Toll-Like Receptors in Hepatitis C Infection:  
Implications for Pathogenesis and Treatment

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Abstract

Hepatitis C infection is a significant global health problem, affecting over 150 million people worldwide. Whilst the critical role of the adaptive immune system in HCV infection is well-established, the importance of the innate immune system in HCV infection has only been recognised in more recent years. Toll-like receptors form the cornerstone of the innate immune response and there is considerable evidence for their crucial role in hepatitis C infection.

This review outlines recent advances made in our understanding of the role of TLR function in HCV infection, exploring how HCV manipulates host immunity to evade immune clearance and establish persistent infection despite leading to inflammatory hepatic damage.
Introduction

Hepatitis C infection is a significant global health problem, affecting over 180 million people worldwide\(^1\). Despite emerging therapies for HCV infection, the sombre prediction is for the health burden from HCV to steadily increase: by 2020, it is projected that untreated patients with HCV liver cirrhosis will double, the number of patients with HCV cirrhosis developing hepatocellular carcinoma will increase by 80% and referrals for liver transplantation for HCV-related liver disease are also predicted to double\(^1,2\). This makes HCV infection a significant global public health issue, with an expected exponential increase in burden of disease over time.

Whilst the critical role of the adaptive immune system in HCV infection is well-established, the importance of the innate immune system in HCV infection has only been recognised in recent years. Toll-like receptors form the cornerstone of the innate immune response and there is considerable evidence for their crucial role in hepatitis C infection.

This review outlines recent advances made in our understanding of the role of TLR function in HCV infection, exploring how HCV manipulates host immunity to evade immune clearance and establish persistent infection despite leading to inflammatory hepatic damage. The potential clinical benefits of therapeutic and screening strategies harnessing TLR function will also be addressed.

The Innate Immune System and Toll-Like Receptors
The innate immune system forms a stereotyped, highly conserved immune response that is the first line of defence against infection and inflammation in an organism. Even though the innate immune system is evolutionarily conserved, it is now recognised to have a critical role in initial host immune defences and leads to appropriate activation of the subsequent adaptive immune response. Innate immune responses are specific, triggered by binding of innate immune receptors to their appropriate ligands, thereby initiating a downstream signalling cascade culminating in upregulation of pro-inflammatory cytokine, chemokine and interferon production. In contrast to adaptive immunity, the innate immune response is rapid in onset and requires no previous exposure to the pathogen.

**Toll-like receptors**

Toll-like receptors (TLRs) are a family of non-clonal, germline encoded, pattern recognition receptors (PRRs) that give the innate immune system considerable specificity for a large range of pathogen classes. To date, there are ten functional TLRs identified in humans (TLR 1-10). Each receptor has two domains: an extracellular leucine rich LRR domain and an intracellular Toll-Interleukin (Il-1 receptor (TIR) domain.

TLRs recognise pathogen associated molecular patterns, or PAMPs, which are highly conserved molecules expressed by classes of invading pathogens. TLR2 and TLR4 also recognise endogenous components derived from dying or damaged host cells (called damage associated molecular patterns, or DAMPs), allowing inflammatory
responses to be initiated by trauma to host cells. Commonly cited PAMPs and DAMPs and their corresponding TLRs are outlined in Table 1.

Greater breadth of specificity of TLR binding is created by dimerization of TLR2 with TLRs 1 and 6, and accessory proteins such as MD2 that bind to TLRs to alter binding specificity. The localisation of TLRs within cells is also important, for example TLRs which bind viral RNA and bacterial DNA are located within endosomes, as these organelles do not contain host RNA or DNA.

There are also other cytosolic pathogen recognition receptors in addition to TLRs which form part of the innate immune system, including the RNA helicases retinoic acid-inducible gene 1 (RIG1), melanoma differentiation-associated gene (MDA)5, and laboratory of genetics and physiology 2 (LGP2) and nucleotide-binding-oligomerization domain (NOD)-like receptors. However, their involvement in HCV infection is beyond the scope of this review.

**Cellular TLR expression**

TLRs are expressed ubiquitously, however levels of expression vary for different cell types. This compartmentalises TLR function by regulating access to TLR ligands for binding and determining the subsequent signalling pathway and inflammatory response that is activated by TLR-ligand interactions. Expression of TLRs by cell type in both peripheral immune cells and liver cells is outlined in Table 2.
The immune system of the liver is highly specialised to prevent constant immune activation in the face of continual bombardment with pathogens, as it receives the entire blood supply of the gastrointestinal tract\textsuperscript{14}. TLR mRNA expression is therefore low in the liver, favouring TLR ligand tolerance, however in pathological conditions TLR expression is induced to allow appropriate TLR activation\textsuperscript{15, 16}.

Two key cell types within the liver that express TLRs and have crucial roles in HCV infection and liver fibrosis are Kupffer cells and hepatic stellate cells. Kupffer cells are resident macrophages expressing TLR2, TLR3, TLR4 and TLR9 and these signalling pathways mediate phagocytosis, antigen presentation and secretion of pro-inflammatory mediators\textsuperscript{17, 18}. TLR-mediated Il-12 and Il-18 from Kupffer cells induces hepatic NK cells to produce IFN\textgamma, which is critical for viral eradication and inhibition of hepatic stellate cells and hepatic fibrogenesis\textsuperscript{19}. Kupffer cells also play a direct role in fibrogenesis, secreting TGF-\beta, matrix metalloproteinases, platelet derived growth factor (PDGF) and reactive oxygen species (ROS) with TLR4 stimulation\textsuperscript{15}.

Hepatic stellate cells (HSCs) are the major fibrogenic cell type in the liver\textsuperscript{20}. When liver injury occurs, quiescent stellate cells become activated fibrogenic myofibroblasts that produce inflammatory mediators and extracellular matrix and collagen, leading to hepatic fibrogenesis\textsuperscript{21, 22}. TLR4 and TLR9 pathways are the most important in HSC activation and fibrogenesis\textsuperscript{23, 24}.

**Toll-like receptor signalling pathways**
When TLRs bind to their appropriate ligand via their leucine rich LRR domain, they initiate a downstream signalling cascade which leads to upregulation of pro-inflammatory cytokine and chemokine production and interferon signalling\textsuperscript{25}. TLRs provide a bridge between innate and adaptive immunity through induction of dendritic cell maturation, antigen presentation and T and B cell recruitment and activation\textsuperscript{15, 26}. These immune responses are critically important in viral infections, including HCV infection.

There are four primary adaptor molecules that bind to intracellular TIR domains of TLRs to transduce signals: MyD88, TIRAP, TRIF and TRAM. In simple terms, MyD88 is the main adaptor protein for all TLRs except TLR3, which uses TRIF\textsuperscript{27}. TIRAP works with MyD88 in TLR2 and TLR4 signalling. TRIF mediates TLR3 and TLR4 anti-viral IFN responses and NFκB activation. TRAM mediates TLR4-TRIF signalling\textsuperscript{15}. The four key signalling pathways that utilise these four adaptor proteins along with other proteins are outlined in Figure 1. A key paradigm in TLR signalling is overlap of signalling pathways and shared pathways of gene transcription, allowing amplification and built-in redundancy of immune responses.

**The MyD88-NF-κB/ AP-1/ IRF5/ p38 pathways**

MyD88 induces proinflammatory and antibacterial gene transcription by activating the NFκB, AP-1, p38 and IRF5 pathways via TLR2, 4 and 5\textsuperscript{28}. Upon stimulation with various ligands IkBs are phosphorylated at serine residues by the IkK complex. This causes degradation of the IkB, allowing NFκB to be released into the nucleus and
bind to the KB site. AP-1 activation in TLR signalling mostly mediated by p30, MAPK and IκK.

The MyD88-IRF7-IFN pathway

TLR7 and TLR9 orchestrate antiviral responses by upregulating gene transcription for IFN-α and IFN-β29. Recruitment of IRF5 then leads to induction of inflammatory cytokines IL6, IL12, p40 and TNFα, but not type I IFN28.

The TRIF-IRF3-IFN pathway

TLR3 and TLR4 stimulation can lead to IFNα and IFNβ production via the TRIF pathway, leading to IκK (noncanonical IκB kinase) and TBK1 (TANK-binding kinase 1) activation which in turn phosphorylate IRF3 and lead to transcription of IRF3 dependent genes30, 31.

The TRIF-NFκB pathway

TLR3 and TLR4 agonists activate TRIF, which in turn can also activate NFκB. TRIF is the only adaptor for TLR3 to activate NFκB pathway. However, TLR4- induced NFκB activation occurs via both TRIF and MyD88.

Control of TLR signalling: Negative Feedback and Tolerance
Due to the potentially deleterious effect of an unchecked pro-inflammatory state, negative feedback exists for TLR signalling and is a critical component of immune activation and modulation\textsuperscript{32}. Perturbation of TLR function can occur at multiple levels in the signalling cascade, including synthesis and expression of signalling receptors and proteins, through proteins that negatively interact with signalling and enhanced ubiquination and degradation of signalling proteins.

Another important mechanism of negative feedback is via tolerance, or reduced subsequent responses from repeated TLR stimulation after initial stimulation of one TLR type. Cross-tolerance also occurs, whereby activation of one TLR pathway can cross-inhibit another via negative feedback\textsuperscript{33}. Potentially, both negative feedback and tolerance can be manipulated by viral infections such as HCV in order to prevent immune clearance.

**The Hepatitis C Virus**

Hepatitis C is a positive strand RNA enveloped flavivirus which was first cloned in 1989\textsuperscript{34}. HCV virions bind to the cell surface and enter cells via receptor-mediated endocytosis. The structure of HCV is outlined in Figure 2. The core and non-structural proteins shown in the diagram are important sequences recognised by PRRs, including TLRs. They are also important inhibitors of TLR signalling\textsuperscript{35,36}.

**General Elements in the Immune Response against HCV Infection**
In order to understand the context of TLR immune responses in HCV infection, it is necessary to consider general features of the immune response against HCV. Fundamentally, T cell responses to HCV are critical for viral eradication and also response to HCV therapy\textsuperscript{37-39}. The balance between Th1 anti-viral and Th2 viral-permissive T cell responses determines viral clearance or persistence and the degree of inflammation and disease progression\textsuperscript{40-43}. CD4+ T cells have a protective effect against liver disease progression in chronic HCV infection and effective CD4+ T cell responses to HCV are required to mount an active cytotoxic CD8+ T cell response for viral eradication\textsuperscript{44-47}. T cell responses to HCV proteins are readily detected early during acute HCV infection, but both CD4+ and CD8+ T cell function is significantly impaired once chronic infection is established, with reduced cytokine production despite ongoing stimulation with circulating HCV antigens\textsuperscript{48-53}.

One of the key determinants of T cell function in HCV infection is the quality of antigen presentation by dendritic cells (DCs), as this determines the number of epitopes recognised by T cells that will engender an anti-viral response\textsuperscript{38, 54, 55}. HCV is associated with a failure of DC function that also leads to impairment in NK cell and NKT cell function, with reduced IFN\textgreek{y} secretion leading to reduced inhibition of HCV replication, reduced inhibition of hepatic stellate cells and greater hepatic fibrosis\textsuperscript{56-58}. Th2-skewed NK cells further downregulate DC function by secreting IL-10 and TGF-\textgreek{b}\textsuperscript{56, 59}. TLRs play a key role in activation of DCs and NK cells and initiate inflammatory cytokine responses in other cell types, including liver cells, which contribute to the appropriate cytokine milieu for DC maturation and T cell activation\textsuperscript{60, 61}. 
TLRs in HCV infection

Arguably, the most important paradigm in the innate immune response against HCV is compartmentalisation. HCV has different effects upon TLR pathway stimulation in various cellular compartments and in this way is able to both stimulate pro-inflammatory cytokine production leading to liver damage, but also evade immune responses to establish viral persistence. A summary of important interactions between HCV viral proteins and TLR signalling pathways are shown in Figure 3 and Table 3.

TLRs in HCV infection: Immune activation

HCV core and non-structural proteins are important PAMPs for TLR2, TLR3, TLR4, TLR7/8 and TLR9. HCV core and NS3 proteins stimulate TLR2 when associated with TLR1 and TLR6 in PBMCs, particularly monocytes and macrophages. TLR2 stimulation leads to production of TNFα, IL-6 and IL-8 via the NFκB, JNK/AP-1, p38 and ERK pathways, with ERK being the dominant pathway for TNFα secretion. Some studies have demonstrated that TLR2 expression by PBMCs is increased in HCV infection and TNFα production can promote TLR2 expression, thereby providing a potential indirect positive feedback loop for TLR2 activation.

TLR4 is also activated by HCV, with NS5A inducing TLR4 expression and thereby increasing IFNα and IL-6 secretion, especially in B cells and hepatocytes. TLR4 also induces IFNβ production, which leads to paracrine IFN production and upregulation of interferon sensitive genes (ISGs) within infected cells and
surrounding tissues. Monocytes in HCV infected patients have impaired tolerance for repeated TLR4 challenge and greater TLR4 expression, leading to higher levels of serum and intra-hepatic TNFα, which contributes to inflammation in HCV infection.

TLR3 is important for its anti-viral immune effects and TLR3 stimulated non-parenchymal liver cells are able to regulate HCV replication through production of IFNβ. TLR3 mRNA is significantly increased in monocytes in chronic HCV infection. An IFN-responsive element has been identified in the promotor region of the TLR3 gene and it therefore seems likely that TLR3 expression is responsive to IFN treatment in HCV infection. mDCs have normal functioning TLR3 and can produce IL-12, IL-6, IL-10, IFNγ and TNFα with TLR3 stimulation despite HCV infection.

HCV genomic RNA has direct immunostimulatory effects on TLR7 and TLR8, leading to IFNα production and activation of IRF7 and NFκB. pDCs can also be activated via TLR7 and TLR9 through the HCV RNA poly-uridine tail. TLR7 activation of hepatocytes also induces IFN-independent anti-viral effects, reducing both HCV RNA levels and NS5A protein expression in cell lines. There is also increased TLR7 and TLR8 expression on monocytes in HCV infection, though the significance of this remains unclear.

**TLRs in HCV: Immune Evasion**

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HCV viral proteins are able to stimulate TLR signalling, which plays an important role in viral immune clearance. However, HCV is able to simultaneously evade immune clearance through specifically targeting and impairing TLR signalling through several mechanisms. Firstly, HCV interferes with signalling via the TRIF-TBK1-IRF3 pathway. The HCV NS3 protein induces degradation of TRIF, whilst the NS3/4A protein impedes IRF3 and NFκB activation by reducing the amount of TRIF in circulation and by generating cleavage products with dominant-negative activity. NS3/4A also interacts directly with TBK1 to reduce TBK1-IRF3 interaction and therefore inhibit IRF3 activation. HCV also interferes with the TLR-MyD88 pathway through NS5A interaction with MyD88 to prevent IRAK1 recruitment and cytokine production in response to ligands for TLR2, TLR4, TLR7 and TLR9.

The HCV lipo-viral particle interferes directly with TLR4 signalling in DCs whilst HCV core protein suppresses TLR4 expression. Cellular expression of TLR2 and TLR4 in mDCs is controversial, being reported as both higher and lower in HCV infection patients compared with healthy controls, though signal transduction of TLR2 and TLR4 in mDCs is certainly impaired in HCV infection. Greater anti-inflammatory IL-10 production by macrophages with TLR2 stimulation has been reported, and may explain the dichotomous effects of TLR2 activation in different cellular compartments.

HCV is able to reduce TLR7 signalling through a myriad of mechanisms. HCV induces increased instability of TLR7 mRNA transcripts whilst the NS5A protein interferes with TLR7 signalling, leading to reduced cytokine responses to
stimulation\textsuperscript{64, 86, 90}. Interestingly, lower TLR7 expression in HCV-infected livers is restored with successful HCV clearance with treatment\textsuperscript{90}. HCV has been shown to regulate TLR9 expression via Elk-1, which is an important signal integration point between TCR and CD28 in Th1 T cell activation\textsuperscript{91}. HCV also impairs TLR9-mediated IFN\textalpha and IFN\beta production and HLA-DR expression by pDCs, associated with impaired activation of naïve T cells\textsuperscript{49}. TLR9 signalling in mDCs is unaffected\textsuperscript{49, 75}.

It is therefore clear that compartmentalisation of effects on TLR function is a key strategy by which HCV is able to evade immune clearance, yet still lead to chronic inflammatory hepatic damage and liver fibrosis.

**Linkage of TLR function and other immune responses in HCV infection**

We can now start to piece together how HCV-mediated alterations in TLR function may contribute to the immune impairments seen in HCV infection that encourage viral persistence.

Activation of TLR2, TLR3 and TLR4 signalling in monocytes, myeloid dendritic cells and liver cells leads to upregulation of pro-inflammatory cytokines and chemokines and recruitment of inflammatory cells to the liver, culminating in cytotoxic and apoptotic death of viral-infected cells and adjacent uninfected cells\textsuperscript{65}. Inflammatory hepatocyte damage stimulates fibrogenesis via hepatic stellate cell activation, culminating in hepatic fibrosis. Fibrogenesis is further augmented by
impaired TLR7/8 signalling in NK cells, which leads in turn to impaired inhibition of hepatic stellate cells. Impaired anti-fibrotic IL-6 production by monocytes with TLR7 and TLR3 stimulation may also contribute. Simultaneously, impaired TLR7/8 and TLR9-mediated interferon production by pDCs leads to impaired antigen presentation by dendritic cells and subsequent defective activation of CD4+ T cells, culminating in impaired T cell responses to HCV antigens, failure of viral clearance and aborted development of lasting immunity.

Clinical applications of TLR function in HCV:

TLR Polymorphisms and clinical outcome prediction

There have been recent considerable advances in our knowledge of TLR function and its role in HCV infection, but a more important question is how this knowledge may be harnessed to improve clinical outcomes.

Pathogen selection pressure has lead to considerably high rates of genetic polymorphism for TLR genes and many of these polymorphisms affect gene function. There has been great interest in exploring relationships between TLR gene polymorphism carriage and clinical disease as SNP detection by PCR is a relatively straightforward technique that could be employed for determining response to therapy and risk of adverse clinical outcomes in HCV infection. A summary of these polymorphisms is outlined in Table 4.

TLR4 gene polymorphism Thr399Gly and co-segregating Asp299Gly have been found to be protective against fibrosis progression in HCV infection, whilst Li et al
also found TLR4 SNPs rs4986791 and rs960312 were associated with increased fibrosis risk\textsuperscript{103}. Carriage of Asp299Gly and Thr399Gly is approximately 8% in Caucasian populations, whilst SNP rs960312 is important for its high prevalence within Asian populations (up to 25%). It has been shown that protective variants lower the apoptotic threshold of hepatocytes, inhibit TLR4 and NFκB signalling and are associated with greater spontaneous apoptosis of hepatic stellate cells\textsuperscript{104}.

By contrast, Eid et al\textsuperscript{105} found that in the post-transplant HCV setting, TLR2 polymorphism Arg753Gln homozygosity was strongly associated with rapid HCV fibrosis progression, but found no association between TLR4 polymorphisms and adverse outcomes.

The TLR7 gene is located on the X chromosome and three SNPs in this gene have been identified with >5% carriage within Caucasian populations: c.1-120T>G (rs2302267), c.32A>T (rs179008, Gln11Leu) and c.2403C>A (rs5743781, Ala448Val)\textsuperscript{106}. In chronic HCV infection, c.1-120T<G was found to be associated with lower levels of hepatic inflammation and fibrosis in males. PBMCs from patients with this genotype had increased IL-6 production in response to TLR7 ligand, providing a mechanistic clue to explain reduced hepatic fibrosis as IL-6 has been shown in various studies to be anti-fibrotic\textsuperscript{92-94}. In contrast, c.32A>T was associated with increased susceptibility to HCV in women, with higher levels of viraemia, more rapid disease progression and failure to respond to interferon-based HCV therapy\textsuperscript{107}. TLR7-mediated IFNα secretion is impaired in these women, whilst TLR7-mediated IL-6 production is preserved\textsuperscript{108}. 
These data collectively demonstrate that TLR2, TLR4 and TLR7 gene SNP detection may eventually provide potential screening tools for adverse outcomes in HCV-infected patients, guiding timing of therapy. However, further validation studies are warranted.

**TLR Therapeutics**

Given the evidence for impairment of TLR function in HCV infection, restoration of TLR function through TLR agonists is a theoretically attractive approach for potential therapy. In particular, restoration of TLR3, TLR7 and TLR9-mediated NK cell and dendritic cell interferon secretion so as to improve antigen presentation and T cell activation is an enticing target for therapy; these effects would not reduce immune responses against other infections, as may be seen if TLR inflammatory pathways were targeted. Importantly, TLR therapies may be less susceptible to viral resistance and broadly active against all HCV genotypes as they do not target HCV proteins directly.

There is evidence that TLR7 agonists are effective at HCV suppression. Isotoribine successfully reduced serum HCV levels in phase I trials, but unfortunately has been removed from further studies due to adverse events; other TLR7 agonists are under development\(^\text{109}\). A TLR9 agonist CPG10101 has also been developed; its administration produced promising reductions in HCV viral load in phase I trials\(^\text{110}\). Isotoribine and CPG10101 both increase interferon secretion, engendering robust polyclonal T cell responses. The side effect profiles of these agents are therefore similar to interferon-based regimens.
TLR4 antagonists have also been developed to dampen tissue-damaging immune responses. They have shown promise in colitis and sepsis trials\textsuperscript{111,112}, but their use in HCV has not yet been explored. Given the protective effect of TLR4 SNPs that lead to blunted TLR4 responses in HCV hepatic fibrosis, these agents may have therapeutic benefit in HCV infection.

Conclusion

The effects of HCV infection on TLR signalling are complex. Compartmentalisation of HCV modulation of TLR signalling means that HCV leads to upregulation of non-specific liver inflammation through stimulation of immune cells in an effort to achieve viral clearance. Conversely, suppression of TLR signalling in key anti-viral immune effector cells, such as dendritic cells, favours inhibition of inflammation which leads to viral persistence and chronic infection. Preliminary evidence suggests that therapeutic strategies harnessing TLR function will prove useful in HCV infection, whilst TLR polymorphisms offer a potential tool for prediction of adverse HCV-related outcomes.
**Figure 1.** Schematic overview of TLR signaling pathways.

Internalised viral PAMPs activate TLR3, TLR7/8 and TLR9 in endosomes, whereas bacterial PAMPs activate TLR1, TLR2, TLR4 and TLR6 from outside the cell. TLRs then interact with adaptor proteins (MyD88, TRIF, TRAM or TIRAP) to induce activation of downstream kinases and transcription factors, leading to upregulation of pro-inflammatory, anti-viral and anti-bacterial genes in the nucleus, including interferon synthesis.

**Figure 2.** Genomic structure of the hepatitis C virus.

Both viral and host cell proteases cleave HCV poly-protein to yield structural and non-structural proteins. These are important pathogen-associated molecular patterns recognised by Toll-like receptors and have several disabling effects on immune function.

**Figure 3.** Positive and negative effects of HCV viral proteins on TLR signaling. HCV core and NS3 proteins stimulate TLR2 and TLR4. HCV dsRNA binds to TLR3. The 3’ UTR tail is a pathogen associated molecular pattern for both TLR7 and TLR9. However, HCV NS3/NS4A degrades TRIF and binds to TBK1, inhibiting IFN production. HCV NS5A also binds to MyD88 to impair TLR2, TLR4, TLR7 and TLR9 signalling.
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PAMP, pathogen associated molecular pattern (exogenous ligand); DAMP, damage associated molecular pattern (endogenous ligand)
**Table 2. TLR Expression Peripheral Blood Immune Cells and Liver Cells**

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<td>Hepatocytes</td>
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<td>Biliary cells</td>
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mDC, myeloid dendritic cell; pDC, plasmacytoid dendritic cell; HSC, hepatic stellate cell
Table 3. Stimulatory and Inhibitory effects of HCV on TLR signalling in different cell types

<table>
<thead>
<tr>
<th>TLR</th>
<th>TLR Stimulation Mechanism</th>
<th>Cell type Description</th>
<th>Effect Description</th>
<th>TLR Inhibition Mechanism</th>
<th>Cell type</th>
<th>Effect Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>TLR2</td>
<td>Core, NS3 (TLR1, TLR6)</td>
<td>Monocytes, Macrophages</td>
<td>↑TLR2 expression, ↑TLR2 activation/cytokine production</td>
<td>HCV lipoparticles</td>
<td>DCs</td>
<td>↓Pro-inflammatory cytokines, ↑IL-10 secretion</td>
</tr>
<tr>
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<td></td>
<td></td>
<td></td>
<td>monocytes</td>
<td>DCs and monocytes</td>
</tr>
<tr>
<td>TLR3</td>
<td>RNA</td>
<td>Monocytes, mDCs, non-parenchymal liver cells</td>
<td>↑TLR3 expression, ↑IFNβ</td>
<td>RNA</td>
<td>Monocytes</td>
<td>↓IRF3 inflammatory cytokines (IL-6)</td>
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<tr>
<td>TLR4</td>
<td>NS5A</td>
<td>Hepatocytes, B cells</td>
<td>↑TLR4 expression, ↑TLR4 activation/cytokine production</td>
<td>HCV lipoparticles</td>
<td>DCs</td>
<td>↓TLR4 expression</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>↑IFNβ/ ISGs, Monocyte tolerance to LPS, ↑Liver fibrogenesis</td>
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</tr>
<tr>
<td>TLR7/8</td>
<td>RNA Poly-U tail</td>
<td>DCs, NK cells, hepatocytes, monocytes</td>
<td>↑Pro-inflammatory cytokines, ↑TLR7/8 expression</td>
<td>NS5A</td>
<td>DCs</td>
<td>↓TLR7/8 expression, ↓TLR7/8 signalling, ↓IRF7</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>monocytes</td>
<td></td>
<td>NK cells</td>
<td>↑Degradation TLR7 liver, ↓IFNaβ, ↓NK cell IFNγ, ↓Inhibition of stellate cells/fibrogenesis</td>
</tr>
<tr>
<td>TLR9</td>
<td>DNA Poly-U tail</td>
<td>DCs</td>
<td>↑Pro-inflammatory cytokines mDCs</td>
<td>DNA Poly-U tail</td>
<td>pDCs</td>
<td>↓IFNa/β, ↓HLA-DR</td>
</tr>
</tbody>
</table>

DC, dendritic cell; mDC, myeloid dendritic cell; pDC, plasmacytoid dendritic cell
Table 4. TLR gene polymorphisms and association with HCV infection

<table>
<thead>
<tr>
<th>TLR</th>
<th>Polymorphism</th>
<th>Estimated carriage</th>
<th>Mutation type</th>
<th>Disease association</th>
</tr>
</thead>
<tbody>
<tr>
<td>TLR2</td>
<td>Arg753Gln</td>
<td>2-10%</td>
<td>Missense</td>
<td>HCV rapid fibrosis post liver transplant</td>
</tr>
<tr>
<td></td>
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<td>caucasian Rare asian</td>
<td></td>
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</tr>
<tr>
<td>TLR4</td>
<td>Asp299Gly and Thr399Ile</td>
<td>8% caucasian</td>
<td>Missense</td>
<td>Reduced fibrosis in HCV</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Co-segregate</td>
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</tr>
<tr>
<td>TLR7</td>
<td>c.1-120T&gt;G (rs2302267)</td>
<td>&gt;5% caucasian</td>
<td>Intron 1 punitive splicing</td>
<td>Reduced HCV fibrosis in males</td>
</tr>
<tr>
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<tr>
<td>TLR7</td>
<td>c.32A&gt;T (rs179008, Gln11Leu)</td>
<td>&gt;5% caucasians</td>
<td>Transversion amino acid change</td>
<td>Increased chronic HCV in women</td>
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<td></td>
<td></td>
<td>Decreased response to IFN-based HCV therapy in women</td>
</tr>
<tr>
<td>TLR7</td>
<td>c.2403C&gt;A (rs5743781, Ala448Val)</td>
<td>&gt;5% caucasians</td>
<td>Non-synonymous alteration exon 3</td>
<td>Increased chronic HCV men and women</td>
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</table>
Viral RNA: TLR3, TLR7/8, TLR9