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Insights into antiviral innate immunity revealed by studying hepatitis C virus

Stacy M. Horner[a,b,*]

[a]Department of Molecular Genetics & Microbiology, Duke University Medical Center, Durham, NC 27710

[b]Department of Medicine, Duke University Medical Center, Durham, NC 27710

Abstract

Experimental studies on the interactions of the positive strand RNA virus hepatitis C virus (HCV) with the host have contributed to several discoveries in the field of antiviral innate immunity. These include revealing the antiviral sensing pathways that lead to the induction of type I interferon (IFN) during HCV infection and also the importance of type III IFNs in the antiviral immune response to HCV. These studies on HCV/host interactions have contributed to our overall understanding of viral sensing and viral evasion of the antiviral intracellular innate immune response. In this review, I will highlight how these studies of HCV/host interactions have led to new insights into antiviral innate immunity. Overall, I hope to emphasize that studying antiviral immunity in the context of virus infection is necessary to fully understand antiviral immunity and how it controls the outcome of viral infection.

Keywords

hepatitis C virus; antiviral immunity; NS3/4A; MAVS; interferon; innate immunity

Introduction

HCV is a positive sense, single-stranded (ss) RNA virus of the genus Hepacivirus and family *Flaviviridae*. HCV infects and replicates in hepatocytes within the human liver. HCV infection can result in liver disease, including fibrosis and cirrhosis, can cause hepatocellular carcinoma, and is the leading indicator for liver transplantation (1). There is no vaccine for HCV; however, recently developed, direct acting antiviral drugs (DAAs) are showing high efficacy towards HCV, although they are incredibly cost-prohibitive (2). HCV isolates have been classified into 7 different genetic groups, referred to as genotypes, based on their sequences and display sequence diversity of greater than 30% (3, 4). The previous standard

*Corresponding Author Stacy M. Horner, Ph.D., Duke University Medical Center, 213 Research Dr., Box 3053 DUMC, Durham, NC USA 27710, Tel: 919-684-1921, Fax: 919-613-8646, stacy.horner@duke.edu.

of care for hepatitis C was treatment with pegylated IFN-α plus ribavirin and resulted in cure rates of only 40–50% for the most difficult to treat HCV genotypes (1 and 4) (5). However, the newest standards of care for HCV involve treatment with these newly developed DAAs, sometimes in IFN-free combinations, leading to cure rates of up to 95% in the controlled settings of clinical trials (2). It is currently unknown if these high cure rates will be maintained following widespread usage of these DAAs. Further, how antiviral resistance will be managed under widespread usage is unknown and needs to be carefully considered.

HCV infection is sensed as foreign or non-self by the host through the antiviral innate immune response. This immune response is triggered shortly after infection in a cell-intrinsic manner by host proteins called pattern recognition receptors (PRRs) that detect specific pathogen-associated molecular patterns (PAMPs) in the virus to activate downstream signaling cascades that drive immunity, including expression of antiviral genes and various cytokines, such as the type I and III interferons and IL-1β. HCV is sensed by multiple PRRs, including members of the RIG-I (retinoic acid-inducible gene I)-like receptors (RLRs), the toll-like receptors (TLRs), and the nucleotide oligomerization domain-like receptors (NLRs) (6). While the subsequent downstream antiviral response can be directly antiviral to limit virus replication and spread, it can also provide signals to the adaptive immune response for full induction of immunity to virus infection. As many viruses, including HCV, have developed effective countermeasures to inactivate this antiviral response, it is clear that the innate immune response plays an important role in determining the outcome of virus infection (7).

The HCV RNA genome is 9.6 kilobases in length and encodes for a single polyprotein that is processed by host and viral proteases into the 10 structural and non-structural proteins of the virus (Figure 1). HCV was discovered in 1989 using modern molecular biology approaches and was found to be the causative agent of non-A non-B hepatitis, first described over ten years earlier (8). Since the initial discovery of HCV, many aspects of the viral life cycle have now been revealed; some of which are now targets for DAAs (9). The initial studies to define the virology of HCV took time to develop because HCV is very difficult to grow in cell culture. For example, it took 10 years after the discovery of HCV to be able to study replicating HCV RNA in a cell culture system (10), and a fully infectious clone of HCV to be used in cell culture was only developed within the last 10 years (11, 12). Current systems for studying HCV have expanded from studying the virus in Huh7 human hepatoma cell lines to using primary human hepatocytes, mice with a chimeric human liver, or mice engineered with various human factors that promote HCV infection (13, 14). Utilization of these systems, including emerging non-chimpanzee animal models for HCV infection (13), will expand our knowledge of the full complement of HCV-host interactions that dictate the outcome of infection.

While we now know many of the important features of both the innate and adaptive immune response to HCV (15), many of these features were unknown when HCV/host interactions were first being studied. These early studies of HCV and antiviral innate immunity were limited by the viral tools and the knowledge of innate immunity available at the time. Even today, we still do not have a full understanding of the complex interactions that govern HCV

interactions with the host innate immune response in the infected liver. This review will feature several key discoveries on antiviral innate immunity in the context of HCV to further illustrate how virology research can elucidate fundamental aspects of host cell biology and antiviral immunity (16).

Early studies on antiviral immunity to HCV focus on PKR

The first hepatitis C therapies utilized the well-known antiviral cytokine IFN-α, which along with IFN-β is a member of the type I IFN family. In cell culture, type I IFN effectively limits HCV replication, however as a therapy in patients, type I IFN-based therapies have varying levels of effectiveness (5). Type I IFN signals through the IFN receptor (IFNAR1 and IFNAR2) to drive JAK/STAT signaling that activates the expression of hundreds of IFN-stimulated genes (ISGs) whose encoded proteins limit virus replication and spread. The antiviral mechanisms of action for many of these ISGs, including how they might be antiviral towards HCV, have not yet been fully described (17–19). The first work demonstrating that HCV induces an innate immune response came from HCV-infection studies in chimpanzees, which found elevated ISGs in the infected chimpanzee livers (20). Not long after that, the first studies suggesting that HCV might have a way to evade some aspects of this host innate immune system were published. These studies evaluating IFN treatment outcomes in Japanese patients infected with a genotype 1b virus found that sequence heterogeneity within the viral NS5A protein at the interferon sensitivity-determining region (ISDR) could predict IFN treatment outcomes (21, 22). While today we know that the NS5A protein plays diverse roles in the viral life cycle, including regulating HCV assembly versus replication (23), these studies on the NS5A ISDR were the first to reveal a virologic function for the NS5A protein. While it's not entirely clear how the sequence variation at the ISDR in NS5A contributes to IFN-based therapy responses amongst the different HCV genotypes or in human populations of different ancestries (24), these studies set the stage for the subsequent work that identified the mechanisms of how HCV antagonizes the antiviral response.

To identify how HCV antagonized the antiviral innate immune response, studies focused on the antiviral effector proteins that had been characterized to date, including the Mx proteins, 2′-5′ oligoadenylate synthestase, RNAseL, and the double-stranded (ds) RNA-activated protein kinase R (PKR). At the time, PKR was the most extensively studied of these antiviral effector proteins. The antiviral activity of PKR is activated by dsRNA, which stimulates its dimerization, autophosphorylation, and phosphorylation of eIF2α, resulting in a global block to cellular translation (25). We now know that PKR-sensing of dsRNA also activates a kinase-independent function of PKR that induces the antiviral IFN response (26). Even in the late 1990s, viral antagonizers of PKR had been described (27). Therefore, because genetic variation within the HCV NS5A protein predicted the IFN-sensitivity of HCV, it seemed likely that HCV also encoded a viral antagonizer of PKR. The most probable candidate was the HCV NS5A protein. Indeed, the NS5A protein did interact with PKR to disrupt its dimerization and ability to catalyze eIF2α phosphorylation (28, 29). Subsequently, it was also shown that the HCV E2 protein also inhibited PKR activation by acting as a pseudosubstrate through its encoded PKR-eIF2α phosphorylation homology domain (30).

The fact that HCV encodes at least two strategies to restrict PKR function would seem to suggest that preventing the inhibition of translation by PKR would be required for effective HCV replication. However, PKR activation does not actually directly regulate HCV translation because translation of the HCV polyprotein is unaffected by eIF2α phosphorylation (31–34). This is because its HCV translation is directed by an internal ribosome entry site (IRES) within its 5′ untranslated region (UTR) that can use eIF2A for translation initiation instead of eIF2α (35). While HCV RNA translation is unaffected by PKR activation and eIF2α phosphorylation, we know that during HCV infection the translation of ISGs and/or IFN is suppressed by PKR activation and the subsequent eIF2α phosphorylation (31, 32). Therefore, in the context of an activated IFN system, PKR activation by HCV may allow HCV to evade the antiviral function of ISGs and therefore be a positive regulator of HCV replication. Based on these findings, there appears to be an unexplained role for NS5A and E2 inhibition of PKR function during HCV infection. As HCV encodes these two PKR-antagonizers, PKR suppression must have some beneficial role in the virus life cycle. It is possible that at early times after infection, before a potent IFN signaling response has been activated, PKR inhibition by HCV proteins could relieve the translational suppression of critical host factors required to promote viral replication. However, we know that at later times after infection when the IFN signaling response is activated, PKR is no longer repressed by the E2 and NS5A proteins perhaps because they are involved in other aspects of the viral life cycle, such as viral assembly. Therefore, at these later times after infection, PKR would be activated and could contribute to the translational suppression of ISGs. More recent work has suggested that PKR, through a kinase-independent mechanism, is also involved in the induction of the signaling cascade that induces type I IFN (26). Therefore, it remains possible that the mechanism for HCV NS5A and/or E2 evasion of PKR has more to do with bypassing this innate immune signaling rather than circumventing the translational suppression function of PKR. Even though PKR was the first antiviral protein studied in the context of HCV, it seems that we still have much more to learn about the role of PKR during HCV infection.

During the initial studies on HCV and PKR, the virologic tools to study if the dsRNA generated during HCV replication was specifically activating PKR and the antiviral immune response had not yet been developed, and so it was unknown if HCV dsRNA was an actual PAMP that activated antiviral immunity. The ability to study HCV replication and how it impacted antiviral immunity finally became possible following the groundbreaking work of the Bartenschlager and Rice labs in developing the HCV subgenomic RNA replicon systems ((10, 36) and reviewed in (14)). These studies utilizing these HCV RNA replicons did not immediately find the dsRNA sensor/PRR for HCV, but rather found that replicating HCV RNA actually inhibited IRF-1 and IRF-3-dependent signaling induced by transfected dsRNA (37, 38). IRF-1 and IRF-3 are transcription factors that contribute to the induction of IFN-β, although IRF-1 is not essential for this induction (39). The HCV block to IRF-I activation was directed by NS5A inhibition of the PKR/IRF-1 signaling axis (37). On the other hand, the HCV-mediated block to IRF-3 activation, which is a seminal finding in the study of HCV/host interactions that regulated innate immunity, was directed by the actions of the HCV NS3/4A serine protease (40). The multifunctional HCV NS3/4A protease is a protein complex between NS3, which contains a serine protease domain and an

NTPase/RNA helicase domain, and its 54-amino acid cofactor NS4A, the membrane targeting subunit of the protease complex (41). NS3/4A is essential for HCV replication, viral polyprotein processing, and even viral assembly (42). NS3/4A blocked IRF-3 activation by preventing both the phosphorylation and its downstream signaling to IFN-β (40). Importantly, it was found that the protease activity of NS3 was responsible for the block in IRF-3 signaling because protease inactivation by mutation or treatment with an NS3-specific protease inhibitor relieved the block to virus-induced antiviral signaling through IRF-3 (40). This data suggested very clearly that NS3/4A was cleaving a host factor to prevent signal transduction to IRF-3 signaling, but the identity of this host factor remained unknown for several more years. Indeed, at this time the PRRs that initiated this signaling had yet to be described, and the virus-activated kinases that phosphorylated IRF-3 (now known as TBK1 and IKK-ε) were just being revealed (43, 44). Nonetheless, these early studies on HCV-innate immune interactions regulated by NS3/4A paved the way for the discovery of the PRRs RIG-I and MDA5, as well as their signaling adaptor protein MAVS, which was eventually found to be the proteolytic target of NS3/4A.

Antiviral innate immune sensing of HCV by RIG-I and MDA5

While PKR was the first described PRR for HCV (and for viruses in general), studies of PKR-deficient mice revealed that PKR-independent antiviral sensing mechanisms existed (45, 46). Soon after these observations, the PRRs that sensed extracellular dsRNA (TLR3) (47) and viral ssRNA (TLR7/8)(48–50) were discovered. However, there still existed an unidentified antiviral PRR, as type I IFN was still induced in response to virus infection in mice and cell lines lacking these TLRs or PKR (51–55). In 2004, Dr. Takashi Fujita and colleagues published their findings on the identification of RIG-I, which is one of the PRRs for intracellular dsRNA (56). RIG-I contains a caspase activation and recruitment domain (CARD) and a DexD/H box RNA helicase that binds to cytoplasmic dsRNA to drive signaling to NF-κB and IRF-3 (reviewed in (57)). A database search of GenBank for other proteins containing CARD motifs led to the discovery of MDA5, another IFN-inducible CARD-containing helicase protein that senses viral dsRNA. The presence of the CARD in RIG-I and MDA5 suggested that these PRRs may propagate their signals through interaction with another CARD-containing protein, as CARD-CARD interactions between proteins were known to regulate apoptotic and innate immune signaling (58). Indeed, the discovery of MAVS, a CARD-containing protein found to interact with both RIG-I and MDA5 to drive antiviral signaling through IRF-3 to IFN-β, proved this hypothesis to be correct (59–62). Importantly for HCV research, MAVS was the sought-after proteolytic target of NS3/4A, and it was found to be cleaved both in human hepatoma Huh7 cell lines and in livers of HCV-infected patients (60, 63–66).

The PRRs RIG-I and MDA5 are now known to differentially recognize distinct PAMPs within RNA viruses (67, 68). In particular, we now know that RIG-I is activated by short dsRNA containing either a 5′ triphosphate motif or a 5′ diphosphate motif (69, 70), while MDA5 senses long dsRNA or even higher order RNA structures that could be viral replication intermediates (71–73). In fact, both RIG-I and MDA5 have now been shown to be important for the innate immune response to HCV (74, 75). The studies that identified

RIG-I and MDA5 as sensors of HCV have led to the discovery of several key properties of antiviral innate immune sensing by these PRRs, described below.

HCV replicates poorly in cell culture, and efficient replication requires cell culture adaptive mutations in the viral RNA (36, 76). Therefore, to study HCV replication it was necessary to identify cell lines highly permissive for HCV replication. To identify these HCV-permissive cell lines, cell lines stably replicating HCV RNA were cured of the replicating HCV RNA using IFN, after which the ability of these IFN-cured cell lines to support HCV replication was tested (77). One particular Huh7 cell line, called Huh7.5, supported very high levels of HCV replication (77). This Huh7.5 cell line was unable to make certain ISGs in response to Sendai virus, which is a paramyxovirus that is normally a potent inducer of type I IFN (74). To identify the missing innate immune factor, a genetic complementation assay to identify cDNA products that could restore antiviral activation of IRF-3 was performed. This assay identified RIG-I as being one of the factors deficient in Huh7.5 cells, and found that Huh7.5 cells contained a single amino acid change in the CARD of RIG-I (T55I) that acts as a dominant negative of RIG-I signaling (74). This study identified RIG-I as a protein that controls innate immunity and permissiveness to HCV infection. The PAMPs that activate RIG-I have now been well-described (reviewed in (78–80)). Studies in HCV contributed to this understanding of the RIG-I PAMPs, as the poly U/UC region at the 3′UTR region of the HCV genome was found to be the HCV PAMP sensed by RIG-I, revealing that RIG-I can bind to RNA in a sequence-specific manner (81, 82). Taken together, HCV studies of innate immunity contributed to the discovery of RIG-I as an antiviral sensor and identified that RIG-I activation by PAMP RNA occurs in an RNA sequence-specific manner.

While RIG-I is essential for the antiviral innate immune response to HCV, a role for MDA5 had been largely over-looked. This was in part because it was known that transfection of *in vitro* transcribed HCV RNA induced signaling to IFN-β in an MDA5-independent manner (81). However, there were several hints suggesting a role for MDA5 in the antiviral response to HCV. The first hint came from the finding that HCV replication was enhanced in the presence of the paramyxovirus V protein, which inhibits MDA5, but not RIG-I function (83–85). In addition, MDA5 gene polymorphisms were found to be associated with spontaneous resolution of HCV infection (86). Now, studies using individual knockdowns of RIG-I and MDA5 have convincingly revealed a role for MDA5 (and confirmed the role of RIG-I) in sensing HCV to activate as antiviral innate immune response (75). Therefore HCV, similar to West Nile virus, is sensed by both MDA5 and RIG-I (87). In the future, it will be important to define the HCV PAMPs sensed by these PRRs during infection and how sensing by both of these PRRs contributes to the resolution of HCV infection. In addition to RIG-I and MDA5, other PRRs have now been identified that sense HCV infection, either directly in the infected cells or in other cells within the liver (88–90). Future studies will undoubtedly provide insights into how these PRRs activate innate immunity to HCV and contribute to the inflammatory responses found in the infected liver.

Studies on HCV evasion of antiviral immunity reveal key features of the anti-HCV innate immune response

While HCV infection is sensed by multiple PRRs that drive induction of the antiviral response and inflammation, HCV has evolved several mechanisms to block these antiviral responses. It is quite likely that this innate immune regulation by HCV contributes to its remarkable success as a viral pathogen by either promoting a cellular state conducive to viral replication or by dysregulating priming of a successful adaptive immune response to HCV (15). HCV has several mechanisms to block antiviral immunity, and this section will focus on the mechanisms by which the HCV NS3/4A protease blocks innate immunity and how this has impacted our understanding of antiviral immunity to HCV (Figure 2). A full review of HCV evasion of innate immunity, including how it inhibits IFN signaling, can be found elsewhere (91–94).

The discovery that the HCV NS3/4A protease cleaves the host protein MAVS to block innate immunity strongly supported the finding of several groups that MAVS is a key antiviral signaling adaptor molecule, especially during HCV infection (59–66). Further studies on NS3/4A have revealed that it cleaves several additional host proteins, highlighting the prominent role of NS3/4A in HCV pathogenesis (42, 95, 96). Importantly, three of these proteins (Riplet, MAVS, and TRIF) targeted by NS3/4A are known innate immune signaling proteins, further supporting the idea that HCV evasion of antiviral immunity is necessary to establish successful infection.

Studies on the molecular mechanisms of how NS3/4A regulates innate immunity have revealed critical aspects of the antiviral response to HCV. NS3/4A cleavage of MAVS during infection inhibits antiviral signaling because this cleavage occurs proximal to the transmembrane domain of MAVS, releasing it from intracellular membranes. The resulting cytoplasmic MAVS is unable to transduce RIG-I/MDA5 signals (reviewed in (42, 94)). Confocal imaging studies have revealed that the multifunctional NS3/4A has several different subcellular localizations, including at the mitochondria, peroxisomes, ER, and mitochondrial-associated ER membranes (MAM) (42, 97, 98). Indeed, we now know that MAVS is also localized to some of these same organelles as NS3/4A. While early studies showed that MAVS was localized to mitochondria (61), in more recent years, we and others have shown that MAVS is not exclusively mitochondrial, but that it is also localized on the MAM and on peroxisomes (98, 99). Quite unexpectedly, in an analysis of the localization of MAVS in isolated subcellular fractions during HCV replication, we found that MAM-localized MAVS was cleaved by NS3/4A, while the mitochondrial-MAVS remained uncleaved during HCV replication (98). While we were unable to determine specifically if NS3/4A targets MAVS localized to peroxisomes, the fact that our confocal imaging analyses revealed that a portion of NS3/4A could be localized to peroxisomes (98), strongly suggests that NS3/4A could also cleave peroxisomal-localized MAVS. This possible cleavage of MAVS at peroxisomes could abrogate peroxisomal-MAVS signaling, recently suggested to induce type III IFNs (100). Further studies to understand why NS3/4A does not target MAVS on the mitochondria are required, and it could be that HCV uses other strategies

independent of NS3/4A to down regulate MAVS signaling of innate immunity from the mitochondria, such as the induction of mitochondrial fission and mitophagy (101).

Why does NS3/4A specifically target the MAM-localized MAVS? Our previous work revealed that MAM/mitochondrial interactions regulate signaling to IFN-β (98). Therefore, it could be that NS3/4A-cleavage of the MAM-localized MAVS blocks antiviral signaling by also regulating membrane interactions between these organelles. MAVS oligomerization, which occurs through the CARD motifs, is also blocked by NS3/4A (102–104). Therefore, we hypothesize that NS3/4A cleavage of MAVS prevents interactions between the CARDs of the MAM-associated MAVS and the mitochondrial-associated MAVS to inhibit antiviral signaling during HCV infection. By studying how the HCV NS3/4A protease targets MAVS, we have learned about new subcellular localizations and signaling functions for MAVS and also about membrane/organelle interactions that regulate the antiviral response to RNA viruses.

Following the discovery that NS3/4A blocked IRF-3 activation of innate immunity, it was also shown that NS3/4A cleaves TRIF (105), the TLR3 adaptor protein that directs dsRNA-induced IRF-3-signaling to IFN-β (106). This cleavage of TRIF prevents polyI:C signaling of the TLR3 pathway, both *in vitro* and during infection (105, 107). The fact that HCV has a mechanism to cleave TRIF and prevents its signaling, strongly suggests that regulation of TRIF must be important for successful HCV infection. This cleavage of TRIF by NS3/4A could prevent TLR3 activation and the resulting inflammation observed during HCV infection (88) or it could alleviate TLR3-independent TRIF signaling of innate immunity (108).

Riplet, another protein in the RIG-I/MAVS signaling pathway is also cleaved by NS3/4A during HCV infection (96). Riplet is an E3 ubiquitin ligase that mediates K63-linked polyubiquitination of the repressor domain of RIG-I to activate RIG-I (109). Riplet contains a canonical NS3/4A serine protease cleavage site, and mutational inactivation of this site prevents its cleavage by NS3/4A (96). This cleavage of Riplet by NS3/4A functionally blocks the Riplet-mediated ubiquitination of RIG-I, inhibiting full RIG-I activation and downstream signaling. As HCV replication is increased following Riplet depletion, Riplet plays an essential function in the antiviral response to HCV (96). Riplet is also inactivated during influenza virus infection, suggesting that Riplet may be a potent antiviral signaling factor to a number of RNA viruses (110). It is not clear why HCV needs to encode a strategy to target Riplet when it already targets MAVS (downstream of Riplet in the antiviral signaling cascade). Understanding the mechanisms that regulate this differential targeting of Riplet and MAVS by NS3/4A will be an important area of future research.

Genome-wide association studies (GWAS) reveal the importance of type III IFNs during HCV infection

It has long been known that hepatitis C patients have varying ability to naturally resolve infection and to respond to IFN-based therapies for HCV (1). Some of these differences can be attributed to the HCV genotype present in the infection. Patients infected with HCV genotypes 2 and 3 have the highest response rates to IFN-based therapies, while those

infected with genotypes 1 and 4 have the lowest response rates (111). However, a large part of the variation in natural and treatment-induced clearance has been attributed to human genetic variation. Ground-breaking GWAS have identified single nucleotide polymorphisms (SNPs) in and around the gene encoding IFNL3 that predict both natural and treatment-induced clearance of HCV (112–116). IFNL3 is a member of the type III IFNs, also known as the IFN-λs, which consist of 4 antiviral cytokines (IFNL 1–4). The type III IFNs signal through their receptor (IL10R2 and IFNLR1) (117) to the JAK/STAT pathway to induce transcriptional activation of ISGs (reviewed in (118)). The type III IFNs appear to drive a more prolonged antiviral response than type I IFN (118, 119). Importantly, the type III IFNs have potent antiviral activity towards HCV (120). Prior to the GWAS that identified SNPs near IFNL3, not much was known about the role of the type III IFNs in antiviral immunity. However, the results of the GWAS strongly implicate type III IFNs as having very important antiviral roles to HCV. These findings propelled the study of the role of type III IFNs in the antiviral response, especially during HCV infection.

While it was initially debated if a SNP in the *IFNL3* locus affected IFNL3 protein expression, multiple studies have now found that the unfavorable haplotype at the *IFNL3* locus results in decreased IFNL3 expression (115, 116, 121–125). Further demonstrating that the protective allele affects the antiviral response, studies in HCV-infected primary human hepatocytes isolated from different donors have found that those that have the protective *IFNL3* allele have an increased ISG response that limits HCV infection (126). In addition to having an obvious role in the innate antiviral response, the type III IFNs have also been recently implicated in contributing to the adaptive immune response (125). A full description of the mechanisms underlying how genetic variation at the IFNL3 locus impacts HCV clearance is beyond the scope of this article, but I will point out that several candidate functional SNPs have been identified that may regulate IFNL function, either of IFNL3 or the newly described IFNL4 (127–131).

The pioneering HCV GWAS highlight a role for the type III IFNs in the antiviral response during HCV infection. While we know a great deal about how type I IFNs are activated and regulated during HCV infection, we know comparatively little about the type III IFNs. We do know that type III IFN is the predominant IFN made during acute HCV infection in hepatocytes (132–134). Additionally, these studies and others have revealed that the Huh7 liver hepatoma cell lines that support HCV replication in cell culture do not actually make much of a type III IFN response following HCV infection (133). A recently described HepG2 cell line does secrete type III IFNs in response to HCV infection, and these type III IFNs limit HCV replication in cell culture (75). Therefore, these studies have revealed that the Huh7-based cell lines that we have used for the last 15 years to study HCV/innate immune interactions actually lack some part of the innate immune response that drives type III IFN induction in response to HCV! It is therefore not that surprising that the HCV field is only now beginning to understand how type III IFNs are induced during HCV infection.

The most relevant cell types that drive expression of the type III IFNs during infection are unknown, and how innate immune sensing of HCV drives type III IFN induction is only beginning to be elucidated. While overexpression of the HCV PAMP (the poly U/UC region in the 3′ UTR of HCV) can induce expression of the type III IFNs in plasmacytoid dendritic

cells (135), it is unknown if this PAMP also induces the type III IFNs in primary human hepatocytes during infection. It appears that the signal transduction cascade that activates type III IFNs in response to HCV in hepatocytes is dependent on both of the PRRs RIG-I and MDA5 (75); and also the signaling adaptor protein MAVS (136). Further, there is evidence that HCV may suppress the long-term induction of the type III IFNs, although this doesn't appear to be as efficient as the ability of the HCV to suppress type I IFN induction in the infected cell (75). Because it has been proposed that MAVS signaling from peroxisomes induces the type III IFNs during virus infection (100), and as we know that some NS3/4A protein is localized to peroxisomes (98), it is possible that NS3/4A could cleave MAVS from peroxisomes to block MAVS signaling to the type III IFNs. Alternatively, HCV induction of host miRNAs (miR-208b and miR-499a-5p) that target the 3′ UTR of *IFNL3* mRNA could prevent full expression of IFNL3 protein during infection (127). Thus, through this strategy HCV could encode a second mechanism to prevent full induction of the type III IFNs. However, this mechanism of IFN-regulation by HCV may be variable depending on the host genetic background, as a SNP (rs4803217) exists within the miRNA targeting site in the 3′ UTR of *IFNL3* that would prevent targeting by the HCV-induced miRNAs (127). Regardless, the fact that HCV seems to have more than one strategy to block type III IFN induction and/or expression supports the idea that viral evasion of the type III IFNs is critical for HCV infection (126, 132).

Summary

Studies of HCV/host interactions during the antiviral innate immune response have led to important discoveries and insights into the field of innate immunity. They have revealed complex roles for PKR in the antiviral response, including a kinase-independent signaling function for PKR. They have contributed to the discovery of RIG-I and provided a more detailed understanding into nature of the RIG-I-activating PAMP RNA. They have solidified the roles of MAVS, TRIF, and Riplet in the innate immune response to HCV, as all three of these proteins are targeted for inactivation by the HCV NS3/4A protease. Studies into the molecular mechanisms of how NS3/4A regulates antiviral signaling have identified new subcellular localizations and signaling functions for MAVS that are leading to a greater understanding of the cell biological organization of the antiviral innate immune response. Finally, HCV/host interaction studies at both the cellular and genetic levels have implicated the type III IFNs as playing a major role in the antiviral response to HCV. I would argue that some of these discoveries would not have been possible, would have been delayed, or their importance would not have been fully realized without HCV research. There are more hidden facets of innate immune signaling that have yet to be discovered, and experimental studies using HCV (and other viruses!) will inform us of the most important aspects of the antiviral innate immune response. If a virus targets it, it must be important! Therefore, it is clear that not only is the field of virology alive and well (16), the field of hepatitis C virology must not be abandoned. Continued research into this fascinating, rapidly evolving RNA virus will undoubtedly lead to more discoveries in innate immunity and also in basic cell biology that will have broad scientific implications.

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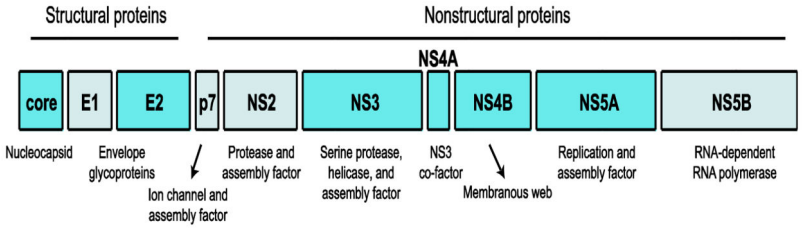

Figure 1. The HCV proteins

The HCV polyprotein is processed into the structural and nonstructural proteins of the virus, as shown here. The HCV proteins that have been implicated in antiviral innate immune evasion, including core, E2, NS3-NS4A, NS4B, and NS5A, are highlighted in blue.

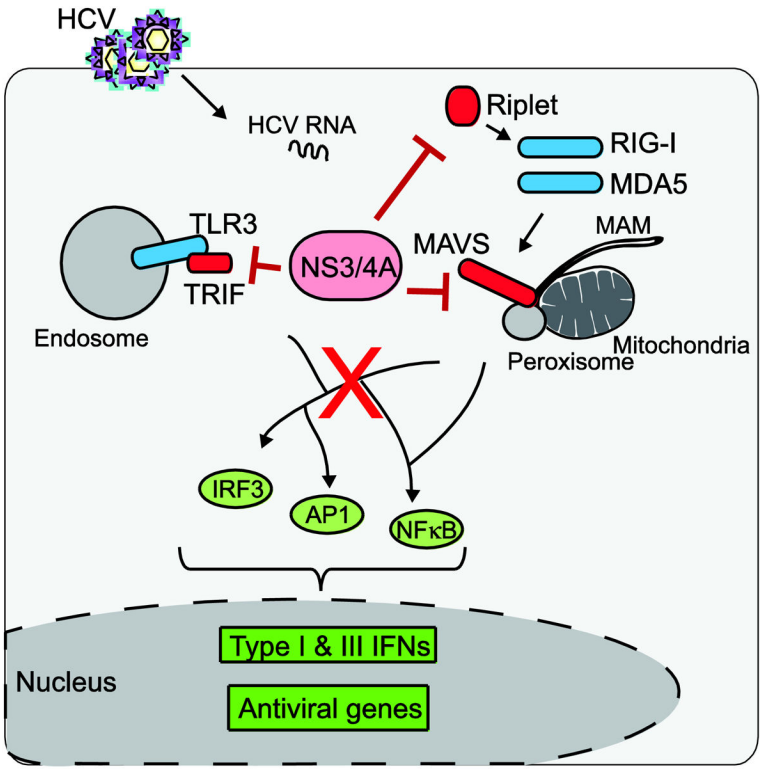

Figure 2. The HCV NS3/4A protein regulates the antiviral innate immune response to HCV

Following HCV infection of hepatocytes, viral pathogen-associated molecular patterns (PAMPs) present in the HCV RNA released into the cytosol are sensed by pattern-recognition receptors, including RIG-I, MDA5, and TLR3 (shown in blue) that signal through the adaptor proteins MAVS and TRIF, respectively, to induce the transcriptional antiviral response (shown in green). Full activation of RIG-I is regulated in part by Riplet, which mediates K63-linked ubiquitination of the repressor domain of RIG-I. The HCV NS3/4A protein blocks this antiviral signaling through cleavage of the host proteins MAVS, Riplet, and TRIF (shown in red).