Author/s:
Howell, J;Angus, P;Gow, P;Visvanathan, K

Title:
Toll-like receptors in hepatitis C infection: Implications for pathogenesis and treatment

Date:
2013-01-01

Citation:
Howell, J., Angus, P., Gow, P. & Visvanathan, K. (2013). Toll-like receptors in hepatitis C infection: Implications for pathogenesis and treatment. Journal of Gastroenterology and Hepatology Australia, 28 (5), pp.766-776. https://doi.org/10.1111/jgh.12170.

Persistent Link:
https://hdl.handle.net/11343/43833

Toll-Like Receptors in Hepatitis C Infection:

Implications for Pathogenesis and Treatment

Jessica Howell[1, 2, 3], Peter Angus[1, 2], Paul Gow[1, 2], Kumar Visvanathan[2, 3, 4].

Liver Transplant Unit, Austin Hospital, Melbourne[1]; Department of Medicine, University of Melbourne[2]; Innate Immune Laboratory, Monash Medical Centre[3], Department of Medicine, St Vincent's Hospital[4], Melbourne, Australia.

Running Title: TLRs in HCV Infection

Article type: Review

Corresponding author: Dr Jessica Howell
Liver Transplant Unit
Austin Hospital
Studley Rd, Heidelberg 3084
Victoria, AUSTRALIA
manukascarlet@yahoo.com
Jess.Howell@austin.org.au
61 3 409433912 (mobile)
61 3 94965000 (business phone)
61 3 94963487 (fax)

Number of Tables: 4
Number of Figures: 3

Author Disclosures: No author had any disclosures to make relevant to this manuscript.

Grant Support: Dr Jessica Howell received scholarship funds for stipend from the Gastroenterological Society of Australia (GESA)

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[3]. 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[4, 5].

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[6]. To date, there are ten functional TLRs identified in humans (TLR 1-10)[7]. Each receptor has two domains: an extracellular leucine rich LRR domain and an intracellular Toll-Interleukin (Il-1 receptor (TIR) domain[8].

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[9]. 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 [10]. 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[11].

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)[12] 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[13]. 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[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[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[17, 18]. TLR-mediated Il-12 and Il-18 from Kupffer cells induces hepatic NK cells to produce IFNγ, which is critical for viral eradication and inhibition of hepatic stellate cells and hepatic fibrogenesis[19]. Kupffer cells also play a direct role in fibrogenesis, secreting TGF-β, matrix metalloproteinases, platelet derived growth factor (PDGF) and reactive oxygen species (ROS) with TLR4 stimulation[15].

Hepatic stellate cells (HSCs) are the major fibrogenic cell type in the liver[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[21, 22]. TLR4 and TLR9 pathways are the most important in HSC activation and fibrogenesis[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[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[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[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[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[28]. Upon stimulation with various ligands IκBs are phosphorylated at serine residues by the IκK complex. This causes degradation of the IκB, 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-β[29]. Recruitment of IRF5 then leads to induction of inflammatory cytokines IL6, IL12, p40 and TNFα, but not type I IFN[28].

The TRIF-IRF3-IFN pathway

TLR3 and TLR4 stimulation can lead to IFNα and IFNβ production via the TRIF pathway, leading to IκK (noncanonical IkB kinase) and TBK1 (TANK-binding kinase 1) activation which in turn phosphorylate IRF3 and lead to transcription of IRF3 dependent genes[30, 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[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[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[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[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[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[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[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[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[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 IFNγ secretion leading to reduced inhibition of HCV replication, reduced inhibition of hepatic stellate cells and greater hepatic fibrosis[56-58]. Th2-skewed NK cells further downregulate DC function by secreting IL-10 and TGF-β[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[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[62, 63]. 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[64], 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[65-68].

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[65]. TLR4 also induces IFNβ production, which leads to paracrine IFN production and upregulation of interferon sensitive genes (ISGs) within infected cells and

surrounding tissues[69]. 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 [64, 70].

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[71, 72]. TLR3 mRNA is significantly increased in monocytes in chronic HCV infection[73]. 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[74]. mDCs have normal functioning TLR3 and can produce IL-12, IL-6, Il-10, IFNγ and TNFα with TLR3 stimulation despite HCV infection[75].

HCV genomic RNA has direct immunostimulatory effects on TLR7 and TLR8, leading to IFNα production and activation of IRF7 and NFκB[76]. pDCs can also be activated via TLR7 and TLR9 through the HCV RNA poly-uridine tail[76-81]. TLR7 activation of hepatocytes also induces IFN-independent anti-viral effects, reducing both HCV RNA levels and NS5A protein expression in cell lines[82]. There is also increased TLR7 and TLR8 expression on monocytes in HCV infection, though the significance of this remains unclear[64].

TLRs in HCV: Immune Evasion

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[83, 84]. NS3/4A also interacts directly with TBK1 to reduce TBK1-IRF3 interaction and therefore inhibit IRF3 activation[85]. 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[86].

The HCV lipo-viral particle interferes directly with TLR4 signalling in DCs whilst HCV core protein suppresses TLR4 expression[64, 87]. 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[49, 56, 88]. 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[89].

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[64, 86, 90]. Interestingly, lower TLR7 expression in HCV-infected livers is restored with successful HCV clearance with treatment [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[91]. HCV also impairs TLR9-mediated IFNα and IFNβ production and HLA-DR expression by pDCs, associated with impaired activation of naïve T cells[49]. TLR9 signalling in mDCs is unaffected[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[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[92-95]. 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[49, 82, 83, 96-99].

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[100, 101]. 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[102], whilst Li et al

also found TLR4 SNPs rs4986791 and rs960312 were associated with increased fibrosis risk[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[104].

By contrast, Eid et al[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)[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[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[107]. TLR7-mediated IFNα secretion is impaired in these women, whilst TLR7-mediated IL-6 production is preserved[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[109]. A TLR9 agonist CPG10101 has also been developed; its administration produced promising reductions in HCV viral load in phase I trials[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[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-baterial 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.

References

1. Gane EJ. The natural history of recurrent hepatitis C and what influences this. Liver Transpl 2008;14 Suppl 2:S36-44.
2. Kim WR. The burden of hepatitis C in the United States. Hepatology 2002;36:S30-4.
3. Barton GM, Medzhitov R. Control of adaptive immune responses by Toll-like receptors. Curr Opin Immunol 2002;14:380-3.
4. Delves PJ, Roitt IM. The immune system. First of two parts. New Eng J Med 2000;343:37-49.
5. Testro AG, Visvanathan K. Toll-like receptors and their role in gastrointestinal disease. J Gastroenterol Hepatol 2009;24:943-54.
6. Medzhitov R, Preston-Hurlburt P, Janeway CA, Jr. A human homologue of the Drosophila Toll protein signals activation of adaptive immunity. Nature 1997;388:394-7.
7. Seki E, Brenner DA. Toll-like receptors and adaptor molecules in liver disease: update. Hepatology 2008;48:322-35.
8. West AP, Koblansky AA, Ghosh S. Recognition and signaling by toll-like receptors. Ann Rev Cell Dev Biol 2006;22:409-37.
9. Lotze MT, Zeh HJ, Rubartelli A, Sparvero LJ, Amoscato AA, Washburn NR, et al. The grateful dead: damage-associated molecular pattern molecules and reduction/oxidation regulate immunity. Immunol Rev 2007;220:60-81.
10. Haziot A, Chen S, Ferrero E, Low MG, Silber R, Goyert SM. The monocyte differentiation antigen, CD14, is anchored to the cell membrane by a phosphatidylinositol linkage. J Immunol 1988;141:547-52.

11. Iwasaki A, Medzhitov R. Toll-like receptor control of the adaptive immune responses. Nat Immunol 2004;5:987-95.

12. Yoneyama M, Kikuchi M, Natsukawa T, Shinobu N, Imaizumi T, Miyagishi M, et al. The RNA helicase RIG-I has an essential function in double-stranded RNA-induced innate antiviral responses. Nat Immunol 2004;5:730-7.

13. Matzinger P. The danger model: a renewed sense of self. Science 2002;296:301-5.

14. Szabo G, Dolganiuc A, Mandrekar P. Pattern recognition receptors: a contemporary view on liver diseases. Hepatology 2006;44:287-98.

15. Schwabe RF, Seki E, Brenner DA. Toll-like receptor signaling in the liver. Gastroenterology 2006;130:1886-900.

16. Crispe IN. Hepatic T cells and liver tolerance. Nat Rev Immunol 2003;3:51-62.

17. Wu J, Lu M, Meng Z, Trippler M, Broering R, Szczeponek A, et al. Toll-like receptor-mediated control of HBV replication by nonparenchymal liver cells in mice. Hepatology 2007;46:1769-78.

18. Seki E, Tsutsui H, Tsuji NM, Hayashi N, Adachi K, Nakano H, et al. Critical roles of myeloid differentiation factor 88-dependent proinflammatory cytokine release in early phase clearance of Listeria monocytogenes in mice. J Immunol 2002;169:3863-8.

19. Tsutsui H, Matsui K, Okamura H, Nakanishi K. Pathophysiological roles of interleukin-18 in inflammatory liver diseases. Immunol Rev 2000;174:192-209.

20. Friedman SL. Hepatic stellate cells: protean, multifunctional, and enigmatic cells of the liver. Physiol Rev 2008;88:125-72.

21. Eng FJ, Friedman SL. Fibrogenesis I. New insights into hepatic stellate cell activation: the simple becomes complex. Am J Physiol Gastrointest Liver Physiol 2000;279:G7-G11.

22. Bataller R, Brenner DA. Liver fibrosis. J Clin Invest 2005;115:209-18.

23. Paik YH, Schwabe RF, Bataller R, Russo MP, Jobin C, Brenner DA. Toll-like receptor 4 mediates inflammatory signaling by bacterial lipopolysaccharide in human hepatic stellate cells. Hepatology 2003;37:1043-55.

24. Watanabe A, Hashmi A, Gomes DA, Town T, Badou A, Flavell RA, Mehal WZ. Apoptotic hepatocyte DNA inhibits hepatic stellate cell chemotaxis via toll-like receptor 9. Hepatology 2007;46:1509-18.

25. Takeda K, Kaisho T, Akira S. Toll-like receptors. Ann Rev Immunol 2003;21:335-76.

26. Schnare M, Barton GM, Holt AC, Takeda K, Akira S, Medzhitov R. Toll-like receptors control activation of adaptive immune responses. Nat Immunol 2001;2:947-50.

27. Mencin A, Kluwe J, Schwabe RF. Toll-like receptors as targets in chronic liver diseases. Gut 2009;58:704-20.

28. Takaoka A, Yanai H, Kondo S, Duncan G, Negishi H, Mizutani T, et al. Integral role of IRF-5 in the gene induction programme activated by Toll-like receptors. Nature 2005;434:243-9.

29. Uematsu S, Akira S. Toll-like receptors and Type I interferons. J Biol Chem 2007;282:15319-23.

30. Fitzgerald KA, McWhirter SM, Faia KL, Rowe DC, Latz E, Golenbock DT, et al. IKKepsilon and TBK1 are essential components of the IRF3 signaling pathway. Nat Immunol 2003;4:491-6.

31. McWhirter SM, Fitzgerald KA, Rosains J, Rowe DC, Golenbock DT, Maniatis T. IFN-regulatory factor 3-dependent gene expression is defective in Tbk1-deficient mouse embryonic fibroblasts. Proc Natl Acad Sci USA 2004;101:233-8.

32. Liew FY, Xu D, Brint EK, O'Neill LA. Negative regulation of toll-like receptor-mediated immune responses. Nat Rev Immunol 2005;5:446-58.

33. Broad A, Kirby JA, Jones DE. Toll-like receptor interactions: tolerance of MyD88-dependent cytokines but enhancement of MyD88-independent interferon-beta production. Immunology 2007;120:103-11.

34. Choo QL, Kuo G, Weiner AJ, Overby LR, Bradley DW, Houghton M. Isolation of a cDNA clone derived from a blood-borne non-A, non-B viral hepatitis genome. Science 1989;244:359-62.

35. Ghany MG, Strader DB, Thomas DL, Seeff LB. Diagnosis, management, and treatment of hepatitis C: an update. Hepatology 2009;49:1335-74.

36. Rosen HR. Clinical practice. Chronic hepatitis C infection. New Eng J Med 2011;364:2429-38.

37. Thimme R, Oldach D, Chang KM, Steiger C, Ray SC, Chisari FV. Determinants of viral clearance and persistence during acute hepatitis C virus infection. J Exp Med 2001;194:1395-406.

38. Lechner F, Wong DK, Dunbar PR, Chapman R, Chung RT, Dohrenwend P, et al. Analysis of successful immune responses in persons infected with hepatitis C virus. J Exp Med 2000;191:1499-512.

39. Nelson DR, Marousis CG, Ohno T, Davis GL, Lau JY. Intrahepatic hepatitis C virus-specific cytotoxic T lymphocyte activity and response to interferon alfa therapy in chronic hepatitis C. Hepatology 1998;28:225-30.

40. Gerlach JT, Diepolder HM, Jung MC, Gruener NH, Schraut WW, Zachoval R, et al. Recurrence of hepatitis C virus after loss of virus-specific CD4(+) T-cell response in acute hepatitis C. Gastroenterology 1999;117:933-41.

41. Kamal SM, Rasenack JW, Bianchi L, Al Tawil A, El Sayed Khalifa K, Peter T, et al. Acute hepatitis C without and with schistosomiasis: correlation with hepatitis C-specific CD4(+) T-cell and cytokine response. Gastroenterology 2001;121:646-56.

42. Sarih M, Bouchrit N, Benslimane A. Different cytokine profiles of peripheral blood mononuclear cells from patients with persistent and self-limited hepatitis C virus infection. Immunol Lett 2000;74:117-20.

43. Sobue S, Nomura T, Ishikawa T, Ito S, Saso K, Ohara H, et al. Th1/Th2 cytokine profiles and their relationship to clinical features in patients with chronic hepatitis C virus infection. J Gastroenterol 2001;36:544-51.

44. Zein NN. Mission poorly accomplished: the protective role of natural killer cells in recurrent hepatitis C after liver transplantation. Liver Transpl 2008;14:4-6.

45. Missale G, Bertoni R, Lamonaca V, Valli A, Massari M, Mori C, et al. Different clinical behaviors of acute hepatitis C virus infection are associated with different vigor of the anti-viral cell-mediated immune response. J Clin Invest 1996;98:706-14.

46. Eckels DD, Wang H, Bian TH, Tabatabai N, Gill JC. Immunobiology of hepatitis C virus (HCV) infection: the role of CD4 T cells in HCV infection. Immunol Rev 2000;174:90-7.

47. Grakoui A, Shoukry NH, Woollard DJ, Han JH, Hanson HL, Ghrayeb J, et al. HCV persistence and immune evasion in the absence of memory T cell help. Science 2003;302:659-62.

48. Villacres MC, Literat O, DeGiacomo M, Du W, Frederick T, Kovacs A. Defective response to Toll-like receptor 3 and 4 ligands by activated monocytes in chronic hepatitis C virus infection. J Viral Hepat 2008;15:137-44.

49. Yonkers NL, Rodriguez B, Milkovich KA, Asaad R, Lederman MM, Heeger PS, Anthony DD. TLR ligand-dependent activation of naive CD4 T cells by plasmacytoid dendritic cells is impaired in hepatitis C virus infection. J Immunol 2007;178:4436-44.

50. Valdez H, Anthony D, Farukhi F, Patki A, Salkowitz J, Heeger P, et al. Immune responses to hepatitis C and non-hepatitis C antigens in hepatitis C virus infected and HIV-1 coinfected patients. AIDS 2000;14:2239-46.

51. Gruener NH, Lechner F, Jung MC, Diepolder H, Gerlach T, Lauer G, et al. Sustained dysfunction of antiviral CD8+ T lymphocytes after infection with hepatitis C virus. J Virol 2001;75:5550-8.

52. Wedemeyer H, He XS, Nascimbeni M, Davis AR, Greenberg HB, Hoofnagle JH, et al. Impaired effector function of hepatitis C virus-specific CD8+ T cells in chronic hepatitis C virus infection. J Immunol 2002;169:3447-58.

53. Ulsenheimer A, Gerlach JT, Gruener NH, Jung MC, Schirren CA, Schraut W, et al. Detection of functionally altered hepatitis C virus-specific CD4 T cells in acute and chronic hepatitis C. Hepatology 2003;37:1189-98.

54. Wertheimer AM, Miner C, Lewinsohn DM, Sasaki AW, Kaufman E, Rosen HR. Novel CD4+ and CD8+ T-cell determinants within the NS3 protein in

subjects with spontaneously resolved HCV infection. Hepatology 2003;37:577-89.

55. McHutchison JG, Bacon BR, Gordon SC, Lawitz E, Shiffman M, Afdhal NH, et al. Phase 1B, randomized, double-blind, dose-escalation trial of CPG 10101 in patients with chronic hepatitis C virus. Hepatology 2007;46:1341-9.

56. Kanto T, Hayashi N. Innate immunity in hepatitis C virus infection: Interplay among dendritic cells, natural killer cells and natural killer T cells. Hepatol Res 2007;37 Suppl 3:S319-26.

57. Guidotti LG, Chisari FV. Noncytolytic control of viral infections by the innate and adaptive immune response. Annu Rev Immunol 2001;19:65-91.

58. Jinushi M, Takehara T, Kanto T, Tatsumi T, Groh V, Spies T, et al. Critical role of MHC class I-related chain A and B expression on IFN-alpha-stimulated dendritic cells in NK cell activation: impairment in chronic hepatitis C virus infection. J Immunol 2003;170:1249-56.

59. Jinushi M, Takehara T, Tatsumi T, Kanto T, Miyagi T, Suzuki T, et al. Negative regulation of NK cell activities by inhibitory receptor CD94/NKG2A leads to altered NK cell-induced modulation of dendritic cell functions in chronic hepatitis C virus infection. J Immunol 2004;173:6072-81.

60. McCoy CE, O'Neill LA. The role of toll-like receptors in macrophages. Front Bio 2008;13:62-70.

61. Reis e Sousa C. Toll-like receptors and dendritic cells: for whom the bug tolls. Semin Immunol 2004;16:27-34.

62. Ishii S, Koziel MJ. Immune responses during acute and chronic infection with hepatitis C virus. Clin Immunol 2008;128:133-47.

63. Rehermann B, Nascimbeni M. Immunology of hepatitis B virus and hepatitis C virus infection. Nat Rev Immunol 2005;5:215-29.

64. Sato K, Ishikawa T, Okumura A, Yamauchi T, Sato S, Ayada M, et al. Expression of Toll-like receptors in chronic hepatitis C virus infection. J Gastroenterol Hepatol 2007;22:1627-32.

65. Machida K, Cheng KT, Sung VM, Levine AM, Foung S, Lai MM. Hepatitis C virus induces toll-like receptor 4 expression, leading to enhanced production of beta interferon and interleukin-6. J Virol 2006;80:866-74.

66. Matsuguchi T, Musikacharoen T, Ogawa T, Yoshikai Y. Gene expressions of Toll-like receptor 2, but not Toll-like receptor 4, is induced by LPS and inflammatory cytokines in mouse macrophages. J Immunol 2000;165:5767-72.

67. Machida K, Cheng KT, Pavio N, Sung VM, Lai MM. Hepatitis C virus E2-CD81 interaction induces hypermutation of the immunoglobulin gene in B cells. J Virol 2005;79:8079-89.

68. Pileri P, Uematsu Y, Campagnoli S, Galli G, Falugi F, Petracca R, et al. Binding of hepatitis C virus to CD81. Science 1998;282:938-41.

69. Taniguchi T, Ogasawara K, Takaoka A, Tanaka N. IRF family of transcription factors as regulators of host defense. Annu Rev Immunol 2001;19:623-55.

70. Dolganiuc A, Norkina O, Kodys K, Catalano D, Bakis G, Marshall C, et al. Viral and host factors induce macrophage activation and loss of toll-like receptor tolerance in chronic HCV infection. Gastroenterology 2007;133:1627-36.

71. Li K, Chen Z, Kato N, Gale M, Jr., Lemon SM. Distinct poly(I-C) and virus-activated signaling pathways leading to interferon-beta production in hepatocytes. J Biol Chem 2005;280:16739-47.

72. Broering R, Wu J, Meng Z, Hilgard P, Lu M, Trippler M, et al. Toll-like receptor-stimulated non-parenchymal liver cells can regulate hepatitis C virus replication. J Hepatol 2008;48:914-22.

73. Dolganiuc A, Garcia C, Kodys K, Szabo G. Distinct Toll-like receptor expression in monocytes and T cells in chronic HCV infection. World J Gastroenterol 2006;12:1198-204.

74. Geiss GK, Carter VS, He Y, Kwieciszewski BK, Holzman T, Korth MJ, et al. Gene expression profiling of the cellular transcriptional network regulated by alpha/beta interferon and its partial attenuation by the hepatitis C virus nonstructural 5A protein. J Virol 2003;77:6367-75.

75. Shiina M, Rehermann B. Cell culture-produced hepatitis C virus impairs plasmacytoid dendritic cell function. Hepatology 2008;47:385-95.

76. Zhang YL, Guo YJ, Bin L, Sun SH. Hepatitis C virus single-stranded RNA induces innate immunity via Toll-like receptor 7. J Hepatol 2009;51:29-38.

77. Decalf J, Fernandes S, Longman R, Ahloulay M, Audat F, Lefrerre F, et al. Plasmacytoid dendritic cells initiate a complex chemokine and cytokine network and are a viable drug target in chronic HCV patients. J Exp Med 2007;204:2423-37.

78. Zhang T, Lin RT, Li Y, Douglas SD, Maxcey C, Ho C, et al. Hepatitis C virus inhibits intracellular interferon alpha expression in human hepatic cell lines. Hepatology 2005;42:819-27.

79. Meier A, Alter G, Frahm N, Sidhu H, Li B, Bagchi A, et al. MyD88-dependent immune activation mediated by human immunodeficiency virus type 1-encoded Toll-like receptor ligands. J Virol 2007;81:8180-91.

80. Judge AD, Sood V, Shaw JR, Fang D, McClintock K, MacLachlan I. Sequence-dependent stimulation of the mammalian innate immune response by synthetic siRNA. Nat Biotechnol 2005;23:457-62.

81. Hornung V, Guenthner-Biller M, Bourquin C, Ablasser A, Schlee M, Uematsu S, et al. Sequence-specific potent induction of IFN-alpha by short interfering RNA in plasmacytoid dendritic cells through TLR7. Nat Med 2005;11:263-70.

82. Lee J, Wu CC, Lee KJ, Chuang TH, Katakura K, Liu YT, et al. Activation of anti-hepatitis C virus responses via Toll-like receptor 7. Proc Natl Acad Sci U S A 2006;103:1828-33.

83. Foy E, Li K, Wang C, Sumpter R, Jr., Ikeda M, Lemon SM, Gale M, Jr. Regulation of interferon regulatory factor-3 by the hepatitis C virus serine protease. Science 2003;300:1145-8.

84. Li K, Foy E, Ferreon JC, Nakamura M, Ferreon AC, Ikeda M, et al. Immune evasion by hepatitis C virus NS3/4A protease-mediated cleavage of the Toll-like receptor 3 adaptor protein TRIF. Proc Natl Acad Sci U S A 2005;102:2992-7.

85. Otsuka M, Kato N, Moriyama M, Taniguchi H, Wang Y, Dharel N, et al. Interaction between the HCV NS3 protein and the host TBK1 protein leads to inhibition of cellular antiviral responses. Hepatology 2005;41:1004-12.

86. Abe T, Kaname Y, Hamamoto I, Tsuda Y, Wen X, Taguwa S, et al. Hepatitis C virus nonstructural protein 5A modulates the toll-like receptor-MyD88-

dependent signaling pathway in macrophage cell lines. J Virol 2007;81:8953-66.

87. Agaugue S, Perrin-Cocon L, Andre P, Lotteau V. Hepatitis C lipo-Viro-particle from chronically infected patients interferes with TLR4 signaling in dendritic cell. PloS one 2007;2:e330.

88. Yakushijin T, Kanto T, Inoue M, Oze T, Miyazaki M, Itose I, et al. Reduced expression and functional impairment of Toll-like receptor 2 on dendritic cells in chronic hepatitis C virus infection. Hepatol Res 2006;34:156-62.

89. Chang S, Dolganiuc A, Szabo G. Toll-like receptors 1 and 6 are involved in TLR2-mediated macrophage activation by hepatitis C virus core and NS3 proteins. J Leuk Biol 2007;82:479-87.

90. Chang S, Kodys K, Szabo G. Impaired expression and function of toll-like receptor 7 in hepatitis C virus infection in human hepatoma cells. Hepatology 2010;51:35-42.

91. Fukuda K, Tsuchihara K, Hijikata M, Nishiguchi S, Kuroki T, Shimotohno K. Hepatitis C virus core protein enhances the activation of the transcription factor, Elk1, in response to mitogenic stimuli. Hepatology 2001;33:159-65.

92. Galun E, Zeira E, Pappo O, Peters M, Rose-John S. Liver regeneration induced by a designer human IL-6/sIL-6R fusion protein reverses severe hepatocellular injury. FASEB 2000;14:1979-87.

93. Zimmers TA, Pierce RH, McKillop IH, Koniaris LG. Resolving the role of IL-6 in liver regeneration. Hepatology 2003;38:1590-1; author reply 1591.

94. Bansal MB, Kovalovich K, Gupta R, Li W, Agarwal A, Radbill B, et al. Interleukin-6 protects hepatocytes from CCl4-mediated necrosis and apoptosis in mice by reducing MMP-2 expression. J Hepatol 2005;42:548-56.

95. Melhem A, Muhanna N, Bishara A, Alvarez CE, Ilan Y, Bishara T, et al. Anti-fibrotic activity of NK cells in experimental liver injury through killing of activated HSC. J Hepatol 2006;45:60-71.

96. Honda K, Yanai H, Negishi H, Asagiri M, Sato M, Mizutani T, et al. IRF-7 is the master regulator of type-I interferon-dependent immune responses. Nature 2005;434:772-7.

97. Grouard G, Rissoan MC, Filgueira L, Durand I, Banchereau J, Liu YJ. The enigmatic plasmacytoid T cells develop into dendritic cells with interleukin (IL)-3 and CD40-ligand. J Exp Med 1997;185:1101-11.

98. O'Doherty U, Peng M, Gezelter S, Swiggard WJ, Betjes M, Bhardwaj N, Steinman RM. Human blood contains two subsets of dendritic cells, one immunologically mature and the other immature. Immunology 1994;82:487-93.

99. Anthony DD, Yonkers NL, Post AB, Asaad R, Heinzel FP, Lederman MM, et al. Selective impairments in dendritic cell-associated function distinguish hepatitis C virus and HIV infection. J Immunol 2004;172:4907-16.

100. Smirnova I, Poltorak A, Chan EK, McBride C, Beutler B. Phylogenetic variation and polymorphism at the toll-like receptor 4 locus (TLR4). Gen Biol 2000;1:RESEARCH002.

101. Ferwerda B, McCall MB, Alonso S, Giamarellos-Bourboulis EJ, Mouktaroudi M, Izagirre N, et al. TLR4 polymorphisms, infectious diseases, and evolutionary pressure during migration of modern humans. Proc Natl Acad Sci U S A 2007;104:16645-50.

102. Huang H, Shiffman ML, Friedman S, Venkatesh R, Bzowej N, Abar OT, et al. A 7 gene signature identifies the risk of developing cirrhosis in patients with chronic hepatitis C. Hepatology 2007;46:297-306.

103. Li Y, Chang M, Abar O, Garcia V, Rowland C, Catanese J, et al. Multiple variants in toll-like receptor 4 gene modulate risk of liver fibrosis in Caucasians with chronic hepatitis C infection. J Hepatol 2009;51:750-7.

104. Guo J, Loke J, Zheng F, Hong F, Yea S, Fukata M, et al. Functional linkage of cirrhosis-predictive single nucleotide polymorphisms of Toll-like receptor 4 to hepatic stellate cell responses. Hepatology 2009;49:960-8.

105. Eid AJ, Brown RA, Paya CV, Razonable RR. Association between toll-like receptor polymorphisms and the outcome of liver transplantation for chronic hepatitis C virus. Transplantation 2007;84:511-6.

106. Schott E, Witt H, Neumann K, Taube S, Oh DY, Schreier E, et al. A Toll-like receptor 7 single nucleotide polymorphism protects from advanced inflammation and fibrosis in male patients with chronic HCV-infection. J Hepatol 2007;47:203-11.

107. Schott E, Witt H, Neumann K, Bergk A, Halangk J, Weich V, et al. Association of TLR7 single nucleotide polymorphisms with chronic HCV-infection and response to interferon-a-based therapy. J Viral Hep 2008;15:71-8.

108. Oh DY, Baumann K, Hamouda O, Eckert JK, Neumann K, Kucherer C, et al. A frequent functional toll-like receptor 7 polymorphism is associated with accelerated HIV-1 disease progression. AIDS 2009;23:297-307.

109. Lee J, Chuang TH, Redecke V, She L, Pitha PM, Carson DA, et al. Molecular basis for the immunostimulatory activity of guanine nucleoside analogs:

activation of Toll-like receptor 7. Proc Natl Acad Sci U S A 2003;100:6646-51.

110. Pappas SC. Good science behind hepatitis C virus antiviral drug development: necessary but not sufficient. Hepatology 2007;46:1317-8.

111. Kanzler H, Barrat FJ, Hessel EM, Coffman RL. Therapeutic targeting of innate immunity with Toll-like receptor agonists and antagonists. Nat Med 2007;13:552-9.

112. Fort MM, Mozaffarian A, Stover AG, Correia Jda S, Johnson DA, Crane RT, et al. A synthetic TLR4 antagonist has anti-inflammatory effects in two murine models of inflammatory bowel disease. J Immunol 2005;174:6416-23.

Table 1. Human TLR receptors and their corresponding ligands

TLR	Ligand	Ligand Type
TLR1	Complexes with TLR2	
TLR2	Lipoproteins (diacylated lipopeptides) and lipoteichoic acid	PAMP
	HMGB1, hyaluronan, heat shock proteins 60 & 70	DAMP
TLR3	double-stranded RNA, particularly viral	PAMP
TLR4	Lipopolysaccharide	PAMP
	HMGB1, hyaluronan, heat shock proteins 60 & 70	DAMP
	Free fatty acids, heparin sulphate, uric acid	
TLR5	Flagellin	PAMP
TLR6	Complexes with TLR2	
TLR7	single stranded RNA, particularly viral	PAMP
TLR8	single stranded RNA, particularly viral	PAMP
TLR9	bacterial unmethylated CpG motif containing DNA	PAMP
	HMGB1	DAMP
TLR10	Unknown	

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

	TLR 1	TLR 2	TLR 3	TLR 4	TLR 5	TLR 6	TLR 7	TLR 8	TLR 9	TLR 10
Monocytes	✓	✓		✓	✓		✓	✓		
mDCs	✓	✓	✓	✓	✓			✓		
pDCs	✓						✓		✓	✓
Neutrophils	✓			✓	✓	✓	✓		✓	✓
Eosinophils	✓						✓			
Mast cells	✓	✓		✓		✓				
Myeloid cells	✓	✓				✓		✓		
NK cells	✓	✓	✓		✓	✓	✓	✓	✓	
T cells	✓	✓	✓	✓	✓		✓	✓	✓	
Tregs	✓	✓		✓	✓	✓	✓	✓		
B cells	✓					✓	✓	✓	✓	✓
Hepatocytes	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Kupffer cells	✓	✓	✓	✓					✓	
HSCs	✓	✓		✓					✓	
Biliary cells	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Endothelial cells	✓	✓	✓	✓	✓	✓	✓	✓	✓	

mDC, myeloid dendritic cell; pDC, plasmacytoid dendritic cell; HSC, hepatic stellate cell

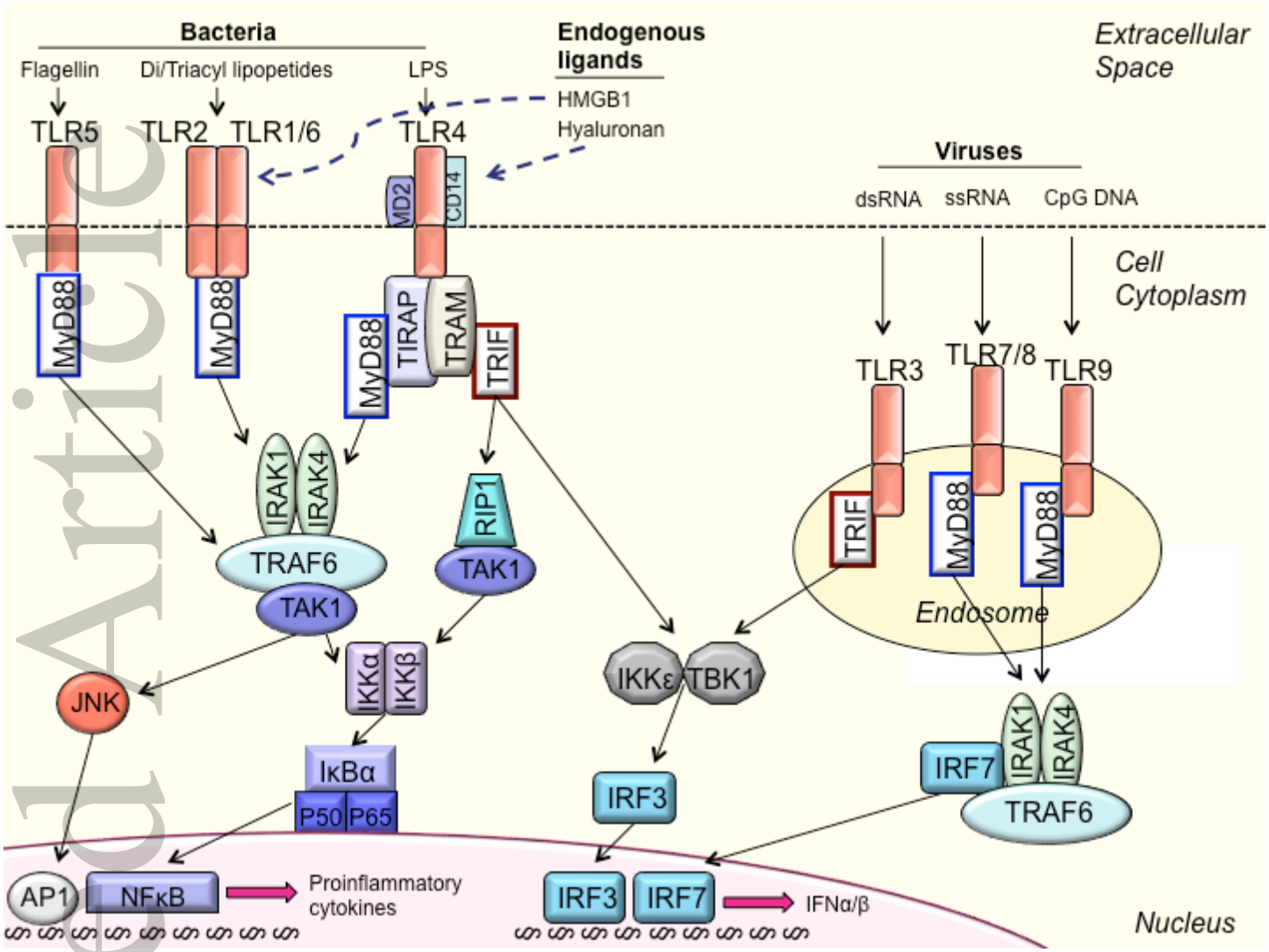
Table 3. Stimulatory and Inhibitory effects of HCV on TLR signalling in different cell types

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

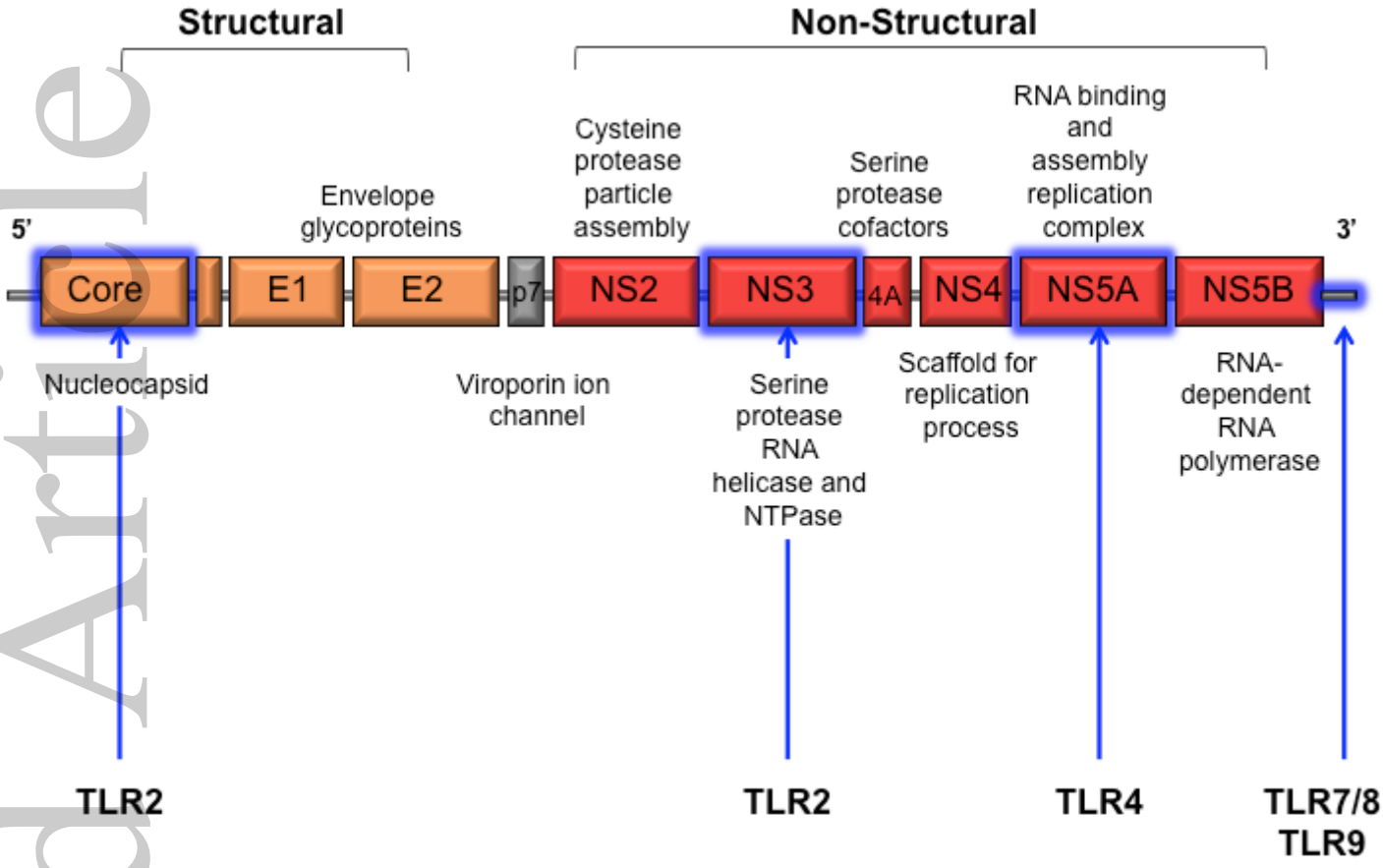
DC, dendritic cell; mDC, myeloid dendritic cell; pDC, plasmacytoid dendritic cell

Table 4. TLR gene polymorphisms and association with HCV infection

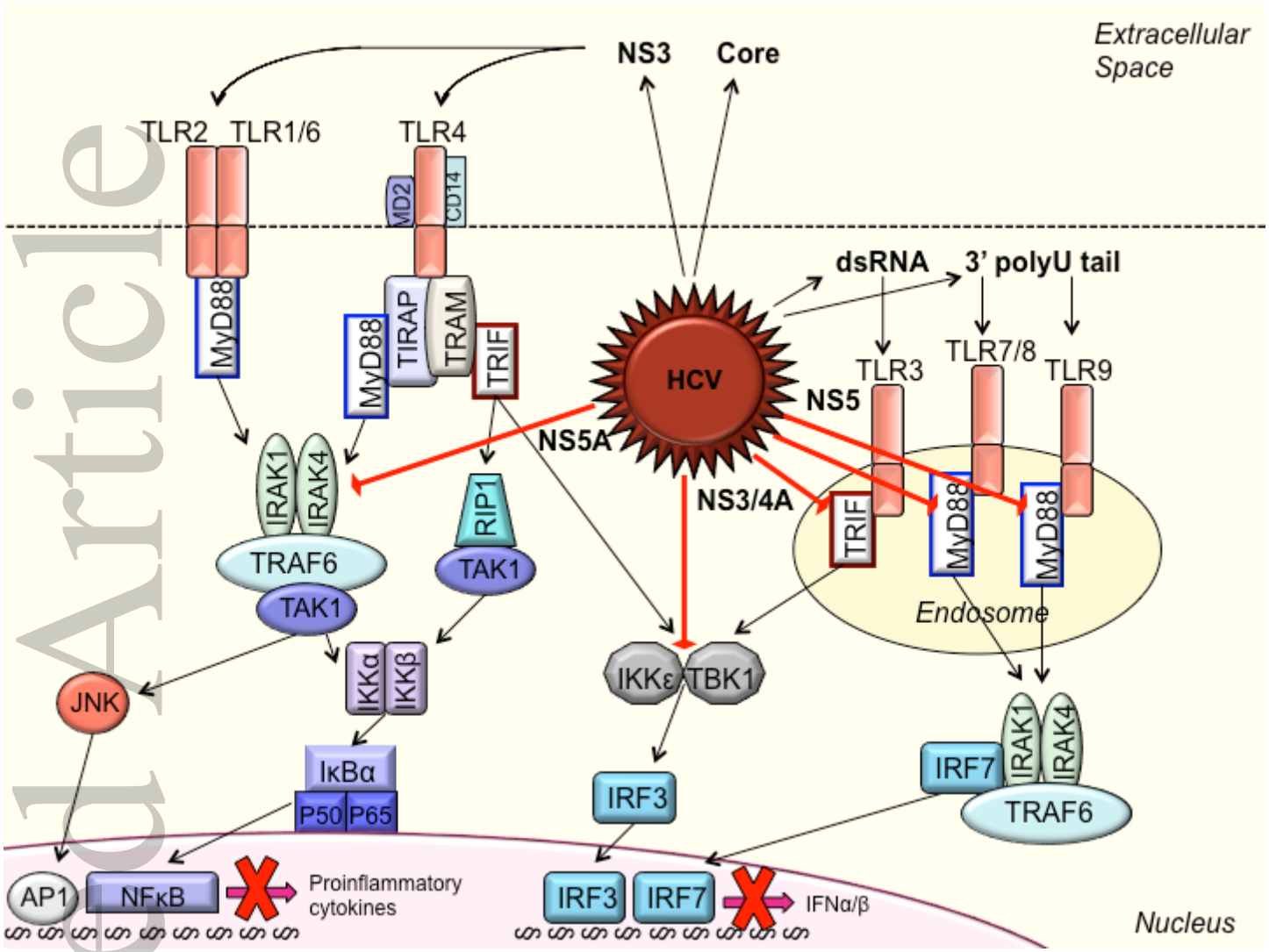
TLR	Polymorphism	Estimated carriage	Mutation type	Disease association
TLR2	Arg753Gln	2-10% caucasian Rare asian	Missense	HCV rapid fibrosis post liver transplant
TLR4	Asp299Gly and Thr399Ile	8% caucasian	Missense Co-segregate	Reduced fibrosis in HCV
TLR7	c.1-120T>G (rs2302267)	>5% caucasians Rare Africans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



jgh_12170_f1.tiff



jgh_12170_f2.tiff



jgh_12170_f3.tiff