World Journal of Gastroenterology

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DOI: 10.3748/wjg.v21.i38.10739

World J Gastroenterol 2015 October 14; 21(38): 10739-10748
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

TOPIC HIGHLIGHT

2015 Advances in Hepatitis C virus

Immune and non-immune responses to hepatitis C virus infection

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Author contributions: Sun J, Rajsbaum R and Yi M analyzed the literature and wrote the manuscript.

Supported by NIH AI109100 (to Sun J), R01AI110358 (to Yi M); and UTMB Institute for Human Infection and Immunity and UT System Rising STAR Award (to Rajsbaum R).

Conflict-of-interest statement: The authors have no conflict of interest to report.

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Received: May 18, 2015
Peer-review started: May 20, 2015
First decision: June 23, 2015
Revised: July 6, 2015
Accepted: September 14, 2015
Article in press: September 14, 2015
Published online: October 14, 2015

Abstract

The host innate and adaptive immune systems are involved in nearly every step of hepatitis C virus (HCV) infection. In patients, the outcome is determined by a series of complex host-virus interactions, whether it is a natural infection or results from clinical intervention. Strong and persistent $CD8^+$ and $CD4^+$ T-cell responses are critical in HCV clearance, as well as cytokine-induced factors that can directly inhibit virus replication. Newly available direct-acting antivirals (DAAs) are very effective in viral clearance in patients. DAA treatment may further result in the down-regulation of programmed death-1, leading to rapid restoration of HCV-specific $CD8^+$ T cell functions. In this review, we focus on recent studies that address the host responses critical for viral clearance and disease resolution. Additional discussion is devoted to the prophylactic vaccine development as well as to current efforts aimed at understanding the host innate responses against HCV infection. Current theories on how the ubiquitin system and interferon-stimulated genes may affect HCV replication are also discussed.

Key words: Hepatitis C virus; Hepatitis; T cell; Direct-acting antiviral; Innate immune response

Core tip: Hepatitis C virus (HCV) is an etiologic agent that can cause severe liver diseases, including chronic hepatitis, liver fibrosis, liver cirrhosis, and hepatocellular carcinoma. Although newly available direct-acting antivirals (DAAs) are very effective in viral clearance in patients, it remains unclear as to how many of the world's infected individuals will benefit from the new DAAs. In this review, we focus on recent studies that address the host responses critical for viral clearance and disease resolution. Additional discussion is devoted to the prophylactic vaccine development and innate responses against HCV infection. Current theories on how the ubiquitin system and interferon-stimulated genes may affect HCV replication are also discussed.

Sun J, Rajsbaum R, Yi M. Immune and non-immune responses to hepatitis C virus infection. *World J Gastroenterol* 2015; 21(38): 10739-10748 Available from: URL: http://www.wjgnet.com/1007-9327/full/v21/i38/10739.htm DOI: http://dx.doi.org/10.3748/wjg.v21.i38.10739

INTRODUCTION

Hepatitis C virus (HCV) is an etiologic agent that can cause severe liver diseases, including chronic hepatitis, liver fibrosis, liver cirrhosis, and hepatocellular carcinoma. Since the first discovery of this virus in 1989 by Choo *et al*[1], much has been learned about viral replication mechanisms and detailed functions of the viral proteins involved in these processes. Such knowledge has accelerated the development of direct-acting antivirals that could cure HCV infections, with much higher efficiency in shortening treatment duration compared to the traditional interferon-α-based therapy[2-6]. On the other hand, the vaccine development front seems to have lagged behind. This is certainly not due to the lack of effort to understand the HCV-induced immunity, but rather to the unique challenges in understanding effective immune responses against HCV.

The first challenge is the virus itself, which could successfully establish a chronic infection in about 80% of infected persons by effectively modulating innate and adaptive immune responses[7,8]. The second challenge is the difficulty in identifying and obtaining samples from acutely infected patients who successfully eliminated the virus due to the lack of distinct symptoms during acute phase of HCV infection. The third challenge is the lack of suitable small animal models that can recapitulate the HCV infection-mediated immune responses in humans. Although chimpanzee model has been the best immunocompetent animal model of HCV infection, with the recent National Institutes of Health (NIH) moratorium of usage of chimpanzee in HCV research, this challenge just got worse. Of note, there are continuing efforts to develop other immunocompetent animal model system[9,10]. Despite these difficulties, new information that could help us eventually control HCV-mediated immune dysregulation keeps emerging.

The goal of this review is to summarize the most up-to-date knowledge regarding both innate antiviral and adaptive immune responses that are available in the literature, to define the successful host responses that could contribute to HCV elimination. In addition, we discuss the implications of effective anti-HCV therapy on HCV-mediated immune modulation and vaccine development.

ADAPTIVE IMMUNE RESPONSES IN HEPATITIS C

Although HCV is capable of interfering with a wide range of host physiological processes, it is readily detected by the host sensing machinery, followed by the triggering of innate cellular responses[11,12], including the production of type Ⅰ interferons (IFN-Ⅰ) and the activation of downstream, antiviral target genes. However, despite these responses, HCV continues to replicate in the liver in the incubation phase. The adaptive immune response to HCV infection develops over several weeks[13]. Although the reasons for this delay are not understood, it is clear that the magnitude, diversity, and quality of the adaptive immune responses are the determinants of the outcome[13]. While this acute immune response has the potential to clear the viral infection, it is unsuccessful at least 50% of the time, and the virus has a very strong propensity to cause chronic infections. Development of chronicity is marked by a dramatic decrease in the activity of $CD8^+$ cytotoxic T lymphocytes (CTL) and $CD4^+$ Th cells in the liver without achieving viral clearance. Interestingly, the T cell dysfunction seems to be restricted to HCV-specific $CD8^+$ T cells, since influenza-specific $CD8^+$ cells were functional in chronic HCV patients[12].

T cell dysregulation in chronic infection

The effector functions of virus-specific $CD8^+$ and $CD4^+$ T cells are critical in viral clearance and disease resolution[14]. Interestingly, virus clearance and disease progression are seemingly mediated by phenotypically distinctive $CD8^+$ CTL populations. The $CD8^+$ CTLs participating in virus clearance expressed high levels of IFN-γ, but low levels of the activation marker CD38 (IFN-γ^{hi} $CD38^{lo}$)[12,13]. In contrast, the CTL involved in liver injury are commonly IFN-γ^{lo} $CD38^{hi}$, and the frequency of these cells tends to increase with the inflammation score (Figure 1, left panel)[15]. Although CTLs can exert limited antiviral activity, they are unable to keep pace with the evolution of HCV. As a result, HCV rapidly accumulates escape mutations in its genome, and persistent viremia ensues[16]. In studies with large cohorts of chronic subjects and spontaneous resolvers, adequate help from $CD4^+$ T cells was found to be essential to promoting immune protection[17]. Among resolvers, the HCV epitopes are presented by multiple alleles of major histocompatibility complex Ⅱ molecules, and nonstructural (NS) protein-directed $CD4^+$ T-cell responses are associated with high levels of IL-2 and IFN-γ[18]. On the other hand, HCV persistence is associated with a high frequency of $CD4^+$ regulatory T cells (Treg) that could directly suppress

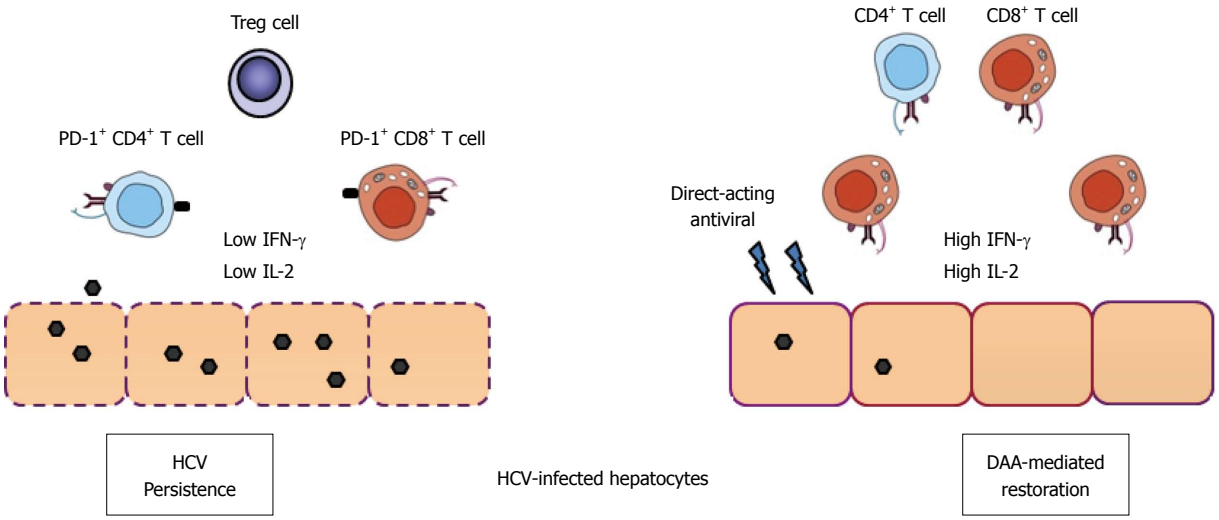

Figure 1 Direct-acting antiviral therapy and immune function restoration in hepatitis C virus infection. In chronic hepatitis C infection, T cells having a narrow repertoire of TCRs mount a weak response to HCV antigens, and their effector functions are often impaired. Many $CD8^+$ and $CD4^+$ T cells express low levels of IFN-γ and IL-2 accompanied by up-regulation of PD-1 molecules in the liver. Development of T regulatory cells and compromised dendritic cell functions also contribute to T cell functional impairment. Recent data suggest that IFN-free direct-acting antivirals not only clear HCV in the majority of patients, but also result in the down-regulation of PD-1, leading to rapid restoration of virus-specific $CD8^+$ T cell functions in patients[28]. HCV: Hepatitis C virus; IFN: Interferon; IL: Interleukin; PD-1: Program death-1; TCR: T cell receptor.

HCV-specific CTL in patients[19]. The resolution of disease is usually associated with the loss of expression of programmed death-1 (PD-1) and decreased functional suppression[17,20]. In addition to Treg cells, high expression of inhibitory receptor-PD-1 on $CD4^+$ and $CD8^+$ T cells also contributes to viral persistence as well as to failure of antiviral therapy (Figure 1, left panel)[21,22].

T cell responses in immunomodulatory and direct-acting antiviral therapies

Until recently, the mainstay of treatment for chronic HCV infection had been pegylated interferon and ribavirin for HCV genotype 1 infection. This treatment resulted in a sustained virologic response (SVR) in 50%-80% of patients with HCV genotype 1 infection (higher SVR among those with genotypes 2 or 3 infections). There has been a clear demonstration that IFN-α, even when it achieved viral clearance, was not able to rescue antiviral T cells from exhaustion due to its pleiotropic effects on T cells[23-25]. To improve the efficacy of hepatitis C therapy, one critical question is whether antiviral T cells are permanently blemished by the sustained expression of PD-1 or other tolerating mechanisms; or alternatively, the removal of IFN-α would promote host recovery and the expansion of T cells with antiviral functions[26,27]. In a recent study involving a clinical cohort undergoing IFN-free therapy, a combination of Faldaprevir (a protease inhibitor) and Deleobuvir (a non-nucleoside polymerase inhibitor) cleared HCV in the majority of patients[28]. Furthermore, viral antigen reduction in response to direct-acting antiviral (DAA) treatment resulted in the down-regulation of PD-1, leading to rapid restoration of HCV-specific $CD8^+$ T cell functions in patients (Figure 1, right panel)[28]. While this study unveils an unexpected benefit of these two DAAs, it is interesting to see whether other DAAs with or without ribavirin are also able to reverse exhaustion and restore full functions of HCV-specific T cells.

HCV PROPHYLACTIC VACCINES

HCV infection is a major public health problem in the world. Although DAAs are a significant advancement for an HCV treatment, it remains unclear as to how many of the world's infected individuals will benefit from the new DAAs. For instance, the high cost of these DAAs will undoubtedly deny their access to low income countries and may also result in selective use in middle and even high income counties[29]. Thus there will likely still be a substantial number of HCV cases, including those individuals who are not screened and unaware of their infection. Additionally, DAAs are not efficacious in those having already developed advanced hepatic cirrhosis, carcinoma and liver failures. Importantly, a recent study showed that persistently HCV-infected chimpanzees cured with DAA maintained narrowly focused stable $CD8^+$ T cell repertoires that were incapable of preventing persistent infection following HCV re-challenge[30]. Thus, vaccination may be necessary even for those individuals who have cleared virus following DAA treatment. For these reasons, an effective prophylactic HCV vaccine remains a critical instrument to halt the global HCV epidemic. Current human and chimpanzee studies suggest that a prophylactic vaccine inducing both protective T cells and broadly-neutralizing antibody (bnAb) responses is important for HCV control and thus highly desirable characteristics for the future vaccine candidates[29].

Circulating HCV is genetically diverse, and therefore a broadly effective vaccine must target conserved T- and B-cell epitopes of the virus. Several prophylactic vaccine candidates based on different strategies and viral targets have been developed in the last two decades. Vaccines aimed to target conserved T cell epitopes are shown to induce vigorous and broadly directed $CD4^+$ and $CD8^+$ T cell responses, and these are well underway in clinical development[31,32]. Despite the molecular trickeries employed by HCV, a number of bnAbs have been identified. The protective role of bnAbs against HCV infection has been demonstrated in chimpanzees, highlighting the possibility of developing a broadly effective vaccine by inducing bnAbs[33,34]. The HCV viral RNA genome encodes two structural envelope glycoproteins, E1 and E2. Although neutralizing antibodies (nAbs) to E1 have also been isolated, E2 is the main target of nAbs in HCV-infected patients[35]. In the last decade, significant discoveries of bnAbs and their structural analysis with antigenic epitopes have been made in the HIV-1 vaccine field; now a similar trend has begun to emerge in the HCV vaccine field. Recently, researchers have combined the latest findings in HCV structural biology and cutting-edge technologies in protein design and next-generation sequencing of Ab repertoires to facilitate HCV immunogen design for the induction of bnAbs in vaccination[35]. The linear HCV epitopes will be grated onto protein scaffolds, which allow epitope presentation in their bnAb-bound conformations. These studies demonstrate the feasibility of generating a highly potent antibody formulation against multiple, conserved neutralizing epitopes on HCV.

HCV AND INNATE IMMUNE RESPONSES

It is now clear that HCV infection induces innate responses capable of limiting virus replication to some extent. However, HCV is still able to establish chronic infections by escaping immune responses. Similar to other viruses, HCV encodes pathogen-associated molecular patterns (PAMPs), which are recognized by the host pattern-recognition receptors (PRRs). Members of the endosomal Toll-like receptor (TLR) family and the cytoplasmic Retinoic acid inducible gene (RIG-I)-like receptors (RLRs) can also recognize HCV PAMPs (Figure 2A). Pathogen recognition by PRRs results in activation of downstream signaling pathways leading to the production of pro-inflammatory cytokines, chemokines, IFN-I and type-Ⅲ IFN (IFN-λ)[36]. IFNs elicit their antiviral activity through the up-regulation of many IFN-I-stimulated genes (ISGs), which act as direct effectors of the antiviral response[37]. HCV-encoded PAMPs recognized by the RIG-I sensor include the polyuridine motif of the HCV genome 3′ non-translated region and its replication intermediate, which binds RIG-I through the 5′ terminal triphosphate on the viral RNA. This signaling induces IFN-I and antiviral ISGs in the liver *in vivo*[38]. HCV PAMPs that are recognized by RIG-I are also produced by cleavage of viral NS5B region of HCV RNA by the IFN-inducible host endoribonuclease RNase L, releasing small structured RNAs with 5′-hydroxyl (5′-OH) and 3′-monophosphoryl (3′-p) groups[39]. Binding of the HCV PAMP induces a conformational change in RIG-I and subsequent ubiquitination by the E3-ubiquitin ligase TRIM25 (Figure 2A). This process and interaction with the chaperone protein 14-3-3e promote recruitment of activated RIG-I to the adaptor protein mitochondria antiviral signaling protein (MAVS) that is anchored to the mitochondria and also located in an intracellular membrane network at the peroxisomes and on mitochondrial- associated membranes[40-43]. Subsequently, different signaling partners are known to be recruited to MAVS, resulting in the activation of the IκB (IKK) and IKK-related kinases[44,45], TBK1 and IKKε, which phosphorylate the transcription factors IRF3 and IRF7 required for IFN-I production as well as IFN-λ[46-48].

Deregulation of innate anti-viral signaling pathways during HCV replication

Both IFN-I and IFN-λ are produced upon innate recognition of viruses, although differential expression has been found in tissues such as the brain upon viral infection[49]. Although signaling occurs through different receptors, both IFN-I and IFN-λ can trigger downstream signaling through phosphorylation of signal transducers and activators of transcription 1 (STAT1) and STAT2, suggesting that both IFN-I and IFN-λ signaling result in induction of the same ISGs. Together, STAT1, STAT2 and IRF9 form the interferon-stimulated gene factor 3 (ISGF3) complex, which is essential for induction of ISGs (Figure 2B)[50]. The tyrosine kinases JAK1 and TYK2, which are both activated by IFN-I and IFN-λ, phosphorylate tyrosine 701 (Y701) on STAT1[51]. In addition, phosphorylation of S708 on STAT1 by the IKKε kinase is also required for the efficient induction of all ISGs in response to IFN-I[52]; however, it is currently unknown whether IFN-λ stimulation also results in activation of IKKε and STAT1-S708 phosphorylation. Furthermore, activation of IKKε during IFN-I signaling also requires binding to unanchored lysine-48 (K48)-linked polyubiquitin chains, which are not covalently attached to any protein[53]. Some evidence suggests that HCV inhibits STAT1 function. For example, by using a microRNA array in human hepatocytes infected with HCV, it was shown that miR-373 is up-regulated in HCV-infected cells (Figure 2B). This microRNA targeted JAK1 and IRF9 and reduced phosphorylation of STAT1. Consistent with this observation, knockdown of miR-373 resulted in the reduction of HCV RNA replication[54]. Furthermore, the core protein of HCV associates with STAT1 and promotes its degradation[55]. Immune cell populations and hepatocytes from HCV^+ patients have reduced STAT1 and STAT3 proteins.

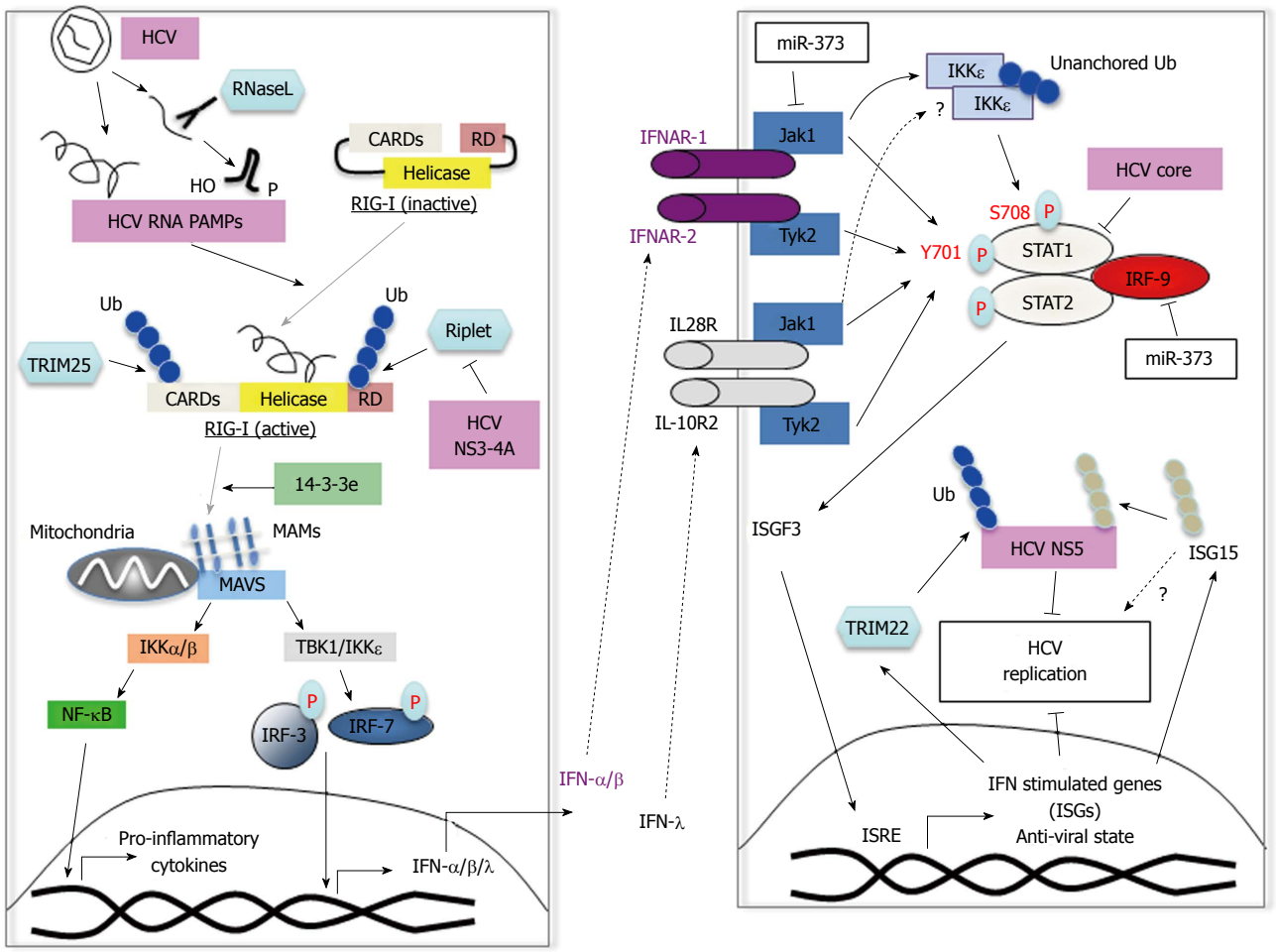

Figure 2 Innate immune response to hepatitis C virus and viral escape mechanisms. A: RIG-I recognizes HCV PAMPs including HCV 5'-triphosphate RNA and small structured RNAs with 5'-hydroxyl (5'-OH) and 3'-monophosphoryl (3'-p) groups, which are cleaved by the host endoribonuclease RNase L. Binding of the HCV PAMP induces a conformational change in RIG-I and subsequent ubiquitination by TRIM25 and Riplet. RIG-I is then recruited to MAVS *via* mitochondria associated membranes (MAMs) and the chaperone protein 14-3-3e. Subsequently, TBK1 and IKKε phosphorylate IRF3 and IRF7 for IFN-I production as well as IFN-λ, and pro-inflammatory cytokines *via* NF-κB activation. The NS3-4A of HCV inhibits Riplet-dependent activation of RIG-I; B: IFN-I and IFN-λ are recognized by the IFNA receptor and IL28/IL-10 receptor respectively. Both trigger downstream signaling through phosphorylation of STAT1 and STAT2. Together, STAT1, STAT2 and IRF9 form the ISGF3 complex, which translocates to the nucleus for induction of antiviral ISGs. The tyrosine kinases JAK1 and TYK2, phosphorylate tyrosine 701 (Y701) on STAT1. In addition, phosphorylation of S708 on STAT1 by the IKKε kinase (activated by unanchored polyubiquitin chains) is also required for ISG induction. It is currently unknown whether IFN-λ stimulation also results in IKKε activation. miR-373 is up-regulated in HCV-infected cells and inhibits JAK1 and IRF9 function resulting in reduced STAT1 phosphorylation. TRIM22, which is induced by IFN-I, inhibits HCV replication probably by a mechanism involving ubiquitination of viral NS5. ISG15, another IFN-I inducible protein can inhibit HCV replication by ISGylation of viral NS5 rendering unstable. ISG15 has also been proposed to have pro-viral roles. The core protein of HCV associates with STAT1 and promotes its degradation. RIG-I: Retinoic acid inducible gene; HCV: Hepatitis C virus; PAMP: Pathogen-associated molecular pattern; IFN: Interferon; IL: Interleukin; ISGF: Interferon-stimulated gene factor; STAT: Signal transducers and activators of transcription; NF-κB: Nuclear factor-kappa B; IKK: Inhibitor of NF-κB kinase.

Furthermore, STAT3 was preferentially ubiquitinated and targeted for proteosomal degradation in the presence of HCV[56]. These observations may explain in part the hypo-responsiveness observed in some patients to IFN-α treatment.

Since IFN-α/β and IFN-λ trigger similar signaling pathways, they are predicted to induce the same group of ISGs. However, in contrast to the widely expressed IFN-I receptor in different cell types, the expression of the IL-10R2 subunit of the IFN-λ receptor appears to be restricted to cell types and tissues, particularly on epithelial cells[49]. This raised the possibility that IFN-λ administration may be a better option to the common therapy for treating HCV infection with pegylated IFN-α (Peg-IFN-α) and ribavirin. It is now known that Peg-IFN-α treatment is only effective in a fraction of HCV-infected individuals with the presence of some side effects. Thus far Peg-IFN-λ has shown promising results[57,58]. Besides the tissue/cell type-specific expression of the IL-10R2 receptor, Peg-IFN-λ may be more effective since, unlike IFN-α treatment[59], Peg-IFN-λ treatment did not lead to refractoriness of JAK-STAT signaling following multiple stimulations[60]. IFN-λ treatment also results in a faster reduction in viral load as compared to those with IFN-α[61]. In addition, HCV infection appears to down-regulate the expression of IFN-I receptor, in contrast to sustained expression of the IL-28 receptor subunit. Global

transcriptome analysis in hepatocytes indicated that IFN-λ stimulation prolonged the expression of various ISGs that are potentially beneficial to antiviral defense mechanisms[62].

In addition to inhibition of STAT-dependent innate immune responses, HCV has also been shown to inhibit TLR3 signaling and IFN-λ production in human hepatoma cell lines, with subsequent reduction in the antiviral ISG56, MxA and OAS-1. NS3/4A, NS5A and NS5B had the ability to inhibit poly I:C-induced IFN-λ1 expression in Huh7 cells[63,64].

Role of ubiquitin and ubiquitin-like molecules in HCV replication

Ubiquitination of proteins is a post-translational process, which has been demonstrated to regulate not only protein stability but also various steps of the signaling pathways in immune regulation and cytokine production[65]. Viruses have adapted to antagonize innate immune responses by using different mechanisms including manipulating the ubiquitin system for their own advantage[66]. For example, it was recently proposed that the Influenza virion carries unanchored polyubiquitin chains that promote virus uncoating by utilizing the host aggresome machinery[67]. At the same time these polyubiquitin chains that are released in the cytoplasm may function as a mechanism to alert the innate cellular response early upon virus entry to the cell[68]. Whether HCV utilizes unanchored polyubiquitin chains for replication remains to be tested. Nevertheless, HCV also has been shown to target the ubiquitin system to escape the innate immune response. HCV NS3-4A proteases inhibit the E3-ubiquitin ligase Riplet, which, together with TRIM25, is required for efficient ubiquitination and activation of RIG-I (Figure 2A)[69]. The ubiquitin system is also utilized by the host to restrict virus replication, and, in fact, may be one of the mechanisms by which IFN therapy limits virus replication in patients. For example the E3-ubiquitin ligase TRIM22, which is highly induced by IFN-I[70-72], may be associated with responsiveness to Peg-IFN-α-2a/RBV combination therapy[73]. A possible mechanistic explanation for these findings is supported by data showing that TRIM22 overexpression inhibits HCV replication and knockdown reduces IFN-induced anti-HCV activity. In addition, TRIM22 appears to promote ubiquitination of HCV-NS5A[74]. Although this study did not elucidate the functional effects of NS5A ubiquitanation, ubiquitin ligases as well as de-ubiquitinating enzymes are emerging as important proteins in controlling HCV replication. In particular, a recent study using an RNA interference (RNAi) screen found a few ubiquitin ligases to be important in HCV replication; the E2-conjugating enzyme UbE2J1, is involved in viral RNA replication; USP11, a de-ubiquitinating enzyme is involved in HCV IRES-mediated translation. In addition, TRIM42, another member of the E3-ubiquitin ligase family of proteins, and UbE2M, another E2 ubiquitin-conjugating enzyme, are also involved at early stages of viral post-entry[75].

Similar to posttranslational covalent modification by ubiquitin, the small ubiquitin-like modifier (SUMO) can also be covalently attached to protein lysines post-translationally, and regulates many different cellular processes. HCV also utilizes this cellular process for its own advantage; the NS5A protein of HCV is SUMOylated, resulting in increased protein stability by inhibiting ubiquitination[76].

ISG15, another ubiquitin-like molecule that can be covalently attached to lysine residues of proteins post-translationally, has been shown to play both positive and negative roles during HCV infection. ISG15 is a highly IFN-inducible gene that has antiviral functions against many viruses[77]. Accordingly, overexpression of ISG15- and ISG15-conjugation enzymes resulted in inhibition of HCV replication. Furthermore, HCV-NS5A protein was ISGylated, and this appeared to decrease NS5A stability[78]. However, in contrast to this study, other studies identified ISG15 as a pro-HCV host factor promoting HCV replication[79,80]. ISG15 may have different effects depending on the cell type or tissue expression. If indeed ISG15 acts as a pro-viral factor, its high induction by IFN treatment may help explain why in some patients IFN-based treatment results in persistent HCV infection. Additional evidence that ISG15 plays a role in HCV replication comes from studies on USP18, which specifically cleaves ISG15 from its cellular targets[81]. USP18 knockout mice are hypersensitive to IFN, with prolonged Jak/Stat signaling[82]. Expression of USP18 is increased in the liver biopsy specimens of patients who do not respond to IFN-α therapy, and siRNA knockdown of USP18 in human cells increases the ability of IFN to inhibit HCV replication as well as to increase cellular protein ISGylation and prolonged STAT1 phosphorylation, and a general enhancement of IFN-stimulated gene expression[83].

To demonstrate that *in vivo* knockdown of ISG15 may be used therapeutically to inhibit HCV replication, Real *et al*[84] used lipid nanoparticles to deliver siRNA specific to ISG15. The treatment resulted in specific reduction of ISG15 expression in the liver *in vivo*, resulting in reduced responses to IFN treatment. This also resulted in a reduction in HCV replication, supporting the role of ISG15 as a pro-viral factor. In addition, ISG15 knockdown revealed five potential candidates as pro-viral factors that depend on ISG15 expression. In particular, knockdown of the ISG15-dependent heterogeneous nuclear ribonucleoprotein K (HnrnpK) also resulted in decreased levels of HCV replication[84].

HCV infection is also known to induce the ubiquitin-dependent degradation of some cellular proteins including the retinoblastoma tumor suppressor protein by viral NS5B[85,86], and the suppressor of cytokine

signaling 3 (SOCS3), which is a negative regulator of the JAK-STAT pathway[87]. HCV viral proteins have also been described to be ubiquitinated and degraded by the proteasome through both ubiquitin-dependent and independent mechanisms (review by Shoji *et al*[88]).

IFN-stimulated genes as antiviral factors to HCV

For decades Peg-IFN-α has been one of the most important therapeutics to control HCV infection in patients, although the exact mechanisms of viral inhibition have remained unclear. It is well established that IFN treatment will induce a large number of ISGs with known antiviral functions, but which ISGs and how they act against HCV remained largely unknown until recently. Some of these ISGs have direct or indirect anti-HCV functions and have been reviewed recently (see Horner *et al*[43]). These include *ADAR*, *DDIT4*, *DDX58* (*RIG-I*), *DDX60*, *EIF2AK2* (*PKR*), *GBP1*, *IFI44L*, *IFI6*, *IFIT1*, *IFIT3*, *IFITM3*, *IRF1*, *IRF7*, *ISG12*, *ISG20*, *MAP3K14* (*NIK*), *MOV10*, *MS4A4A*, *MX1* (*MxA*), *NOS2*, *NT5C3*, *OAS1*, *OASL*, *PLSCR1*, *RNASEL*, *RSAD2* (*viperin*), *SSBP3*, and *TRIM14*[43]. Many of these genes were found by using lentiviral vectors expressing 389 selected ISGs in Huh-7.5 cells, a RIG-I-defective derivative of Huh-7 cells. Although most of the ISGs showed some degree of inhibition of HCV replication, RIG-I, MDA5, IRF1 and IRF7, which are genes involved in signaling to produce IFN-I, were the strongest inhibitors of HCV[37]. Other studies showed the IFN-induced transmembrane protein 1 (IFITM1) as an inhibitor of HCV[89,90]. In addition, another screen identified several antiviral ISGs induced by IFN-α and IFN-γ using an RNAi[91]. IFITM1 is highly induced by both IFN-I and IFN-γ has been shown to inhibit different viruses including West Nile virus, Influenza, HIV and HCV[89,92]. IFITM1 accumulates at hepatic tight junctions in HCV-infected human patient liver during IFN therapy and interacts with the HCV co-receptors CD81 and occludin, blocking viral entry[90]. ISG56 was also shown to inhibit HCV replication[89]. Additional ISGs have been reported to inhibit HCV. ISG20, and PKR are reported to inhibit HCV RNA synthesis[93]. Recently, the Cholesterol-25-hydroxylase (CH25H), a 31.6-kDa endoplasmic reticulum-associated enzyme that catalyzes oxidation of cholesterol to 25-hydroxycholesterol (25HC), was also shown to inhibit HCV. CH25H is an ISG that is induced in many tissues upon *in vivo* exposure to TLR ligands and IFN stimulations[94]. 25HC has also been reported to possess anti-HCV activity[95]. CH25H can interact with the NS5A protein of HCV and inhibit its dimer formation, which is essential for HCV replication[96].

In summary, although in recent years there have been great advances in our understanding of the anti-HCV functions of ISGs, many of these studies still fail to take into consideration the physiological conditions in which the virus replicates, as well as relevant immune cell types that are localized in the liver. Furthermore, it remains unclear as to how to induce these genes with exogenous treatments, or how to deliver lentiviral vectors containing specific ISG as potential antiviral treatments. Thus, additional studies are required using novel *in vivo* models combined with biochemical methods to identify the molecular mechanisms of antiviral functions.

CONCLUSION

The recent availability of highly effective DAAs against HCV infection brings hope for HCV eradication. However, initial reports suggest that DAA alone may not be enough to achieve this goal. Instead, HCV eradication will ultimately require the boosting of favorable innate and adaptive immune responses and ultimately vaccine development. Based on our knowledge of antiviral immune responses, the raising of effective antiviral responses against HCV will require agents or vaccine candidates that promote innate antiviral signaling and enhance both CD4 and CD8 responses effectively without inducing exhausted phenotypes and bnAb that could neutralize multiple genotypes of HCV. There is no doubt that effective immune-modulators against HCV infection will be available someday as a result of our continued efforts to understand HCV-induced immune regulation.

ACKNOWLEDGMENTS

The authors wish to express gratitude to their trainees, as well as Ms. Mardelle Susman for assistance with manuscript preparation.

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P- Reviewer: Takahashi T **S- Editor**: Ma YJ **L- Editor**: A
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ISSN 1007-9327