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Title:

Hepatitis C Virus NS5B polymerase primes innate immune signaling

Yu G, He G, Li C, Tang M, Grivennikov S, Tsai W, et al. Hepatic expression of HCV RNA-dependent RNA polymerase triggers innate immune signaling and cytokine production. *Molecular Cell* 2012 in press (Reprinted with permission.)

Abstract

Innate immunity controls pathogen replication and spread. Yet, certain pathogens, such as Hepatitis C Virus (HCV), escape immune elimination and establish persistent infections that promote chronic inflammation and related diseases. Whereas HCV regulatory proteins that attenuate antiviral responses are known, those that promote inflammation and liver injury remain to be identified. Here, we show that transient expression of HCV RNA-dependent RNA polymerase (RdRp), NS5B, in mouse liver and human hepatocytes results in production of small RNA species that activate innate immune signaling via TBK1-IRF3 and NF- κ B and induce cytokine production, including type I interferons (IFN) and IL-6. NS5B-expression also results in liver damage.

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Comment

Chronic hepatitis C virus (HCV) infection is one of the leading causes of liver disease worldwide and results in liver fibrosis, cirrhosis and hepatocellular carcinoma. Understanding the underlying mechanisms of HCV induced liver injury is critical for tailoring optimal therapies with minimal adverse effects. Cytotoxicity in chronic HCV is mediated on the one hand by direct effects of the virus on the cell and on the other hand by host inflammatory responses.

For instance endoplasmic reticulum (ER) and oxidative stress upon virus infection directly damage infected cells, cause hepatocyte apoptosis and trigger liver inflammation (1). Similarly, HCV-dependent changes in cellular metabolic pathways, most notably lipid metabolism, contribute to the natural course of chronic hepatitis C (2).

A second factor in HCV-triggered liver injury is the host immune response, which gives rise to chronic inflammation. In infected hepatocytes replication of HCV plus strand RNA genome generates double stranded RNA intermediates with 5' tri-phosphate motifs. These motifs as well as the poly-uridin motif within the 3' non-translated region of the HCV genome are typical non-self molecular patterns (3, 4) which are sensed by so called cellular pattern recognition receptors (PRRs). These PRRs include cellular proteins as the Toll-like receptors (TLRs), the retinoic acid inducible gene I-like (RIG-I), the nucleotide oligomerization domain-like (NOD) and the C-type lectin receptors. In case of HCV particularly the cytosolic RIG-I, senses these viral RNA species and

induces signaling, which culminates in the production of interferon β (IFN- β). In turn, secreted IFN- β binds to the type I interferon receptor, which elicits Janus kinase and signal transducer and activator of transcription (JAK/STAT) signaling to induce expression of several hundred interferon-stimulated genes (ISGs). These ISGs include pro-inflammatory cytokines, MHC genes as well as effector proteins, which establish an intracellular antiviral state and shape innate and adaptive immune responses (5).

Clearly, in most HCV patients these immune responses are not sufficient to eliminate the virus during the acute and later stages of infection. At least in part this seems to be due to viral immune evasion strategies. For instance, HCV counteracts innate immune sensing by cleavage and inactivation of the RIG-I and TLR-3 adaptors mitochondrial antiviral signaling protein (MAVS, also known as IPS-1 or Cardif) and TIR-domain-containing adapter-inducing IFN- β (TRIF), respectively, through its NS3-4A protease (6, 7). It is noteworthy that viral interference appears to be incomplete, since HCV induces considerable levels of IFN- β and ISGs in acutely infected chimpanzees and humans (8, 9). The balance between innate immune sensing and viral countermeasures is thought to shape the character and degree of the initial antiviral immune response and consequently liver damage.

While the interference of viral NS3-4A protease with immune signaling is well established it remained largely unexplored if viral proteins are directly involved in eliciting liver inflammation. Here we comment on an exciting article that highlights a novel facet of how HCV triggers innate immunity. Yu et al. have undertaken a conclusive set of experiments indicating that the HCV RNA dependent RNA polymerase (RdRp) NS5B uses cellular RNA templates to produce small dsRNAs. These activate signaling through TANK-binding kinase 1 (TBK1), interferon regulatory factor-3 (IRF-3) and nuclear factor kappa-light-chain-enhancer of activated B cells (NF- κ B) leading to the secretion of proinflammatory cytokines including IFN- β and interleukin 6. Strikingly, expression of NS5B in mice causes liver damage suggesting that this feature of NS5B may contribute to liver inflammation and tissue damage in chronic HCV patients (10).

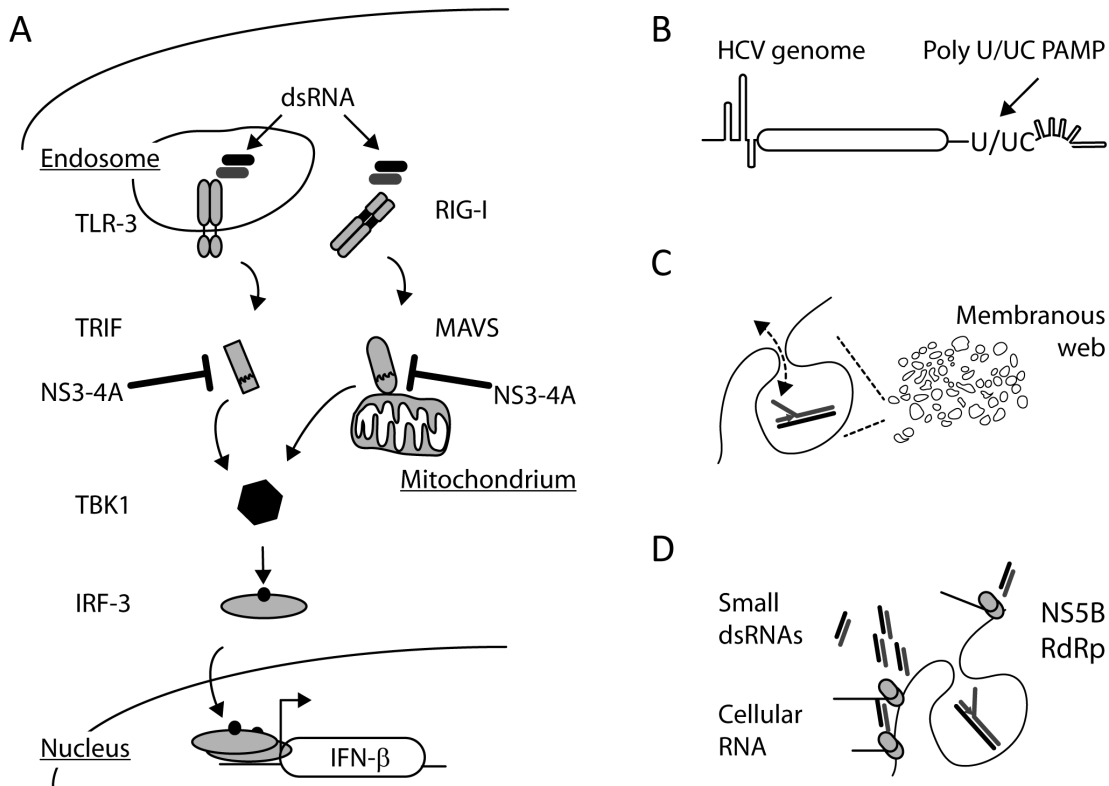


Figure 1: Generation and signaling of double stranded RNA (dsRNA) in HCV infected cells. (A) Simplified signaling cascade resulting in production of interferon β (IFN- β). Double stranded RNA is recognized by endosomal and cytoplasmic pattern recognition receptors Toll-like receptor 3 (TLR-3) and retinoic acid inducible gene I (RIG-I), respectively. The signal is relayed via TIR-domain-containing adapter-inducing interferon- β (TRIF) and mitochondrial antiviral signaling protein (MAVS, also known as IPS-1 or Cardif) adaptor proteins to the TANK-binding kinase 1 (TBK1). Interferon regulatory factor-3 (IFR-3) is phosphorylated, translocates to the nucleus and induces the IFN- β promoter. Note that both TRIF and MAVS are targets of the NS3-4A protease. (B-C) Viral molecular patterns like the poly U/UC tract of HCV (4), double stranded genome stretches and/or replication intermediates trigger cellular pattern recognition receptors. (D) The NS5B RNA-dependent RNA polymerase (RdRp) utilizes cellular RNA templates to produce small dsRNA molecules which trigger MAVS and TBK1-dependent IFN- β secretion (Yu et al.).

Previously, two independent groups had shown that ectopic expression of HCV NS5B in immortalized human liver cells or 293T cells induces IFN- β secretion via TLR-3 and RIG-I (11, 12). However, the detailed mechanism and the relevance for HCV pathogenesis remained elusive. Yu et al. now expressed a HCV subgenomic replicon consisting of 5'- and 3'-non-translated regions and the coding region of NS3 to NS5B in mouse livers using hydrodynamic delivery and observed elevated levels of IFN- β and IL-6 in liver and serum, respectively. Using adenoviral delivery of HCV non-structural proteins, they showed that enzymatically active NS5B was necessary and sufficient for cytokine production. Moreover, NS5B expression induced elevated alanine aminotransferase (ALT) levels in mice indicating liver damage. The authors extended their findings of NS5B-induced IFN- β and IL-6 production to primary mouse hepatocytes,

immortalized human hepatocytes and HCVcc infected Huh-7 cells. Cytokine production in mouse hepatocytes was dependent on MAVS, TBK1 and IRF-3. Collectively, the authors established that catalytically active NS5B when expressed in murine or human liver cells produced short dsRNA fragments from host template RNA. These dsRNAs were capable of triggering RIG-I signaling and cytokine secretion and caused liver damage in mice.

Rapid initiation of innate immunity triggered by virus sensing is crucial for protective immunity. HCV, in turn, antagonizes innate immune sensing through the proteolytic activity of NS3-4A. In addition, HCV induced membrane alterations, generally termed as the membranous web, likely not only serve as membrane scaffold for optimal genome replication but also to hide double stranded replication intermediates from surveillance by cytosolic pattern recognition receptors (13). Both mechanisms could explain why Yu et al. only observed 2- to 4-fold increases in cytokine expression in mice using HCV replicons containing NS3-4A, while delivery of NS5B alone resulted in a 10- to 20-fold IFN mRNA induction.

Yu et al. showed that the viral RdRp produces small dsRNA molecules even in the absence of viral genome template. Notably, NS5B catalyzes RNA synthesis in the absence of a specific primer and (at least *in vitro*) without template selectivity (14, 15). Although NS5B is localized in membrane protected HCV replication complexes, it is conceivable that host templates are also amplified in infected cells. In fact, in replicon cells, a more than 1000-fold excess of NS5B over viral RNA was noted and less than 5% of NS5B molecules were actively engaged in genome synthesis and protected from proteolytic digestion (i.e. within the membrane enclosed replication complex) (16). Still, in the context of full length virus infection it remains to be shown whether the stoichiometry of NS5B and viral versus cellular RNA templates as well as the localization of polymerase and template favors a role for cellular dsRNA in activating the RIG-I pathway.

If a similar situation applies *in vivo*, the study of Yu et al. raises several questions. Why does HCV produce an excess of NS5B with its potential danger of synthesizing immune activating molecules? Could the cellular dsRNAs have a functional role for the virus? The authors sequenced small RNAs from NS5B expressing mouse livers and observed a bias towards non-coding RNAs. Possibly, this might be a mechanism by which HCV increases the abundance of regulatory RNAs. Alternatively, host dsRNAs could be an unwanted side product. In this context, differential activities of NS5B from diverse HCV strains, as seen for J6 and JFH-1 (17), might translate into differential production of dsRNA molecules, inflammation, and liver damage. Intriguingly, in the light of clinical applications of NS5B inhibitors the findings suggest that polymerase inhibition not only has anti-viral effects but may also reduce the risk of tissue damage. In the era of directly acting antivirals the study thus highlights that targeting viral proteins may have beneficial effects beyond mere restriction of virus propagation.

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