VOLUME 29 NUMBER 1 January 2023

pISSN 2287-2728
eISSN 2387-285X

CLINICAL and MOLECULAR HEPATOLOGY

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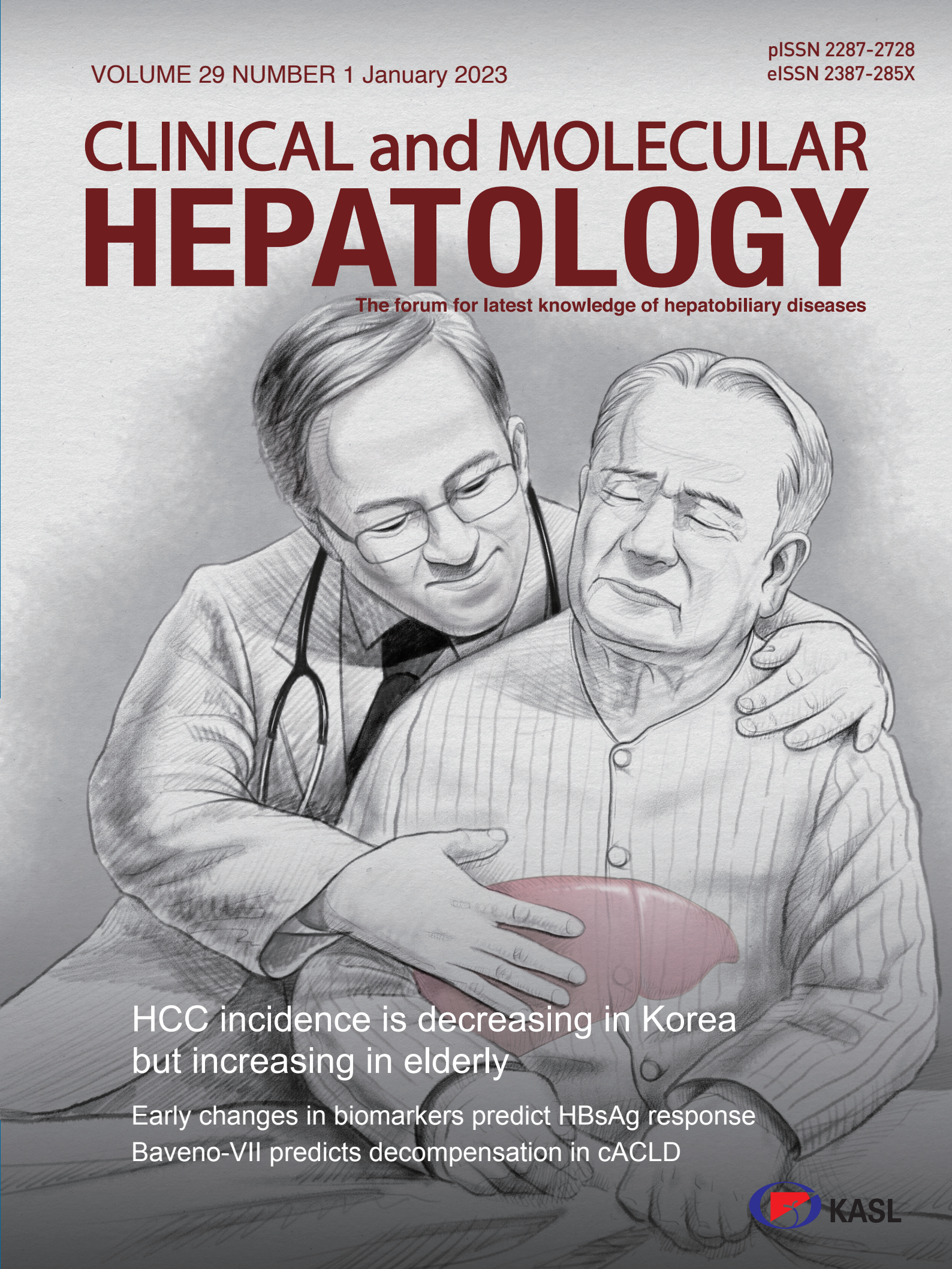

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pISSN 2287-2728
eISSN 2287-285X

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https://doi.org/10.3350/cmh.2022.0032
Clinical and Molecular Hepatology 2023;29:65-76

Review

Hepatocytes infected with hepatitis C virus change immunological features in the liver microenvironment

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Hepatitis C virus (HCV) infection is remarkably efficient in establishing viral persistence, leading to the development of liver cirrhosis and hepatocellular carcinoma (HCC). Direct-acting antiviral agents (DAAs) are promising HCV therapies to clear the virus. However, recent reports indicate potential increased risk of HCC development among HCV patients with cirrhosis following DAA therapy. $CD8^+$ T-cells participate in controlling HCV infection. However, in chronic hepatitis C patients, severe $CD4^+$ and $CD8^+$ T-cell dysfunctions have been observed. This suggests that HCV may employ mechanisms to counteract or suppress the host T-cell responses. The primary site of viral replication is within hepatocytes where infection can trigger the expression of costimulatory molecules and the secretion of immunoregulatory cytokines. Numerous studies indicate that HCV infection in hepatocytes impairs antiviral host immunity by modulating the expression of immunoregulatory molecules. Hepatocytes expressing whole HCV proteins upregulate the ligands of programmed cell death protein 1 (PD-1), programmed death-ligand 1 (PD-L1), and transforming growth factor β (TGF-β) synthesis compared to those in hepatocytes in the absence of the HCV genome. Importantly, HCV-infected hepatocytes are capable of inducing regulatory $CD4^+$ T-cells, releasing exosomes displaying TGF-β on exosome surfaces, and generating follicular regulatory T-cells. Recent studies report that the expression profile of exosome microRNAs provides biomarkers of HCV infection and HCV-related chronic liver diseases. A better understanding of the immunoregulatory mechanisms and identification of biomarkers associated with HCV infection will provide insight into designing vaccine against HCV to bypass HCV-induced immune dysregulation and prevent development of HCV-associated chronic liver diseases. **(Clin Mol Hepatol 2023;29:65-76)**

Keywords: Hepatitis C; Hepatocellular carcinoma; Immunity; Cell communication; Exosomes

INTRODUCTION

The hepatitis C virus (HCV) is a serious and growing worldwide threat to human health, having already infected approximately 3% of the world's population (>180 million people).[1] HCV transmission can often be linked to a blood-borne route, such as intravenous drug use or medical procedures. HCV infection is almost invariably associated with viral persistence, leading to development of hepatocellular carcinoma (HCC), as well as, autoimmune diseases such as mixed

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Editor: Won-Il Jeong, Korea Advanced Institute of Science and Technology, Korea

Received : Feb. 5, 2022 / **Revised :** Jul. 26, 2022 / **Accepted :** Aug. 11, 2022

cryoglobulinemia.[2] Direct-acting antiviral agents (DAAs) are promising HCV therapies to clear the virus. However, recent reports indicate a potential increased risk of HCC development among HCV-infected patients with cirrhosis following DAA therapy.[3,4] Unfortunately, development of a vaccine against HCV infection has failed, and no vaccine is currently available.

Since HCV was identified as the causative agent of non-A, non-B hepatitis, the immune responses to HCV infection have been examined in detail.[5-8] It is notable that immune responses to HCV are significantly impaired. First, the appearance of HCV-specific antibody response is delayed and is detectable on 2–4 months after viral infection.[9] Second, T cell responses to HCV have been demonstrated with multiple antigenic stimulations.[10] Importantly, early and sustained $CD4^+$ and $CD8^+$ T-cell responses are crucial for controlling HCV infection,[11] but the magnitude of T-cell responses is dramatically decreased in chronic hepatitis C patients compared to that in acute hepatitis C patients. This suggests that HCV may employ mechanisms to evade or possibly suppress host T-cell responses. It is important to understand how chronic HCV infection dampens T-cell responses against HCV infection and develop vaccine against HCV.

Numerous studies have reported that HCV actively suppresses the immune response by altering the differentiation of innate immune cells, resulting in the impairment of subsequent robust antiviral adaptive responses. Moreover, $CD4^+$ $CD25^+$ regulatory T-cells (Tregs) have been consistently shown to be expanded in patients with chronic infection.[6,12,13] $CD4^+CD25^+$ Tregs play a pivotal role in maintaining immune homeostasis and controlling excessive immune responses. The immunoregulatory cytokines, transforming growth factor β (TGF-β) and interleukin (IL)-10, are crucial for the induction and maintenance of Tregs. TGF-β is involved in the generation of inducible Tregs and the maintenance of Treg function.[14] IL-10 is a critical factor for sustaining FoxP3 expression.[15] In addition, these cytokines have been reported to be secreted during HCV infection and have polymorphisms that correlate with HCV clearance.[16]

Molecular biological studies of HCV have shown that it is a positive-stranded RNA virus related to the *Flaviviridae* family.[17] The viral genome encodes a single polyprotein of approximately 3,000 amino acids (aa) processed by host and viral proteases to form non-structural and structural proteins including a nucleocapsid (core) and two envelope proteins. The primary site of HCV replication is in hepatocytes. HCV life cycle involves multiple steps to generate infectious virus and lipid droplet formation is crucial for viral RNA replication (Fig. 1). Viral tropism seems to be determined by initial interaction of HCV glycoproteins with dendritic cell-specific intercellular adhesion molecule-3-grabbing non-integrin (SIGN) and lymph node-SIGN on the surface of liver endothelial cells and antigen-presenting cells. This interaction is followed by binding to CD81, SR-B1, and/or heparin sulfate on the cell surface of hepatocytes.[18] Although there is evidence for HCV replication at extrahepatic sites including B-cells, the vast majority of HCV replication and protein expression occur in hepatocytes.[19] Recently, it has been reported that hepatocytes are capable of exerting immunoregulatory function. Notably, HCV-infected hepatocytes interact with immune cells present in the liver microenvironment and suppress host immune responses. In this review article, we discuss the contribution of HCV-infected hepatocytes to regulate host immune responses during HCV infection and the molecular mechanism for their immunoregulatory function.

IMMUNOLOGICAL FEATURES OF HEPATOCYTES UPON ENCOUNTER WITH VIRAL INFECTION

Hepatocytes are not traditionally regarded as key players in mounting immune response. However, they have the ability to produce a large variety of cytokines and chemokines.

Abbreviations:

AIH, autoimmune hepatitis; ASC, caspase recruitment domain; ATP, adenosine triphosphate; DAA, direct-acting antiviral agent; EVs, extracellular vesicles; GC, germinal center; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; HSC, hepatic stellate cell; HTA, host-targeting antiviral; IFN, interferon; IL, interleukin; LSEC, liver sinusoidal endothelial cell; MDSC, myeloid-derived suppressor cell; MIP-1α, macrophage inflammatory protein-1 α; miRNA, microRNA; mRNA, messenger RNA; MVs, microvesicles; NAFLD, nonalcoholic fatty liver disease; NLRP3, nucleotide-binding oligomerization domain-like receptor family pyrin domain containing 3; PAMP, pathogen-associated molecular pattern; Panx1, pannexin 1; PD-1, programmed cell death protein 1; PD-L1, programmed death-ligand 1; RANTES, regulated upon activation, normal T cell expressed and secreted; ROS, reactive oxygen species; RUNX1, runt-related transcription factor1; RUNXOR, RUNX1 overlapping RNA; SIGN, specific intercellular adhesion molecule-3-grabbing non-integrin; Tfr, T follicular regulatory; TGF-β, transforming growth factor β; TLR, toll-like receptor; Treg, regulatory T-cells

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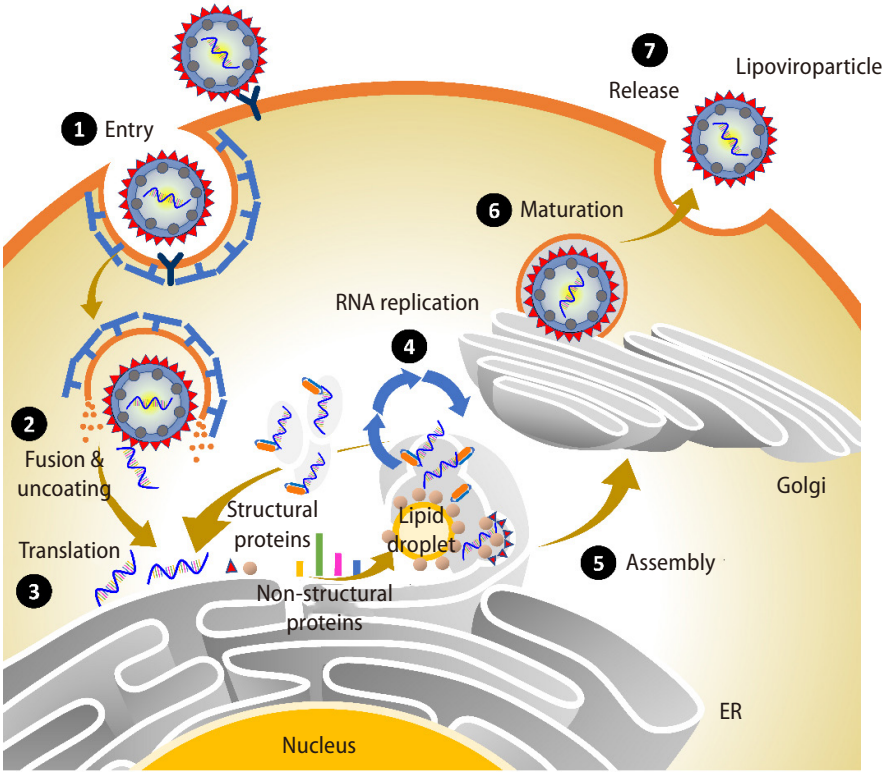

Figure 1. Hepatitis C virus life cycle occurs via 7 steps; entry, fusion & uncoating, translation, replication, assembly, maturation, and release. Formation of lipid droplet is crucial for viral RNA replication. ER, endoplasmic reticulum.

Thus, in the liver microenvironment, the cellular interaction between lymphocytes and hepatocytes might take place due to the fenestrated structure of hepatic sinusoids, combined with the lack of basal membrane and the low blood flow. Current techniques available for the *in vivo* analysis of acute HCV infection are limited because the chimpanzee is the only animal susceptible to a natural HCV infection. The *in vitro* tissue culture of HCV has been used for studying the interaction of infected hepatocytes with immune cells. HCV infection leads to hepatocyte damages that initiate hepatic inflammatory responses by recruiting immune cells (i.e., myeloid and T-cells) at the site of infection.[20] Secretion of immune mediators from infected hepatocytes and immune cells is involved on the activation of hepatic stellate cells (HSCs) and the development of liver fibrosis (Fig. 2).

Immune mediators produced from infected hepatocytes

The HCC cell line, Huh7, is established from HCC and commonly used for *in vitro* studies. Following HCV infection, Huh7 cells are able to produce IL-7, IL-15, and TGF-β, and their expression does not change with IL-1α exposure.[21,22] Other cytokines and chemokines, such as tumor necrosis factor, IL-1β, regulated upon activation, normal T cell expressed and secreted (RANTES), macrophage inflammatory protein-1 α (MIP-1α), and IL-8, are also produced by hepatocytes, and their productions are increased in response to pro-inflammatory IL-1α activation. In addition, HCV infection is associated with the activation of inflammasomes such as nucleotide-binding oligomerization domain-like receptor family pyrin domain containing 3 (NLRP3), apoptosis-associated speck-like protein containing a caspase recruitment domain (ASC), caspase-1, and release of IL-1β secretion.[23] Many of these cytokines and chemokines are important to $CD4^+$ T-cell survival and differentiation. For example, RANTES is $CD4^+$ T-cell recruiting cytokine and contributes to development of Th1 response.[24] While IL-15 enhances Th1 cytokine production and promotes development of an effector phenotype,[25] TGF-β has a negative influence on effector T-cell function and is known to be involved in Treg cell and Th17 cell development. Development of HCV replicon (genotype 1a)[26] as

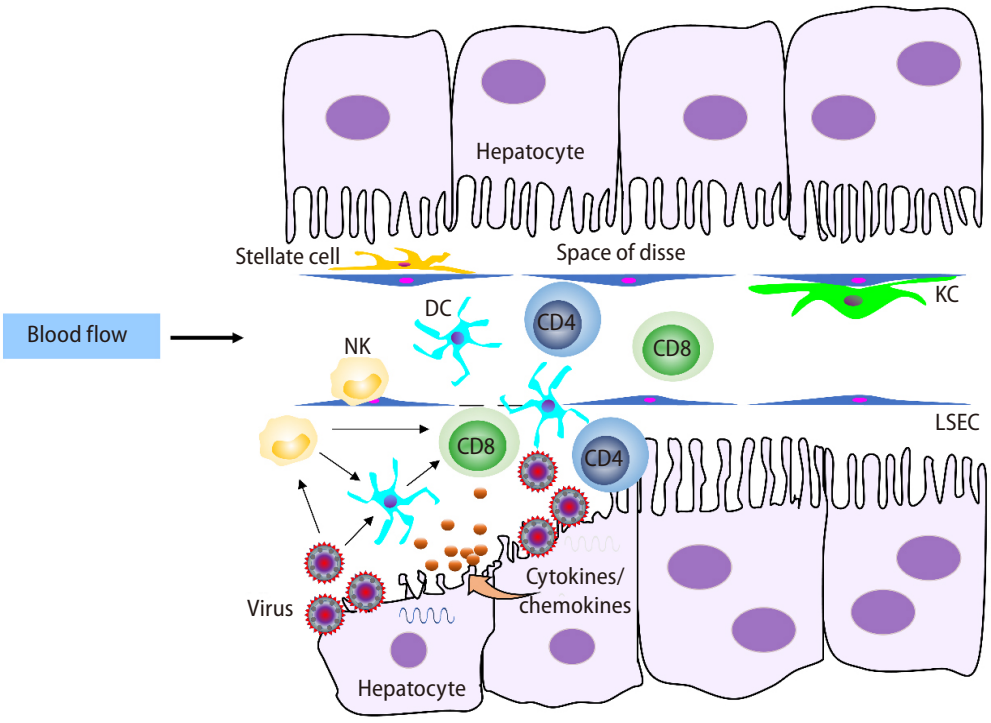

Figure 2. Interaction between virus-infected hepatocyte and immune cells. Hepatitis C virus infection and replication in hepatocytes promote the production of cytokines/chemkines leading to recruit immune cells. The excessive cytokines cause hepatic inflammation in the liver and exacerbate tissue damage and liver disease progression. DC, dendritic cell; KC, Kupffer cell; NK, natural killer cell; LSEC, liver sinusoidal endothelial cell.

well as replicating JFH1 virus (genotype 2a)[27] represent a major advancement for studying the interaction of HCV-infected hepatocytes and the host immune system. Successful replication of the viral genome has been superior in the HCC cell line Huh7.5. Huh7.5 cells were generated from HCV-positive parental cell, Huh7, which was cured of HCV using interferon-α treatment. These cells were subsequently receptive to HCV replication such that HCV RNA and proteins could be detected soon after transfection with HCV replicons.[26] Studies on cytokine analysis using HCC cells have been validated in primary hepatocytes following HCV infection.

Programmed cell death protein 1 (PD-1) and PD-1 ligand expression

PD-1 is a receptor for the programmed death-ligand 1 (PD-L1) and PD-L2, and plays a role in dampening host immune responses. Specifically, T-cells activation increased the level of PD-1 expression and engagement of PD-1 with its ligand inhibits their activation, proliferation, and cytokine secretions.[28-30] Leukocytes, a number of soft tissues, and endothelial cells constitutively express low levels of PD-L1, but induce the expression of PD-L2 under the inflammatory condition.[28] Inflammatory cytokines, including interferon (IFN)-γ, up-regulate PD-L1 and PD-L2 expression on a variety of epithelial cells and leukocytes.[31] The PD-1 pathway is associated with outcome of human disease severity (e.g., autoimmune diseases, cancer). PD-1 ligand expression is seen in a variety of cancers, often correlating with worse cancer outcome. Immuntherapy based on PD-1 blockade has been developed to treat cancer patients.

The pathogenic role of the PD-1 pathway has been demonstrated in the progression of chronic liver diseases by determining the modulation of the inhibitory PD-1 ligands in the liver with chronic inflammation. Chronically damaged livers provide ample opportunity for lymphocyte modulation via PD-1/PD-1 ligand ligation. Indeed primary human hepatocytes as well as Kupffer cells, stellate cells, T-cells, myeloid cells, and liver sinusoidal endothelial cells (LSECs) express PD-L1 and PD-L2.[32] At the messenger RNA (mRNA) level, chronic

https://doi.org/10.3350/cmh.2022.0032

hepatitis C and autoimmune hepatitis (AIH) patients have increased levels of PD-L1 and PD-L2 mRNA compared to those with normal livers. Multiple studies found that blocking PD-1 and PD-L1 interactions on leukocytes from hepatitis B virus (HBV)- or HCV-infected patients restored T-cell function *in vitro*.[33,34]

Cellular location of PD-1 and its ligand expression, has been identified by histologic studies on liver biopsies from chronic hepatitis B, chronic hepatitis C, AIH, and nonalcoholic fatty liver disease (NAFLD) patients as well as individuals with normal liver histology. The presence of the normal control group enabled to differentiate baseline tolerogenic features of the liver from those modulated during chronic liver damage. The increased numbers of $CD3^+$ T-cells were detected in chronic hepatitis B, chronic hepatitis C, and AIH livers and significant portions of intrahepatic lymphocytes from these patient groups expressed PD-1. LSECs, Kupffer cells, and intrahepatic leukocytes expressed PD-L1 and PD-L2 while hepatocytes also expressed PD-L1 and PD-L2 under inflammation. These studies confirm that PD-L1 and PD-L2 expression on parenchymal and non-parenchymal cells can deliver a negative signal to T-cells, dampening their responses. Moreover, the necroinflammatory levels associated with chronic hepatitis B, chronic hepatitis C, and AIH were correlated with increased PD-L1 and PD-L2 on leukocytes, Kupffer cells, and LSECs. However, early-stage NAFLD patients did not demonstrate significant increases in $CD3^+$ lymphocyte infiltrates, PD-1 or PD-L1 and PD-L2 expression, suggesting that inflammation rather than liver damage itself leads to the expression of PD-1 and PD-1 ligands.

Induction of Treg driven by TGF-β secreted from HCV-infected hepatocytes

Impaired antiviral $CD8^+$ and $CD4^+$ Th1 T-cell responses are associated with persistence of HCV infection.[35] Although failure of T-cell responses might occur as a result of mutation in viral antigens.[36,37] and upregulation of negative costimulatory PD-1 and CTLA-4 pathways,[38,39] HCV infection generates a direct mechanism to generate $CD4^+CD25^+FoxP3^+$ Tregs to inhibit T-cell responses. Notably, an increase in the number and functionality of Tregs has been detected in chronic HCV patients as compared to those with resolved infection.[6,40,41] The increased frequency of Tregs observed in chronic HCV patients might arise from expansion of thymic-derived natural Tregs or from *de novo* induction from naïve T-cells. The mechanism underlying induction of Tregs during HCV infection remains unclear.

Notably, HCV protein expression within hepatocytes alters the function of $CD4^+$ T-cells and could contribute to development of Tregs.[42] By using an HCV whole protein-expressing hepatoma line (Huh7.5-FL), studies have been conducted to examine contribution of infected hepatocytes on $CD4^+$ T-cell dysfunction. $CD4^+$ T-cell responsiveness, as measured by IFN-γ production, was diminished in co-culture with Huh7.5-FL compared to controls. Importantly, $CD4^+$ T-cells in contact with Huh7.5-FL adopted a Treg phenotype ($CD25^+FoxP3^+$ $CTLA\text{-}4^+LAP^+$) and developed the ability to suppress effector T-cell proliferation. The role of hepatocytes in Treg development was clarified by finding that Huh7.5-FL produced more TGF-β than control hepatocytes. Moreover, intracellular expression of an HCV core is known to enhance TGF-β1 mRNA production by the hepatoma cell line HepG2.[43,44] These provide evidence that the site of HCV infection (i.e., hepatocytes) plays a pivotal role in impairing the antiviral T-cell response by the induction of Tregs via TGF-β production.

CELLULAR CROSSTALK VIA EXOSOMES RELEASED BY HCV-INFECTED HEPATOCYTES

Cells exchange information through release of soluble factors or by direct interaction. Several reports demonstrate that cells can also communicate by circular membrane fragments called extracellular vesicles (EVs).[45] Normal or diseased cells release different types of EVs, including microvesicles (MVs) and exosomes, depending on their cellular origin. Exosomes (40–100 nm) are formed by the fusion between multivesicular bodies and the plasma membrane, while MVs (100–2,000 nm) bud directly from the plasma membrane. Exosomes have been shown to provide a means of intercellular communication as contributing factors in the development of several diseases by the spread of proteins, mRNAs, and microRNAs (miRNAs).[45] During virus infection, exosomes released from virus-infected cells contain viral proteins, viral RNAs, and certain specific miRNAs that are able to spread the infection and alter the cellular response in uninfected target cells during the immune response and pathogenesis.

Exosomes secreted from HCV-infected hepatocytes play a critical role in promoting intercellular crosstalk with liver non-

parenchymal cells.[46,47] HCV infection may directly activate a signaling network in hepatocytes, promoting release of immunoregulatory molecules packaged into exosomes, leading to intercellular communication inducing for activation of fibrotic macrophages and LSEC (Fig. 3). Recently, accumulating evidence demonstrates that exosomes and exosomal miRNAs from HCV-infected hepatocytes lead to polarization and differentiation of macrophages and mediate pro-fibrotic responses in HSC and T follicular regulatory (Tfr) cells expansion.[48-50] This suggests that development of liver disease involves intercellular communication during HCV infection. Interestingly, some studies have reported increased release of specific miRNAs, such as miR-122 in HCV infection.[51] Recently, potential cellular and molecular mechanisms of HCV-mediated secretions of exosome and exosomal miRNAs have been elucidated.[52]

Exosomes containing immunoregulatory molecules

Numerous studies have been conducted to identify contents of exosomes secreted from HCV-infected hepatocytes and define their biological function. Interestingly, HCV exosomes play a role in regulating host immune responses and facilitating development of persistent HCV infections and chronic liver diseases. HCV-dependent elevated reactive oxygen species (ROS) levels and induction of autophagy are related to exosomes derived from the endosomal pathway.[53] Toll-like receptor (TLR) 7 and TLR8 are present in intracellular vesicles from HCV-infected hepatocytes and macrophages.[54] TLR is a type of pattern-recognition receptor in the immune system recognizing pathogen-associated molecular patterns (PAMPs) and exerts a broad spectrum of innate immunity. Exosomes containing single-strand HCV RNA have been shown to affect differentiation of monocytes to fibrogenic macrophages in a TLR7/8-dependent manner.[48] TLR3 activation was reduced under influence of viral dsRNA contained in exosomes secreted from HCV-positive cells, showing a novel mechanism to evade the host immune response in virus persistence.[55]

Moreover, HCV exosomes isolated from infected hepatocytes contain TGF-β at the surface of exosomes. TGF-β is important for induction and expansion of Tfr cells, a subset of Tregs.[56] Increased Treg responses are a prominent feature in

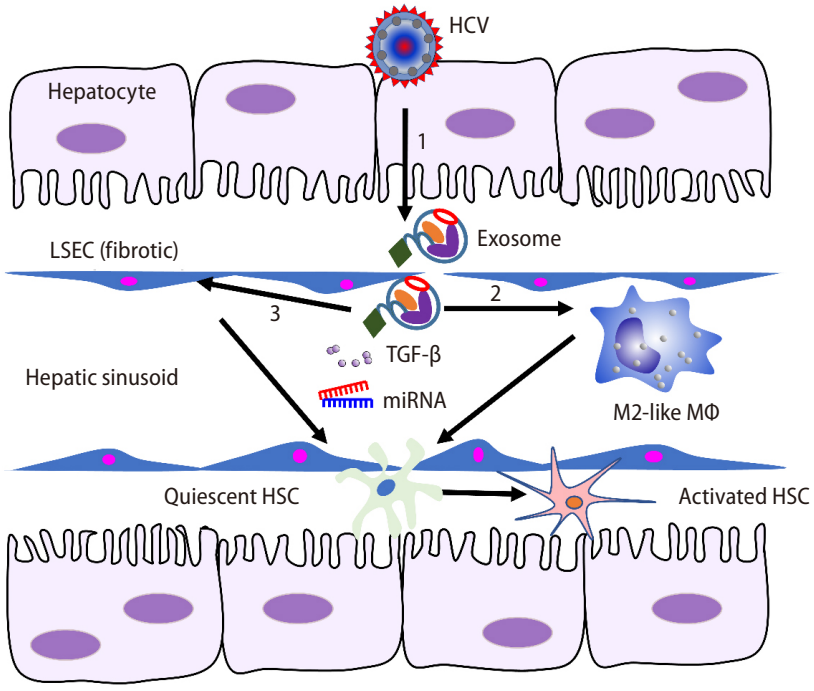

Figure 3. Schematic diagram of HCV exosomes. Exosomes released by HCV-infected hepatocytes promote intercellular crosstalk with Mϕ and LSEC leading to stellate cell activation. HCV, hepatitis C virus; LSEC, liver sinusoidal endothelial cells; TGF-β, transforming growth factor β; miRNA, microRNA; HSC, hepatic stellate cell.

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HCV infection such that Tregs are increased in both number and function in chronic hepatitis C patients and are positively correlated with viral load.[6,7] Furthermore, abundant Tregs are found in the livers of chronic hepatitis C patients.[57,58] Recently Tfr cells have been identified for functional regulation of germinal center (GC) responses by limiting Tfh cells and B cells.[59,60] Tfrs are reported to be increased in the circulation of chronic hepatitis C patients.[61] Tfrs are identified by the expression of follicular markers CXCR5 and PD-1 and regulatory markers CD25 and Foxp3. This allows Tfrs to co-migrate with Tfh to control GC responses.[62] Interestingly, lymphoid follicles, containing T- and B-cells, are commonly observed in the livers of HCV-infected patients and exhibit signs of GC-like architecture.[63,64] Recent studies have identified the presence of Tfh within the livers of HCV-infected patients.[65]

Exposure of $CD4^+$ T-cells to TGF-β-containing exosomes from HCV-infected hepatocytes led to a significant increase in Tfrs. This study has been done by culturing exosomes isolated from HCV-infected primary hepatocytes with pre-activated $CD4^+$ T-cells. Moreover, depletion of $CD14^+$ monocytes prior to co-culture of infected hepatocytes with PBMCs did not affect the ability of infected hepatoma cells to drive Tfr expansion but monocytes are not required for expansion of Tfr cells. Importantly, expansion of Tfr cell is accompanied by acquisition of an enhanced regulatory phenotype and leads to the functional suppression of Tfh cells. Increases in Tfr responses are driven by a novel pathway involving the release of TGF-β-containing exosomes from HCV-infected hepatocytes. These findings highlight the accumulation of Tfrs in the livers of HCV-infected patients, potentially inhibiting protective Tfh and B-cell responses at the site of infection, and contributing to viral persistence.

Exosomes containing miRNAs

Several studies report the expression and biological activity of various miRNAs in HCV infection-associated exosomes. In the exosomes of HCC patients, miR-21-5p, miR-10b-5p, miR-221-3p, and miR-223-3p were significantly upregulated compared to the non-HCC individuals.[66] miR-19a and miR-192 from exosomes secreted from HCV-infected hepatocytes were internalized into HSCs and induced HSC activation by triggering STAT3-mediated TGF-β signaling.[50,67] HCV-induced exosomal miR-122/let-7b/miR-206 induced activation of B-cells associated with mixed cryoglobulinemia.[68] A link between the runt-related transcription factor1 (RUNX1)/RUNX1 overlapping RNA (RUNXOR) and the STAT3/miR-124 pathway regulated differentiation of myeloid-derived suppressor cells (MDSCs) during chronic HCV infection, and expression of miR-124 was negatively correlated with expression of STAT3.[69] In addition, a pilot study on expression profiles of exosomal miRNAs in HCV-infected patients has identified various miRNAs related to other diseases.[70]

Molecular mechanism of exosome release from infected hepatocytes

The exosome plays a critical role in mediating the cellular communication.[50] Syntenin has been reported to be involved on the secretion of E2 via exosomes. E2 is a viral envelope glycoprotein that forms a heterodimer and mediates viral entry.[71] The release of MVs or exosomes can be stimulated by

Table 1. Candidate biomarkers of HCV exosom miRNAs

Biomarker	Responses of each markers in HCV	Reference
miR-21-5p, miR-10b-5p, miR-221-3p, miR-223-3p	Increased	66
miR-19a	Increased	50
miR-192	Increased	81
miR-124	Decreased	69,82
miR-885-5p, miR-365	Increased	70
miR-627-5p, miR-221	Decreased	70
miR-155	Increased	76
miR-122, let-7b, miR-206	Increased	68,77
miR-199a	Increased	77

HCV, hepatitis C virus; miRNA, microRNA.

stress signals, including DNA damage, intracellular calcium, and extracellular adenosine triphosphate (ATP).[72] Exosome release can occur by an ESCRT-dependent or ESCRT-independent pathway. Moreover, exosome release is induced by extracellular ATP that is associated with purinergic receptor activation.

Pannexin 1 (Panx1) is a transmembrane channel that mediates ATP release. Panx1 is activated by the stretch of the plasma membrane during changes in osmolality or mechanical injuries or by proteolysis via caspase-3 and -7 during early apoptosis.[73] The ATP released by Panx1 activation can bind to the purinergic receptor, leading to a calcium influx.[74] Expression of Panx-1 and purinergic receptor was increased in HCV-infected hepatocytes.[75] However, participation of Panx1 pathway-mediated exosome release in viral infection has not been well elucidated. Our studies demonstrate that secretions of exosomes and specific miRNAs are associated with the Panx1/purinergic receptor pathway in HCV-infected hepatocytes.[52] Notably, Panx1 inhibitors prevented release of exosomes from HCV-infected hepatocytes.

DEVELOPMENT OF FUTURE THERAPEUTICS TO TREAT CHRONIC LIVER DISEASE

Therapeutic interventions to develop drugs for halting the liver disease progression and vaccine for preventing HCV infection have met with limited success. It is vital to understand the pathogenesis of HCV infection and the mechanism of virus-induced immune suppression leading to the establishment of persistent infection. The studies described in this review article provide novel and important information on the role of HCV-infected hepatocytes in the pathogenesis of HCV infection and inhibition of T-cell function. Results of these studies contribute to advance the understanding of impaired T-cell responses via interactions between hepatocyte and T-cell. Thus these data should stimulate development of novel vaccine strategies for this important human pathogen.

Furthermore, miRNA-containing exosomes have been reported as biomarkers for diagnosis of HCV. Table 1 summarizes miRNAs identified as biomakers associated with HCV infection. Exosomes containing miR-19a and miR-192 were observed in the serum of HCV patients and presented as a new marker.[50] An increase of exosome miR-885-5p and miR-365 but a decrease of exosome miR-627-5 and miR-221 showed characteristic of HCV-infection among other miRNAs.[70] Expression of exosome miR-155 was reduced after rituximab treatment in HCV patients.[76] In particular, serum miR-122 and miR-199a are potential biomarkers reflecting therapeutic efficacy against HCV infection.[77] Potential mechanisms of HCV anti-viral therapy involve therapeutic agents directly acting on the virus, IFN-dependent/independent therapeutics, and host-targeting antivirals (HTAs). miR-122 increases viral replication by directly binding to two conserved flanking regions of the 5′ UTR of HCV RNA and acts as HTA against HCV replication.[78] Miravisen, miR-122 antisense blocker, has been developed as a latest HTA.[79] In addition, treatment of syntenin, a protein involved in the exosome secretion pathway, has recently been introduced,[80] but like the above-mentioned treatment, there are few reports of its clinical test results yet. Nevertheless, these studies are important for developing therapeutics to target HCV-infected hepatocytes and prevent development of HCV-associated chronic liver diseases.

CONCLUSION

In summary, HCV-infected hepatocytes play a pivotal role in changing immunological features in the liver microenvironment. Through cellular and molecular mechanisms, HCV-infected hepatocytes dampen intrahepatic T-cell responses directly via increased expression of PD-L1 or indirectly by releasing immunoregulatory molecules such as TGF-β. Future studies are needed to develop immune-based therapeutics to treat chronic liver diseases associated with HCV infection. In addition, the markers in the various immunological mechanisms presented in this review can be used in future research on immune-based therapeutics.

Authors' contribution

SJP contributed to manuscript research and writing. YSH contributed to conceptualization of review article and critical review/editing of the manuscript.

Acknowledgements

This paper is dedicated to the memory of Hugo Rosen, MD who has done pioneering studies on chronic liver diseases and liver immunology. This work was supported by the grant from the National Institute of Health (DK122737 to YSH).

Conflicts of Interest

The authors have no conflicts to disclose.

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