive immune response in case of viral hepatitis.

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