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Commentary

Pushing to a cure by harnessing innate immunity against hepatitis C virus

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ABSTRACT

Hepatitis C virus (HCV) causes 350,000 deaths and infects at least 3 million people worldwide every year. Currently no vaccine has been developed. Direct-acting antiviral (DAA) drugs with high efficacy for suppressing HCV infection have recently been introduced into the clinic. While DAAs initially required combination therapy with type-1 interferon (IFN) administration for full efficacy and to avoid viral resistance to treatment, new DAA combinations show promise as an IFN-free regimen. However, IFN-free DAA therapy is in its infancy, still to be proven and today is cost-prohibitive for the patient. A major goal in HCV therapy to remove or replace IFN with DAAs or an alternative therapeutic to render virologic response with continued virus sensitivity to DAAs, thus facilitating a cure for infection. Recent advances in our understanding of innate immune responses to HCV have identified new therapeutic targets to combat HCV infection. We discuss how the targeting of innate immune response factors can be harnessed with DAAs to produce new generations of DAA-based HCV therapeutics. This article forms part of a symposium in *Antiviral Research* on "Hepatitis C: next steps toward global eradication."

1. Introduction

HCV is a highly successful virus that establishes chronic infection in the majority of acutely infected people. HCV is distributed globally as six major genotypes, and is spread through parenteral exposure to infected blood. Chronic infection and persistent viremia then facilitate continual virus transmission from infected individuals; such that nearly 200 million people now have chronic HCV infection (Horner and Gale, 2013). The standard of care for HCV infection has been based on pegylated IFN-α with ribavirin. This regimen is curative as a sustained virologic response (SVR) in approximately 40–50% of HCV patients infected with HCV genotypes 1 or 4 (Soriano et al., 2009). Recently, direct-acting antiviral (DAA) agents have been approved for clinical use and function to suppress HCV replication by blocking the activity of the viral nonstructural (NS) 3 protease (called NS3/4A) (Scheel and Rice, 2013) and preventing viral protein processing. However, this regimen alone or when administered with additional similar DAAs can result in the outgrowth of drug-resistant viral variants or "quasispecies" owing to the error-prone nature of HCV RNA replication (Franco et al., 2007, 2008). To alleviate this problem, DAAs are currently approved for use as combination therapy with pegylated IFN-α and ribavirin to suppress the outgrowth of therapy-resistant HCV quasispecies and render enhanced virologic response (Halfon and Locarnini, 2011).

While this improvement in HCV therapeutics in combining a single DAA with IFN is encouraging, these therapies do not achieve SVR in all cases. Moreover, the use of pegylated IFN continues to elicit adverse effects associated with IFN-based treatments, reducing patient compliance and overall efficacy of therapy. Thus, there is a need for DAA combinations that target additional virologic activities of HCV-encoded enzymes and protein components. The recent clinical trial demonstrations of combination DAA therapy targeting the NS5B protein or NS3/4A along with NS5A and NS5B proteins show high SVR in a complete IFN-free regimen. While these DAA regimens are powerful and exciting they are not fully proven and problematically will remain cost-prohibitive to the patient, thus underscoring a need for continued development of effective and affordable therapy adducts for HCV treatment (Au and Pockros, 2013a).

As an innate immune cytokine and therapeutic, IFN has been heavily studied, and as a result, the mechanisms of virus-induced IFN production and actions of interferon-stimulated genes (ISG)s that have antiviral action to suppress HCV replication and spread (Horner and Gale, 2013) are well-defined. These studies have informed specific strategies for therapeutic targeting the innate

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immune response as a possible component of anti-HCV therapy to offer alternatives to systemic IFN exposure (Tarr et al., 2012). Therapeutics that target innate immunity could effectively induce antiviral actions through innate immune responses that operate independently of IFN and/or within the framework of endogenous IFN production that would be localized rather than systemic, thus limiting exposure and possible adverse effects when used with DAA-based therapy regimens. Such innate immune therapeutics and their potential benefits in HCV therapy are discussed in this review.

2. Overview of innate immune response to hepatitis C virus

HCV is hepatotropic and infects hepatocytes, the chief epithelial cell of the liver. During acute infection the immune response to HCV is first induced on the molecular level directly within the infected hepatocyte by the recognition of HCV as non-self through specific pattern recognition receptors (PRRs). The main PRR for cellular recognition of HCV is the retinoic acid inducible gene-I (RIG-I) protein, while Toll-like receptor 3 (TLR3), and protein kinase PKR also have been shown to mediate non-self recognition of specific HCV products (Horner and Gale, 2013). Pathogen-associated molecular patterns (PAMPs) inherent within the HCV molecular structure are detected by these PRRs, each which induces signaling cascades that drive antiviral activity through activation of latent transcription factors, interferon regulatory factor (IRF)3 and NF-κB, and others, to drive virus-induced gene expression, including the expression and production of IFN. When secreted from the infected cell, IFN binds to specific receptors within the local tissue to activate the JAK/STAT signaling pathway and induce the expression of hundreds of ISGs. ISG products include pro-inflammatory cytokines as well as antiviral and immune-modulatory proteins that suppress HCV infection and support the onset and function of the adaptive immune response (Horner and Gale, 2013) (Fig. 1).

As a major PRR of HCV recognition, RIG-I serves to recognize a PAMP motif within the HCV RNA that is embedded in the poly U/UC ribonucleotide rich 3′ nontranslated region (NTR) of the viral genome and harbors exposed 5′ triphosphate (Saito et al., 2008; Schnell et al., 2012; Uzri and Gehrke, 2009). Upon HCV PAMP binding, RIG-I undergoes a conformational change, oligomerization, and translocation from the cytocol to intracellular membranes (Horner et al., 2011; Liu et al., 2012a), where it interacts with the MAVS adaptor protein to drive the formation of a signalosome to induce signaling activation of IRF-3 and NF-κB. The mechanisms by which RIG-I and its downstream effector molecules induce pro-inflammatory and antiviral responses are multifactorial and include RIG-I post-translational modifications as well as interaction with specific co-factors, including TBK1 activation (Barral et al., 2009; Liu et al., 2012a; Nakhaei et al., 2009; Gack et al., 2007; Hayakawa et al., 2011; Wies et al., 2013). In contrast to HCV activation of RIG-I, which occurs immediately upon infection, transcriptional responses induced by the endosomal sensor TLR3 occur days later (Wang et al., 2009; Li et al., 2012). TLR3 detects HCV double stranded (ds) RNA replication intermediates, and signals IRF3 and NF-κB transcriptional activity through TIR-domain containing adaptor inducing interferon-β (TRIF) and activation of TBK1. In contrast, dsRNA at the HCV internal ribosomal entry site (IRES) activates the non-traditional PRR protein kinase PKR dependent activation of MAVS, TNF-associated factor 3, IRFs, and NF-κB to induce IFN and ISG production (Arnaud et al., 2011; Shimoike et al., 2009). Other studies have found that HCV Core and nonstructural proteins are able to activate other members of the TLR family in both infected cells and bystander cells, including TLR2 and TLR4, while HCV genomic RNA can stimulate TLR7 and TLR8 expressed in myeloid cells (Howell et al., 2013). Overall, HCV stimulation of TLR signaling likely contributes to the amplification and enhancement of the innate immune response triggered by RIG-I while serving to regulate the function of immune cells that express specific TLRs (Kawai and Akira, 2011; Loo and Gale, 2011).

RIG-I and other PRRs that mediate HCV recognition function cooperatively to mediate host antiviral and pro-inflammatory host defenses. Studies of acute HCV infection in humans and chimpanzees have found that primary acute HCV infection can be cleared spontaneously in the presence of high initial viremia within the window of the innate immune response (Liu et al., 2012b; Su et al., 2002) and that while the extent of ISG induction correlates with HCV viral load, the onset of ISG expression marks the points of viral suppression. These studies underscore that HCV infection stimulates innate immune induction and that the resulting innate immune response can suppress infection. Harnessing the function of PRRs and the innate immune response could therefore offer effective strategies for therapy adducts to control HCV infection.

3. HCV evasion of innate immunity

Despite the ability of PRRs to initiate a strong antiviral host response to acute HCV infection, HCV has inherent evasion strategies that allow the virus to circumvent innate immune actions and establish chronic infection in approximately 70–80% of infected individuals (Table 1). These evasion strategies particularly target specific PRR pathways and their innate immune effectors.

3.1. HCV NS3/4A targets MAVS and TRIF

NS3/4A is a protease complex that proteolytically processes the HCV polyprotein and also functions to evade host immune responses by cleaving and rendering ineffective key host proteins necessary to mount an effective immune response. NS3/4A effectively blocks RIG-I signaling through cleavage of MAVS to prevent PRR function and downstream signal transduction of innate immunity. Indeed, MAVS cleavage fragments have been detected in liver samples of chronically infected HCV patients who also have lower levels of IFN-β activation (Bellecave et al., 2010; Loo et al., 2006). Cleavage of MAVS by NS3/4A may also ablate PKR signaling of innate immunity after engagement of HCV dsRNA (Arnaud et al., 2010; Dabo and Meurs, 2012). TRIF, the TLR3 signaling adaptive protein, is another proteolytic target of NS3/4A. Notably, TRIF protein levels decrease overall during HCV infection in vitro, but direct evidence of TRIF proteolysis in vivo has yet to be shown (Li et al., 2005). Thus, the proteolytic targeting of host proteins by NS3/4A blocks PRR signaling otherwise induced by the HCV PAMP/PRR interaction during acute infection. The outcome of these actions of NS3/4A is to attenuate virus-induction of IRF-3 and NF-κB activation in the infected cell, thus hindering innate immune defenses and suppressing IFN and ISG expression to support HCV progression to chronic infection.

3.2. Accomplices of innate immune regulation by HCV

In addition to NS3/4A actions on the host cell to suppress PRR signaling by RIG-I and TLR3, other viral proteins have been implicated in innate immune regulation through targeting specific factors of innate immune signaling. As described below, multiple strategies are employed by HCV to suppress specific arms of the larger innate immune program of the cell, thus securing HCV to sustain a chronic infection.

3.3. NS4B: blocking TBK1 activation of IRF-3

The HCV viral protein NS4B has been shown to suppress innate immune responses by blocking the activation of IKK/TANK-binding

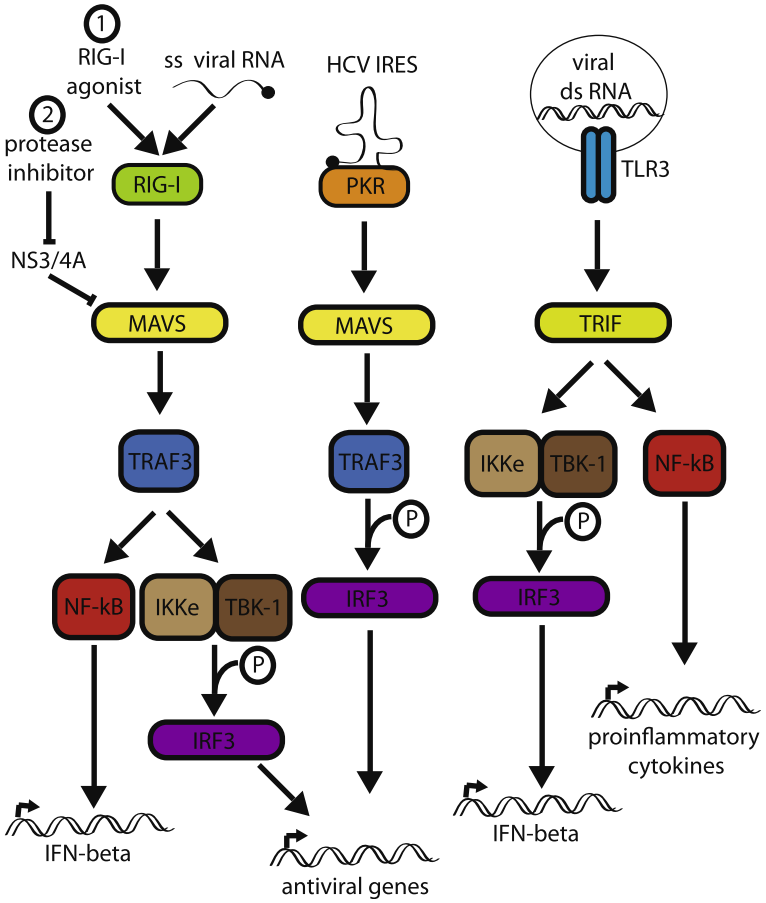

Fig. 1. Innate immune signaling in response to HCV infection and targeting RIG-I in combination with DAAs as innate immune therapy against HCV. HCV is detected by PRRs RIG-I, PKR, and TLR3 and leads to downstream signaling to activate IRF-3 and NF-kB transcriptional activation of IFN-beta, antiviral genes, and pro-inflammatory cytokines that limit infection and enhance the immune response to HCV. RIG-I agonists could enhance therapeutic efficacy of DAAs by activating the RIG-I signaling pathway while the NS3/4A protease inhibitor functions to remove virus negative modulation of MAVS activity to restore signaling through the RIG-I pathway.

Table 1
Mechanisms by which HCV evades host innate immune responses.

Host target	Viral protein	Mechanism	References
MAVS	NS3/4A	Proteolysis of MAVS blocks RIG-I and PKR pathways to induce IFN and antiviral activity	Bellecave et al. (2010) and Loo et al. (2006)
TRIF	NS3/4A	Proteolysis of TRIF blocks TLR3 pathway to induce IFN and antiviral activity	Li et al. (2005)
PKR	NS5A, E2	Binding to PKR inhibits PKR dimerization and kinase activity	Gale et al. (1998), Pavio et al. (2002) and Taylor et al. (1999)
SOCS3	Core	Inhibit phosphorylation and subsequent activation of STAT1, a co-activator of IFN-induced transcription	Bode et al. (2003)
PP2Ac	Undetermined viral proteins	Hypermethylation of STAT-1 to increase STAT-1 inhibitor PIAS association with STAT-1 and decrease STAT-1 binding to DNA	Duong et al. (2004)
STING and TBK1	NS4A	Blocks interaction of TBK1 and STING to prevent IRF-3 phosphorylation	Ding et al. (2013)
IFNL3 mRNA	miR-208b and miR-499a-5p	Target and destabilize IFNL3 mRNA transcripts	McFarland et al. (2014)

kinase 1 (TBK-1) and the phospho-dependant activation of IRF-3. NS4B prevents TBK-1 activation by preventing the scaffolding protein STING/MITA from interacting with TBK-1 to bring it within proximity to IRF-3 (Ding et al., 2013). This blockade provides an NS3/4-independent mechanism for preventing innate immune induction but has yet to be validated in human patient liver.

3.4. Targeting of the JAK/STAT pathway

A number of studies in cell lines and transgenic mouse models have found that HCV can interfere with IFN signaling by antagonizing the JAK/STAT pathway of IFN receptor signaling (Blindenbacher et al., 2003; Heim et al., 1999; Luquin et al., 2007) through various mechanisms. One study reported that HCV Core protein increases the STAT inhibitor SOCS3, which functions to inhibit tyrosine phosphorylation of STAT1 by JAKs (Bode et al., 2003). Another study found that HCV induced the transcriptional repressor protein phosphatase PP2Ac. PP2Ac functions to inhibit ISG transcription by increasing the methylation of STAT-1 and association of protein inhibitor of activated STAT-1 (PIAS1), thereby decreasing the IFN-induced binding of STAT-1 to DNA (Duong et al., 2004). JAK-STAT regulation by these processes could serve to disrupt autocrine

IFN signaling to support virus replication and spread in the liver, and to suppress the therapeutic actions of IFN in current HCV treatment regimens.

3.5. Inhibition of PKR

HCV impact on PKR function is contextual and has been controversial. On one hand, HCV activation of PKR induces phosphorylation of the host eukaryotic initiation factor 2, a subunit (eIF2α) to suppress RNA translation and attenuate very early ISG expression (Garaigorta and Chisari, 2009) (Arnaud et al., 2010), although this impact may be very transient. Other evidence has indicated virus-mediated activation of PKR may actually enhance HCV replication, though the mechanisms of this enhancement were not defined (Kang et al., 2009). These outcomes contrast with reports that HCV can suppress PKR by the binding of HCV NS5A protein to inhibit PKR dimerization (Gale et al., 1998) and E2-mediated suppression of PKR kinase activity (Pavio et al., 2002; Taylor et al., 1999). In each case the studies were conducted prior to the creation of an in vitro HCV RNA replication system, and since then the inhibition of PKR has been shown to stimulate protein synthesis of surrogate RNA virus infection in the context of ectopic expression of the HCV protein.

While the impact of HCV on PKR activity appears contradictory from these many studies, it should also be noted that these studies varied greatly from another in use of cell culture models, and in the more recent studies, HCV expression/replication systems. Thus it has been suggested that HCV impact on PKR activity is temporal during the course of infection (Horner and Gale, 2013; Thimme et al., 2012). In support of this notion, PKR has been shown to provide innate immune signaling function early in infection through interaction with MAVS, thus extending and/or diversifying RIG-I induction of innate immunity. In this respect, dsRNA activation of PKR or inhibition of PKR by NS5A or E2 would function to alter the translational control and innate immune signaling properties of PKR to impact HCV infection at multiple points, including viral protein synthesis and restriction of viral replication.

4. Therapeutic targets of innate immunity

The innate immune response suppresses viral infection directly, drives further inflammatory responses, and shapes adaptive immune responses to control virus infection and provide long-term protective immunity. Thus, targeting the innate immune response either as a post-infection therapeutic or as a HCV vaccine adjuvant could have powerful application in the clinic to treat both acute and chronic HCV infections as well as to prevent new infections. Overall, an innate immune targeted therapy would either specifically stimulate PRRs or their downstream effector molecules or antagonize pathway inhibitors to ultimately induce pro-inflammatory and antiviral responses to control infection. Such therapies could function by directly enhancing the innate response to infection or by tilting the innate/adaptive immune interface in favor of driving a more potent adaptive immune response. Moreover, by targeting specific PRRs, such as RIG-I and TLR3, and their downstream pathways that function specifically in host response to RNA viruses such as HCV, these therapeutics will drive a more specific response that is inherently designed to combat RNA viral pathogens. Below we describe specific innate immune components as viable targets for novel HCV therapeutic development (Table 2).

4.1. Targeting PRRs and effectors

The direct targeting of PRRs, including RIG-I and TLRs, as well as their modulators and downstream effector molecules with specific agonists, is expected to induce virus-response antiviral genes, IFN and pro-inflammatory cytokines for adaptive immune cell recruitment and modulation. As proof of principle, Bedard and colleagues recently implemented a high-throughput approach and identified small molecule agonists of the RIG-I like receptor pathway to activate IRF-3 and downstream pro-inflammatory responses (Bedard et al., 2012). These agonists have demonstrated effective antiviral activity in vitro against various RNA viruses, including HCV. Likewise, TLR agonists, particularly those that activate TLR7/8 and TLR3, have the capacity to induce innate immune/IFN responses that could likewise enhance adaptive immunity to HCV infection (Bergmann et al., 2011; Boonstra et al., 2012; Fidock et al., 2011; Horsmans et al., 2005; Pockros, 2010; Wang et al., 2009). Importantly, TLR and RLR pathways can be activated through the use of a variety of ligands, including synthetic TLR ligands, imiquimod (TLR7 ligand), synthetic RNA molecules (TLRs and RLRs), and small molecule compounds (TLRs and RLRs).

4.1.1. RLR pathway

Innate immune signaling by the RLRs involves a variety of co-factors that impart modulation of RLR activity to regulate the RIG-I or MDA5 interaction with MAVS as well as to control RLR effector actions to activate IRF-3 and NF-κB (Loo and Gale, 2011). Agonist co-factors that impart RIG-I signaling to MAVS include: TRAF3/6, caspase 8/10, RIP-1, Fas-associated death domain (FADD) and TNF receptor death domain (TRADD), and ZAPS, among others (Hayakawa et al., 2011; Kawai et al., 2005; Michallet et al., 2008; Paz et al., 2011; Rajput et al., 2011; Saha et al., 2006; Sears et al., 2011; Yoshida et al., 2008). Moreover, an additional set of signaling proteins serve as RLR co-factors to transduce signaling from MAVS downstream to activate IRF3 and NF-κB (Loo and Gale, 2011). In particular these include TBK1/IKK-ε, and IKKα/IKKβ, protein kinases and associated partners (Fitzgerald et al., 2003; Sharma et al., 2003; Taylor and Mossman, 2013).

Importantly, RLR signaling is subject to regulation by negative cofactors. This list of RLR signaling antagonists include RNF125, ISG15, DAK, Atg12–Atg5, and possibly NLRX1. The expression or activity of these negative cofactors typically is regulated late in the temporal progression of the innate immune response wherein they function to mediate homeostatic control of the proinflammatory actions of RLR signaling (Allen et al., 2011; Arimoto et al., 2007, 2008; Diao et al., 2007; Kim et al., 2008; O'Neill, 2008; Soares et al., 2013; Takeshita et al., 2008). Notably, these RLR cofactors or antagonists include specific enzymatic or allosteric activities that govern RLR pathway signaling, identifying each as possible drug targets for the control of innate immune signaling by RLRs (Loo and Gale, 2011). One attractive notion for therapeutic regulation of RLR activity is to consider strategies to modulate the ubiqination status of RIG-I, whose signaling activity is acutely modulated by reversible ubiquitination by the TRIM25 protein and specific deubiquitinases (Barral et al., 2009; Nakhaei et al., 2009; Zeng et al., 2010; Gack et al., 2007). In this sense, the modulation of RIG-I can be considered to enhance its antiviral activity toward suppressing HCV infection.

4.1.2. TLR pathway

Similar to RLR targeting, TLR pathway-based therapeutics present the opportunity to induce downstream antiviral effector molecules for the suppression of HCV. Most relevant to this approach are TLR3 and TLR7/8, the two TLR pathways that are induced by PAMPs from RNA virus infection (Howell et al., 2013). In addition to the specific TLRs themselves, key enzymatic or allosteric nodes of these pathways include the TRIF/TRAM adaptor proteins and the IKKε/TBK1 protein kinases for TLR3; and the MyD88 adaptor protein with the protein kinases IRAK1 and IRAK4 as well as the TRAF6 scaffold protein to transduce TLR7/8-mediated signals. Since

Table 2
Targets for innate immune therapy against HCV.

Target	Mechanism of therapeutic action	References
RIG-I, TLR3, TLR7/8	Direct activation of PRRs to induce IRF3/7 transcriptional activity	Bedard et al. (2012), Bergmann et al. (2011), Boonstra et al. (2012), Fidock et al. (2011), Horsmans et al. (2005), Pockros (2010) and Wang et al. (2009)
MAVS, TRAF3/6, caspase 8/10, RIP-1, (FADD), (TRADD), TBK1/IKK-ε, IKKα/IKKβ, IRF-3	Direct activation of RIG-I pathway to induce IFN-mediated pro-inflammatory immunity and antiviral genes	Loo and Gale (2011), Hayakawa et al. (2011), Kawai et al. (2005), Michallet et al. (2008), Paz et al. (2011), Rajput et al. (2011), Saha et al. (2006), Sears et al. (2011), Yoshida et al. (2008), Fitzgerald et al. (2003), Sharma et al. (2003) and Taylor and Mossman (2013)
RNF125, ISG15, DAK, Atg12–Atg5, NLRX1, caspase-1, RIG-I deubiquinating enzymes	Antagonists of negative regulators of RLR signaling enhance pathway activity	Allen et al. (2011), Arimoto et al. (2007, 2008), Diao et al. (2007), Kim et al. (2008), O'Neill (2008), Soares et al. (2013), Takeshita et al. (2008), Barral et al. (2009), Nakhaei et al. (2009), Zeng et al. (2010) and Gack et al. (2007)
MyD88, IRAK1/IRAK4/TRAF6, IRF7	Direct activation of TLR pathways to induce IFN-mediated pro-inflammatory immunity and antiviral genes	Reviewed in Mifsud et al. (2014) and O'Neill et al. (2013)
JAK1, JAK2, TYK2, STAT1, ISGF3	Agonists of IFN pathway signaling to enhance ISG transcription	Reviewed in Heim, 2013
SOCS, USP18, PIAS1, TcPTP	Antagonists of negative regulators of IFN signal transduction enhances IFN signaling activity	Reviewed in Heim, 2013
HCV ISGs (ADAR, IFIT1, IFIT3, IFITM1, IFITM3, ISG20, OAS1, RNASEL, RSAD2 (viperin), OAS1L	Agonists or upregulation of ISGs to induce HCV antiviral activity	Reviewed in Horner and Gale (2013)
Targets that overcome innate immune tolerance	Render IFN therapy effective by alleviating IFN tolerance	Lau et al. (2013)
HCV microRNAs: miR280b, miR-499	Restore IFNL3 expression that is downregulated by HCV miRNAs	McFarland et al. (2014)

TLRs are widely expressed by myeloid cells, including plasma cytoid dendritic cells (pDC)s, such strategies would serve to activate IRF3 and IRF7 transcriptional activity in monocytes, macrophages and pDCs for induction of IFN and proinflammatory cytokine production to overall suppress HCV infection while enhancing the adaptive immune response. Consideration of these approaches however must be weighted with caution, as inflammatory stimulants can present adverse outcomes associated with induced immune pathology (Fidock et al., 2011; Pockros, 2010).

4.2. Targeting newly-emerged regulation of IFN-L

Interferon-λ3 (IFNL3), also referred to as IL-28B, has been linked to HCV patient response to therapy through genome-wide association studies and has emerged as a new basis for treatment therapy (Hayes et al., 2012). IFNL3 is a potent antiviral cytokine that is induced in both hematopoietic and nonhematopoietic cells (Stone et al., 2013; Witte et al., 2010). IFNL3 receptors are present on liver cells, including hepatocytes such that IFNL3 can inhibit HCV replication (Robek et al., 2005). While IFN-α and IFNL3 induce similar sets of ISGs, the host response to IFNL3 differs from IFN-α. IFNL3 signaling is cell type-specific (Sommereyns et al., 2008) and induces a steady increase of ISGs compared to the rapid peak and decline of ISGs induced by IFN-α (Marcello et al., 2006). Control of IFNL3 expression may determine HCV patient response to treatment. Many studies have positively correlated IFNL3 genotype and IFNL3 expression with HCV clearance (Fukuhara et al., 2010; Langhans et al., 2011; Raglow et al., 2013; Suppiah et al., 2009; Tanaka et al., 2009). Clinical benefit has also been demonstrated in trials that have shown the effectiveness of pairing IFNL3 treatment with DAAs (Ghany et al., 2011).

Three single nucleotide polymorphisms (SNPs) near IFNL3, the gene which encodes IFNL3, were found to be strongly associated with natural clearance of HCV (Rauch et al., 2010; Thomas et al., 2009) and indicative of patient response to HCV therapy (Ge et al., 2009; Rauch et al., 2010; Suppiah et al., 2009; Tanaka et al., 2009). Recently, McFarland et al. found that SNP rs4803217, which is located in the UTR of IFNL3, can regulate IFN-λ3 expression in hepatocytes by impacting IFNL3 transcript stability (McFarland et al., 2014). Importantly, substitution from the less favorable allele "T" to the more favorable "G" allele at this SNP, prevented AU-rich element (ARE)-mediated decay (AMD) of IFNL3 mRNA and post-transcriptional regulation by HCV induced micro-RNAs, miR-208b and miR-499. Remarkably, targeting of these two HCV-induced mi-RNAs using inhibitory RNA ligands could restore IFNΛ3 expression and suppress HCV in patients that have the unfavorable SNP. Thus, therapeutics directed toward suppressing the induction or actions of the miR-208b and miR-499 may offer the benefit of restoring IFNL3 efficacy to restrict HCV infection.

4.3. Targeting specific ISGs

IFN therapy induces a large number of anti-HCV ISGs (Sarasin-Filipowicz et al., 2008), which likely contributes to generating an effective therapeutic response. While the function of some of the ISGs that can suppress HCV infection during an IFN response (natural or therapeutic application) are known, a the specific function of most have yet to be elucidated (reviewed in (Horner and Gale, 2013)). Regardless, therapies that induce the expression and/or activities of specific ISGs that have proven anti-HCV function would be expected to have clinical application as HCV therapy adducts. A problem to consider for this approach however is revealed by in vitro studies that have applied single ISG knockdown versus combinatorial knockdown of multiple ISGs together, showing that efficient suppression of HCV replication by IFN is mediated by the actions of multiple ISGs who products impart antiviral activity against specific steps of viral replication or through mitigating specific interactions of HCV with other cellular factor requisites of HCV infection (Li et al., 2009; Schoggins et al.,

2011, 2013). Thus, it is unlikely that any single ISG determines the outcome of IFN therapy during HCV infection (Metz et al., 2012). By extension, ISG therapeutic approaches will need to target groups of ISGs with anti-HCV activity in order to capture their likely synergistic actions as HCV antivirals.

4.4. Modulating innate immune tolerance to restore IFN responses

Recent studies to examine the hepatic innate immune response in patients who fail IFN therapy have revealed that nonresponse to IFN correlates with a high pre-therapy ISG expression levels in the liver, while patients that favorably respond to IFN therapy exhibit low or no ISG expression pretherapy (Dill et al., 2011; McGilvray et al., 2012; Sarasin-Filipowicz et al., 2008). Upon IFN therapy, the nonresponder patients then exhibit virtually no increase in ISG expression while responder patients exhibit large induction of ISGs expression owing to their pre-therapy set point levels. A recent study shed light on this disparity between patient groups to show that hepatic macrophages or "Kupffer cells" can produce a constitutive level of IFN-β to drive high ISG expression set point in the hepatocytes of these nonresponder patients. Because many ISG products are toxic to the cell when constitutively expressed, this study concluded that under high ISG set point conditions the hepatocyte develops a state of tolerance to ISG actions and thus becomes refractory to mediate antiviral actions typically driven by IFN signaling (Lau et al., 2013). The major findings of this study were that during HCV infection in the liver, Kupffer cells are a local source of IFN that promote basal expression of ISG in hepatocytes of nonresponders and that this constitutive exposure to IFN causes a state of innate immune tolerance to ISG function. Thus, chronically infected patients may not respond to therapy due to local IFN production by Kupffer cells that promotes hepatic innate immune tolerance. In this light, strategies that disrupt the virus-host interactions that induce innate immune tolerance could be developed to improve responsiveness to IFN therapy. However, additional studies are needed to understand the mechanism of IFN-β induction that drives hepatic innate immune tolerance such that specific factors involved in this process could be targeted for negative regulation to render low pretherapy ISG levels for improved IFN therapy outcomes.

5. Combination approaches: DAAs with innate immunity therapeutics

Increased understanding of the HCV life cycle has led to the development of direct-acting antivirals (DAAs) that target specific HCV nonstructural proteins to disrupt viral infection and replication. These new inhibitors fall within four classes: protease inhibitors, NS5A inhibitors, NS5B nucleoside inhibitors, and NS5B non-nucleoside inhibitors (Au and Pockros, 2013a). As described below, these classes demonstrate differences in efficacy, tendency to develop resistant virus, and toxicity. Only one class, the protease inhibitors, impacts innate immune responses to HCV infection.

5.1. Protease inhibitors

Most DAA protease inhibitors target NS3 within the NS3/4A protease by acting as peptidomimetic active site inhibitors (Aghemo and De Francesco, 2013) (which function to impair protease post-translational processing of viral proteins and cleavage of MAVS and TRIF by NS3/4A. Thus, the protease inhibitor class of DAAs simultaneously impairs viral replication and restores innate immune signaling through lifting a virus-mediated block of PRR signaling. While the first generation of protease inhibitors had limited efficacy when applied alone, due to the development of viral resistance and drug side effects, second-wave protease inhibitors are now in development that elicit less viral resistance and side effects but will still be applied theraptically with pegylated IFN to treat chronic HCV infection (Clark et al., 2013). Additional drawbacks to the protease inhibitor class of DAAs are that they are limited in efficacy largely to HCV genotype 1, thus restricting their application to treat all HCV genotypes (Aghemo and De Francesco, 2013). More recently, allosteric PIs that bind the interface between the NS3 helicase and protease domains have been discovered to render similar inhibition of HCV genotypes 1, 3a, 5, and 6, but not genotypes 2a and 4 (Saalau-Bethell et al., 2012). Consideration is given to linking a RIG-I or TLR3 signaling agonist with DAAs that target and suppress the HCV NS3/4A protease, to dually relieve the HCV-mediated blockade on RIG-I or TLR3 signaling while activating each to drive a potent innate immune response. In terms of restoration of RIG-I signaling by NS3/4A inhibitor DAAs, this application has actually been shown to suppress HCV replication directly within hepatocytes (Foy et al., 2003). Such a combination could offer intracellular actions to directly suppress HCV replication through the actions of RIG-I-responsive genes. Antiviral action attributed to RIG-I signaling also offers a potential and highly specific replacement of IFN with RIG-I agonists as a therapy adduct that would offer antiviral activity while suppressing the outgrowth of NS3 protease inhibitor-resistant HCV variants, rendering a therapy combination that overall enhances the efficacy of DAA treatment (Johnson et al., 2007; Loo et al., 2006) (Fig. 1).

5.2. Other DAAs

The NS5A class of DAA inhibitors function to bind the viral NS5A protein and impair its ability to support HCV RNA replication (Yang et al., 2011). When applied alone as a monotherapy this DAA class has limited efficacy due to the development of viral resistance and drug toxicity (Au and Pockros, 2013b; Fridell et al., 2011) and must be applied as a combination therapy to reduce outgrowth of resistance variants. As DAAs have been developed that target the HCV NS5B protein, the RNA-dependent RNA polymerase of HCV, therapy combinations of DAAs are expected to offer increased efficacy with reduced viral resistance. Indeed, a recent study of combination DAA treatment of NS5A and NS5B inhibitors with ribavirin in patients infected with HCV genotype 1 showed that such combinations can actually achieve 100% efficacy after 12 weeks of treatment to drive HCV to undetectable levels in selected patient cohorts (Gane et al., 2013). The response to combination DAA therapy among all HCV patients and viral genotypes will undoubtedly be variable to reveal an additional need for therapy adducts to sustain a high efficacy for each regimen. Of note is that toxicity is variable from low to adverse among combination DAA-treated patients, underscoring the importance of defining a novel treatment regimen that will achieve sustained responses to suppress HCV while supporting patient compliance to remain on therapy for an eventual cure of HCV infection (Au and Pockros, 2013b). The development of specific innate immune therapeutics to combine with DAAs could serve to achieve these goals as host-targeted therapy adducts. Such a strategy offers the combination of targeting both virus and host, thus blocking viral replication while stimulating host antiviral programs and immunity. As various HCV proteins can antagonize innate immune defenses (Horner and Gale, 2013), the action of DAAs to reduce viral load and HCV protein abundance in the infected hepatocytes mediates relief of host defenses from viral control. Thus, the addition of an innate immune therapeutic that stimulates intracellular antiviral defenses to the DAA therapy regimen will facilitate host-mediated HCV suppression, driving to the cure of HCV infection.

6. Innate immune modulators for HCV vaccine adjuvants

Currently, no vaccine is available for the prevention of HCV infection. The development of HCV vaccines is essential for controlling global HCV infection, as DAA therapeutics do not impart immune protection against HCV infection. HCV vaccines could be used both to prevent infection and in combination with DAAs to provide an IFN-free therapy regimen for treatment of chronic infection with the goal of establishing an immune response to drive an HCV cure. Specifically, HCV vaccines could be used in patients with chronic infection to drive down viral load through vaccine-specific induced immune responses. Studies of HCV-infected chimpanzees, and of individuals who spontaneously recovered from infection after acute exposure to the virus, indicate that an effective vaccine will likely need to induce multifunctional CD4+T cells, cross-genotype CD8+T cells, and cross-genotype neutralizing antibodies (Cooper et al., 1999; Shoukry et al., 2003; Walker, 2010; Yu and Chiang, 2010). While various vaccine strategies have been attempted using recombinant E1 and E2 proteins, synthetic peptides, DNA, and various prime-boost regimens, none have been effective (reviewed in Liang, 2013). One major impediment contributing to the lack of success of HCV vaccines is the use of vaccine adjuvants with poor potency. Improving adjuvant potency will enhance vaccine efficacy by better priming antigen presenting cells to direct the development of effective cellular and humoral immune responses to HCV antigens.

Agonists of the innate immune system have the potential to serve as strong adjuvants for vaccines. The pro-inflammatory genes induced by PPR signaling pathways induce chemokine and cytokines that promote the recruitment and maturation of DCs to the area of infection, or in the case of a vaccine, to the site of vaccine administration. Activated DCs then direct the development of adaptive responses that ultimately function to mediate long-lasting immunity. Indeed, TLR agonists are being developed as adjuvants (Lahiri et al., 2008) and are present in the AS04 adjuvant systems currently included in the approved HPV vaccine (Markowitz et al., 2007; Roteli-Martins et al., 2012). As a molecular sensor for HCV PAMPs, RIG-I agonists also have the potential to be developed as specific, potent adjuvants for HCV vaccines, and are currently in development (Bedard et al., 2012). Thus, with their antiviral and immune-modulatory activities, innate immune agonists offer broad application to the treatment and prevention of HCV infection.

7. Future perspectives on innate immune modulators and HCV therapeutics

Whether used alone as antiviral therapeutics, in combination therapy with DAAs, or as adjuvants in HCV vaccine applications, innate immune modulators will offer host-targeted antiviral treatment regimens against HCV infection. By harnessing the potency of the innate immune system to mediate intracellular and tissue antiviral responses, while also enhancing adaptive immunity, innate immune modulators have the potential to definitively transform HCV and antiviral therapies for the control of infection but without the limitations of viral resistance outgrowth.

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