Hepatic cell injury during HCV infection: a review on the role of various host factors

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ABSTRACT

The present review deals with the interaction of hepatitis C virus (HCV) with various host factors and the underlying mechanisms involved in liver pathology during HCV infection. The persistence of virus after HCV infection causing chronic hepatitis in high majority of patients was explained in terms of host immune response varying with mutating nature of HCV virus. High scale variability in HCV genome arising of frequent mutation favours HCV to escape immune mediated eradication and to persist in host causing continued liver cell injury. HCV shows pathogenic effect via its role in several metabolic changes and inducing reactive phenomenon including hepatic steatosis, oxidative stress and insulin resistance in HCV infected patients. The structural and non-structural components of HCV virus, particularly, HCV-core, NS3 and NS5A proteins were involved in causing all these reactive state. Moreover, HCV-genotypes showed their varying effect on overall HCV pathogenesis. The HCV core protein from HCV-genotype-3 had close association with causing hepatic steatosis, increasing oxidative stress and inducing insulin resistance in HCV infected patients. All these reactions, i.e. hepatic steatosis, oxidative stress and insulin resistance play important role in progression / regression of disease. However, many more studies are still needed to understand it in full measure for developing an effective anti-viral or anti-infection therapy.

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patients develop an acute HCV infection with resolution of disease in a shorter time period, majority of HCV infected patients run a chronic course with persistence of virus for decades and finally development of end stage liver diseases including cirrhosis and hepatocellular carcinoma (HCC). This may be better explained by a sequence of reactions involving onset of HCV and response of host immune system to counter the onset.

**Innate Immunity**

Innate immunity appears essential and first line defense for control of HCV infections like for several other viral infections. During HCV infection, cells produce Type I interferon (IFN) as a result of innate immune signaling events. The secreted IFN induce the cells to resist infection, check viral replication, promote adaptive immunity and activation of Natural Killer (NK) cells, Dendritic Cells (DC) and Kupffer cells etc. Intracellular innate immunity is triggered through host recognition of viral macromolecular motifs, known as pathogen-associated molecular patterns (PAMPs) as non-self by cellular pathogen recognition receptors (PRRs) including Toll-like receptors (TLRs) and Retinoic acid-inducible gene-I (RIG-I) like receptors (RLRs). In hepatocytes RIG-I binds PAMP on HCV-RNA and activates interferon regulatory factor-3 (IRF-3) for expression of IFN-α/β and anti-viral/ interferons stimulated genes (ISGs) that control viral infection. IFN remain major part of innate immunity and are regularly produced in response to viral infection both within infected cells and bystander cells. PAMP-RIG-I interaction is most upstream to intracellular immunity to produce ISGs and IFN. The secreted IFN and cytokines are responsible for activation of NK, DC and Kupffer cell etc. These cells plays significant role in mounting T/B cell response, including cirrhosis and hepatocellular carcinoma (HCC). This notion is also supported by the observations that interferon treatment is effective in HCV-infected persons in whom it increases NK cell activity. Surprisingly, HCV has evolved multiple strategies to evade the immune challenge. For this, the non-structural proteins of HCV i.e. NS3 and NS4A form a complex which activates NS protease domain to target cleavage of IPS-1. After cleavage, IPS-1 can no longer signal downstream to activate IRF-3 and NFκB and the infected cells no longer produce IFN-β or express ISGs. Thus HCV virions defend them from RIG-I mediated pathway.

In majority of HCV infected patients (70-80%), HCV can effectively evade innate immunity resulting in persistent viral infection. This is so because HCV has evolved to counteract the RIG-1 pathway and thus evade the immune challenge. For this, the major role of CTL is the elimination of cells infected with virus. Several viruses have evolved mechanisms to avoid recognition by CTL. They either reduce the expression of MHC molecules or prevent the viral peptide from presentation at the cell surface. CTL plays a part in viral eradication.

Cytotoxic T Lymphocytes

During viral infection, T cells recognize viral peptides presented by Major Histocompatibility Complex (MHC) molecules on infected cells. Cellular and viral molecules partly degraded by proteasomes are transported to the endoplasmic reticulum and get associated with MHC molecules which are finally transported to cell surface. These are reviewed by T cells for recognition and their action. Most cytotoxic T lymphocytes (CTL) are CD8+ and recognize antigen presented on MHC class I molecules. However, about 10% of MHC-restricted CTL are CD4+ which recognizes antigen presented on class II molecules. The major role of CTL is the elimination of cells infected with virus. Several viruses have evolved mechanisms to avoid recognition by CTL. They either reduce the expression of MHC molecules or prevent the viral peptide from presentation at the cell surface. CTL plays part in viral eradication.

The destruction of HCV-infected hepatocytes releases HCV fragments; these fragments are taken up by myeloid DCs, consequently activating the DCs. These DCs migrate to the draining lymph nodes and express HCV antigens on human leukocyte antigen (HLA) class II molecules. Then, they enhance expression of costimulatory molecules (CD80, CD86) that interact with and activate antigen-specific helper T (Th) cells. In turn, the activated Th cells promote the maturation of DCs by the expression of CD40 ligand and TNF-α.

Dendritic cells induce T-cell activation upon maturation by up-regulation of the expression of their surface molecules, with enhanced antigen presentation capacity and through the increased production of cytokines that stimulate T-cell activation. IL-12 has been shown to play an important role in stimulating IFN-γ production from activated T cells and thus, induces the development of type 1 (Th1) immune response characteristic of CTL activation.

Mature DCs stimulate specific CTLs by antigen presentation on HLA class I molecule and enhance the expression of costimulatory molecules. Cytokines such as IL-2 and IL-12 produced by Th1 cells and DCs further promote CTL activation. These CTLs infiltrate the liver and recognize HCV antigens presented on the surface of HCV-infected hepatocytes together with HLA class I molecules. Then, the effector CTLs release perforin, granzyme, and TNF-α, or express Fas ligand, and initiate a direct attack on HCV-infected hepatocytes.

Type I IFNs produced by HCV-infected hepatocytes and plasmacytoid DCs (PDC) suppress viral replication by inducing enzymes such as 2′-5′ oligoadenylate synthetase (OAS) and RNA-dependent protein kinase (PKR) in hepatocytes. The plasmacytoid DC recognizes HCV infection through toll-like receptor (TLR)-7, which interacts with single-stranded RNA. The TLR-signaling upregulates PDC-Triggering Receptor Expressed on Myeloid Cells (PDC-TREM) on the cell surface, and PDC-TREM-dependent signal induces further production of IFN-α. Activated OAS destroys viral RNAs, whereas PKR inhibits forming polymers of viral mRNA.

When appropriate CTL responses are induced in hosts, HCV eradication is achieved. However, HCV-specific CTL responses are usually not strong enough to eradicate the virus, hence contributing to persistent infection.
It has become increasingly clear that successful clearance of HCV virus during the acute HCV infection depends on the generation of a vigorous and sustained Th1 type immune response. Patients who can mount strong Th1 response showed efficient viral clearance and a self-limited course of disease. In contrast, those who showed defect in IL-12 and IFN-γ production invariably led to viral persistence and chronic hepatitis. Maturation of DC with E2 protein strongly induces IL-12 production from these cells. It is important that an overwhelming majority of the infected persons fail to control the infection and develop a chronic infection with a variable degree of hepatitis and viremia. Experimental studies have demonstrated that HCV preferentially induces the expression of antigen processing and IFN-stimulated genes in the infected livers.

Impaired function of DCs, which play the crucial role of antigen-presenting cells in inducing immunity, may be responsible for the impaired immune responses. It has been reported that the HCV core, E1, and NS3 proteins inhibit DC maturation. HCV is thought to infect DCs through the binding of HCV E2 protein and thereby suppress DC function. The virus-specific CTL kill not only virus-infected cells but also contribute to virus control by noncytolytic mechanisms by secreting cytokines, e.g., IFN-γ, IFN-α/β, and tumor necrosis factor α (TNF-α), which induce an antiviral state in host cells. This makes uninfected cells resistant to infection and frees them from virus by stopping viral replication. The progression of the majority of the infected persons to chronic infection suggests the inability of the antiviral immunity to contain this infection. There may be several reasons for this failure, including emergence of escape variants as a result of a high rate of virus mutations, a decreased production of antiviral cytokines or "stunning" of HCV-specific CTL, a compromised cytolysic potential of the CTL, and antagonistic peptides. The HCV genome in single host is a dynamic population of different but closely related genomes, designated quasispecies. Hyper variable region-1 (HVR-1) is one of the main contributors to these genetically related variants. In acute resolving hepatitis, HVR-1 shows very little variation in genetic variants, as compared to that in chronic hepatitis. HVR-1 induces anti-HCV neutralizing antibodies and HVR-1 specific CD4+ and CD8+ T cells. Using the responding host cellular immune response differentially, HVR-1 favours viral escape. HVR-1 variations result from the action of a continuous immune-driven positive selection, probably controlled by humoral immune responses. Thus, HVR-1 complexity could represent a virus adaptive strategy to escape the continuous selective process mediated by anti-HVR1 antibodies. HCV clearance is associated with a vigorous HCV specific CD4+ and CD8+ T cell response in the acute phase of infection. In contrast, viral persistence is associated with a weak and dysfunctional virus specific T cell response. Several possible mechanisms of T cell failure and HCV immune evasion have been proposed and include T cell dysfunction and the emergence of viral escape mutations.

Recently, the possible role of different regulatory T cell populations in HCV persistence has also been suggested. There are reports showing higher frequency of CD4+CD25+ regulatory T cells in the blood and CD4+FoxP3+ T cells in the liver of chronically HCV infected patients. CD4+CD25+ regulatory T cells suppress HCV specific CD8+ T cell and CD4+ T cell proliferation as well as CD8+ T cell IFN-γ secretion in a dose-dependent and unspecific manner. Treg cells secrete IL-10 and Transforming Growth Factor-β (TGF-β) after HCV antigen stimulation to show Treg cell mediated suppression of virus specific T cell responses. CD4+CD25+ T cells obtained from chronically HCV infected patients demonstrated more suppressive activity against HCV specific CD8+ T cells compared to Treg cells isolated from acute HCV infected patients. However the suppressive effect observed in patients who successfully cleared the virus was still significant. Furthermore, another study showed that the frequency of CD4+CD25+FoxP3+ T cells and their suppressive capacity against virus specific T cell responses were as high in HCV recovered chimpanzees as in persistently HCV infected chimpanzees. Induction of Treg cells by HCV antigens was demonstrated first time by a response of CD4+ T cell to HCV core protein. HCV specific IL-10 secreting T cells were detected in the blood of chronic HCV infected persons. These regulatory TR1 cells recognized the same epitopes on the core protein as IFN-γ producing TH1 cells. The regulatory CD8+ T cells may play an important role in chronic HCV infection. It is supported by the observation that HCV specific CD8+CD25+FoxP3+ T cells from blood of chronically infected patients suppress HCV specific T cell responses via transforming growth factor-β (TGF-β) secretion. The blockade of TGF-β markedly enhanced the HCV specific IFN-γ secretion by CD4+ and CD8+ T cells. The presence of Treg cells, especially in the liver, may also protect the host from tissue damage.

Another important impact of chronic HCV infection on adaptive T cell response is the exhaustion or impairment of HCV-specific CD8+ T cells antiviral function. During chronic HCV infection, CD8+ T cells show their failure to proliferate or secrete antiviral cytokines including interferon-γ (IFN-γ). This phenomenon is promoted by lack of CD4+ T cells and expression of immunomodulatory cytokines like IL-10. The major cause of HCV specific CD8+ T cells impairment is ascribed to expression of inhibitory receptor like Programmed Death-1 (PD-1), Lymphocyte-Activation Gene-3 (LAG-3, a protein related to CD4), CTLA-4 (a member of CD28 receptor family), T-cell immunoglobulin mucin-3 (TIM-3) and 2B4 etc. on HCV-specific CD8+ T cells in blood and liver. Expression of these inhibitory receptors is associated with low levels of CD127 expression and impaired proliferation and differentiation of T cells. Thus, different mechanism contribute to the dysfunction of HCV-specific CD8+ T cells in chronic HCV infection.

HCV Associated Oxidative Stress

Oxidative stress is supposed to be an important part of HCV-induced liver pathogenesis. In studies conducted to explore the role of different molecular components of HCV structure in modulating oxidative stress, it was noticed that HCV-core protein present within the outer membrane of mitochondria induce oxidation of glutathione and promotes Ca2+ uptake into mitochondria. Clement et al. explained the molecular mechanism by a schematic diagram and demonstrated that following glutathione oxidation, there is increased reactive oxygen species (ROS) production by mitochondrial electron transport complex I and II. The HCV non-structural protein NS5A promotes ROS production in the membrane of endoplasmic reticulum (ER) by activating the release of Ca2+ from ER, thereby inducing oxidative stress. NS3 protein induces ROS production by activation of NADPH oxidase.
That HCV infection causes increased ROS production and consequent oxidative stress is evident by presence of markers of increased oxidative stress in the blood. Levels of 8-hydroxy deoxyguanosine and 4-Hydroxy-2-nonenal are increased in HCV infection.71,72 Similarly, few studies have shown reduced levels of glutathione, possibly used up by antioxidant enzyme glutathione peroxidase, during HCV infection. In yet another study, the serum level of thioredoxin, marker of oxidative stress, was significantly reduced in HCV infection.73-75

Although, presence of oxidative stress has been noted in other hepatitis like hepatitis B also, however, there is a remarkable increase in Oxidative Stress (OS) in HCV infection.71 Several studies conducted at molecular level have shown that structural components of HCV induces an effective OS.71 HCV-core and non-structural components NS3 and NS5A proteins directly induce OS.76-79 Core protein is involved in OS generation via oxidation of mitochondrial GSH and uptake of Ca²⁺ into mitochondria79-80 thus, changing the permeability of its membrane.81 As a result, electron transport complex I increases production of ROS and redistributes cytochrome from mitochondria to cytosolic fraction.64 NS5A is associated with membrane of ER82 as mentioned above. NS5A, simultaneously activates even signal transducers transcription and nuclear factor κB (NFκB).83 All these activations lead to inflammation, immune response and apoptosis.83,84 Similarly, NS3 triggers ROS by activating NADPH oxidase 2 in mononuclear and polymorphonuclear phagocytes85 that increase role of apoptosis of hepatocytes.85 Thus, it is concluded that during HCV infection, the structural and non-structural components of HCV induce significant increase in OS that help in liver damage by following several mechanisms.

HCV Induced Steatosis

Steatosis, a condition with extra fat deposit in liver, is a state leading to liver injury. There are several factors responsible for causing steatosis, including alcohol consumption, obesity, diabetes, etc.86-88 Studies on steatosis in relation to hepatotropic viruses demonstrated that HCV infection directly causes steatosis in some patients. When these patients are treated with antivirals, steatosis usually disappears. Not only this, there are reports indicating reappearance of steatosis with relapse of infection after end of therapy.89 Studies in experimental animals have shown that HCV-core protein promotes steatosis in liver.90-91 Furthermore, when steatosis was studied in relation to HCV-genotypes, it was noticed that although steatosis is induced by all HCV-genotypes, it appears more prominent and frequent with HCV-genotype 3 infection.92-95 Genotype-3 shows direct involvement in accumulation of triglyceride in hepatocyte. In those patients carrying genotype-3 infection, there is a good correlation between level of steatosis and HCV replication94,96 and presence of HCV-core in liver.97 Also steatosis disappears in patient with genotype-3 when treated successfully by anti-viral therapy as compared to those with non-genotype-3 who remain steatotic.98-99 Steatosis reappears with relapse of infection,99 clearly supports that HCV-genotypes particular have more steatogenic potential. Subsequent studies100 indicated that genotype-3 interferes with VLDL secretion. Core protein, which promotes lipid accumulation in hepatocytes,90,91,101,102 proves more efficient from genotype-3 as compared core from genotype-1. Core protein inhibits microsomal triglyceride transfer protein (MTP) activity, a key protein involved in VLDL assembly, thus leading to steatosis.

Based on various reports, it was concluded that HCV causes steatosis in three different ways: (i) Impaired secretion of lipids from hepatocyte, (ii) Increased de novo synthesis of Free Fatty Acid (FFA) and (iii) Impaired FA degradation. The first aspect of HCV-induced steatosis was proposed as due to the impaired secretion of VLDL. To substantiate it, reports from different studies demonstrated decreased level of Apoprotein B (Apo B) and cholesterol in chronic HCV infected patients.103,104 Their low levels pointed towards HCV disturbing the assembly and secretion of VLDL from the liver. It was further supported by some experimental studies in transgenic mice expressing HCV core protein. These mice had impaired VLDL and Apo-B secretion104 as compared to non-transgenic mice. Another important aspect in this relation was the evidence of increased de novo synthesis of FFA under the effect of HCV infection. In this context, it is suggested that HCV upregulated the Sterol Regulatory Element Binding Protein-1c (SREBP-1c) signaling pathway105 with NS2 and NS4B proteins inducing SREBP at transcriptional level,105-106 It was also induced by expression of HCV core protein.32 Similarly, investigations on sub-cellular localization of HCV proteins in cells transacted with JFH1 RNA50 demonstrated core localized to lipid droplets (LDs). Core enhances LDs formation. These studies conducted on JFH1 also indicate genotype-2 to show its involvement in LDs formation and disturbing lipid metabolism. Few studies in chimpanzees infected with HCV also demonstrated that HCV increase activity of lipogenic enzymes like ATP citrate lyase.107 HCV-core, in particular, activates and helps in cellular lipid synthesis,107 possibly via its binding with retinoid receptor. However, there is possibility that other viral protein also help in hepatic steatosis via neolipogenesis.

The third important aspect of HCV-induced steatosis is an impaired Fatty Acid (FA) degradation by HCV. Expression of HCV-core protein is reported to reduce the expression of peroxisome proliferation activated receptor-α (PPARα), a nuclear receptor involved in FA degradation and down regulation of mitochondria β-oxidation.108 Genotype-3 shows significant down-regulation of PPARα as compared to genotype-1.109-110 It is again HCV-core protein that down regulates PPARα and so, is more effective when from genotype-3 as compared to genotype-1. Core protein from genotype-3 also down-regulated the PPARγ and upregulated suppressor of cytokine signaling-7 (SOCS-7) in Human Hepatoma cells (HuH-7).111 All these data clearly support that HCV-core protein may modulate the expression of various genes responsible for FA degradation via down regulation of PPARs.

HCV Induced Insulin Resistance (Ir)

HCV is reported to influence several metabolic pathways to increase steatosis, fibrosis, inflammation, apoptosis and insulin resistance112-114 during disease course. Insulin resistance plays an important role in liver pathogenesis by HCV infection. It has been observed that IR increases the de novo lipogenesis i.e. FA synthesis via over expression and maturation of SREBP-1c, which in turn increases the activities of lipogenic enzymes including Acetyl CoA carboxylase and FA synthase. At the same time, intermediates of triglyceride biosynthesis also activate inhibitors of insulin signaling. For example, activation of protein kinase C (PKC)-E by phosphorylating insulin receptor substrate (IRS-1) and thus inhibiting phosphatidylinositol 3,4,5 triphosphate (PIP3),115 inhibiting Akt translocation by ceramides etc.116 HCV-core protein, either by its direct interaction with insulin signaling pathway or via an increased secretion of TNF-
\( \alpha \) is considered to be causing IR.\(^{117-118}\) The HCV core can activate inhibitors of insulin signaling including mammalian target of rapamycin (mTOR)\(^{111}\) and SOCS-3 and C-Jun Nterminal kinase (JNK).\(^{119-120}\) The activation of JNK by HCV core may follow a direct or indirect proinflammatory cytokine mediated mechanism. In conclusion, HCV infection leads to IR in infected patients. IR can lead to steatosis and vice-a-versa and in either case, liver pathology is increased.

**Impact**

The overall impact of host factors including immune response, oxidative stress, steatosis and IR caused or promoted by HCV infection leads to cause liver damage in different proportion depending on their collective effect. IR promotes steatosis and reduces the response to treatment than steatosis.\(^{121}\) Though, it is not yet clear, it is assessed that it may be possibly due to deregulation of SOCS-3.\(^{122}\) Most studies support the theory that IR is an important factor to be considered in HCV infection, both for liver fibrosis and anti-viral treatment. And so, correction of IR appears to be more promising. Another important aspect coming out of all studies till date is the differential inducement of all these causative factors by different HCV-genotypes. At present, there is only preliminary information available regarding the outcome of all these conditions in relation to HCV-genotypes. Once the real impact of each disease causing condition with HCV-genotypes and its isotypes is well established, the preliminary screening for HCV-genotypes may help a lot in predicting the progression of disease and response to treatment, particularly with viewing the impact of above factors.

**Conclusion**

All above studies finally conclude that it is not any single cause of liver pathogenesis during HCV infection. In fact, host immune response to HCV related peptides and interruptions in the pathways of normal cellular metabolism by all these viral components have a collective role against virus and infected cells. Whereas host immunity is noted to be very crucial and deciding for the acute / chronic course of disease, an inducement of oxidative stress, hepatic steatosis and insulin resistance by HCV protein become the major underlying contributing factors for cellular damage. With an established fact that HCV-genotypes respond differently to anti-viral treatment, their variable role in pathogenic changes becomes an important tool for future research to design therapeutic strategies according to their pathogenic potentials. Though, these studies address some aspects of HCV pathogenesis, however, there is still a lot to unravel the total mystery for an effective anti-HCV therapeutic measure based on well defined pathogenic changes during HCV infection.

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**References**


