Chapter 2

# Hepatitis C Virus and Inflammation

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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-$\alpha$) 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-$\alpha$ 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-$\alpha$ 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-$\alpha$, IL-6 and IL-1$\beta$ 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-1$\beta$ 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-1$\beta$ and IL-18 | [15, 18, 38–43] |
| **AIM2** | VACV, HBV, HPV and mCMV | Cytoplasmic DNA | IL-1$\beta$ and IL-18 | [14, 16, 17, 44] |
| **RIG-I** | Influenza virus, HCV, Rabies virus, JEV, RSV | RNA | Type I IFNs, IL-1$\beta$ and IL-18 | [6, 9, 45, 46] |
| **IFI16** | KSHV, EBV, HSV-1 | Nuclear DNA | Type I IFNs, IL-1$\beta$ | [47–49] |

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

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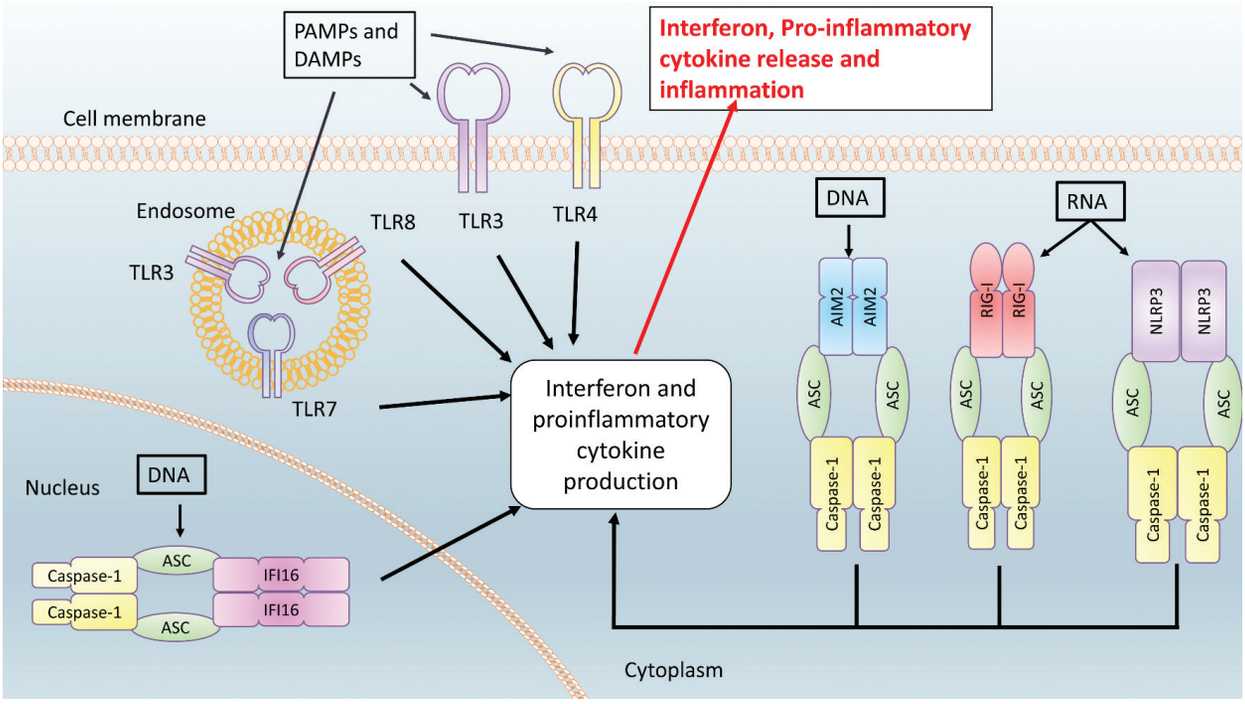


**Figure 1.** Inflammasome activation during viral infection. Infection with viruses leads to inflammasome activation. Depending on the type of nucleic acid composition of the invading pathogen different types of inflammasomes are activated. TLRs do not form inflammasome but do sense PAMPs and DAMPs 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

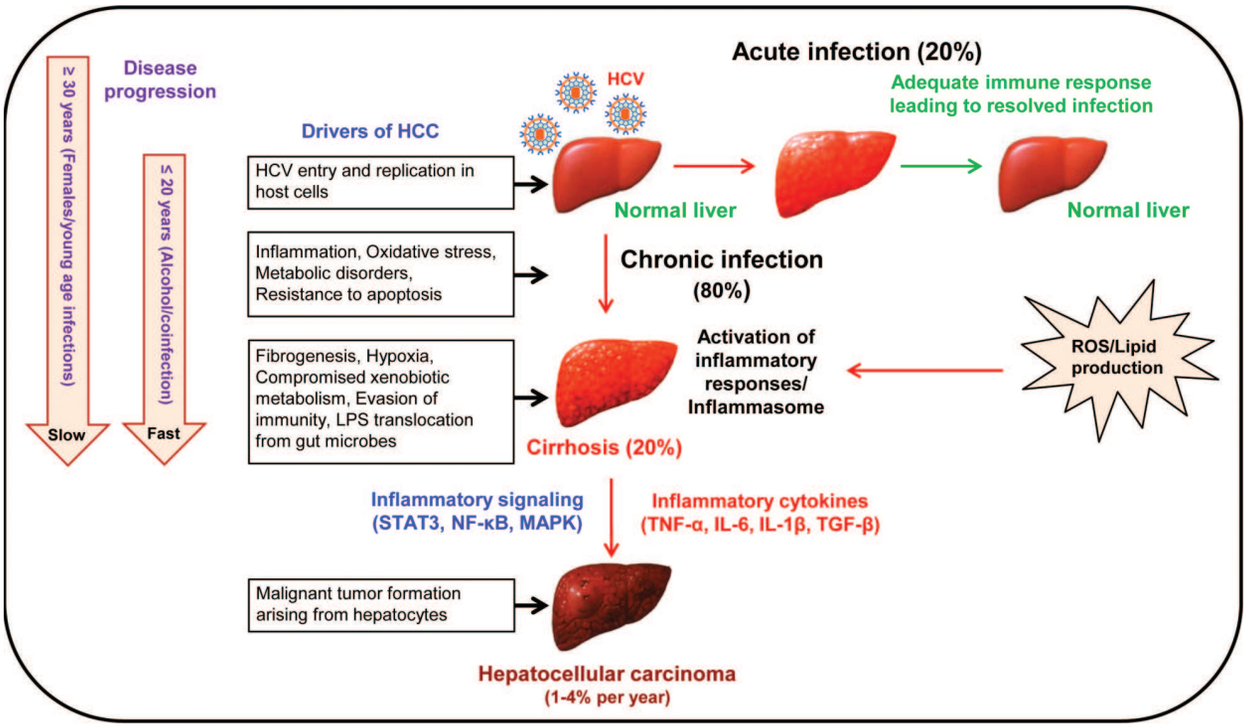


**Figure 2.** Schematic diagram representing different stages of HCV-induced liver disease progression.

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-$\alpha$, IL-6 and IL-1$\beta$ [80]. These inflammatory events make the HSCs highly responsive to the transforming growth factor $\beta$ (TGF-$\beta$) [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-1$\beta$ 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-1$\alpha$ and MIP-1$\beta$, 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-$\alpha$ and IL-1$\beta$ [94]. The HCV-NS5A protein has been shown to induce high levels of pro-inflammatory chemokine IL-8 to inhibit IFN-$\alpha$ thus facilitating the viral replication despite IFN $\alpha/\beta$ 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-$\beta$, IL-6, TNF-$\alpha$, 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

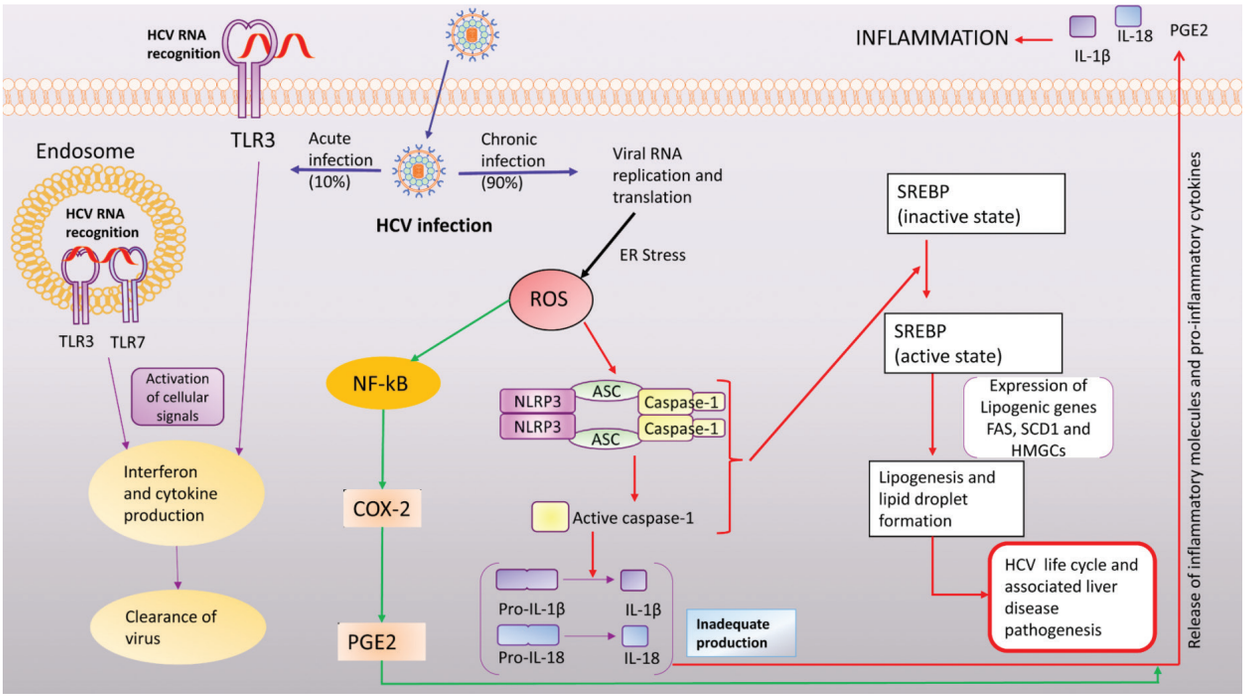


**Figure 3.** HCV-induced inflammasome regulates liver disease pathogenesis.

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-$\alpha$, IL-1, IL-6 and TGF-$\beta$ 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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## References

[1] Nathan C. Points of control in inflammation. Nature. 2002;**420**(6917):846-852

[2] Medzhitov R, Janeway Jr CA. Decoding the patterns of self and nonself by the innate immune system. Science. 2002;**296**(5566):298-300

[3] Janeway Jr CA, Medzhitov R. Innate immune recognition. Annual Review of Immunology. 2002;**20**:197-216

[4] Wilkins C, Gale Jr M. Recognition of viruses by cytoplasmic sensors. Current Opinion in Immunology. 2010;**22**(1):41-47

[5] Kawai T, Akira S. TLR signaling. Seminars in Immunology. 2007;**19**(1):24-32

[6] Kato H, Sato S, Yoneyama M, Yamamoto M, Uematsu S, Matsui K, et al. Cell type-specific involvement of RIG-I in antiviral response. Immunity. 2005;**23**(1):19-28

[7] Ting JP, Lovering RC, Alnemri ES, Bertin J, Boss JM, Davis BK, et al. The NLR gene family: A standard nomenclature. Immunity. 2008;**28**(3):285-287

[8] Roberts TL, Idris A, Dunn JA, Kelly GM, Burnton CM, Hodgson S, et al. HIN-200 proteins regulate caspase activation in response to foreign cytoplasmic DNA. Science. 2009; **323**(5917):1057-1060

[9] Pothlichet J, Meunier I, Davis BK, Ting JP, Skamene E, von Messling V, et al. Type I IFN triggers RIG-I/TLR3/NLRP3-dependent inflammasome activation in influenza A virus infected cells. PLoS Pathogens. 2013;**9**(4):e1003256

[10] Garlanda C, Dinarello CA, Mantovani A. The interleukin-1 family: Back to the future. Immunity. 2013;**39**(6):1003-1018

[11] Martinon F, Tschopp J. Inflammatory caspases and inflammasomes: Master switches of inflammation. Cell Death and Differentiation. 2007;**14**(1):10-22

[12] Martinon F, Burns K, Tschopp J. The inflammasome: A molecular platform triggering activation of inflammatory caspases and processing of proIL-beta. Molecular Cell. 2002; **10**(2):417-426

[13] Brodsky IE, Monack D. NLR-mediated control of inflammasome assembly in the host response against bacterial pathogens. Seminars in Immunology. 2009;**21**(4):199-207

[14] Rathinam VA, Jiang Z, Waggoner SN, Sharma S, Cole LE, Waggoner L, et al. The AIM2 inflammasome is essential for host defense against cytosolic bacteria and DNA viruses. Nature Immunology. 2010;**11**(5):395-402

[15] Burdette D, Haskett A, Presser L, McRae S, Iqbal J, Waris G. Hepatitis C virus activates interleukin-1beta via caspase-1-inflammasome complex. The Journal of General Virology. 2012;**93**(Pt 2):235-246

[16] Hornung V, Ablasser A, Charrel-Dennis M, Bauernfeind F, Horvath G, Caffrey DR, et al. AIM2 recognizes cytosolic dsDNA and forms a caspase-1-activating inflammasome with ASC. Nature. 2009;**458**(7237):514-518

[17] Reinholz M, Kawakami Y, Salzer S, Kreuter A, Dombrowski Y, Koglin S, et al. HPV16 activates the AIM2 inflammasome in keratinocytes. Archives of Dermatological Research. 2013;**305**(8):723-732

[18] Kuriakose T, Kanneganti TD. Regulation and functions of NLRP3 inflammasome during influenza virus infection. Molecular Immunology. 2017;**86**:56-64

[19] Poeck H, Bscheider M, Gross O, Finger K, Roth S, Rebsamen M, et al. Recognition of RNA virus by RIG-I results in activation of CARD9 and inflammasome signaling for interleukin 1 beta production. Nature Immunology. 2010;**11**(1):63-69

[20] Sun P, Fernandez S, Marovich MA, Palmer DR, Celluzzi CM, Boonnak K, et al. Functional characterization of ex vivo blood myeloid and plasmacytoid dendritic cells after infection with dengue virus. Virology. 2009;**383**(2):207-215

[21] Tsai YT, Chang SY, Lee CN, Kao CL. Human TLR3 recognizes dengue virus and modulates viral replication in vitro. Cellular Microbiology. 2009;**11**(4):604-615

[22] da Conceicao TM, Rust NM, Berbel AC, Martins NB, do Nascimento Santos CA, Da Poian AT, et al. Essential role of RIG-I in the activation of endothelial cells by dengue virus. Virology. 2013;**435**(2):281-292

[23] Nasirudeen AM, Wong HH, Thien P, Xu S, Lam KP, Liu DX. RIG-I, MDA5 and TLR3 synergistically play an important role in restriction of dengue virus infection. PLoS Neglected Tropical Diseases. 2011;**5**(1):e926

[24] Zhang L, Wang A. Virus-induced ER stress and the unfolded protein response. Frontiers in Plant Science. 2012;**3**:293

[25] Carroll TP, Greene CM, O'Connor CA, Nolan AM, O'Neill SJ, McElvaney NG. Evidence for unfolded protein response activation in monocytes from individuals with alpha-1 antitrypsin deficiency. Journal of Immunology. 2010;**184**(8):4538-4546

[26] Eizirik DL, Miani M, Cardozo AK. Signalling danger: Endoplasmic reticulum stress and the unfolded protein response in pancreatic islet inflammation. Diabetologia. 2013; **56**(2):234-241

[27] Smith JA. A new paradigm: Innate immune sensing of viruses via the unfolded protein response. Frontiers in Microbiology. 2014;**5**:222

[28] Schreck R, Rieber P, Baeuerle PA. Reactive oxygen intermediates as apparently widely used messengers in the activation of the NF-kappa B transcription factor and HIV-1. The EMBO Journal. 1991;**10**(8):2247-2258

[29] Wang G, Zhang J, Li W, Xin G, Su Y, Gao Y, et al. Apoptosis and proinflammatory cytokine responses of primary mouse microglia and astrocytes induced by human H1N1 and avian H5N1 influenza viruses. Cellular & Molecular Immunology. 2008;**5**(2):113-120

[30] Wang G, Li R, Jiang Z, Gu L, Chen Y, Dai J, et al. Influenza virus induces inflammatory response in mouse primary cortical neurons with limited viral replication. BioMed Research International. 2016;**2016**:8076989

[31] Eliopoulos AG, Stack M, Dawson CW, Kaye KM, Hodgkin L, Sihota S, et al. Epstein-Barr virus-encoded LMP1 and CD40 mediate IL-6 production in epithelial cells via an NF-kappaB pathway involving TNF receptor-associated factors. Oncogene. 1997; **14**(24):2899-2916

[32] Mosialos G, Birkenbach M, Yalamanchili R, VanArsdale T, Ware C, Kieff E. The Epstein-Barr virus transforming protein LMP1 engages signaling proteins for the tumor necrosis factor receptor family. Cell. 1995;**80**(3):389-399

[33] Maggio E, van den Berg A, Diepstra A, Kluiver J, Visser L, Poppema S. Chemokines, cytokines and their receptors in Hodgkin's lymphoma cell lines and tissues. Annals of Oncology. 2002;**13**(Suppl 1):52-56

[34] Punj V, Matta H, Schamus S, Yang T, Chang Y, Chaudhary PM. Induction of CCL20 production by Kaposi sarcoma-associated herpesvirus: Role of viral FLICE inhibitory protein K13-induced NF-kappaB activation. Blood. 2009;**113**(22):5660-5668

[35] Ensoli B, Sturzl M. Kaposi's sarcoma: A result of the interplay among inflammatory cytokines, angiogenic factors and viral agents. Cytokine & Growth Factor Reviews. 1998; **9**(1):63-83

[36] Falasca K, Ucciferri C, Dalessandro M, Zingariello P, Mancino P, Petrarca C, et al. Cytokine patterns correlate with liver damage in patients with chronic hepatitis B and C. Annals of Clinical and Laboratory Science. 2006;**36**(2):144-150

[37] Shukla R, Yue J, Siouda M, Gheit T, Hantz O, Merle P, et al. Proinflammatory cytokine TNF-alpha increases the stability of hepatitis B virus X protein through NF-kappaB signaling. Carcinogenesis. 2011;**32**(7):978-985

[38] Allen IC, Scull MA, Moore CB, Holl EK, McElvania-TeKippe E, Taxman DJ, et al. The NLRP3 inflammasome mediates in vivo innate immunity to influenza A virus through recognition of viral RNA. Immunity. 2009;**30**(4):556-565

[39] McRae S, Iqbal J, Sarkar-Dutta M, Lane S, Nagaraj A, Ali N, et al. The hepatitis C virus-induced NLRP3 inflammasome activates the sterol regulatory element-binding protein

(SREBP) and regulates lipid metabolism. The Journal of Biological Chemistry. 2016; **291**(7):3254-3267

[40] Chen W, Xu Y, Li H, Tao W, Xiang Y, Huang B, et al. HCV genomic RNA activates the NLRP3 inflammasome in human myeloid cells. PLoS One. 2014;**9**(1):e84953

[41] Rajan JV, Rodriguez D, Miao EA, Aderem A. The NLRP3 inflammasome detects encephalomyocarditis virus and vesicular stomatitis virus infection. Journal of Virology. 2011; **85**(9):4167-4172

[42] Lawrence TM, Hudacek AW, de Zoete MR, Flavell RA, Schnell MJ. Rabies virus is recognized by the NLRP3 inflammasome and activates interleukin-1beta release in murine dendritic cells. Journal of Virology 2013;**87**(10):5848-5857

[43] Negash AA, Ramos HJ, Crochet N, Lau DT, Doehle B, Papic N, et al. IL-1beta production through the NLRP3 inflammasome by hepatic macrophages links hepatitis C virus infection with liver inflammation and disease. PLoS Pathogens. 2013;**9**(4):e1003330

[44] Wu DL, Xu GH, Lu SM, Ma BL, Miao NZ, Liu XB, et al. Correlation of AIM2 expression in peripheral blood mononuclear cells from humans with acute and chronic hepatitis B. Human Immunology. 2013;**74**(5):514-521

[45] Saito T, Owen DM, Jiang F, Marcotrigiano J, Gale Jr M. Innate immunity induced by composition-dependent RIG-I recognition of hepatitis C virus RNA. Nature. 2008; **454**(7203):523-527

[46] Loo YM, Fornek J, Crochet N, Bajwa G, Perwitasari O, Martinez-Sobrido L, et al. Distinct RIG-I and MDA5 signaling by RNA viruses in innate immunity. Journal of Virology. 2008;**82**(1):335-345

[47] Ansari MA, Singh VV, Dutta S, Veettil MV, Dutta D, Chikoti L, et al. Constitutive interferon-inducible protein 16-inflammasome activation during Epstein-Barr virus latency I, II, and III in B and epithelial cells. Journal of Virology. 2013;**87**(15):8606-8623

[48] Ansari MA, Dutta S, Veettil MV, Dutta D, Iqbal J, Kumar B, et al. Herpesvirus genome recognition induced acetylation of nuclear IFI16 Is essential for its cytoplasmic translocation, inflammasome and IFN-beta responses. PLoS Pathogens. 2015;**11**(7):e1005019

[49] Johnson KE, Chikoti L, Chandran B. Herpes simplex virus 1 infection induces activation and subsequent inhibition of the IFI16 and NLRP3 inflammasomes. Journal of Virology. 2013;**87**(9):5005-5018

[50] Kufer TA, Fritz JH, Philpott DJ. NACHT-LRR proteins (NLRs) in bacterial infection and immunity. Trends in Microbiology. 2005;**13**(8):381-388

[51] He Y, Hara H, Nunez G. Mechanism and regulation of NLRP3 inflammasome activation. Trends in Biochemical Sciences. 2016;**41**(12):1012-1021

[52] Hornung V, Bauernfeind F, Halle A, Samstad EO, Kono H, Rock KL, et al. Silica crystals and aluminum salts activate the NALP3 inflammasome through phagosomal destabilization. Nature Immunology. 2008;**9**(8):847-856

[53] Cruz CM, Rinna A, Forman HJ, Ventura AL, Persechini PM, Ojcius DM. ATP activates a reactive oxygen species-dependent oxidative stress response and secretion of proinflammatory cytokines in macrophages. The Journal of Biological Chemistry. 2007;**282**(5):2871-2879

[54] Ichinohe T, Pang IK, Iwasaki A. Influenza virus activates inflammasomes via its intracellular M2 ion channel. Nature Immunology. 2010;**11**(5):404-410

[55] Kaushik DK, Gupta M, Kumawat KL, Basu A. NLRP3 inflammasome: Key mediator of neuroinflammation in murine Japanese encephalitis. PLoS One. 2012;**7**(2):e32270

[56] Ermler ME, Traylor Z, Patel K, Schattgen SA, Vanaja SK, Fitzgerald KA, et al. Rift Valley fever virus infection induces activation of the NLRP3 inflammasome. Virology. 2014;**449**:174-180

[57] Muruve DA, Petrilli V, Zaiss AK, White LR, Clark SA, Ross PJ, et al. The inflammasome recognizes cytosolic microbial and host DNA and triggers an innate immune response. Nature. 2008;**452**(7183):103-107

[58] Nour AM, Reichelt M, Ku CC, Ho MY, Heineman TC, Arvin AM. Varicella-zoster virus infection triggers formation of an interleukin-1beta (IL-1beta)-processing inflammasome complex. The Journal of Biological Chemistry. 2011;**286**(20):17921-17933

[59] Askari A, Nosratabadi R, Khaleghinia M, Zainodini N, Kennedy D, Shabani Z, et al. Evaluation of NLRC4, NLRP1, and NLRP3, as components of inflammasomes, in chronic hepatitis B virus-infected patients. Viral Immunology. 2016;**29**(9):496-501

[60] Yu X, Lan P, Hou X, Han Q, Lu N, Li T, et al. HBV inhibits LPS-induced NLRP3 inflammasome activation and IL-1beta production via suppressing the NF-kappaB pathway and ROS production. Journal of Hepatology. 2017;**66**(4):693-702

[61] Kolakofsky D, Kowalinski E, Cusack S. A structure-based model of RIG-I activation. RNA. 2012;**18**(12):2118-2127

[62] Yoneyama M, Kikuchi M, Natsukawa T, Shinobu N, Imaizumi T, Miyagishi M, et al. The RNA helicase RIG-I has an essential function in double-stranded RNA-induced innate antiviral responses. Nature Immunology. 2004;**5**(7):730-737

[63] Fernandes-Alnemri T, Yu JW, Juliana C, Solorzano L, Kang S, Wu J, et al. The AIM2 inflammasome is critical for innate immunity to *Francisella tularensis*. Nature Immunology. 2010;**11**(5):385-393

[64] Pan X, Xu H, Zheng C, Li M, Zou X, Cao H, et al. Human hepatocytes express absent in melanoma 2 and respond to hepatitis B virus with interleukin-18 expression. Virus Genes. 2016;**52**(4):445-452

[65] Choubey D. Interferon-inducible Ifi200-family genes as modifiers of lupus susceptibility. Immunology Letters. 2012;**147**(1-2):10-17

[66] Uchida K, Akita Y, Matsuo K, Fujiwara S, Nakagawa A, Kazaoka Y, et al. Identification of specific autoantigens in Sjogren's syndrome by SEREX. Immunology. 2005;**116**(1):53-63

[67] Mondini M, Vidali M, Airo P, De Andrea M, Riboldi P, Meroni PL, et al. Role of the interferon-inducible gene IFI16 in the etiopathogenesis of systemic autoimmune disorders. Annals of the New York Academy of Sciences. 2007;**1110**:47-56

[68] Kerur N, Veettil MV, Sharma-Walia N, Bottero V, Sadagopan S, Otageri P, et al. IFI16 acts as a nuclear pathogen sensor to induce the inflammasome in response to Kaposi Sarcoma-associated herpesvirus infection. Cell Host & Microbe. 2011;**9**(5):363-375

[69] Dutta D, Dutta S, Veettil MV, Roy A, Ansari MA, Iqbal J, et al. BRCA1 regulates IFI16 mediated nuclear innate sensing of herpes viral DNA and subsequent induction of the innate inflammasome and interferon-beta responses. PLoS Pathogens. 2015;**11**(6):e1005030

[70] Wang J, Alexander J, Wiebe M, Jones C. Bovine herpesvirus 1 productive infection stimulates inflammasome formation and caspase 1 activity. Virus Research. 2014;**185**:72-76

[71] Chevaliez S, Pawlotsky JM. HCV genome and life cycle. In: Tan SL, editor. Hepatitis C Viruses: Genomes and Molecular Biology. Norfolk (UK): Horizon Bioscience; 2006

[72] MacCallum PR, Jack SC, Egan PA, McDermott BT, Elliott RM, Chan SW. Cap-dependent and hepatitis C virus internal ribosome entry site-mediated translation are modulated by phosphorylation of eIF2alpha under oxidative stress. The Journal of General Virology. 2006;**87**(Pt 11):3251-3262

[73] Jaafar ZA, Oguro A, Nakamura Y, Kieft JS. Translation initiation by the hepatitis C virus IRES requires eIF1A and ribosomal complex remodeling. eLife. 2016;**5**:e21198

[74] Bradrick SS, Walters RW, Gromeier M. The hepatitis C virus 3'-untranslated region or a poly(A) tract promote efficient translation subsequent to the initiation phase. Nucleic Acids Research. 2006;**34**(4):1293-1303

[75] Appel N, Schaller T, Penin F, Bartenschlager R. From structure to function: New insights into hepatitis C virus RNA replication. The Journal of Biological Chemistry. 2006;**281**(15):9833-9836

[76] Pradere JP, Kluwe J, De Minicis S, Jiao JJ, Gwak GY, Dapito DH, et al. Hepatic macrophages but not dendritic cells contribute to liver fibrosis by promoting the survival of activated hepatic stellate cells in mice. Hepatology. 2013;**58**(4):1461-1473

[77] Tanaka H, Fujita N, Sugimoto R, Urawa N, Horiike S, Kobayashi Y, et al. Hepatic oxidative DNA damage is associated with increased risk for hepatocellular carcinoma in chronic hepatitis C. British Journal of Cancer. 2008;**98**(3):580-586

[78] Di Bisceglie AM. Natural history of hepatitis C: Its impact on clinical management. Hepatology. 2000;**31**(4):1014-1018

[79] Nunez O, Fernandez-Martinez A, Majano PL, Apolinario A, Gomez-Gonzalo M, Benedicto I, et al. Increased intrahepatic cyclooxygenase 2, matrix metalloproteinase 2, and matrix metalloproteinase 9 expression is associated with progressive liver disease in chronic hepatitis C virus infection: Role of viral core and NS5A proteins. Gut. 2004; **53**(11):1665-1672

[80] Huang YS, Hwang SJ, Chan CY, Wu JC, Chao Y, Chang FY, et al. Serum levels of cytokines in hepatitis C-related liver disease: A longitudinal study. Zhonghua Yi Xue Za Zhi (Taipei). 1999;**62**(6):327-333

[81] Matsuzaki K. Modulation of TGF-beta signaling during progression of chronic liver diseases. Frontiers in Bioscience (Landmark Ed). 2009;(14):2923-2934

[82] Okumoto K, Hattori E, Tamura K, Kiso S, Watanabe H, Saito K, et al. Possible contribution of circulating transforming growth factor-beta 1 to immunity and prognosis in unresectable hepatocellular carcinoma. Liver International. 2004;**24**(1):21-28

[83] Teicher BA. Malignant cells, directors of the malignant process: Role of transforming growth factor-beta. Cancer Metastasis Reviews. 2001;**20**(1-2):133-143

[84] Gong G, Waris G, Tanveer R, Siddiqui A. Human hepatitis C virus NS5A protein alters intracellular calcium levels, induces oxidative stress, and activates STAT-3 and NF-kappa B. Proceedings of the National Academy of Sciences of the United States of America. 2001;**98**(17):9599-9604

[85] Qadri I, Iwahashi M, Capasso JM, Hopken MW, Flores S, Schaack J, et al. Induced oxidative stress and activated expression of manganese superoxide dismutase during hepatitis C virus replication: Role of JNK, p38 MAPK and AP-1. Biochemical Journal. 2004;**378**(Pt 3):919-928

[86] Moriya K, Fujie H, Shintani Y, Yotsuyanagi H, Tsutsumi T, Ishibashi K, et al. The core protein of hepatitis C virus induces hepatocellular carcinoma in transgenic mice. Nature Medicine. 1998;**4**(9):1065-1067

[87] Okuda M, Li K, Beard MR, Showalter LA, Scholle F, Lemon SM, et al. Mitochondrial injury, oxidative stress, and antioxidant gene expression are induced by hepatitis C virus core protein. Gastroenterology. 2002;**122**(2):366-375

[88] Gotoh M, Sakamoto M, Kanetaka K, Chuuma M, Hirohashi S. Overexpression of osteopontin in hepatocellular carcinoma. Pathology International. 2002;**52**(1):19-24

[89] Patouraux S, Bonnafous S, Voican CS, Anty R, Saint-Paul MC, Rosenthal-Allieri MA, et al. The osteopontin level in liver, adipose tissue and serum is correlated with fibrosis in patients with alcoholic liver disease. PLoS One. 2012;**7**(4):e35612

[90] Iqbal J, McRae S, Banaudha K, Mai T, Waris G. Mechanism of hepatitis C virus (HCV)-induced osteopontin and its role in epithelial to mesenchymal transition of hepatocytes. The Journal of Biological Chemistry. 2013;**288**(52):36994-37009

[91] Iqbal J, McRae S, Mai T, Banaudha K, Sarkar-Dutta M, Waris G. Role of hepatitis C virus induced osteopontin in epithelial to mesenchymal transition, migration and invasion of hepatocytes. PLoS One. 2014;**9**(1):e87464

[92] Basu A, Meyer K, Lai KK, Saito K, Di Bisceglie AM, Grosso LE, et al. Microarray analyses and molecular profiling of Stat3 signaling pathway induced by hepatitis C virus core protein in human hepatocytes. Virology. 2006;**349**(2):347-358

[93] Nishitsuji H, Funami K, Shimizu Y, Ujino S, Sugiyama K, Seya T, et al. Hepatitis C virus infection induces inflammatory cytokines and chemokines mediated by the cross talk between hepatocytes and stellate cells. Journal of Virology. 2013;**87**(14):8169-8178

[94] Hosomura N, Kono H, Tsuchiya M, Ishii K, Ogiku M, Matsuda M, et al. HCV-related proteins activate Kupffer cells isolated from human liver tissues. Digestive Diseases and Sciences. 2011;**56**(4):1057-1064

[95] Polyak SJ, Khabar KS, Rezeiq M, Gretch DR. Elevated levels of interleukin-8 in serum are associated with hepatitis C virus infection and resistance to interferon therapy. Journal of Virology. 2001;**75**(13):6209-6211

[96] Li K, Foy E, Ferreon JC, Nakamura M, Ferreon AC, Ikeda M, et al. Immune evasion by hepatitis C virus NS3/4A protease-mediated cleavage of the Toll-like receptor 3 adaptor protein TRIF. Proceedings of the National Academy of Sciences of the United States of America. 2005;**102**(8):2992-2997

[97] Spanakis NE, Garinis GA, Alexopoulos EC, Patrinos GP, Menounos PG, Sklavounou A, et al. Cytokine serum levels in patients with chronic HCV infection. Journal of Clinical Laboratory Analysis. 2002;**16**(1):40-46

[98] Ramadori G, Saile B. Inflammation, damage repair, immune cells, and liver fibrosis: Specific or nonspecific, this is the question. Gastroenterology. 2004;**127**(3):997-1000

[99] Antonelli A, Ferri C, Ferrari SM, Ghiri E, Goglia F, Pampana A, et al. Serum levels of proinflammatory cytokines interleukin-1beta, interleukin-6, and tumor necrosis factor alpha in mixed cryoglobulinemia. Arthritis and Rheumatism. 2009;**60**(12):3841-3847

[100] Basha HI, Subramanian V, Seetharam A, Nath DS, Ramachandran S, Anderson CD, et al. Characterization of HCV-specific CD4+Th17 immunity in recurrent hepatitis C-induced liver allograft fibrosis. American Journal of Transplantation. 2011;**11**(4):775-785

[101] Kasprzak A, Zabel M, Biczysko W, Wysocki J, Adamek A, Spachacz R, et al. Expression of cytokines (TNF-alpha, IL-1alpha, and IL-2) in chronic hepatitis C: Comparative hybridocytochemical and immunocytochemical study in children and adult patients. The Journal of Histochemistry and Cytochemistry. 2004;**52**(1):29-38

[102] Moschen AR, Fritz T, Clouston AD, Rebhan I, Bauhofer O, Barrie HD, et al. Interleukin-32: A new proinflammatory cytokine involved in hepatitis C virus-related liver inflammation and fibrosis. Hepatology. 2011;**53**(6):1819-1829

[103] Knolle PA, Gerken G. Local control of the immune response in the liver. Immunological Reviews. 2000;**174**:21-34

[104] Waris G, Siddiqui A. Hepatitis C virus stimulates the expression of cyclooxygenase-2 via oxidative stress: Role of prostaglandin E2 in RNA replication. Journal of Virology. 2005;**79**(15):9725-9734

[105] Choi J, Lee KJ, Zheng Y, Yamaga AK, Lai MM, Ou JH. Reactive oxygen species suppress hepatitis C virus RNA replication in human hepatoma cells. Hepatology. 2004; **39**(1):81-89

[106] Bowman T, Garcia R, Turkson J, Jove R. STATs in oncogenesis. Oncogene. 2000;**19**(21): 2474-2488

[107] Waris G, Turkson J, Hassanein T, Siddiqui A. Hepatitis C virus (HCV) constitutively activates STAT-3 via oxidative stress: Role of STAT-3 in HCV replication. Journal of Virology. 2005;**79**(3):1569-1580

[108] Poli G. Pathogenesis of liver fibrosis: Role of oxidative stress. Molecular Aspects of Medicine. 2000;**21**(3):49-98

[109] Vidali M, Tripodi MF, Ivaldi A, Zampino R, Occhino G, Restivo L, et al. Interplay between oxidative stress and hepatic steatosis in the progression of chronic hepatitis C. Journal of Hepatology. 2008;**48**(3):399-406

[110] Presser LD, McRae S, Waris G. Activation of TGF-beta1 promoter by hepatitis C virus-induced AP-1 and Sp1: Role of TGF-beta1 in hepatic stellate cell activation and invasion. PLoS One. 2013;**8**(2):e56367

[111] Szabo G, Chang S, Dolganiuc A. Altered innate immunity in chronic hepatitis C infection: Cause or effect? Hepatology. 2007;**46**(4):1279-1290

[112] Wang Y, Li J, Wang X, Ye L, Zhou Y, Thomas RM, et al. Hepatitis C virus impairs TLR3 signaling and inhibits IFN-lambda 1 expression in human hepatoma cell line. Innate Immunity. 2014;**20**(1):3-11

[113] Wang N, Liang Y, Devaraj S, Wang J, Lemon SM, Li K. Toll-like receptor 3 mediates establishment of an antiviral state against hepatitis C virus in hepatoma cells. Journal of Virology. 2009;**83**(19):9824-9834

[114] Loo YM, Owen DM, Li K, Erickson AK, Johnson CL, Fish PM, et al. Viral and therapeutic control of IFN-beta promoter stimulator 1 during hepatitis C virus infection. Proceedings of the National Academy of Sciences of the United States of America. 2006; **103**(15):6001-6006

[115] Saito T, Hirai R, Loo YM, Owen D, Johnson CL, Sinha SC, et al. Regulation of innate antiviral defenses through a shared repressor domain in RIG-I and LGP2. Proceedings of the National Academy of Sciences of the United States of America. 2007;**104**(2):582-587

[116] Liu HM, Loo YM, Horner SM, Zornetzer GA, Katze MG, Gale Jr M. The mitochondrial targeting chaperone 14-3-3epsilon regulates a RIG-I translocon that mediates membrane association and innate antiviral immunity. Cell Host & Microbe. 2012;**11**(5):528-537

[117] Loo YM, Gale Jr M. Immune signaling by RIG-I-like receptors. Immunity. 2011;**34**(5): 680-692

[118] Gack MU, Shin YC, Joo CH, Urano T, Liang C, Sun L, et al. TRIM25 RING-finger E3 ubiquitin ligase is essential for RIG-I-mediated antiviral activity. Nature. 2007; **446**(7138):916-920

[119] Baril M, Racine ME, Penin F, Lamarre D. MAVS dimer is a crucial signaling component of innate immunity and the target of hepatitis C virus NS3/4A protease. Journal of Virology. 2009;**83**(3):1299-1311

[120] Horner SM, Liu HM, Park HS, Briley J, Gale Jr M. Mitochondrial-associated endoplasmic reticulum membranes (MAM) form innate immune synapses and are targeted by hepatitis C virus. Proceedings of the National Academy of Sciences of the United States of America. 2011;**108**(35):14590-14595

[121] Csak T, Ganz M, Pespisa J, Kodys K, Dolganiuc A, Szabo G. Fatty acid and endotoxin activate inflammasomes in mouse hepatocytes that release danger signals to stimulate immune cells. Hepatology. 2011;**54**(1):133-144

[122] Boaru SG, Borkham-Kamphorst E, Tihaa L, Haas U, Weiskirchen R. Expression analysis of inflammasomes in experimental models of inflammatory and fibrotic liver disease. Journal of Inflammation (Lond). 2012;**9**(1):49

[123] Shrivastava S, Mukherjee A, Ray R, Ray RB. Hepatitis C virus induces interleukin-1beta (IL-1beta)/IL-18 in circulatory and resident liver macrophages. Journal of Virology. 2013;**87**(22):12284-12290

[124] Sun Q, Gao W, Loughran P, Shapiro R, Fan J, Billiar TR, et al. Caspase 1 activation is protective against hepatocyte cell death by up-regulating beclin 1 protein and mitochondrial autophagy in the setting of redox stress. The Journal of Biological Chemistry. 2013;**288**(22):15947-15958

[125] Taxman DJ, Holley-Guthrie EA, Huang MT, Moore CB, Bergstralh DT, Allen IC, et al. The NLR adaptor ASC/PYCARD regulates DUSP10, mitogen-activated protein kinase (MAPK), and chemokine induction independent of the inflammasome. The Journal of Biological Chemistry. 2011;**286**(22):19605-19616

[126] Li YF, Nanayakkara G, Sun Y, Li X, Wang L, Cueto R, et al. Analyses of caspase-1-regulated transcriptomes in various tissues lead to identification of novel IL-1beta-, IL-18- and sirtuin-1-independent pathways. Journal of Hematology & Oncology. 2017;**10**(1):40

[127] Guarda G, Braun M, Staehli F, Tardivel A, Mattmann C, Forster I, et al. Type I interferon inhibits interleukin-1 production and inflammasome activation. Immunity. 2011; **34**(2):213-223

[128] Marcucci F, Mele A. Hepatitis viruses and non-Hodgkin lymphoma: Epidemiology, mechanisms of tumorigenesis, and therapeutic opportunities. Blood. 2011;**117**(6):1792-1798

[129] Ishizaka N, Ishizaka Y, Takahashi E, Tooda E, Hashimoto H, Nagai R, et al. Association between hepatitis C virus seropositivity, carotid-artery plaque, and intima-media thickening. Lancet. 2002;**359**(9301):133-135

[130] Adinolfi LE, Restivo L, Zampino R, Guerrera B, Lonardo A, Ruggiero L, et al. Chronic HCV infection is a risk of atherosclerosis. Role of HCV and HCV-related steatosis. Atherosclerosis. 2012;**221**(2):496-502

[131] Tillmann HL. Hepatitis C virus infection and the brain. Metabolic Brain Disease. 2004; **19**(3-4):351-356

[132] Chen KJ, Tseng CK, Chang FR, Yang JI, Yeh CC, Chen WC, et al. Aqueous extract of the edible Gracilaria tenuistipitata inhibits hepatitis C viral replication via cyclooxygenase-2 suppression and reduces virus-induced inflammation. PLoS One. 2013;**8**(2):e57704

[133] Wang Y, Gao J, Zhang D, Zhang J, Ma J, Jiang H. New insights into the antifibrotic effects of sorafenib on hepatic stellate cells and liver fibrosis. Journal of Hepatology. 2010;**53**(1):132-144

[134] Cohen-Naftaly M, Friedman SL. Current status of novel antifibrotic therapies in patients with chronic liver disease. Therapeutic Advances in Gastroenterology. 2011;**4**(6):391-417

[135] Sandler NG, Koh C, Roque A, Eccleston JL, Siegel RB, Demino M, et al. Host response to translocated microbial products predicts outcomes of patients with HBV or HCV infection. Gastroenterology. 2011;**141**(4):1220-1230. (e1-e3)

[136] Dammacco F, Tucci FA, Lauletta G, Gatti P, De Re V, Conteduca V, et al. Pegylated interferon-alpha, ribavirin, and rituximab combined therapy of hepatitis C virus-related mixed cryoglobulinemia: A long-term study. Blood. 2010;**116**(3):343-353

[137] Dinarello CA. A clinical perspective of IL-1beta as the gatekeeper of inflammation. European Journal of Immunology. 2011;**41**(5):1203-1217

[138] Uribe M, Go VL. Corticosteroid pharmacokinetics in liver disease. Clinical Pharmacokinetics. 1979;**4**(3):233-240

[139] Malekzadeh R, Nasseri-Moghaddam S, Kaviani MJ, Taheri H, Kamalian N, Sotoudeh M. Cyclosporin A is a promising alternative to corticosteroids in autoimmune hepatitis. Digestive Diseases and Sciences. 2001;**46**(6):1321-1327

[140] Johnson PJ, McFarlane IG, Williams R. Azathioprine for long-term maintenance of remission in autoimmune hepatitis. The New England Journal of Medicine. 1995; **333**(15):958-963

[141] Manns MP, Woynarowski M, Kreisel W, Lurie Y, Rust C, Zuckerman E, et al. Budesonide induces remission more effectively than prednisone in a controlled trial of patients with autoimmune hepatitis. Gastroenterology. 2010;**139**(4):1198-1206

[142] Larsen FS, Vainer B, Eefsen M, Bjerring PN, Adel Hansen B. Low-dose tacrolimus ameliorates liver inflammation and fibrosis in steroid refractory autoimmune hepatitis. World Journal of Gastroenterology. 2007;**13**(23):3232-3236

[143] Tacke F. Cenicriviroc for the treatment of non-alcoholic steatohepatitis and liver fibrosis. Expert Opinion on Investigational Drugs. 2018;**27**(3):301-311

[144] Rice LM, Padilla CM, McLaughlin SR, Mathes A, Ziemek J, Goummih S, et al. Fresolimumab treatment decreases biomarkers and improves clinical symptoms in systemic sclerosis patients. The Journal of Clinical Investigation. 2015;**125**(7):2795-2807

[145] Bansal R, Nagorniewicz B, Prakash J. Clinical advancements in the targeted therapies against liver fibrosis. Mediators of Inflammation. 2016;**2016**:7629724

[146] Kessoku T, Imajo K, Honda Y, Kato T, Ogawa Y, Tomeno W, et al. Resveratrol ameliorates fibrosis and inflammation in a mouse model of nonalcoholic steatohepatitis. Scientific Reports. 2016;**6**:22251

[147] Burmester GR, Panaccione R, Gordon KB, McIlraith MJ, Lacerda AP. Adalimumab: Long-term safety in 23 458 patients from global clinical trials in rheumatoid arthritis,

juvenile idiopathic arthritis, ankylosing spondylitis, psoriatic arthritis, psoriasis and Crohn's disease. Annals of the Rheumatic Diseases. 2013;**72**(4):517-524

[148] Tindall E. Celecoxib for the treatment of pain and inflammation: The preclinical and clinical results. The Journal of the American Osteopathic Association. 1999;**99** (11_suppl):S13-SS7

[149] Ko WK, Lee SH, Kim SJ, Jo MJ, Kumar H, Han IB, et al. Anti-inflammatory effects of ursodeoxycholic acid by lipopolysaccharide-stimulated inflammatory responses in RAW 264.7 macrophages. PLoS One. 2017;**12**(6):e0180673

[150] Liang TJ, Yuan JH, Tan YR, Ren WH, Han GQ, Zhang J, et al. Effect of ursodeoxycholic acid on TGF beta1/Smad signaling pathway in rat hepatic stellate cells. Chinese Medical Journal. 2009;**122**(10):1209-1213

[151] Hernandez E, Correa A, Bucio L, Souza V, Kershenobich D, Gutierrez-Ruiz MC. Pentoxifylline diminished acetaldehyde-induced collagen production in hepatic stellate cells by decreasing interleukin-6 expression. Pharmacological Research. 2002; **46**(5):435-443

[152] Austin AS, Mahida YR, Clarke D, Ryder SD, Freeman JG. A pilot study to investigate the use of oxpentifylline (pentoxifylline) and thalidomide in portal hypertension secondary to alcoholic cirrhosis. Alimentary Pharmacology & Therapeutics. 2004;**19**(1):79-88

[153] Raetsch C, Jia JD, Boigk G, Bauer M, Hahn EG, Riecken EO, et al. Pentoxifylline downregulates profibrogenic cytokines and procollagen I expression in rat secondary biliary fibrosis. Gut. 2002;**50**(2):241-247