Chapter 2

Hepatitis C Virus and Inflammation

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Additional information is available at the end of the chapter

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Abstract

Inflammation is often a rapid coordinated response generated in the host against evading microbial infections or tissue injury. Microorganisms like bacteria and viruses instigate inflammation mediated by pro-inflammatory cytokines and activate cascade of signaling events leading to the recruitment of inflammatory cells (neutrophils and macrophages). Although the main function of inflammation is the resolution of infection, several viruses, including the hepatitis C viruses (HCV) have evolved to utilize this host response and make the cellular environments conducive to infection. In majority of infected individuals, HCV causes persistent chronic liver inflammation leading to development of liver cirrhosis and hepatocellular carcinoma. HCV induces reactive oxygen species (ROS) and activates nuclear factor-κB (NF-κB) leading to the activation of cyclooxygenase-2 (Cox-2) that ultimately produces prostaglandin-E2 (PGE2), thus enhancing inflammatory process. Interestingly, HCV further activates NACHT, LRR, and PYD domains-containing protein 3 (NLRP3) inflammasome (a multiprotein complex) by recruiting adaptor protein apoptosis-associated speck-like protein containing a carboxy-terminal CARD (ASC) which are involved in activation of caspase-1 leading to production of interleukin-1beta (IL-1β) and interleukin-18 (IL-18). In this chapter we have highlighted the recent advancements in HCV-induced inflammatory responses and discussed potential future directions to understand the role of inflammation during HCV infection.

Keywords: PAMP, DAMP, TLR, NLRP3, AIM2, RIG-I, IFI16, inflammation, inflammasome, IL-Iβ, Caspase-1, HCV, HBV, herpesvirus

1. Introduction

Inflammation, often triggered by harmful stimuli such as tissue injury and pathogenic infections, is an adaptive response that underlies a wide variety of both physiological and

pathological processes [1]. Inflammation can be acute or chronic. Acute inflammation is generally induced by tissue injury, noxious compounds or invasion of pathogens with general clinical signs like swelling, redness, pain and heat at the site of the insult. Acute inflammation is the initial response of body during which, the small immune-mediating molecules called anaphylatoxins are recruited to site where it stimulates mast cells to release histamine, serotonin and prostaglandins. This event is followed by vasodilation to allow immune cells such as the neutrophils to rush to the site to respond to the causative agent. During the acute stage, the inflammation remains a beneficial process to heal and provide relief within few days. Chronic inflammation, however, lasts for weeks, months or even years and cause tissue damage. At the chronic stage, the inflammation becomes a problem rather than solution to infection or disease. In contrast to acute inflammation, the chronic inflammation is generally seen in viral infections and other hypersensitive disorders where the inflammation is persistent for a longer duration. During chronic inflammation, the primary immune cells are macrophages and T lymphocytes which play crucial roles by producing cytokines and other enzymes that are detrimental to cells. Several studies have focused on the chronic inflammation that occurs during type-2 diabetes, cardiovascular and autoimmune diseases and during localized chronic inflammation that occurs due to chronic infections. In spite of so much advancements made in inflammation biology, the causes and mechanistic details are still partly understood and need an in-depth analysis to completely unravel the mystery.

During pathogenic invasion, the host immune system initiates an immediate defense mechanism. The pathogens are recognized by the pattern-recognition receptors (PRR) [2] that identify pathogen-associated molecular patterns (PAMPs) [3] and danger-associated molecular patterns (DAMPs) to rapidly activate the innate arm of the host immune system, including the secretion of chemokines and cytokines [4]. The PRRs, like the Toll-like receptors (TLRs) [5] are present on the plasma membrane and in the endosomes while the RIG-I-like receptors (RLRs) [6], NOD-like receptors (NLRs) [7] and AIM2-like receptors (ALRs) [8] reside in the cytoplasm. During viral infections, the viral RNA is sensed by TLR3, TLR7 and TLR8, and viral DNA is sensed by TLR9. Similarly, viruses are also recognized by soluble sensors such as the RNA-sensing RIG-like helicases (RIG-I and MDA5) or the DNA-sensing PRRs (DAI and AIM2). The viral RNA in cytoplasm is detected by the helicase domain of either RIG-I or MDA5 followed by the exposure of the caspase recruitment domain (CARD) to interact with the N-terminal of mitochondrial adaptor protein (MAVS). This CARD-CARD interaction leads to dimerization of MAVS in the mitochondria to form the MAVS signalosome which further activates the NF-κB, production of type I interferons (IFNs) and the secretion of proinflammatory cytokines (IL-1β and IL-18) and chemokines [9, 10]. The maturation of IL-1β and IL-18 depends on the proteolytic cleavage of the pro-form of caspase-1 to release the active forms of IL-1β and IL-18 [11]. The formation of the active caspase-1 (p10/p20) is often regulated by multi-protein complexes called the inflammasomes [12].

Several distinct inflammasomes including the NLRP3 inflammasome, the absent in melanoma 2 (AIM2) inflammasome, the γ-interferon-inducible protein 16 (IFI16) inflammasome and the RIG-I inflammasomes have been identified to be activated during specific viral and bacterial infections [13]. Several viruses such as vaccinia virus (VACV) [14], HCV [15], hepatitis B virus (HBV) [16], human papillomavirus [17], mouse cytomegaloviruses (mCMV) [14, 16], influenza

virus [9, 18] and Vesicular stomatitis viruses (VSV) [19] have been reported to activate inflammasomes. In this book chapter, we have reviewed the role of inflammation and discussed the detailed mechanism of activation, following viral invasions, specifically during HCV infection.

2. Overview of inflammatory response to viral infections

2.1. Virus-induced inflammatory response

Inflammation is very crucial in maintaining the homeostasis that's altered during any exogenous stimuli such as the tissue injury or a pathogenic infection. Several viruses are known to induce inflammatory response. The virus is sensed by TLRs (TLR3/7, TLR8/9), RLRs (RIG-I and MDA5) and RNA-dependent protein kinases (PKR), to induce the production of inflammatory mediators and IFNs. The dsRNA is usually sensed through RIG-I and/or TLR3 in the monocytes, macrophages and non-immune cells (endothelial cells, epithelial cells and hepatocytes) whereas in plasmacytoid dendritic cells, TLR7 is highly expressed and acts as the major ssRNA sensor [20–23]. The activation of RLRs and TLRs then promote the secretion of IFNs and proinflammatory cytokines. The inflammation is further amplified when the proinflammatory cytokines and chemokines, such as IL-6, IL-8, tumor necrosis factor alpha (TNF-α) and Rantes starts recruiting other cell types to the infected tissue. These events not only contribute in the control of virus replication but also significantly enhance the inflammatory responses and disease severity.

The endoplasmic reticulum is the major site for protein synthesis including viral protein synthesis that disturbs the ER homeostasis and causes ER stress [24]. The main stress response pathway in the ER is the unfolded protein response (UPR) which has been linked to enhanced cytokine (TNF-α and IL-6) production due to activation of NF-κB and pro-inflammatory transcription factors [25, 26]. Thus the UPR pathway serves as the internal danger signal and compliments the cellular viral sensors to boost subsequent antiviral response [27]. Since the ER stress in the absence of any viral infection also leads to production of IL-1β secretion and cell death, it would be interesting to investigate further if there is a crosstalk between the UPR pathway and inflammasome activation during viral infection. The mitochondrial stress has also been associated with formation of ROS that can result in the activation of NF-κB, Cox-2, PGE2, IL-6 and activating protein-1 (AP-1), that subsequently up-regulate antioxidants and inflammatory pathways, including the ISGs [28].

Several viruses such as influenza viruses (human H1N1 and avian H5N1) have been shown to infect the microglia, astrocytes and neuronal cell lines and produce pro-inflammatory cytokines, ultimately leading to cell apoptosis [29]. A recent study also showed that influenza virus infection of mouse primary cortical neurons enhanced the mRNA levels of inflammatory cytokines, chemokines, and type I IFNs [30]. The Epstein–Barr virus (EBV) also triggers the TNF-α signaling by its LMP1 protein, activating NF-κB and resulting in production of IL-6 and subsequently a number of pro-inflammatory and immune stimulatory cytokines [31–33]. Similarly, the KSHV encodes several genes specially the viral Fas-associated death domain-like IL-1-converting enzyme inhibitory protein (vFLIP) that induce NF-κB activation that subsequently upregulates the chemokine CCL20 and its receptor CCL6. The CCL20 then recruits dendritic

cell and lymphocyte and thus contributes to the inflammatory infiltrate in the Kaposi's sarcoma lesions [34, 35]. In case of hepatitis B and C viruses, the liver cancer develops due to years of inflammation, oxidative stress (OS) and cell death leading to chronic liver damage. The liver infiltrating lymphocytes contributes majorly in the production of pro-inflammatory cytokines such as TNF-α, IL-6 and IL-1β during chronic HBV/HCV infection [36, 37].

2.2. Virus-induced inflammasomes

Several viruses like the influenza viruses, Respiratory syncytial virus (RSV), hepatitis B and C viruses, Dengue virus and herpesviruses have been reported to induce inflammation and activate the inflammasomes (**Table 1**). Few viruses are cleared, while a majority of viruses that cause chronic infection and cancer tend to utilize the inflammasome complex and the cellular milieu for their survival and have successful infection. The various inflammasomes that gets activated during different viral invasions are shown in **Table 1** and **Figure 1**.

The inflammasomes further contribute in secretion of inflammatory cytokines during viral infections. The following inflammasomes have been widely discussed during viral infections:

2.2.1. NLRP3 inflammasome

The NLRP3 inflammasome is the best-studied inflammasome and is known to be activated by viruses belonging to different families, suggesting a common pathway for detection of viruses and appropriate response by the host cells. NLRP3 is a multi-domain protein comprising of the N-terminal caspase recruitment domain (CARD), a PYD, a central nucleotide-binding and oligomerization domain (NACHT) (also termed NOD) and the C-terminal leucine-rich repeats (LRRs) [50]. The N-terminal domain helps in signal transduction by interacting with other CARD or PYD-containing proteins. The central NACHT domain serves as the scaffold protein and helps in oligomerization, thus activating the inflammasome. The LRRs are believed to act as ligand sensors. The formation of NLRP3 inflammasome induces the activation of caspase-1 and production of mature IL-1β and IL-18 [11]. NLRP3 inflammasome has been shown to be activated by ATP mediated efflux of PAMPs [51], lysosome/cathepsin B [52] and Ca^{2+}/ROS [53]. Viruses from different families are known to activate and modulate NLRP3 inflammasomes.

PRR	Pathogens	PAMPs recognized	Cytokines expression modulated	Refs
NLRP3	Influenza virus, Sendai virus, Vaccinia virus, HCV, RSV, VSV and Rabies virus	RNA	IL-1β and IL-18	[15, 18, 38–43]
AIM2	VACV, HBV, HPV and mCMV	Cytoplasmic DNA	IL-1β and IL-18	[14, 16, 17, 44]
RIG-I	Influenza virus, HCV, Rabies virus, JEV, RSV	RNA	Type I IFNs, IL-1β and IL-18	[6, 9, 45, 46]
IFI16	KSHV, EBV, HSV-1	Nuclear DNA	Type I IFNs, IL-1β	[47–49]

Table 1. Virus-induced inflammasome activation and modulation of cytokines.

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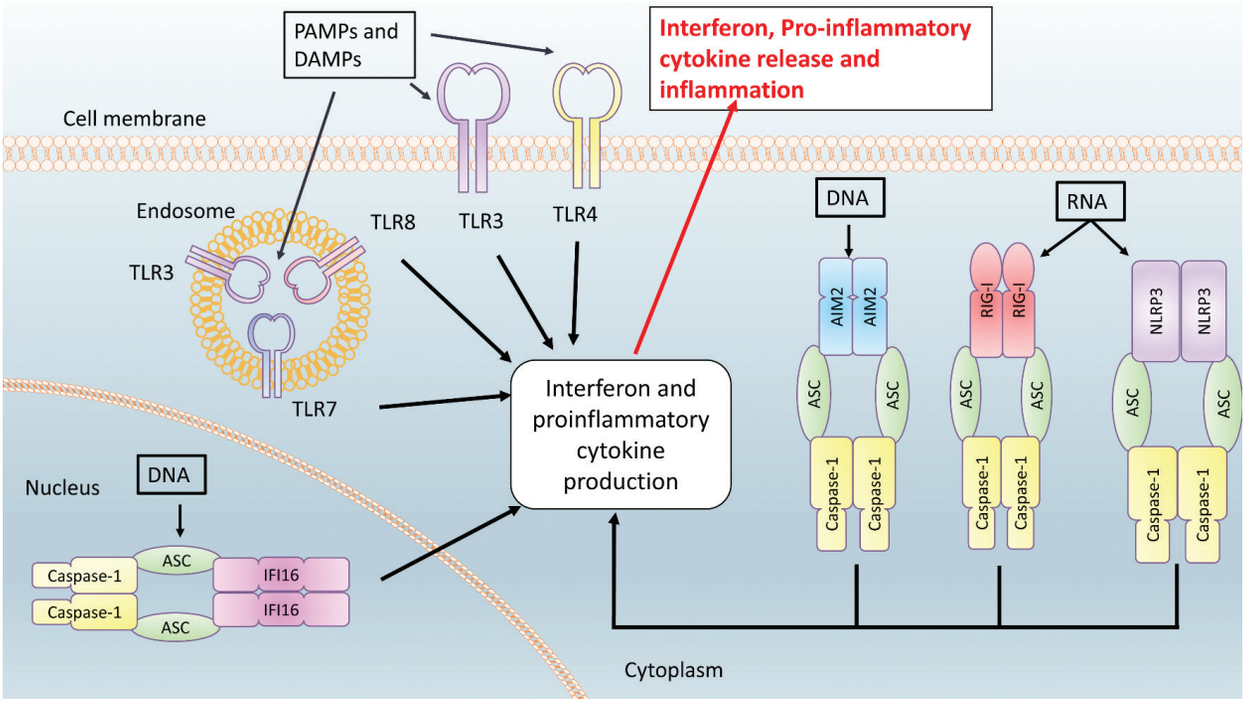

Figure 1. Inflammasome activation during viral infection. Infection with viruses leads to inflammasome activation. Depending on the type of nucleic acid composition of the invading pathogen different types of inflammasomes are activated. TLRs do not form inflammasome but do sense PAMPs and DAMPs associated with pathogens and its associated products. TLRs are located on either the cell membrane (TLR3 and TLR4) or endosome (TLR7 and TLR8). Sensing of PAMPs and DAMPs by TLRs activates cellular pathways which leads to the production of IFNs and proinflammatory cytokines. IFI16 detects DNA in the nucleus and is activated through formation of a complex formed with ASC and caspase-1. Similarly, AIM2 also detects pathogen DNA in the cytoplasm and forms an inflammasome with ASC and caspase-1. Whereas, RIG-I and NLRP3 both sense RNA PAMPs from pathogens, and similar to IFI16 and AIM2, form an inflammasome complex with adaptor ASC and effector caspase-1. Formation of inflammasome complex leads to its activation and release of IFN and proinflammatory cytokines which ultimately causes inflammation.

Influenza viruses are the most common activators of NLRP3 inflammasome [38]. Studies have further shown that the influenza virus proton-specific ion channel M2 protein activates NLRP3 inflammasome in the acidic trans-Golgi network [54]. The hepatitis C virus (JFH-1) also activates the NLRP3 inflammasome in Huh7.5 cells and THP-1 macrophages and leads to the production of IL-1β [15, 43]. The ROS inhibitor diphenyleneiodonium (DPI) has been shown to inhibit the HCV-induced IL-1β production [43]. Thus HCV has been shown to activate the NLRP3 inflammasomes both through the HCV genomic RNA and ROS model. Others viruses like the Rabies virus [42], modified vaccinia virus [14], Japanese encephalitis virus [55] and Rift Valley fever viruses [56] are also shown to induces IL-1β production and NLRP3 inflammasome activation.

Apart from RNA viruses, the DNA viruses are also reported to activate NLRP3 inflammasome. The Herpes simplex virus 1 (HSV-1) infection triggers the association of ASC with NLRP3 along with the production of mature caspase-1 and IL-1β in the human foreskin fibroblasts [49]. Adenovirus activates IL-1β secretion in monocytic cells. The transfected adenoviral DNA was known to activate the inflammasome which was NLRP3 independent, however later in a study, it was observed that adenoviral infection could activate the NLRP3 inflammasome, thus suggesting that NLRP3 inflammasome activation could be dependent on the

route of viral DNA. The study further showed that NLRP3 knockout mice showed decreased IL-1β induction in response to adenoviral infection thus indicating the possibility of other sensors identifying transfected adenoviral DNA in previous studies [57]. In another study, the Varicella-Zoster Virus (VZV) was also demonstrated to activate the NLRP3 followed by recruitment of ASC and caspase-1 in monocytic and melanoma cell lines and in skin xenografts [58]. Few studies have shown the relation of NLRP3 in HBV infections, however the results does not directly correlate the increased expression of NLRP3 in CHB patients with HBV-DNA copy number. Hence the increase in NLRP3 may be due to an indirect effect of HBV such as the liver damage [59]. Another recent study has shown that HBV-HBeAg suppressed the LPS-induced activation of the NLRP3 inflammasome and production of IL-1β by suppressing the NF-κB pathway and ROS production [60]. Since studies about the activation of NLRP3 during HBV infection are still progressing, it would be interesting to understand how HBV modulates inflammasomes for its propagation.

2.2.2. RIG-I inflammasome

The RIG-I, a member of the RLR family, contains two N-terminal CARDs that recruits several adaptor proteins, a central RNA helicase domain that has an ATPase activity and a C-terminal regulatory domain (CTD) that binds to the dsRNA to collectively induce the type I IFN production [61]. The RIG-I has been shown to recognize the dsRNA replication intermediates of several RNA viruses [62]. Influenza virus, HCV, Sendai virus, New castle disease virus, rabies virus and RSV showed defective IFN production in the absence of RIG-I [6]. The role of RIG-I as inflammasome activator has been shown in a study that was conducted with rhabdovirus VSV infection in murine dendritic cells in which there was RIG-I dependent production of IL-1β and IL-18 via NF-κB, caspase-1, and caspase-3 activation. The knockdown of RIG-I in mice inhibited the secretion of IL-1β [19]. Another study however showed conflicting results in which the infection with VSV was shown to be activated by NLRP3 and not by RIG-I [41]. These contrary results highlight the possible dual role of RIG-I in the inflammasome and type 1 IFN pathways. A study conducted with influenza virus infection in the primary human bronchial epithelial cells demonstrated both RIG-I-dependent priming of the NLRP3 inflammasome as well as direct RIG-I-mediated inflammasome activation [9]. Thus extensive research is still needed to analyze the roles of RIG-I during viral infections.

2.2.3. AIM2 inflammasome

The AIM2 is a member of the interferon (IFN)-inducible protein with a 200 amino acid repeat family (also known as the HIN200 family of IFI200 family) containing an N-terminal PYD and a C-terminal HIN200 domain. The family includes at least six members in mice (IFI202, IFI203, IFI204, IFI205, PYHIN1 and AIM2) and four members in humans (IFI16, MNDA, IFIX and AIM2). Studies have demonstrated that AIM2 senses the cytoplasmic bacterial, viral, or even the host double-stranded DNA (dsDNA) [8, 16]. The AIM2 utilizes its PYD domain to interact with ASC and recruit caspase-1 for the AIM2 inflammasome formation and IL-1β and IL-18 secretion [16]. AIM2 has been shown to be required for activation of caspase-1 during the VACV and MCMV infection in cell culture system but not during the HSV-1 infection [14, 63]. The sensing of VACV

and MCMV but not HSV-1 indicates that few viruses have evolved to block the AIM2 mediated recognition of their genome and downstream signaling. It has been further shown that AIM2$^{-/-}$ mice infected with MCMV were defective in IL-18 and IFN-γ production as compared to their control littermates [14]. The human hepatocytes have also been shown to express AIM2. An *in vitro* study has shown that the AIM2 senses the hepatitis B virus in hepatocytes and increases the production of IL-18. Further, the study showed that the expression of AIM2 in chronic hepatitis B (CHB) patients was higher than that of controls and which positively correlated to the severity of liver inflammation [64]. In another study conducted on peripheral blood mononuclear cells (PBMCs) from patients with acute hepatitis B (AHB) and CHB during different clinical phases, the expression of AIM2, IL-1β, and IL-18 was observed to be significantly high in AHB compared with expression in CHB patient samples [44]. The low expression in CHB patients also suggests that AIM2 may be associated with the chronic development of hepatitis [44]. It would be interesting to study if all the family of DNA viruses is sensed by the AIM2 inflammasomes.

2.2.4. IFI16 inflammasome

Similar to AIM2, the IFI16 belongs to the ALR family however they differ in their cellular localization. The former is strictly cytosolic while the latter is mainly localized in the nucleus due to its nuclear localizing sequence (NLS). Since both AIM2 and IFI16 recognizes DNA, these sensors are also reported to get activated by self-DNA, potentially leading to various autoimmune and auto inflammatory diseases such as lupus pathogenesis [65], Sjögren's syndrome [66] and systemic sclerosis [67]. The IFI16 is also known to sense viral DNA during infection. A study conducted on KSHV has shown that IFI16 recognized the viral DNA in the nucleus and later translocated to cytoplasm only in infected cells [68]. Upon recognition of the KSHV genome, the IFI16 is acetylated in the nucleus and later redistributed to the cytoplasm with the help of BRCA1 [48, 69]. Among others, the herpes simplex virus 1 (HSV-1), Epstein–Barr virus (EBV), and bovine herpesvirus 1 (BoHV-1) are also reported to activate the IFI16-ASC inflammasomes and produce inflammatory cytokine IL-1β [47, 49, 70].

3. Hepatitis C virus and liver inflammation

Hepatitis C virus is a hepatotropic virus, belongs to the *Flaviviridae* family. It is a positive sense single-stranded RNA virus. The RNA genome is present in an icosahedral structure made up of core proteins, which is further encapsulated in lipid bilayer which contains E1/E2 glycoproteins in a heterodimer on the membrane [71]. The RNA genome contains a 5'UTR which has an internal ribosomal entry site (IRES) and is required for cap–independent translation [72, 73]. On the other hand, the 3'UTR consists of mainly a poly (U/UC) tract and X-tail which have been shown to be required for replication of viral RNA [74, 75]. In between the two UTRs exists the genomic region which translates into a 3000aa polyprotein which is cleaved by host peptidases and viral proteins to form structural (core, E1 and E2) proteins, p7 and non-structural (NS2, NS3, NS4A, NS4B, NS5A, and NS5B) proteins. The virus is known to cause chronic infections in liver and eventually cancer (**Figure 2**). HCV causes chronic inflammation leading to liver fibrosis, steatosis, cirrhosis and finally hepatocellular carcinoma (HCC).

Inflammation is a crucial physiological event that occurs during chronic HCV infection. Chronic inflammation is defined by the persistence of inflammatory cells and destruction of liver cells. The liver cells have a unique regenerative capacity and can replace a significant loss of liver cells by compensatory proliferation. However, the chronic liver damage and regeneration results in scarring of liver called liver fibrosis. The fibrotic stage is characterized by the activation of HSCs and extracellular matrix (ECM) secretion. The liver fibrosis is also enhanced due to promotion of activated hepatic stellate cells (HSCs) survival in a NF-κB dependent manner by the KCs and recruited macrophages [76]. The ROS released by KCs and NADPH oxidase stimulated ROS production in HSCs and hepatocytes, result in robust induction of OS leading to DNA damage, enhanced expression of proinflammatory genes, fibrogenesis and malignancy [77]. The fibrotic stage gradually progresses to late stage of fibrosis called cirrhosis, which is the hallmark of an irreversible advanced stage liver injury. At this stage the dense bands of fibrotic scar develops into abnormal nodules of hepatocytes, resulting mainly from regenerative hyperplasia, separated by fibrous tissues. The disease progression eventually leads to the loss of normal functionality of liver such as xenobiotic metabolism and the metabolism of carbohydrates, proteins and other crucial molecules. In case of HCV infection, the complication progresses as a mild liver disease for 15–20 years after which a substantial number of individuals develop liver cirrhosis with clinical complications such as ascites, variceal hemorrhage and hepatic encephalopathy [78]. The ultimate complication of cirrhosis is the development of hepatocellular carcinoma.

In HCV infected individuals, besides a local inflammation in the liver, a mild systemic inflammation is also observed due to increased pro-inflammatory cytokine serum levels and

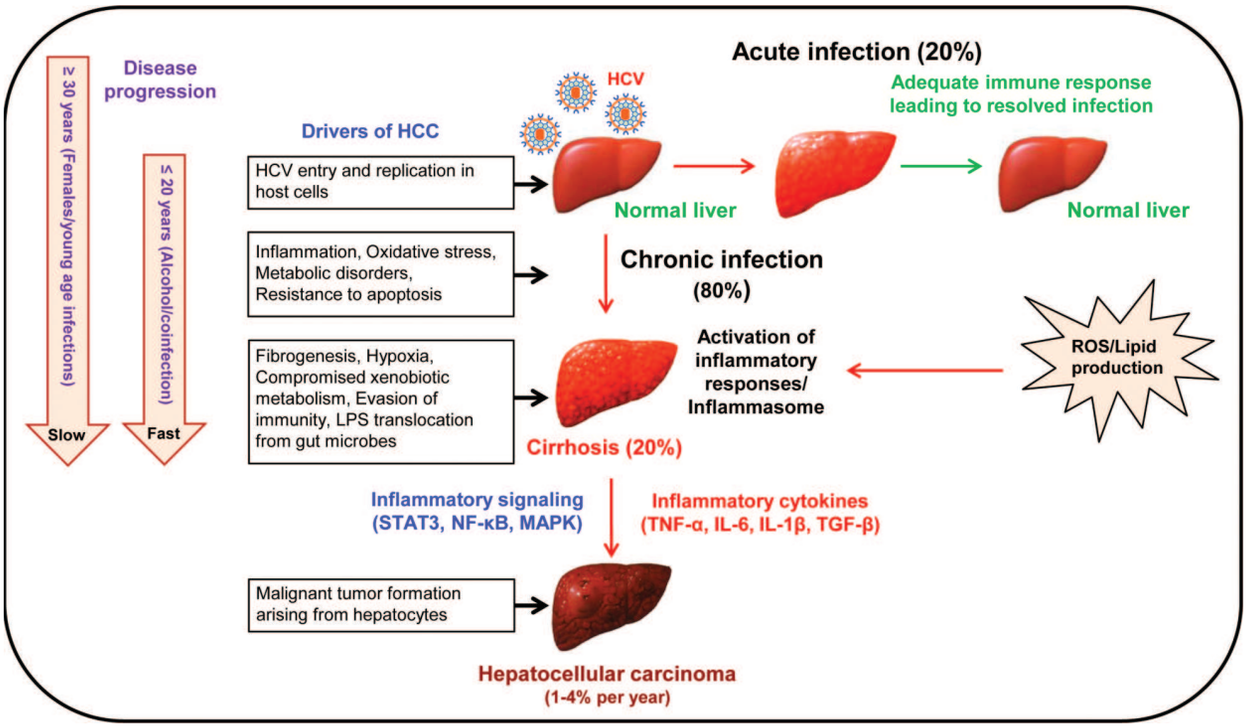

Figure 2. Schematic diagram representing different stages of HCV-induced liver disease progression.

activation of blood monocytes. The OS generated during chronic infection also plays key roles in the development of local and systemic inflammation. HCV proteins activate several pathways responsible for increased inflammatory response. The NS5A, for example, promotes upregulation of Cox-2 which contributes to chronic inflammation and fibrosis through production of various prostaglandins [79]. The chronic liver damage due to continuous inflammatory response (various inflammatory cytokines) and OS for several years ultimately leads to liver cancer [36]. Similarly, HCV infection also leads to an enrichment of proinflammatory cytokines in the liver cells ultimately leading to increased secretion of TNF-α, IL-6 and IL-1β [80]. These inflammatory events make the HSCs highly responsive to the transforming growth factor β (TGF-β) [81] that promotes hepatic fibrogenesis and eventually the progression and prognosis of HCC [82, 83]. HCV also induces the ER stress that increases the intracellular ROS levels which ultimately leads to increase in inflammatory gene expression by activation of NF-κB, AP-1 and STAT3 [84, 85]. A study has shown that the HCV core induces lipid accumulation leading to increased ROS production and inflammation ultimately promoting the HCC in transgenic mice [86, 87]. Osteopontin (OPN) is a cytokine that either remain intracellular or is secreted to allow both autocrine and paracrine signaling. Studies have shown the correlation of hepatic inflammation with increased expression of OPN [88, 89]. Recent studies have also shown that OPN is a crucial player during HCV infection and plays roles in epithelial to mesenchymal transition of hepatocytes [90, 91].

3.1. Role of various cytokines in HCV-induced inflammation

Cytokines belong to a large group of proteins that are secreted from specific cells of the immune system and perform a wide range of biological functions including innate and acquired immunity, hematopoiesis and inflammation. They mainly include the interleukins, chemokines, IFNs, TNF etc. Viral proteins and dsRNA from HCV triggers the induction of proinflammatory cytokines and chemokines. HCV core protein has been shown to induce inflammatory cytokines through the STAT3 signaling pathway [92]. A study further showed that a cross-talk existed between the HSCs and HCV-infected hepatocytes. The IL-1β secreted by HSCs co-cultured with the hepatocytes, ignited the production of several pro-inflammatory cytokines and chemokines, such as IL-6, IL-8, MIP-1α and MIP-1β, by the hepatocytes [93]. The HCV proteins (NS3, NS4 and NS5) are also reported to induce the human Kupffer cells (KCs) to synthesize inflammatory cytokines such as TNF-α and IL-1β [94]. The HCV-NS5A protein has been shown to induce high levels of pro-inflammatory chemokine IL-8 to inhibit IFN-α thus facilitating the viral replication despite IFN α/β induction [95]. *In vitro* studies have shown that IL-10 production is regulated by HCV structural proteins to inhibit IL-12 production in myeloid cells. This also correlated with reduced IL-12 levels observed in chronic hepatitis C patients [96]. Serum cytokine levels were evaluated in HCV patients, and it was observed that both T helper (Th) 1 and Th2 lymphocytes were highly associated with chronic HCV infection [97]. This lead to the increased production of IL-2, IL-4, and IL-6 cytokines in all chronic active hepatitis patients [97]. Liver fibrosis has been shown to progress due to the persistent inflammation activating the HSCs, myofibroblasts, and fibroblasts which are regulated by pro-inflammatory cytokines such as TGF-β, IL-6, TNF-α, CCL21, and platelet-derived growth factor (PDGF) [98]. The HCV related mixed cryoglobulinemia

(MC) (MC + HCV) is an extrahepatic disease associated with HCV infection. In a study, the MC + HCV was shown to express significantly higher mean IL-1β, IL-6, and TNF-α levels than the controls or the HCV patients [99]. A recent study has shown the importance of Th17/IL-17 axis in HCV-induced chronic hepatitis and progression to cirrhosis. It promotes the recruitment of inflammatory cells and cytokines IL-6 and IL-23. A similar observation was also made in HCV patients with orthotopic liver transplantation (OLT). The recipients with HCV-induced allograft fibrosis or cirrhosis presented with higher levels of HCV-specific Th17 cells along with proinflammatory mediators (IL-17, IL-1β, IL-6, IL-8, and MCP-1) [100]. In a study conducted to analyze the expression of cytokines in HCV infected patients, it was observed that TNF-α expression was localized mainly in liver sinusoidal cells (macrophages, endothelial cells) and a high proportion of hepatocytes demonstrated expression of TNF-α, IL-1α, and IL-2 [101]. IL-32 has also been shown to be expressed by human hepatocytes and hepatoma cells and is involved in HCV-associated liver inflammation [102]. In addition, IL-32 was found to be constitutively expressed in the human hepatoma cells and was observed to be upregulated by IL-1β and TNF-α [102].

3.2. HCV-induced oxidative stress adds to inflammatory response

Oxidative stress plays a significant role in HCV-induced liver damage. HCV infection has also been reported to activate the liver-residing macrophages- Kupffer cells (KC) and result in ROS production. The activated KCs enhance the production of TNF-α and ROS as a mechanism to cope with HCV infection by killing hepatocytes [103]. HCV has also been shown to induce OS through calcium signaling [84, 104, 105]. The HCV infection also induces ROS that stimulates the NF-κB to activate Cox-2. This event ultimately leads to overexpression of Cox-2 thereby increasing the levels of pro-inflammatory molecules, PGE_2 (**Figure 3**) [104]. The ROS also activates a transcription factor, STAT-3, that controls important cellular processes required for cell survival, proliferation, differentiation and oncogenesis [106] and constitutive activation of NF-κB and STAT-3 by HCV has been shown to be involved in acute and chronic liver disease associated with HCV infection [107]. ROS has also been shown to increase the proliferation of HSCs as well as TGF-β and collagen synthesis to promote fibrogenesis [108]. Hepatic steatosis, reported in more than 50% of HCV-infected patients, has also been linked to OS in CHC patients infected with HCV genotype non-3 [109]. The HCV-infected human hepatoma cells enhance the expression of TGF-β1 by induction of transcription factors AP-1, Sp1, NF-κB and STAT-3 via OS [110].

3.3. Role of inflammasomes in HCV-induced inflammatory response

HCV infection in liver cells stimulates host responses which triggers PRRs to recognize HCV components. Recognition usually occurs through TLR3 and TLR7 on either the cell surface or the endosomal compartments during HCV infection (**Figure 3**) [111]. TLR expression and recognition of HCV associated PAMPs has led to production of IFN as well as activation of NF-κB mediated inflammatory molecules which ultimately cause inflammation. TLR3 signaling pathway is led by TIR-domain-containing adaptor-inducing interferon-B (TRIF) which activates IRF-3 and NF-κB which produces pro-inflammatory cytokines, chemokines and type I IFN. Even though TLR3 expression was observed in HCV infected cells it was identified that the downstream signaling is impaired by HCV non-structural proteins NS3/4A, NS5A

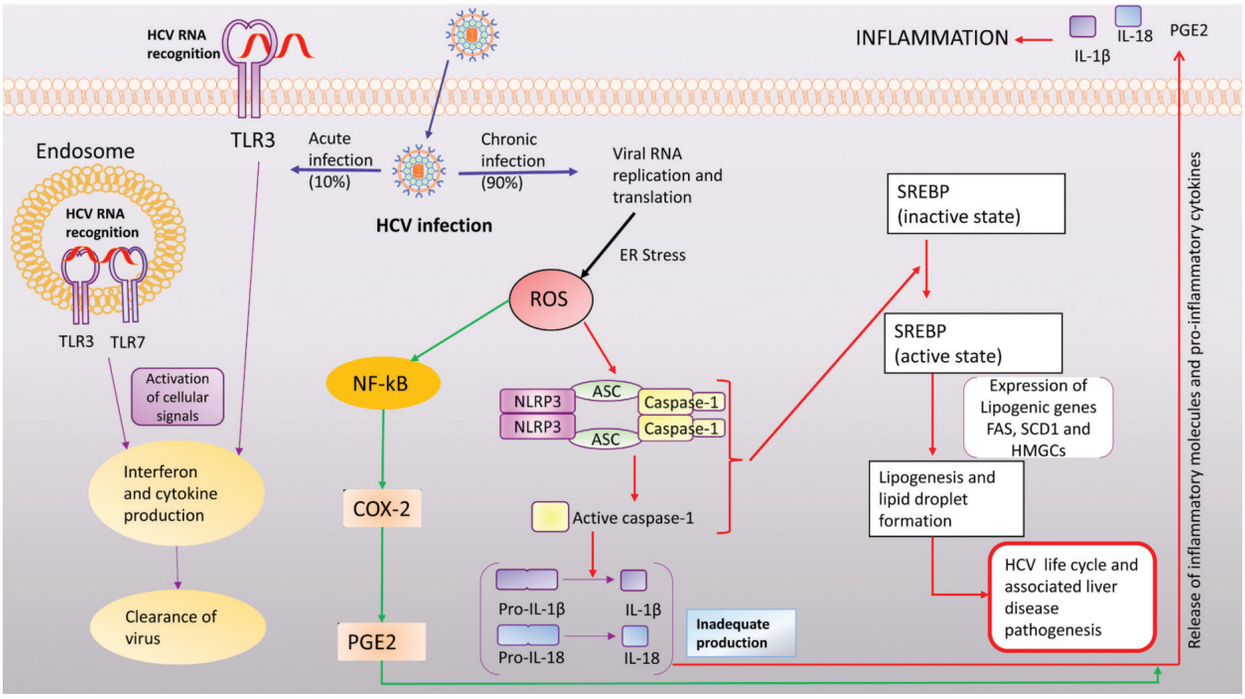

Figure 3. HCV-induced inflammasome regulates liver disease pathogenesis.

and NS5B [112] and also by decreasing the expression of TLR3 adaptor TRIF [113]. TLR7 activation leads to formation of a complex with MyD88, TRAF6, IRAK4 and IRAK1, which further activates IRF7 and induces interferon signaling.

During HCV infection HCV PAMPs are not only recognized by TLRs but also by RIG-I. It has been observed that HCV dsRNA is recognized by RIG-I during initial hours of HCV viral infection [114]. dsRNA binding to RIG-I initiates an interaction between 14-3-3ε and E3-ubiquitin ligase TRIM25 [115, 116]. This interaction leads to another interaction of RIG-I with MAVS, which contributes to IRF3 and NF-κB signalosome activation and production of IFNs [117, 118]. It was identified by Baril et al. that HCV prevents further signal transduction of RIG-I through proteolytic cleavage of MAVS by HCV NS3/NS4A protease [119]. MAVS cleavage results in disruption of RIG-I mediated IFN production during HCV infection [120].

HCV has also been shown to activate NLRP3 inflammasome in infected liver cells. A study has shown that HCV increases NLRP3 expression in liver [121]. In another study Burdette et al. for the first time showed induction and assembly of NLRP3 inflammasome in human hepatoma cells infected with HCV (JFH-1) (**Figure 3**) [15]. The study demonstrated that NLRP3, upon sensing the HCV, recruits an adaptor protein ASC for the assembly of the inflammasome complex. The study also highlighted that the activation of IL-1β in HCV infected cells was achieved by proteolytic processing of pro-caspase-1 into mature caspase-1 [15] and siRNA mediated cleavage of NALP3, ASC and caspase-1 abrogated the IL-1β secretion suggesting that HCV infected hepatoma cells (epithelial) activates NLRP3 inflammasome [15]. In another study by Boaru et al., it was shown that NLRP3 inflammasome was prominently assembled in liver sinusoidal endothelial cells and KCs, moderately in cultured HSCs and periportal myofibroblasts and almost absent in primary hepatocytes [122]. Studies have also shown that NLRP3 inflammasome was

not activated in human hepatoma cells or primary hepatocytes [43, 123]. The possible reason for not observing the inflammasome in primary hepatocytes could be explained by the fact that the authors relied on the detection of mature IL-1β and IL-18. There are other studies that support that hepatocytes express and also activates the inflammasome complex, however do not secrete detectable amounts of IL-1β and IL-18 as compared to immune cells [124, 125]. This also suggests that the activation of inflammasome in epithelial cells might be performing cytokine independent functions. Negash et al. also showed that KCs were the major IL-1β-producing cell population during HCV infection and that the serum levels of IL-1β were significantly increased in patients with CHC [43]. They also showed that exposure of THP1 cells to HCV-induced IL-1β production and secretion via NLRP3 inflammasome pathway. All these events lead to enhanced proinflammatory cytokine and immune-regulatory gene expression [43]. In another study, Chen et al. reported that HCV-induced ROS production activated the NLRP3 inflammasome and subsequent IL-1β secretion [40]. Similarly, Shrivastava et al. also showed that the inflammatory cytokines IL-1β and IL-18 were produced through the activation of NF-κB pathway and induction of ROS. In THP-1 cells they observed that the production of these cytokines was through the NLRP3 inflammasome activation and caspase-1 cleavage [123]. Interestingly, caspase-1 activation has been shown to not only result in pro-inflammatory cytokine production but also regulation of many other cellular pathways. A study by Li et al. identified 40 genes regulated by caspase-1 in various tissues [126]. Previously, Grucel et al. showed caspase-1 induced activation of sterol regulatory element binding proteins (SREBP) in response to bacterial pore forming toxins. Thus, the contradicting results observed for the NLRP3 inflammasome activation in human hepatocytes cells and immune cells could be due to the possibility that activation of the NLRP3 inflammasome leads to regulation of other cellular genes or pathways other than production of pro-inflammatory cytokines. Therefore, the recent study from our lab has shown that HCV exploits the NLRP3 inflammasome to activate the SREBPs and host lipid metabolism for liver disease pathogenesis (**Figure 3**) [39]. In addition, IFN has been shown to inhibit NLRP3 inflammasome by blocking the caspase-1 dependent IL-1β maturation [127]. Thus therapeutically targeting NLRP3 inflammasome complex or IL-1β could provide better interventions in managing liver inflammation in CHC patients.

4. Therapeutic approaches to manage HCV-induced inflammation

HCV has been linked to several other diseases including the lymphoproliferative diseases [128], cardiovascular diseases [129], and atherosclerosis [130], and neuropsychiatric symptoms [131]. Since inflammation plays a key role in disease progression in chronic hepatitis C patients, a therapeutic method to anti-inflammatory approach would result in better management of the disease. Chen et al. have shown the beneficial effect of the aqueous extract of an edible seaweed *Gracilaria tenuistipitata* in inhibition of HCV replication by suppressing the Cox-2 protein and thus reducing inflammatory response [132]. Sorafenib is a chemotherapeutic agent that has been shown to inhibit the Raf/ERK pro-inflammatory and pro-fibrotic signaling pathways [133]. Similarly animal model have been used to show the effect of TNFα inhibitors on reduction of IL-6 and TGF-β [134], however the efficacy of such anti-inflammatory drugs will need extensive research owing to the risk of interference with the IFN therapy prescribed for HCV

Drugs	Disease	Role	Refs
Pre-existing treatments			
Sorafenib	Hepatocellular carcinoma	Inhibits Raf/ERK	[130]
Corticosteroids	Liver disorders	Anti-inflammatory	[138]
Cyclosporine	Autoimmune hepatitis	Calcineurin inhibitor, reduces cytokines, inhibits TGF-β and IL-4	[139]
Azathioprine	Autoimmune hepatitis	Anti-inflammatory	[140]
Budesonide	Autoimmune hepatitis	Anti-inflammatory synthetic corticosteroid	[141]
Tacrolimus	Autoimmune hepatitis	Calcineurin inhibitor	[142]
Emerging or possible treatments for liver inflammation			
Cenicriviroc	Non-alcoholic steatohepatitis (NASH) and liver fibrosis	Inhibits chemokine receptors CCR2/CCR5	[143]
Fresolimumab	Systemic sclerosis	Neutralizes TGF-β	[144]
Pioglitazone	Hepatic steatosis due to HIV/HCV infections	Acts as a PPARγ agonist, helps in reduction of ROS	[145]
Glycyrrhizin	Chronic hepatitis C and F2/F3 liver fibrosis	Anti-oxidant	[145]
Resveratrol	Non-alcoholic steatohepatitis (NASH)	Anti-oxidant	[146]
Humira	Certain arthritis such as rheumatoid and psoriatic	TNF-α blockers	[147]
Celecoxib	Pain and inflammation	Cox-2 inhibitor	[148]
Canakinumab	Acute and chronic non-infectious inflammatory diseases	IL-1β inhibitor	[135]
Pentoxifylline	Liver fibrosis, Non-alcoholic steatohepatitis (NASH), Primary biliary cirrhosis (PBC), Alcoholic liver disease	TNFα suppressing phosphodiesterase inhibitor	[136, 137]
Ursodeoxycholic acid	Primary biliary cirrhosis (PBC), Autoimmune hepatitis	Decreases TGF-β signaling and oxidative stress, TNF-α, IL-1α, IL-1β, and IL-6, IL-10 NF-κB	[149, 150]

Table 2. Pre-existing and emerging or possible treatments used against hepatic inflammation observed in various liver diseases.

mediated hepatitis. Microbial translocation in HCV infected resident KCs could also serve as a good platform to minimize the LPS-induced inflammasome response [135]. Dammacco et al. in their study showed that triple therapy with pegylated IFN-α, ribavirin, and rituximab (RTX) to patients with HCV-related cryoglobulinemia gave significantly better results than those who only got pegylated IFN-α and ribavirin [136]. Since IL-1β is directly involved in inflammatory response, and hence Canakinumab, a human monoclonal antibody that selectively inhibits IL-1β was shown to inhibit many inflammatory biomarkers [137].

Pentoxifylline (PTX) is a methylxanthine derivative with a variety of anti-inflammatory and antifibrotic effects, has been shown to be effective in liver diseases like the alcoholic liver disease [151], fibrosis/cirrhosis [152]. The drug also decreases the levels of TNF-α, IL-1, IL-6 and TGF-β which holds significant therapeutic potential [153]. There are few preexisting and possible emerging therapies against hepatic inflammation and liver disease available which are listed in **Table 2**.

5. Conclusions

Inflammation is a crucial part of human immune response that kicks into high gear during any tissue injury or invasion of harmful bacteria and viruses. When a cell dies, it stimulates a number of processes including the rapid recruitment of innate immune components from blood to generate an inflammatory response. This is a double-edged sword that in one hand protects and heals the injured tissues while on the other hand cause significant damage and disease progression. Both bacterial and viral infections have been well recognized as potent source of inflammation. Various studies have shown that these pathogens induce inflammation and in some cases the inflammation is continuous for several years ultimately contributing to cancer. With some oncogenic viruses, the unceasing inflammation significantly contributes to tumor formation. Growing evidences support the crucial role of HBV- and HCV-induced inflammatory responses in liver for both the reversal of disease as well as pathogenesis of hepatic and extrahepatic diseases. The persistent HCV infection leads to chronic inflammation which has been shown to be the primary cause of liver fibrosis and cancer. More importantly the epithelial cells mediate the progression from fibrotic to carcinogenic stage. It has been shown that during the chronic HCV infection, the hepatocytes show a transition from pSmad3C pathway, characteristics of mature epithelial cells, to JNK/pSmad3L pathway which favors the liver fibrosis and also increase the risk of cancer. Several studies have shown the roles of inflammatory mediator such as the IL-6, Cox-2, NF-κB and more recently the activation of inflammasomes, as major contributors in HCV pathogenesis. The HCV-induced inflammation still needs more studies to better elucidate the treatment options and to date, the novel therapeutic targets for inflammation, seems to be a good option for better management of disease, especially in non-responders to the standard antiviral treatment.

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Conflict of interest

None.

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