Innate Immunity in Hepatitis C Virus Infection

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Activation and viral control of the innate immune response are hallmarks of hepatitis C virus (HCV) infection and are major determinants of spontaneous clearance or progression to chronic infection and liver disease. In this review, we provide a contemporary overview of how HCV is sensed by the host cell to trigger innate immune activation and the mechanisms deployed by the virus to evade this response. Type I and III interferons (IFNs) are crucial mediators of antiviral innate immunity against HCV, and we specifically highlight the importance of IFN- λ host genetics for the outcome of HCV infection. Last, we focus on the proinflammatory responses elicited by HCV infection and describe our current understanding of how interleukin (IL)-1 β signaling and cross talk between the IL-1 β and IFN signaling pathways lead to sustained inflammation and increased risk of liver pathology.

Hepatitis C virus (HCV) typically mediates persistent infection unless fully interrupted by direct-acting antiviral (DAA) therapy (Smolders et al. 2019). Viral persistence is associated with chronic inflammatory liver disease such as fibrosis and liver cancer (Wang et al. 2020). Innate immune regulation and the hepatic inflammatory responses are major features of persistent HCV infection that underlie chronic hepatitis and liver disease. In this review, we provide a contemporary and comprehensive overview of these processes in the pathophysiology of HCV infection.

HEPATIC INNATE IMMUNITY

The innate immune response represents our first line of defense against microbial infection.

During a virus infection, innate immunity is triggered within the infected cell when specific pathogen recognition receptors (PRRs) of the host cell recognize and bind to non-self-macromolecular products of an invading virus termed pathogen-associated molecular patterns (PAMPs). Viral nucleic acid represents a major PAMP of non-self-recognition by PRRs including retinoic acid-inducible gene-I (RIG-I)-like receptors (RLRs) and Toll-like receptors (TLRs), as well as other PRRs that drive innate immunity and inflammation (Chow et al. 2018; Streicher and Jouvenet 2019). In particular, the RLR pathway plays a prominent role in sensing of HCV through the actions of RIG-I. RIG-I is the charter member of the RLR family of proteins that includes RIG-I, melanoma differentiation antigen 5 (MDA5), and Laboratory of Ge-

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netics and Physiology 2 (LGP2). The RLRs are cytosolic RNA helicases (Yoneyama et al. 2004; Chow et al. 2018). RIG-I and MDA5 harbor tandem caspase activation and recruitment domains (CARDs) that facilitate signaling through CARD–CARD interaction with the signaling adaptor protein, mitochondrial antiviral-signaling protein (MAVS) (Loo et al. 2006). LGP2 lacks CARDs and is thought to function as a regulator of the RLR signaling pathway (Yo-

neyama et al. 2004). During acute HCV infection, RIG-I recognizes viral RNA as non-self, serving to trigger downstream signaling through MAVS leading to innate immune activation (Fig. 1).

THE RLR PATHWAY

RIG-I mediates the recognition of RNA that has free and exposed 5' triphosphate with double-

Figure 1. Sensing and innate immune response to hepatitis C virus (HCV). During acute HCV infection in the hepatocyte, the cytosolic RIG-I-like receptor (RLR), retinoic acid-inducible gene-I (RIG-I), recognizes 5'-triphosphate double-stranded RNA (dsRNA) motifs and/or polyuridine (poly(U)) motifs within HCV RNA. Upon pathogen-associated molecular patterns (PAMPs) binding, RIG-I translocates to the mitochondria-associated membranes (MAMs) where it interacts with the adaptor molecule mitochondrial antiviral-signaling protein (MAVS). Interaction of RIG-I with MAVS at the MAM signaling platform recruits and activates the downstream transcription factors IRF-3 and NF-κB, resulting in the induction of antiviral and immune modulatory type I (interferon [IFN]-α/β) and III (IFN-λ) IFNs. Secreted IFNs bind their cognate receptors IFN-αR and IFN-λR on the cell surface. This autocrine and paracrine IFN signaling engages the JAK/STAT signaling cascade downstream of the IFN receptor and mediates activation and nuclear translocation of the trimeric ISGF3 transcription factor complex, which binds to IFN-stimulated response elements (ISREs) in the promoter regions and induces expression of hundreds of IFN-stimulated genes (ISGs) to establish an antiviral state. The viral NS3/4A protease accumulates in the infected cell and targets and cleaves MAVS, releasing it from the MAM and thus inactivating the RLR pathway. This process allows HCV to evade intracellular innate immunity to mediate persistent viral replication and chronic infection.

stranded RNA (dsRNA) and/or polyuridine (poly(U))-rich motifs. Together, these features of HCV RNA mark it as non-self and facilitate recognition and direct binding by RIG-I to the viral RNA (Foy et al. 2005; Sumpter et al. 2005). RIG-I specifically recognizes the 5'ppp of the viral genome RNA in combination with the 3' untranslated region (UTR) poly(U)-rich motif (Saito et al. 2008). During viral RNA replication dsRNA structures within the 5' end of the HCV genome mediate a "kissing loop" interaction with dsRNA structures in the 3' end of the genome, which brings the 5'ppp into close proximity to the poly(U) motif, comprising the PAMP that is recognized by RIG-I (Friebe et al. 2005; Schnell et al. 2012). Upon PAMP binding, RIG-I hydrolyzes ATP and undergoes a major conformation change that places it in a "signaling-on" conformation, thus allowing RIG-I to mediate cofactor interactions and formation of a RIG-I-translocon that chaperones RIG-I interaction with MAVS on mitochondria-associated membranes (MAMs). The MAM acts as an innate immune signaling platform that allows signaling molecules interaction that directs the downstream activation of interferon (IFN) regulatory factor-3 (IRF-3) and NF- κ B. IRF-3 induces the expression of many target genes including antiviral and immune modulatory genes, whereas NF- κ B directs the expression of a variety of inflammatory mediators including interleukin 1 β (IL-1 β), as discussed below. IRF-3 and NF- κ B together participate in the induction of type I and III IFN expression (described below). IFNs are major antiviral factors secreted from the infected cell to signal locally and systemically through binding the cognate IFN receptor and driving signaling that induces hundreds of IFN-stimulated genes (ISGs). ISG products have antiviral, inflammatory, and immunomodulatory functions that together restrict virus replication and spread and serve to polarize the adaptive immune response for effector actions against infection (Schoggins 2019). Problematically, HCV targets and inactivates the RLR pathway early during acute infection, thus evading intracellular hepatic innate immune defenses.

INTRACELLULAR INNATE IMMUNE EVASION BY HCV

HCV replication is mediated through the actions of the viral nonstructural (NS) proteins and host cofactors (Tabata et al. 2020). The viral NS proteins include the NS3/4A serine protease that imparts processing of the viral polyprotein for liberation of NS proteins to establish the viral replicase (Bartenschlager 1999). Importantly, NS3/4A also is shown to target and cleave MAVS in vitro in HCV infection models and in vivo in the liver of chronically HCV-infected patients (Levitzki et al. 1991). NS3/4A cleavage of MAVS releases it from intracellular membranes. MAVS must be anchored on the MAM or outer mitochondria membrane for RLR signaling to occur. Thus, once MAVS is cleaved by the HCV NS3/4A protease, the RLR pathway is rendered inactive within the infected cell, allowing HCV to replicate and spread through the liver (Lau et al. 2008). HCV mediates cell-to-cell spread through tight junction molecules that serve as receptors for the virus (Zeisel et al. 2018; Gerold et al. 2020). When initiating infection in neighboring hepatocytes, the RLR pathway is again activated via RIG-I sensing of HCV PAMP, leading to IFN expression and secretion. As HCV continuously spreads from cell to cell, this process drives de novo IFN production and ISG expression, placing the liver in a continuous stage of innate immune stimulation and activation (Wilkins et al. 2013). Although RLR signaling is then effectively shut off in the infected cell from NS3/4A-mediated cleavage of MAVS, the spread of HCV through the liver continues to drive this state of hepatic innate immune activation. HCV has also been shown to be sensed by TLR3 at least in vitro wherein TLR3 signaling can drive innate immune activation. In vitro studies show that the viral NS3/4A protease can target and cleave the cellular TRIF protein, an essential cofactor of TLR3 signaling (Li et al. 2005; Wang et al. 2009). This process abrogates the TLR3 pathway concomitant with disruption of RLR signaling by the NS3/4A protease (Ferreon et al. 2005). However, the relevance of TLR3 regulation by HCV has not been demonstrated

J. Schwerk et al.

in vivo, and it is not known whether TLR3 actually senses HCV PAMPs to direct innate immune activation in the infected human liver.

INTERFERONS IN HCV INFECTION

The continuous hepatic innate immune activation during HCV infection plays an important role in recruiting immune cells to the liver, including myeloid cells and lymphocytes (Lau et al. 2013). Dendritic cell (DC) recruitment to the HCV liver provides a source of IFN wherein HCV RNA can be encountered by infiltrating DCs through exosomes containing the HCV RNA that are shed by infected hepatocytes (Dreux et al. 2012). This process results in DC activation and the production and secretion of IFN into the hepatic environment (Longatti et al. 2015). Moreover, the IFN production driven by acute RLR signaling serves to activate resident hepatic cells including stellate cells, liver macrophages, or Kupffer cells (KCs), endothelial cells and others (Lin et al. 2014). Indeed, transcriptional profiling studies of liver gene expression in HCV patients show a remarkable and complex gene expression signature marked by ISGs, inflammatory genes, immune regulatory genes, and metabolic genes that underlie the development of tissue fibrosis and liver disease (Helbig et al. 2005). ISGs often dominate the gene expression signature of the chronic HCV liver owing to the ongoing production of IFN as the virus triggers RIG-I signaling during the spread of infection and from the actions of infiltrating myeloid cells that produce IFN (Lau et al. 2008, 2013). Both type I (IFN- α/β) and type III (IFN- λ) IFNs are produced by these cells in HCV-infected liver. IFNs act to bind to their respective cognate receptors—IFN- α R and IFN- λ R—on the cell surface. Engagement of the receptors by IFN activates the JAK/STAT signaling cascade downstream of the receptor, which results in formation and nuclear translocation of the heterotrimeric transcription factor complex ISGF3 consisting of STAT1, STAT2, and IRF-9. ISGF3 binds to promoter regions of numerous ISGs (Fig. 1). Only a handful of ISGs have been functionally described as antiviral effector molecules, such as *IRF1*, *OAS1*, *IFITM1*,

IFI44L, *OASL*, and *RNaseL*, some of which act directly as antiviral against HCV and other viruses (Schoggins et al. 2011; Metz et al. 2013). For years, the standard of care for all HCV patients prior to the introduction of DAA, which are small-molecule antiviral drugs against HCV, was a combination therapy of pegylated IFN- α with ribavirin.

SIMILARITIES AND DISTINCT ANTIVIRAL PROPERTIES OF TYPES I AND III INTERFERONS

Although type I IFNs were first described more than six decades ago (Isaacs and Lindenmann 1957), it was not until 2003 that the family of type III IFNs was discovered (Kotenko et al. 2003; Sheppard et al. 2003). One of the major differences between the two IFN families is the tissue restriction of their cognate receptors. The heterodimeric IFN- α R, consisting of the IFN- α R1 and IFN- α R2 receptor subunits, is ubiquitously expressed. In contrast, expression of the IFN- λ R, composed of the IFN- λ R1 and IL-10R2 subunits, is restricted to cells of the mucosal epithelium and hepatocytes to provide antiviral protection at these sites (Sommereyns et al. 2008). Recent studies have shown that certain immune cells such as neutrophils and DCs are responsive to IFN- λ (Broggi et al. 2017; Hemann et al. 2019).

Type I and III IFNs induce the expression of a mostly overlapping set of ISGs (Zhou et al. 2007; Ank et al. 2008). Nevertheless, differences in gene induction patterns between the two IFN families lead to distinct functional consequences (Marcello et al. 2006; Jilg et al. 2014; Hong et al. 2016; Forero et al. 2019). The temporal pattern of ISG expression upon type III IFN sensing is delayed and sustained, whereas type I IFN elicits an early but transient ISG induction (Fig. 2; Marcello et al. 2006; Jilg et al. 2014; Forero et al. 2019). An important distinction between the two IFN families is that IFN- λ does not promote inflammatory responses that type I IFN evokes at epithelial barrier surfaces (Davidson et al. 2016; Galani et al. 2017). This distinct response is attributed to the decreased induction of the transcription factor IRF-1 through limited

Figure 2. Differential interferon (IFN)-stimulated gene (ISG) expression kinetics of type I and type III IFN. Type I (IFN- α/β) and type III (IFN- λ) IFNs confer differential ISG expression kinetics in epithelial cells upon engagement of their respective receptors. The response induced by type I IFN is immediate and short-lived. In contrast, type III IFN-induced ISG expression is delayed, but sustained. This division of labor between type I and type III IFN results in optimal establishment of an antiviral state.

formation of STAT1 homodimers by IFN- λ and a simultaneously increased induction of factors associated with tissue repair (Fig. 3; Forero et al. 2019). These unique properties of IFN- λ have long been considered a major advantage over pegylated IFN- α , which is routinely used in combination with ribavirin in the clinics to treat chronic HCV infection, as it would act on a more restricted pool of cell types (including hepatocytes) and thereby reduce the adverse side effects caused by pegylated IFN- α treatment. The potential of IFN- λ as a far more efficient alternative for treatment of chronic HCV infection over type I IFN therapy has been further highlighted by the advent of genome-wide association studies showing strong correlation between single-nucleotide polymorphisms (SNPs) in the *IFNL* locus and outcome of HCV infection (Ge et al. 2009; Suppiah et al. 2009; Tanaka et al. 2009; Thomas et al. 2009; Rauch et al. 2010).

IMPORTANCE OF INTERFERON- λ GENETICS IN HCV INFECTION

Soon after its initial discovery, the antiviral potential of type III IFN was explored and showed similar potential as type I IFN (Dumoutier et al. 2004; Ank et al. 2006). Several studies have

shown that SNPs in the *IFNL* locus strongly associate with spontaneous and type I IFN therapy-induced HCV clearance. Several independent HCV infection cohorts have replicated the genetic associations with *IFNL* SNPs (Ge et al. 2009; Suppiah et al. 2009; Tanaka et al. 2009; Thomas et al. 2009; Rauch et al. 2010; Prokunina-Olsson et al. 2013). Although the importance of individual *IFNL* SNPs as predictors of HCV susceptibility and clearance of infection is contentious, there is a uniform consensus that SNPs in the *IFNL* locus are the best predictors for outcome of HCV infection. Because associations of *IFNL* SNPs with HCV susceptibility are strong, many groups including ours, have been in the hunt to identify the functional SNP that affects HCV clearance.

Three SNPs in the *IFNL* locus seem to exhibit features of functionality (Fig. 4). The most obvious candidate SNP (rs368234815) in the *IFNL4* gene is a dinucleotide variant that confers the protein-coding potential of *IFNL4* (Bibert et al. 2013; Prokunina-Olsson et al. 2013; Aka et al. 2014). Interestingly, contrary to the conventional protective antiviral function of IFNs, the SNP variant predicting to encode functional IFN- $\lambda 4$ protein, associates with increased risk of HCV persistence, whereas

Figure 3. Type I, but not type III interferon (IFN), promotes inflammatory responses at the site of infection. The proinflammatory gene signature induced by type I IFN (IFN- α/β) is caused by increased induction of *IRF1* through type I IFN-mediated STAT1 homodimer formation. In contrast, lower abundance of surface IFN- λ R expression leads to insufficient activation of STAT1 homodimer formation required for induction of *IRF1* and downstream inflammatory genes.

patients with the noncoding *IFNL4* SNP variant clear the virus (Aka et al. 2014; Meissner et al. 2014; Lu et al. 2015a,b; O'Brien et al. 2015; Peiffer et al. 2016). Another SNP in *IFNL4* (rs117648444) causes a P70S substitution that leads to decreased activity of IFN- λ 4 protein and correlates with increased clearance of HCV infection in patients (Prokunina-Olsson et al. 2013; Terczynska-Dyla et al. 2014). This puzzling observation, combined with the inability to detect IFN- λ 4 protein in liver biopsies of

HCV patients, complicates the functional significance of *IFNL4* in HCV infection and clearance. Moreover, as IFNs are known to clear HCV infection, the paradoxical activity of *IFNL4* is in stark contrast to biological studies showing that recombinant IFN- λ 4 protein has a similar antiviral activity to IFN- λ 3 (Hamming et al. 2013; Hong et al. 2016). However, compared to *IFNB* and *IFNL1-3* mRNAs, the induction of full-length *IFNL4* mRNA is barely detectable. Furthermore, unlike other *IFNL* genes, *IFNL4* is

Figure 4. Functional single-nucleotide polymorphisms (SNPs) in the human *IFNL* locus. Several SNPs in the human *IFNL* locus have been shown to be functional. The dinucleotide variant rs368234815 in the *IFNL4* gene confers protein-coding potential and associates with decreased clearance of hepatitis C virus (HCV) infection. The P70S substitution (rs117648444) leads to decreased activity of recombinant interferon (IFN)- λ 4, which associates with improved HCV infection outcome. The SNP rs4803217 located in the 3' untranslated region (UTR) of the *IFNL3* gene affects stability of *IFNL3* mRNA through AU-rich elements (AREs) and microRNA (miRNA) binding sites.

alternatively spliced to produce several nonfunctional mRNA isoforms and intron-retained isoforms that probably undergo nonsense-mediated decay (Hong et al. 2016). Finally, *IFNL4* mRNA is not efficiently loaded onto polyribosomes for translation, because of inefficient polyadenylation of the mRNA (Hong et al. 2016). Based on these observations, one hypothesis is that the host has adopted multiple measures to suppress IFN- λ 4 protein expression. Our study provides mechanistic evidence that humans do not express functional IFN- λ 4 protein irrespective of the *IFNL4* SNP genotype, suggesting that the antiviral activity against HCV is mediated through IFN- λ 1-3 and type I IFN. Because the *IFNL4* genotype predicted to produce functional IFN- λ 4 protein is in strong linkage with the less favorable *IFNL3* genotypes at rs12979860 and rs4803217 (Lu et al. 2015b), it is possible that the association of *IFNL4* genotype with HCV persistence is based on an indirect effect of less favorable *IFNL3* genotypes. Nevertheless, the SNPs in *IFNL4* are strong predictors for HCV clearance or persistence in patients and further research efforts are required to identify the functional mechanism(s) behind *IFNL4* SNP genotype and outcome of HCV infection (Prokunina-Olsson et al. 2013).

The other SNP in the *IFNL* locus that was predicted to have a functional consequence on HCV infection is in the 3' UTR of *IFNL3* mRNA (Fig. 4). We found that the SNP rs4803217 influenced the stability and turnover of *IFNL3* mRNA through AU-rich elements (AREs) and microRNA (miRNA) binding sites encoded in the *IFNL3* 3' UTR (McFarland et al. 2014). HCV-induced miRNA expression such as miR-208b and miR-499a-5p target miRNA

binding sites in the *IFNL3* 3' UTR, thereby mediating instability through ARE-mediated decay. As the SNP rs4803217 lies in the miRNA seed region, the HCV-induced miRNAs target and repress the risk allele of *IFNL3* (T/T) but not the protective allele (G/G). Specific inhibition of HCV-induced miR-208b and miR-499a-5p led to a significant decrease of HCV replication (McFarland et al. 2014). Furthermore, HCV-induced type I IFN amplifies miR-208b and miR-499a-5p expression in hepatocytes. In patients with the unfavorable *IFNL3* (T/T) genotype, this would further suppress IFN- λ 3 protein expression, making this genotype particularly unfavorable during type I IFN therapy. By contrast, patients with the favorable *IFNL3* (G/G) genotype would escape this posttranscriptional regulation and have increased expression of IFN- λ 3. Additionally, HCV-induced miRNAs miR-208b and miR-499a-5p also negatively regulate expression of *IFNAR1* mRNA of the type I IFN receptor subunit, thereby dampening type I IFN signaling in response to HCV infection (Jarret et al. 2016). The fact that treatment of HCV-infected hepatocytes with recombinant type I IFN led to increased expression of miR-208b and miR-499a-5p than just HCV infection alone, explains why chronic HCV patients with the unfavorable *IFNL3* (T/T) genotype respond poorly to type I IFN therapy.

HCV-INDUCED LIVER INFLAMMATION

Although recent advances in drug discovery and development have shifted the standard of care for HCV patients to DAAs with much success, future setbacks can be expected because of mutations in HCV and emergence of DAA

resistance variants. Thus, a more detailed understanding of the inherent innate immune responses and the molecular mechanisms underlying the effect of host genetic polymorphisms to outcomes of HCV infection are essential to tailor adequate treatment strategies in the future. DAA therapy is instrumental to HCV cure but has not proven to facilitate the repair of a diseased liver from HCV infection. Indeed, HCV directs a chronic inflammatory disease that is marked by local hepatic production of IL-1 β (Negash and Gale 2015). Although the innate immune response, as discussed above, is rapidly deployed within the liver during an infection to restrict and clear pathogens such as viruses (Akira et al. 2006; Thompson et al. 2011; Liaskou et al. 2012), liver inflammation (hepatitis) within the hepatic microenvironment during HCV infection occurs as an orchestrated and progressive event whose outcome is conferred by signaling cross talk including intercellular cross talk conferred by intrahepatic cells (Ishii and Koziel 2008; Kumar et al. 2013; Park and Rehermann 2014; Negash and Gale 2015). IL-1 β is a key proinflammatory factor that has been implicated in HCV-induced hepatitis (Burdette et al. 2012; Negash et al. 2013; Shrivastava et al. 2013; Chattergoon et al. 2014; Farag et al. 2017; Molyvdas et al. 2018). IL-1 has diverse biological activity and is a pivotal player in immune regulation during acute and chronic viral infection (Dinarello 2002; Ichinohe et al. 2009; Rajan et al. 2011; Ramos et al. 2012). As one of the early proinflammatory cytokines produced during an infection, its production increases the production of other proinflammatory mediators, promotes the migration of leukocytes, induces growth factor production for the maturation of immune cells, and imparts immune polarization (Negash and Gale 2015).

IL-1 exists as two forms within the body, IL-1 α and IL-1 β (Dower et al. 1986; Sims et al. 1989). They both engage IL-1 receptor 1 (IL-1R) to induce NF- κ B activation and drive the expression of inflammatory genes including IL-1. Although IL-1 α is readily produced by many cell types in response to stimuli (Di Paolo and Shayakhmetov 2016), IL-1 β production is tightly controlled at the expression, processing, and

maturation stages. The inflammasome is a cytoplasmic, multiprotein-containing, and oligomerizing signaling complex that leads to the maturation and release of bioactive IL-1 β (Martinon et al. 2002; Jin and Flavell 2010). The inflammasome complex is composed of a NOD-like receptor such as NLRP3, adaptor protein ASC, and the effector protease caspase-1. Initiation of NLRP3 inflammasome signaling is triggered by a priming step or “signal-one,” which leads to the transcriptional induction of NLRP3 and IL-1 β . To promote mature and active IL-1 β production and release, an inflammasome-activating signal called “signal-two” is required. PAMP/PRR interaction leads to signal-one activation to produce immature pro-IL-1 β protein. On the other hand, signal-two activation is triggered by diverse cellular responses, often induced by the ensuing infection, such as potassium efflux, calcium signaling, and endosomal rupture. To date, several inflammasomes are activated in response to viruses, bacteria, and particulate matters such as monosodium urate and asbestos. In particular, the well-characterized NLRP3 inflammasome has been shown to respond to diverse stimuli including viral infections such as with HCV (Rajan et al. 2011; Elliott and Sutterwala 2015).

Expression of the key inflammasome components occurs in most cell types and tissues, including the liver. The liver shows an overall low-level expression of *NLRP3* mRNA, but high hepatic caspase-1 expression, the initiator caspase of NLRP3 inflammasome activation (Lech et al. 2010; Csak et al. 2011). Moreover, hepatocytes and stellate cells express inflammasome components (Masumoto et al. 2001; Anderson et al. 2004; Watanabe et al. 2009). Several studies implicated IL-1 β in HCV-associated liver inflammation. Early studies (Tanaka et al. 2003; Wang et al. 2003) found that in HCV-infected patients a polymorphism at the *IL1B* locus conferred high risk for developing HCV-mediated hepatocellular carcinoma (HCC). Further studies revealed that IL-1 β protein can be detected in sera of HCV-infected individuals (Bortolami et al. 2008; Baranova et al. 2011; Negash et al. 2013). Gene expression profile analyses revealed that IL-1 β expression is linked to

HCV-induced severe liver disease. The HCV-infected cirrhotic liver contains a high level of IL-1 β , whereas the cytokine is low or not detected in infected individuals with only mild liver disease. These in vivo findings lead to further research to characterize the signaling events of HCV-induced IL-1 β production as a mechanism by which HCV-associated hepatic inflammation is established.

Although active HCV replication can be detected only in hepatocytes, inflammasome activation by HCV in hepatocytes is only weakly induced. Instead, hepatic macrophages respond robustly to HCV by producing IL-1 β (Negash et al. 2013; Shrivastava et al. 2013; Chattergoon et al. 2014). Within the hepatic environment initiation of inflammatory responses within the liver is carried out by KCs (Krenkel and Tacke 2017; Li et al. 2017), liver-resident macrophages. The KCs are the chief producers of inflammatory mediators. Immunohistochemistry analysis identified that macrophages including KCs are the major producers of IL-1 β within HCV-infected liver as compared to healthy uninfected liver. Similarly, stimulation with HCV of various immune cell subsets such as monocytes, natural killer (NK) cells, and T cells isolated from peripheral blood revealed that only monocytes and macrophages responded to HCV by producing IL-1 β . HCV triggers NLRP3 inflammasome activation to induce IL-1 β release from KCs or liver-infiltrating macrophages. Macrophages were found to engulf HCV virions leading to sensing of HCV and triggering of IL-1 β release. Induction of *IL1B* mRNA and downstream production of immature of IL-1 β protein was shown to be triggered by TLR7-mediated recognition of HCV RNA and signaling through MyD88 in macrophages (Negash et al. 2013). Two of the HCV proteins, p7 and core, have been shown to serve as NLRP3 inflammasome agonists. The HCV viroporin, p7, from different genotypes is indeed able to drive NLRP3 activation (Farag et al. 2017). A more recent report showed that the HCV core protein is a major virion component that is able to trigger NLRP3 inflammasome assembly and activation in macrophages (Negash et al. 2019). The HCV core protein activates the NLRP3 in-

flammasome through modulation of intracellular calcium signaling pathways. IL-18 is another cytokine that belongs to the IL-1 family and is regulated by the inflammasome pathway (Dinarello 1999; Vecchiet et al. 2005; Chattergoon et al. 2011). Like IL-1 β , IL-18 is detected in HCV-infected individuals and remains high in chronic infection, underscoring its important role in HCV pathogenesis (Fig. 5).

IL-1 β and IFN cross talk sustain the inflammatory response. As a major proinflammatory mediator, IL-1 β directs a signaling cascade that induces the expression of hundreds of genes in responding cells (Negash et al. 2013; Aarreberg et al. 2018). Many of these gene products function to amplify and diversify the inflammatory response by activating and recruiting immune cells. Analysis of the gene expression profile of blood and liver from chronic HCV patients revealed a strong inflammatory signature concomitant with increased IL-1 β levels (Negash et al. 2013; Khatun and Ray 2019). Remarkably, many ISGs were present and highly expressed in a manner linked with IL-1 β levels. Analysis of liver tissue from these patients demonstrated the presence of type I IFN within KCs of the chronic HCV liver, the same cells that produce and respond to IL-1 β , suggesting that KCs or macrophages in particular might respond to either cytokine to program rounds of signaling by the other cytokine. In this respect, recent studies have shown that IL-1 β signaling in macrophages, hepatocytes, and other epithelial cells leads to the release of mitochondrial DNA from the mitochondria of the responding cell. This process likely occurs through IL-1 β -mediated damage and permeabilization of mitochondria leading to accumulation of mitochondrial DNA in the cell cytoplasm. Genetic studies then showed that the cytoplasmic mitochondrial DNA is sensed by the cGAS protein leading to production of cGAMP and activation of the STING adaptor protein (Aarreberg et al. 2019). This process then leads to IRF-3 and NF- κ B activation, thus mediating IFN production. Whereas typically IFN is produced acutely in response to PRR signaling in virus infection, the IL-1 β -driven induction of IFN during virus infection occurs later, as it is dependent on

Figure 5. Hepatic inflammation during hepatitis C virus (HCV) infection. Hepatic macrophages (Kupffer cells [KCs]) produce and secrete inflammatory mediators such as IL-1 β , IL-18, and TNF- α through combined activation of the endosomal TLR7/MyD88 pathway by HCV RNA and activation of the NLRP3 inflammasome by the HCV viroporin p7 and core proteins. High expression of IL-1 β is a hallmark of HCV-infected cirrhotic liver and a key contributor to hepatic inflammation and HCV-induced liver disease.

IL-1 β production following NLRP3 inflammasome activation (Aarberg et al. 2018, 2019), thus establishing a cytokine cross talk circuit to amplify and extend IFN induction while mediating inflammatory signaling. When active in the HCV liver, this process contributes to continued inflammation linked with liver damage and continuous ISG expression. As a result, the liver was shown to become refractory to the actions of IFN because of obtaining a state of “innate immune tolerance” that facilitates cell survival to overcome the otherwise hepatotoxicity mediated by constitutive ISG expression and inflammatory response (Lau et al. 2013). Although innate immune tolerance is thought to protect the liver tissue from inflammatory cell death, the outcome is that the cells develop tolerance and become refractory to the antiviral actions of IFN. Indeed, analysis of liver gene expression profiles in patients with chronic HCV who were undergoing IFN therapy showed that the unresponsive liver failed to induce ISG expression beyond the level of expression mediated through cytokine cross talk that was ongoing (Lau et al. 2013). The process of innate immune tolerance might impart reduced

efficacy to IFN therapy for treatment of HCV infection.

FUTURE CONSIDERATIONS

HCV infection causes an inflammatory disease of the liver. In the era of DAAs, current therapy effectively overcomes the obstacle of innate immune tolerance and nonresponse to IFN to mediate HCV cure (Sandmann et al. 2019). However, the incidence of HCV infection among high-risk groups continues to present a public health concern (Wu et al. 2019), and coinfections with HCV, including HIV and hepatitis B virus (HBV), present a mounting concern, as DAA therapy does not prevent re-infection upon subsequent HCV exposure and does not offer treatment for HIV or HBV (Abutaleb and Sherman 2018; Mücke et al. 2018). More importantly, HCV clearance with DAA therapy, although efficient, enigmatically does not prevent “cured” liver from progressing to severe liver disease such as cirrhosis and/or liver cancer. In addition, liver-specific interventions to disrupt inflammasome activation and/or IL-1 β actions are needed for treatment of acute

and chronic liver inflammatory disease linked with new or reacquisition of HCV infection, thus protecting the liver tissue from damage that could otherwise independently propagate hepatic inflammation. Moreover, it is not clear whether the diseased liver can fully repair itself once HCV is cured, nor is it known whether the hepatic inflammatory response has returned to homeostatic and normal levels present in healthy non-HCV liver. Long-term monitoring of patient liver status following HCV cure will ascertain whether the cure fully turns off the inflammatory response to return the diseased liver to a normal state. Finally, as noted above, DAA therapy can drive the outgrowth of drug-resistant virus, although this is a very rare outcome (Bradshaw et al. 2019; Costa et al. 2019). DAA therapy is still not universally effective against all HCV genotypes (Hayes et al. 2019; Paolucci et al. 2019), and IFN therapy is variably used as the treatment for chronic HCV infection in countries outside of the United States and Western Europe (Esmat et al. 2014; Asselah and Bourlière 2015). Studies comparing DAAs and IFN in the treatment of HCV infection and reinfection should be considered to determine the best approach for therapy against difficult-to-treat HCV. Understanding how to target and modulate innate immunity and the actions of IFN and ISGs remains essential for improving IFN therapy as adduct for treatment of HCV infection. Thus, improving both DAA therapy and IFN therapy will impart success against difficult-to-treat HCV cases and will support the use of IFN-based therapy across patient populations in which DAA therapy is not yet widely applied.

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