# Hepatitis C Virus

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Contributor: Juan Unfried

HCV leads to chronic infection in many patients that may progress to liver cirrhosis and hepatocellular carcinoma (HCC). The interferon (IFN) response is a critical component of the antiviral innate immune response against HCV infection. IFN signaling promotes the expression of many factors that can block the viral replication cycle. These IFN-induced antiviral factors can act at every level of HCV infection by decreasing viral entry, replication, transcription, translation, packaging and release. However, the antiviral state can generate significant collateral damage to the cell, requiring very tight control over the magnitude and duration of the IFN response. This is partially achieved through IFN-mediated negative self-regulation that helps in the termination of the IFN response and the return to homeostasis. However, these negative regulatory mechanisms can be hijacked by HCV to increase viral replication and promote productive infections.

Keywords: type I IFN ; HCV

## 1. Hepatitis C Virus (HCV)

HCV infection leads to the death of over half a million people every year [1][2]. Although several antiretroviral agents have been recently developed that impede viral replication and lead to viral clearance in most patients, the high prevalence of the infection (around 2% of the world population), the high number of undiagnosed patients and the slow progression to fatal symptoms, makes HCV infection a major gastrointestinal health problem [3]. After viral entry, HCV produces an acute infection that can be cleared spontaneously in some patients (15-50% of cases) or it may progress to a chronic infection in others (55-85%) [4]. Most chronically infected patients respond to the infection with sustained liver inflammation and liver injury that may cause liver fibrosis, cirrhosis, and, in some cases, hepatocellular carcinoma (HCC) [5]. These are the primary causes of death after HCV infection.

A careful description of the viral particle and the mechanisms that allow viral replication can be found in any of the several excellent reviews about HCV published recently [6]. Briefly, HCV belongs to the Flaviviridae family of positive, single-stranded, enveloped RNA viruses. The viral particle is small in size (40-80 nm) and circulates in the blood bound to lipoproteins and lipid particles, which can help viral evasion from the immune system and efficient infection of target hepatocytes [7]. At the hepatocyte surface, HCV binds to different receptors, including LDLR (low-density lipoprotein receptor) and CLDN1 (Claudin-1), and is transported to the cytoplasm by endocytosis [8]. Once in the endosome, the low pH helps viral uncoating, and the viral genome is free to bind to the endoplasmic reticulum, where the formation of a membranous web helps viral replication [9]. Prior to replication, the viral genome is translated from a 5´ internal ribosome entry site (IRES) by cap-independent translation [10]. Translation produces a polyprotein that is cleaved into three structural proteins (core protein and E1 and E2 glycoproteins) and seven non-structural proteins (p7, NS2, NS3, NS4A, NS4B, NS5A, and NS5B) that are required for polyprotein processing, viral replication, packaging and release, and for blocking the cellular antiviral response.

## 2. HCV and the Antiviral Response

Efficient HCV replication requires that viral proteins block type I IFN (interferon) synthesis and signaling pathways. These are key routes of the type I IFN response, one of the primary innate immune weapons against microbe infection. IFNs have been classified in different groups according to the cellular receptor that they bind: type I (which includes IFNα and β), type II (IFNγ), and type III (IFNλ). However, there is a significant overlap in the genes that are induced after activation of the different IFNs [11]. A systematic description of the IFN response can be found in any of the outstanding reviews published recently [12][13]. In this excerpt, we describe the relationship between HCV and type I IFN.

The synthesis of type I IFN is the result of the activation of cellular sensors by PAMPs (pathogen-associated molecular patterns) such as DNA, RNA, or LPS (lipopolysaccharide). In the case of HCV, viral RNA is sensed shortly after infection by the canonical sensor RIG-I (retinoic acid-inducible gene I, which binds to the viral genome), by the non-canonical protein kinase R (PKR, which recognizes the viral 5´UTR) or by the DEAD-box helicase 3 X-linked (DDX3X, activated by

the viral 3´UTR) [14][15][16][17]. RIG-I and the canonical sensor TLR3 (toll-like receptor 3) can also be activated by the viral dsRNA (double-stranded RNA) produced during replication [18][19]. In addition, it has been recently shown that the canonical sensor MDA5 (melanoma differentiation-associated protein 5) can also bind viral RNA and activate IFN [20]. Activated sensors can bind MAVS (mitochondrial antiviral signaling protein) and TRIF (Toll-Interleukin Receptor-domain-containing adapter-inducing interferon-β), which trigger the induction of transcription factors NF-κB (nuclear factor κb) and IRF (interferon regulatory factors), in charge of activating IFN synthesis.

IFN signaling induces the expression of numerous genes with potent antiviral functions at different levels. Secreted type I IFN binds to IFNAR (IFN-α/β receptor), activating JAK/STAT (Janus kinase/signal transducers and activators of transcription) and phosphorylation, dimerization, and nuclear translocation of STAT1 and STAT2 (signal transducer and activator of transcription 1 and 2). Once in the nucleus, STAT1/STAT2 are bound by IRF9 to constitute the ISGF3 (IFN-stimulated gene factor 3) complex, in charge of activating the transcription of the vast ISG (IFN-stimulated genes) repertoire [21][22]. Several ISGs have been shown to decrease HCV replication by affecting viral infection (Mx, TRIM, IFITM, CH25H), viral RNA translation, replication, stability (OAS, IFIT, GBP1), or virus packaging and release (tetherin/BST2, viperin) [23][24]. These effects are reinforced by ISGs that contribute to IFN synthesis or signaling. Indeed, expression of PKR, STAT1, STAT2, or IRFs is also induced by IFN, resulting in a positive loop that amplifies the IFN response. Interestingly, several ISGs inhibit the IFN response to allow the cell to return to homeostasis [25][26]. One of them is SOCS3 (suppressor of cytokine signaling 3) which blocks JAK activity and STAT binding. Therefore, these inhibitory ISGs can be considered as actual proviral factors induced by IFN.

Activation of some of these inhibitory ISGs is one of the mechanisms employed by HCV to counteract the IFN pathway. Viral core protein induces SOCS3 and PP2A (Protein Phosphatase 2), which also blocks STAT1 function [27][28][29]. In addition, several HCV proteins have evolved to block the function of specific antiviral ISGs or to block IFN synthesis: MAVS and TRIF are cleaved by the viral NS3-NS4A protease, and RIG-I pathway is blocked by HCV-mediated induction of autophagy [30][31][32]. Despite this and against initial expectations, many patients with active HCV infections have high levels of ISG mRNAs [33]. One possibility to explain this observation is that ISG mRNAs are not efficiently translated in HCV-infected cells due to PKR activation. Although PKR activation by the viral genome may seem to work against the HCV cell cycle, it is an excellent weapon to increase viral replication. PKR activates the IFN synthesis pathway but also phosphorylates eIF2α (eukaryotic translation initiation factor 2 alpha) leading to inhibition of cap-dependent translation. Since viral translation is cap-independent, it is not affected by PKR action [34]. Instead, translation of newly transcribed ISG mRNAs will be abrogated. Similar to PKR, two other ISGs with a proviral function in HCV infection are ISG15 and DDX3X, whose activation results in increased levels of lipogenic genes required for viral packaging [17][35][36]. ISG15 is particularly interesting. ISG15 is required for protein ISGylation, an IFN-induced ubiquitin-like process that serves to modify newly-translated proteins co-translationally [35]. After infection, high levels of viral proteins need to be translated, and viral protein ISGylation should alter viral protein stability or structure, and its function [37]. However, ISG translation is also mandatory in newly infected cells. In fact, RIG-I ISGylation affects its functionality and leads to reduced IFN response [35][38]. Therefore, the battle between viral replication and the antiviral response causes severe collateral damage over cellular and viral proteins, as ISGylation compromises their stability and function, and PKR blocks their translation. Under such circumstances, it is plausible that cellular and viral evolution has fostered the development of non-coding RNAs that are resistant to such protein-hostile environments and could function to modulate viral replication and the antiviral response [39]

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