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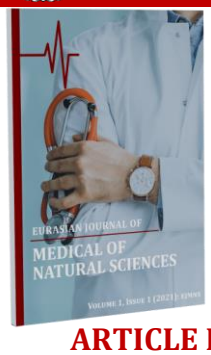

HEPATITIS C VIRUS: MECHANISMS TO ESCAPE CELLULAR INNATE IMMUNE RESPONSES

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ABSTRACT

Hepatitis C virus (HCV) infections are a significant global health concern, representing the primary cause of chronic hepatitis and liver disease. While current treatments can be effective, they are costly and often come with adverse side effects, highlighting the pressing need for new therapeutic strategies and a preventive vaccine. A deeper understanding of the interactions between HCV and host cells is critical for elucidating disease progression and may reveal novel cellular targets for antiviral drug development. This review examines the relationship between HCV and the host cell's innate immune system, detailing the cellular pathways activated in response to infection and the strategies employed by the virus to evade these intracellular defenses.

Introduction

Hepatitis C virus (HCV) remains one of the most prevalent infectious agents worldwide, significantly contributing to liver-related illness and mortality and posing a major global public health challenge. Although current treatments can cure over 95% of diagnosed cases, access to medical care is often limited, and no effective vaccine exists to prevent new infections. Furthermore, since acute HCV infection is usually asymptomatic, nearly 70% of patients progress to chronic infection, which may ultimately lead to chronic hepatitis, liver fibrosis, cirrhosis, and even hepatocellular carcinoma.

HCV, a member of the Hepacivirus genus within the Flaviviridae family, is classified into eight genotypes that vary in geographic distribution, clinical symptoms, and response to therapy. Previously, treatment commonly relied on a combination of pegylated interferon-α (Peg-IFN-α) and ribavirin. In this regimen, Peg-IFN-α induces an antiviral state in host cells, while ribavirin works synergistically—modulating interferon-stimulated gene expression and promoting error catastrophe through mutagen incorporation. However, this treatment often led to significant side effects. Today, direct-acting antiviral agents (DAAs) have largely replaced older therapies. These agents, which target different viral proteins and are used in combination to optimize viral clearance, are grouped into three main classes. Early DAAs, such as protease inhibitors targeting the NS3-4A complex, were most effective when used with interferons, particularly in patients with genotype 1 infections. Newer DAAs targeting NS3-4A now offer broader genotype coverage, while drugs aimed at NS5A provide extensive

coverage despite some issues with resistance. Additionally, DAAs targeting NS5B are divided into nucleoside and non-nucleoside analogues, with nucleoside analogues being especially potent and broadly effective, and possessing a high barrier to resistance. Despite the effectiveness of these treatments, the elimination of HCV is unlikely to be achieved through therapy alone, and the virus's extensive genetic variability continues to complicate vaccine development.

Upon entering host cells, HCV rapidly activates the intracellular innate immune response. This activation is mediated by pattern recognition receptors (PRRs) that detect key viral components, or pathogen-associated molecular patterns (PAMPs). These receptors, which can be found in the cytosol, attached to cellular membranes, or secreted into bodily fluids, primarily recognize viral nucleic acids with distinct signatures that distinguish non-self from self. The engagement of these PAMPs triggers signaling cascades that culminate in the production of interferons (IFNs) and interferon-stimulated genes (ISGs), which interfere with multiple stages of the viral life cycle.

Understanding the methods by which HCV circumvents these cellular defenses is critical for improving current treatments, mitigating liver disease, and developing innovative antiviral therapies. In this review, we examine the interplay between HCV and the host's intracellular innate immune system, emphasizing the virus's evasion strategies and discussing their implications for infection outcomes and disease pathogenesis.

Hepatitis C Virus

HCV is an enveloped, positive-sense single-stranded RNA virus that mainly targets hepatocytes in humans and chimpanzees. It can also infect other cell types, including B cells and dendritic cells. The virus's genome is approximately 9.6 kilobases long and consists of a single open reading frame that encodes a polyprotein of around 3000 amino acids, flanked by 5' and 3' untranslated regions.

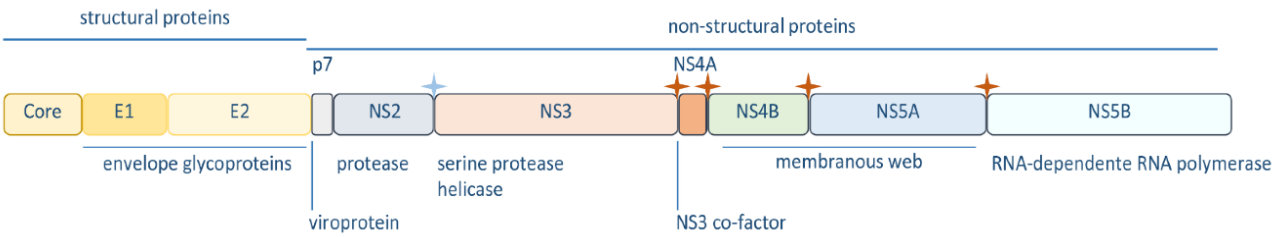

Figure 1. Schematic representation of the hepatitis C virus (HCV) polyprotein referring to the individual proteins and their relevance for the HCV life cycle. The stars indicate cleavage sites of auto-proteases—blue star: NS2-3; red star: NS3-4A. N-terminal processing is accomplished by host proteases.

NS4B expression causes modifications in intracellular membranes, resulting in the formation of sponge-like structures closely linked to the rough endoplasmic reticulum. This network, known as the membranous web, serves as the site for viral genome replication. Meanwhile, NS5B functions as the RNA-dependent RNA polymerase responsible for generating a complementary negative-strand RNA intermediate, which then acts as a template for amplifying the viral positive-strand genome. Due to the lack of proofreading activity in

NS5B, replication is prone to numerous nucleotide substitutions, leading to a high mutation rate. As a result, infected individuals harbor a diverse collection of viral microvariants surrounding a dominant master sequence, commonly referred to as quasispecies. The presence of these quasispecies is associated with various biological properties, such as altered tissue tropism and carcinogenic potential, and has been linked to differences in treatment outcomes, including resistance to interferon and direct-acting antivirals.

HCV viral particle assembly is intricately linked to cellular lipid metabolism. Once the core protein undergoes post-translational modifications, it is transported to lipid droplets (LDs), prompting its relocation to a perinuclear area. These lipid droplets then migrate closer to the endoplasmic reticulum (ER) and the membranous webs—sites where viral genome replication occurs. This proximity enables the core protein to interact with the viral genome, thereby promoting nucleocapsid formation. Current models of virus envelopment propose that nucleocapsids enter the ER lumen, where they acquire their lipid envelope and viral glycoproteins via integration into the very low-density lipoprotein (VLDL) pathway. Finally, HCV virions are released through the endosomal secretory pathway, a process regulated by the endosomal sorting complex required for transport (ESCRT), and this occurs without inducing cell lysis.

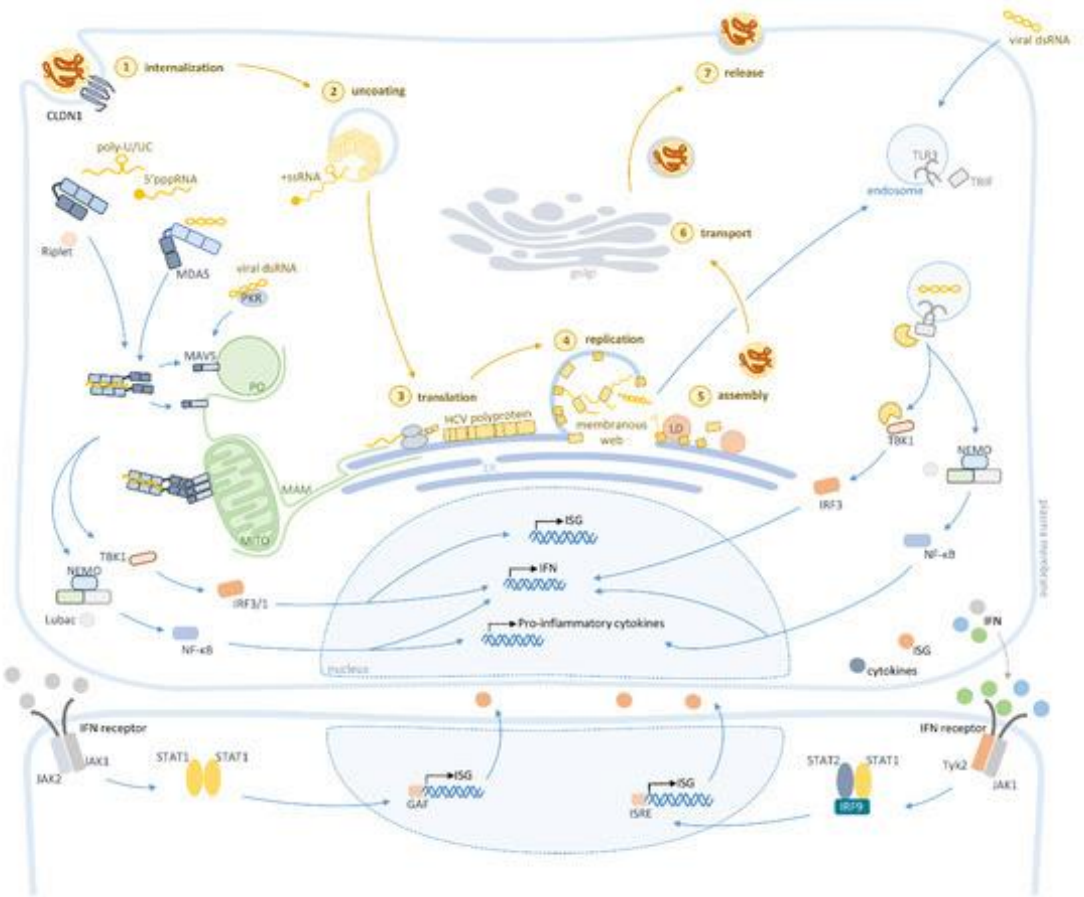

Figure 2. HCV first attaches to cellular receptors and is internalized via endocytosis (1). Following entry, uncoating occurs, releasing the viral genome into the cytoplasm (2). At the endoplasmic reticulum (ER), the viral RNA is translated into a polyprotein that is

subsequently cleaved—both co- and post-translationally—into 10 distinct viral proteins (3). The expression of these proteins drives the formation of a membranous web, an essential structure for viral replication (4). HCV then exploits the host's lipid transport system for viral assembly (5) and uses the endosomal secretory pathway to reach the plasma membrane, where virions are released (6-7). Concurrently, intracellular sensors such as RIG-I, MDA5, and PKR, along with the membrane-bound TLR3, detect viral RNA and trigger downstream signaling that leads to the production of interferons and proinflammatory cytokines.

Activation of Intracellular Innate Immunity by HCV

HCV infection is primarily detected by cytosolic pattern recognition receptors (PRRs) of the RIG-I-like receptor (RLR) family—DExD/H-box helicases that identify various forms of viral RNA. Additionally, viral RNA is recognized by TLR3, an endosomal membrane receptor from the type I transmembrane receptor family that detects a broad array of pathogen-associated molecular patterns.

Upon HCV infection, RIG-I is rapidly triggered by the detection of 5′-triphosphate RNA and poly-U/UC sequences located in the 3′ untranslated region of the viral genome. The role of MDA5 in sensing HCV RNA remains a subject of debate; while some studies suggest that MDA5 contributes more robustly to RLR signaling than RIG-I, others indicate that MDA5 is activated predominantly by the double-stranded RNA replication products generated during infection. Regardless, both RIG-I and MDA5 bind the viral RNA backbone and activate the mitochondrial antiviral signaling protein (MAVS) via their CARD domains. This binding induces a conformational change in MAVS, leading to the formation of stable, prion-like aggregates that amplify the antiviral signal by recruiting additional MAVS molecules. The subsequent polymerization of MAVS attracts tumor necrosis factor receptor-associated factors (TRAF2, TRAF5, and TRAF6), which are essential for activating the TBK1 and IκB kinase (IKK) complexes. These kinases then phosphorylate IRF3 and NF-κB; phosphorylated IRF3 dimerizes and moves into the nucleus, where it promotes the expression of interferons, cytokines, and interferon-stimulated genes (ISGs).

Later in the infection, TLR3 becomes activated by recognizing double-stranded RNA intermediates that accumulate during HCV replication. Once engaged, TLR3 signals through the adaptor protein TRIF, leading to the activation of IRF3 and NF-κB and, consequently, the production of both interferons and inflammatory cytokines.

The signaling cascades initiated by RIG-I and TLR3 ultimately converge on the secretion of interferons, which, through autocrine and paracrine mechanisms, activate the JAK/STAT pathway. This activation induces the expression of a range of ISGs—including RIG-I, PKR, OAS, MHC class I, and others—that contribute to the antiviral response.

In the canonical pathway, interferons bind to their respective receptors, leading to the phosphorylation of associated JAK kinases. These kinases then phosphorylate specific STAT proteins: type II interferons trigger the formation of STAT1 homodimers (gamma interferon-activated factor, GAF), which stimulate ISGs containing gamma interferon-activated sites (GAS), while type I and type III interferons promote the heterodimerization of STAT1 and STAT2. Together with IRF9, these form the IFN-stimulated gene factor 3 (ISGF3) complex, which drives the expression of ISGs harboring interferon-stimulated response elements

(ISREs). Recent findings have also highlighted a more intricate, non-canonical regulation of the JAK/STAT pathway.

Innate Immune Evasion Mediated by HCV

Antiviral defenses against HCV are activated almost immediately upon infection, even before the virus has undergone extensive replication. During acute HCV infection, and even when initial viremia is high, the virus can sometimes be spontaneously cleared, emphasizing the critical role of a rapid response by pattern recognition receptors and the innate immune system. However, about 70% of HCV-infected individuals fail to control the virus, leading to chronic infection, which indicates that the virus can effectively disrupt host antiviral defenses.

HCV produces a relatively small number of proteins, each of which serves multiple functions during infection. In addition to their essential roles in viral replication, proteins such as core, E2, NS3-4A, and NS5A are also key factors in the virus's ability to evade the immune response, as depicted in Figure 3.

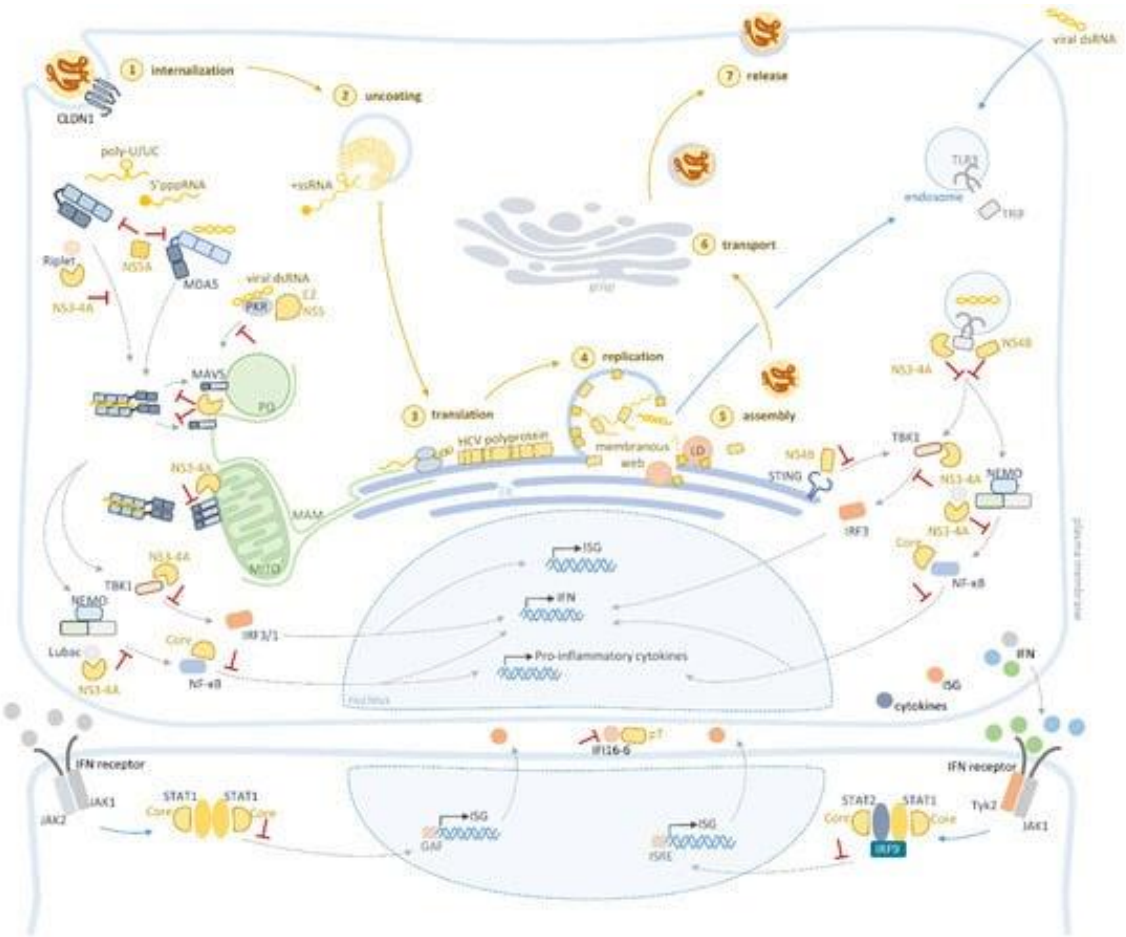

Figure 3. HCV proteins—including NS3-4A, E2, NS5A, NS4B, p7, and core—interfere with various steps in the signaling pathways of pattern recognition receptors that detect HCV. By downregulating the production of interferons, proinflammatory cytokines, and interferon-stimulated genes, these viral proteins effectively inhibit the intracellular antiviral response.

4.1. NS3-4A The HCV NS3-4A protease is a complex comprising the serine protease NS3 and the cofactor NS4A, which contains a transmembrane domain that anchors NS3 to cellular

membranes and facilitates the complex's dimerization (see Figure 1). In addition to its pivotal roles in viral replication and assembly, NS3-4A is crucial for evading the host's antiviral defenses.

NS3-4A is widely recognized for cleaving MAVS at the membranes of mitochondria, peroxisomes, and mitochondrial-associated membranes. This cleavage releases the cytoplasmic domain of MAVS, disrupting downstream signal transduction and leading to reduced expression of interferons (IFNs) and interferon-stimulated genes (ISGs). Clinical observations in HCV-infected livers have confirmed that patients with cleaved MAVS exhibit lower IFN levels.

Furthermore, NS3-4A targets TRIF, the adaptor protein essential for TLR3 signaling, thereby suppressing IFN production and ISG expression. Because HCV is primarily detected by RIG-I-like receptors (RLRs) and TLR3 (refer to Figure 2), the cleavage of these adaptor proteins is vital for inhibiting the host's initial antiviral response. By interfering with both pathways, NS3-4A can also help prevent excessive inflammation and impede chemokine induction, which delays immune cell recruitment.

Beyond these key adaptors, NS3-4A disrupts other components of the innate immune response. Following activation of RLRs or TLR3 by HCV, signaling converges on IRF3—a transcription factor activated by the kinase TBK1. The NS3 helicase domain binds TBK1, preventing its interaction with IRF3, thereby inhibiting IRF3's nuclear translocation and subsequent IFN induction.

NS3-4A also targets Riplet, an E3 ubiquitin ligase essential for RIG-I activation, by cleaving it and reducing its cellular levels, which in turn impairs RIG-I polyubiquitination. Recent studies have pinpointed the NS4A Y16F residue as a key factor in inhibiting Riplet without affecting MAVS cleavage. This impairment of Riplet activity, and the subsequent inhibition of IRF3 activation and IFN production, appears to occur independently of the RIG-I/MAVS pathway.

In addition, several reports indicate that HCV can inhibit TNF-α-mediated activation of NF-κB, though the precise mechanism remains unclear. One study demonstrated that NS3 binds to the linear ubiquitin chain assembly complex (LUBAC), which is responsible for the polyubiquitination of NEMO—a crucial step for NF-κB activation—resulting in reduced expression of inflammatory cytokines and contributing to HCV persistence by dampening immune responses.

Moreover, NS3-4A has been shown to promote STAT1 degradation, even though no direct interaction between the two has been detected.

4.2. NS4B

NS4B has been found to suppress TLR3-mediated signaling by reducing TRIF protein levels through a caspase 8-dependent mechanism. Interestingly, although HCV is an RNA virus, NS4B also interacts with STING—an adaptor protein located on the ER membrane that is crucial for initiating type I interferon responses to viral DNA. While it remains uncertain if the STING pathway is directly activated during HCV infection or significantly contributes to the antiviral response, its activation has been shown to restrict viral replication in infected cells. Moreover, the effectiveness of the STING antiviral response appears to vary with different HCV genotypes, and NS4B's ability to inactivate STING also differs among these

genotypes. Although the interplay between the RIG-I/MAVS and STING pathways is still under debate, current evidence suggests that crosstalk between these signaling mechanisms may play a role in antiviral immunity against both RNA and DNA viruses, including HCV.

4.3. NS5A and E2

NS5A and E2 contribute to HCV's immune evasion by targeting PKR, a kinase that phosphorylates eIF2α and functions as a sensor for double-stranded RNA. PKR plays a dual role: it can trigger a shutdown of host protein synthesis, which favors viral persistence, while also inhibiting the expression of host factors critical for HCV replication and inducing the production of interferon-beta and ISGs. HCV circumvents the antiviral effects of PKR through the actions of NS5A and E2, and it can also induce PKR phosphorylation to further inhibit the translation of antiviral proteins. In addition, NS5A has been shown to interact with NAP1L1, a chaperone that shuttles between the nucleus and cytoplasm and regulates various cellular processes. In genotype 2 infections, this interaction leads to the sequestration and proteasomal degradation of NAP1L1, preventing its nuclear translocation and downregulating genes essential for innate immune responses mediated by RIG-I and TLR3. NS5A is also implicated in dampening the activation of type I and type III interferons—possibly by binding to viral RNA and shielding it from detection by sensors like RIG-I and MDA5.

4.4. Core

The HCV core protein is involved in multiple stages of the viral life cycle and influences various host functions, including cell growth and apoptosis. Its overexpression has been linked to malignant transformation in experimental models, such as the development of hepatocellular carcinoma in transgenic mice. The role of the core protein in immune evasion is somewhat controversial, particularly regarding its effects on the NF-κB pathway. Some studies indicate that core protein overexpression inhibits NF-κB signaling, thereby suppressing inflammatory responses, while others suggest it activates NF-κB to promote the expression of proinflammatory cytokines—a hallmark of HCV pathogenesis. These conflicting results may arise from differences in experimental approaches and cell models. Furthermore, the core protein has been shown to interfere with the JAK/STAT pathway, which is essential for mediating responses to interferon signaling. Early reports suggested that core binds to STAT1, leading to its degradation or hindering its ability to bind DNA. More recent work proposes that the core protein differentially regulates IFN-α and IFN-γ signaling and is associated with reduced phosphorylation of STAT2, a phenomenon observed in HCV-infected patients.

4.5. p7

Research using genome-wide mutagenesis to explore HCV's anti-interferon strategies has revealed that p7—a viral membrane-spanning protein that functions as an ion channel and is crucial for virion production—also plays a role in immune evasion. Studies found that HCV mutants with defective p7 are more vulnerable to the overexpression of IFI16, an interferon-inducible protein whose precise function is not fully understood. Further investigations suggest that p7 interacts with IFI16 and causes depolarization of the mitochondrial membrane potential, which likely impairs IFI16 function. In addition, accumulating evidence shows that HCV alters host microRNA expression to modulate a variety of cellular functions. For example, the virus induces miR-208b and miR-499a-5p,

which downregulate IFNL3—a member of the interferon-lambda family—thus aiding viral escape from innate immunity. HCV has also been reported to upregulate selenoprotein P, a hepatokine linked to insulin resistance and type 2 diabetes; this protein appears to bind and inhibit RIG-I activity, with its overexpression correlating with poorer treatment outcomes. Interestingly, some HCV double-stranded RNA intermediates are released in extracellular vesicles, a mechanism that may contribute to reduced TLR3 activation.

Conclusions

The host's innate immune system responds to HCV infection by inducing a range of type I and type III interferon-stimulated genes. However, this response only partially controls the infection and fails to clear the virus in most patients, primarily because HCV has evolved multiple strategies to counteract these antiviral defenses. These evasion mechanisms, mediated by various viral proteins, target distinct cellular antiviral signaling pathways, collectively allowing the virus to effectively escape the intracellular immune response. We have outlined the currently understood evasion strategies of HCV (summarized in Table 1 and illustrated in Figure 3), though additional mechanisms are likely to be discovered in the future.

Direct-acting antivirals (DAAs) have transformed HCV treatment and offer highly effective therapeutic options. Nevertheless, their high cost limits accessibility for many patients, and concerns remain about drug resistance and adverse side effects. Consequently, there is an urgent need for an effective vaccine to prevent HCV infection and ultimately eradicate the disease. In addition, new therapies are required to reduce the morbidity and mortality associated with HCV-induced liver disease. Further research into the interactions between HCV and host intracellular immune signaling is critical to improve our understanding of the transition from acute to chronic infection, elucidate the role of innate immunity in hepatic inflammation, and identify novel cellular targets for future antiviral therapeutics.

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