Immune evasion strategies of Hepatitis C virus (HCV)

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Abstract:

Standing high level of the innate and adaptive immune resistance, hepatitis C virus (HCV) causes chronically persisting infection in most exposed individuals. Chronic replication is evidence of a multi-layered immune-evasion program commencing at the time pathogen symmetry and continuing across antigen exposure, effector-cell function, and antibody recognition and so on. Key signaling nodes in Type I/III IFN induction and response pathways are subverted by viral nonstructural proteins (most notably NS3/4A, NS4B and NS5A), and structural elements of the E1/E2 envelope glycoproteins together with lipaviroparticle configuration minimize sensitivity to neutralizing antibodies. Concurrently, HCV promotes a qualitative defect of cellular immunity through T cell exhaustion, regulatory networks and perturbation of dendritic cell (DC) and NK cell function. In this review, we have integrated the current knowledge of HCV immune evasion mechanisms and how they work together to promote persistence and discuss their implications for vaccine design and immunotherapeutic strategies.

Hepatitis C virus (HCV), a poorly cytopathic single-stranded RNA virus, persists in the majority of individuals who encounter the virus although HCV-specific innate and adaptive immune responses are frequently vigorous, reflecting pathogens with extraordinary mechanisms to elude host immune control. Persistence is organized by a hierarchical program that functions from initial pathogen sensing to effector and antibody-mediated immunity. HCV nonstructural (NS) proteins, mainly NS3/4A, NS4B, and NS5A, inhibit major players of innate immunity signal pathways by PRRs and IFN activators, MAVS, TRIF, STING, and PKR to diminish IFN induction while reprograming antiviral effector responses. Simultaneously, HCV undermines early antiviral control through the circumvention of natural killer cell function and dampening dendritic cell maturation and antigen presentation to generate a tolerogenic

immune environment. Chronic antigen exposure additionally drives functional exhaustion of virus-specific T cells by inhibitory receptor pathways such as PD-1, curbing effective cellular immunity. At the humoral level, broad glycan shielding and structural flexibility of the E1/E2 viral envelope glycoproteins, packaging with host lipoproteins as lipoviroparticles, and immune evasion through antibody-resistant cell-to-cell transmission provide strong dampening effects on neutralizing antibody responses. Here we provide an overview of current concepts of how these evasion strategies act in concert and redundancy to secure viral persistence, summarized from mechanistic understanding. Knowledge of this global evasion network could have implications in the rational design of HCV vaccines and diagnostic immunotherapies for preventing immune escape and achieving persistent protection.

Keywords: HCV, immune evasion, interferon, MAVS, TRIF, STING, glycan shield, lipoviroparticle, T cell exhaustion, PD-1 open in new, neutralizing antibodies

Introduction:

Hepatitis C virus (HCV) is a positive sense single stranded RNA that belongs to the family Flaviviridae [1]. It is still a leading cause of chronic liver disease globally. Although the introduction of direct-acting antivirals (DAAs) has revolutionized HCV management and allowed high rates of cure, millions of individuals become newly infected each year, rendering no licensed prophylactic vaccine. What characterizes HCV infection is its high tendency to establish a chronic infection: although some infected patients are able to spontaneously clear the virus during acute phase, the majority of them develop chronic overall disease that may evolve into liver fibrosis, cirrhosis and hepatocarcinoma.

HCV is a positive-strand RNA virus (Flaviviridae) that has a remarkable ability for persistence and immune evasion, manifested clinically by low rates of spontaneous clearance and the difficulty in creating an effective, broadly protective vaccine. A common theme is redundancy: several viral proteins interfere with multiple host defense pathways and the structural/biophysical properties of virions limit immune recognition. Traditional profiles of HCV intracellular immune evasion were based on

blocking signaling, targeting antiviral effectors, and persistent viral genetic heterogeneity providing for immunologic pressure-generated escape mutants [1].

This clinical result suggests not immune ignorance but an extraordinary capacity of the virus to circumvent, subvert and compossh host immune defenses. HCV viral infection induces potent innate (e.g. pattern recognition receptor [PRR,] type I and III interferon) and adaptive (e.g., natural killer [NK] cell recruitment, antiviral T- and B-cell priming) immune responses in the body. In infected-and-clearing individuals, such responses are organized, persistent and characterized by long-lasting antiviral pressure. Chronic infection, however, is associated with qualitative impairment in several immune compartments, more consistent with the notion that we are dealing not with an insufficiently stimulated but rather actively manipulated immune response to HCV. Because of their significance, it is unlikely that these evasion strategies occur coincidentally and are redundant; rather, they represent a multilayered and temporally staged program that nullifies immune responses from the first instance of viral recognition to persisting adaptive immunity.

Regarding innate immunity, HCV directly disrupts PRR pathways responsible for sensing viral RNA and triggering IFN-dependent responses. The viral nonstructural proteins, in particular the NS3/4A protease complex, NS4B and NS5A can block major signaling adaptors and kinases such as MAVS, TRIF, STING, TBK1 and PKR. By cleaving or functionally inactivating these proteins, the virus dampens IFN induction and signaling activity, impairs the establishment of an antiviral state, and reshapes downstream effector pathways to enable ongoing viral replication or assembly. These strategies spotlight that HCV not only suppresses immune pathways, but also hijacks and repurposes host antiviral effectors for its own benefit. In addition, to intracellular innate sensing, early antiviral effector and antigen-presenting cells may be the targets of HCV. NK cell activity is suppressed by direct binding of the viral envelope glycoprotein E2 with its receptor CD81, which results in mitigation of cytotoxicity and cytokine expression. Meanwhile, dendritic cell maturation and antigen presentation are inhibited, resulting in tolerogenic signaling microenvironments that compromise effector T cell activation. These are early perturbations that set the future course of adaptive immunity and decrease the opportunities for sterilizing immune control.

Chronic Ag exposure also induces severe dysfunction in the virus-specific T cell pool. HCV-specific $CD8^+$ and $CD4^+$ T cells gradually become exhausted with persistent expression of inhibitory receptors, such as PD-1, reduced effector function, altered metabolic profiles, and compromised memory formation. Regulatory mechanisms mediated by tolerogenic dendritic cells and regulatory T cells also attenuate antiviral immunity, to promote a state of immune adaptation promoting viral persistence rather than elimination. At the humoral level, HCV uses several mechanisms to escape neutralizing antibodies. The heavily glycosylated E1/E2 envelope glycoprotein complex confers a high degree of conformational plasticity, which conceals many of its conserved epitopes by 'glycan-shielding' and restructuring. In vivo, HCV is a lipoviroparticle that circulates along with host lipoproteins and apolipoproteins, especially ApoE which in turn masks viral epitopes altering antibody access. Furthermore, HCV can also be transmitted via direct cell-to-cell passage, a pathway that is partially resistant to antibody-mediated neutralization and may allow viral propagation even in the face of circulating neutralizing antibodies.

Collectively, these tactics reveal that immune evasion by HCV is not the product of one bullet but encompasses a systems level plan with simultaneous interference at signaling and effector levels of innate sensing, cellular immunity and humoral recognition. This redundancy and overlap can help explain why natural infection often does not generate protective immunity, as well as why vaccine development has been difficult. We here synthesize existing mechanistic knowledge on HCV immune escape in innate, cellular and humoral arms of immunity and highlight how these strategies work together to sustain chronic infection. Knowledge of this coordinated escape network is critical for informing the rational design of new-generation HCV vaccines and immunotherapies that can effectively evade immune evasion and provide long-term immunity.

Methods (literature search strategy)

We performed a narrative review of available peer-reviewed literature dealing with this mechanistic HCV immune evasion. (1) a core set of primary mechanistic studies demonstrating viral antagonism of innate sensing and Ib), (2) structural/immunological studies associated with escape from antibody, and (3)

clinical/immunophenotyping studies documenting an association between persistence and T cell exhaustion/regulatory pathways. Recent review articles were referred for the mechanistic context [2].

Evasion of innate immunity

Blunting IFN induction by targeting pattern-recognition receptor signaling NS3/4A cleavage of MAVS and TRIF

The complex web of pattern-recognition receptors is a key component in the initiation of innate immune responses, predominantly through the generation of interferons that are essential for antiviral immunity [3]. Nevertheless, viruses have developed sophisticated strategies to overcome these host defenses by exploiting the PRRs and their downstream signaling pathways, which allows the initiation of viral immune escape and replication in the organism [4,5]. This arms race between host and pathogen emphasises the importance of a deeper knowledge of viral evasion for antiviral therapy [6]. Boiled immune response The fine balance between positive and negative regulation to pattern recognition receptor-mediated host immune responses determines a favourable outcome of the host [7]. Viruses do so by a variety of means, including the direct manipulation PTMs necessary for PRR activation or the suppression of accessory cell components necessary for normal receptor activity [8].

Several viruses, such as influenza A virus, Flaviviridae family members, SARS-CoV-2 and Zika virus [9], down-regulate the antiviral response by directly targeting the type I IFN signaling pathways through non-structural proteins. The NS3/4A protease is an innate adversary of HCV. It inhibits RIG-I–like receptors signaling by cleaving MAVS (also known as IPS-1/Cardif/VISA) to block downstream IRF3/NF-κB activation and type I IFN production [10]. Furthermore, the proteolytic cleavage of TRIF (TICAM-1) by NS3/4A alone is sufficient to suppress dsRNA-mediated antiviral gene expression [11]. The former generally block both cytosolic and endosomal RNA sensing pathways, restricting early responses by the host to IFN.

NS4B antagonism of STING/TBK1 axis

Outside of NS3/4A, HCV NS4B blocks the downstream of pathway by directly interacting with STING (also called MITA) and inhibiting its ability to activate TBK1

efficiently, which results in a repression of IRF3 activation and IFN production [12]. NS4B also inhibits STING upregulation and signaling, thereby offering genotype-selective and pathway-layered antagonism [13].

This immune evasion is important for viral replication, because it directly disrupts the host's natural antiviral responses mediated by the type I interferon (IFN) pathway [14, 15]. For example, NS4B of several different RNA viruses (e.g., HCV as well as flaviviridae that includes the DENV and WNV) was shown to be a strong inhibitor of STING and activated TANK-binding kinase 1 (TBK1)-mediated signaling that effectively suppresses IFN-I production [16,17]. Such antagonism can occur via variety of mechanisms, for example by blocking the direct mechanism of TBK1 autophosphorylation or competitive binding to STING that prevents downstream signaling cascades [15,18]. For example, Hepatitis C virus NS4B has been demonstrated to directly inhibit the interaction between STING and TBK1, thereby disrupting the assembly of the essential signaling complex that is necessary for IFN-β production [17]. In genotype 2a hepatitis C virus, the inhibitory impact of NS4B on STING is more evident [c4] and interrupting the interaction between STING and TBK1 is one of its important strategies to immune evasion [14].

Another is NS1, in addition to Zika virus NS4B and inhibits TBK1 phosphorylation and interferon expression also probably by compete with TBK1 directly [18]. Likewise, NS4B proteins of Dengue virus (serotypes 1/2/4) and West Nile virus was shown to suppress TBK1 phosphorylation leading to inhibition of IFN-β induction [19]. The range of such antagonistic measures is even broader, and encompasses the interference with other key immune regulators by viral proteases (e.g. NS3/NS4A complex that drastically suppresses kinase activities of IKKα and IKKβ), which results in further impairment of the antiviral armament of the host [20]. These complex strategies demonstrate the sophisticated ploy used by flaviviruses to counteract the innate immune system in order to promote viral replication and spread. A better understanding of these complex molecular interactions with NS4B and their contribution to the STING/TBK1 axis will be critical for developing new specifically targeted antiviral therapeutics to these immune evasion strategies [14,21,22]. More detailed studies to define the exact binding sites and conformational changes induced by NS4B in STING and TBK1 might lead to small molecule inhibitors that restore

normal stimulation. These inhibitors could potentially restore activation of the host innate immune response, which limits viral replication and reduces disease severity.

NS4B-mediated TRIF degradation (TLR3 pathway)

HCV also inhibits TLR3-mediated responses by a mechanism not involving NS3/4A cleavage. NS4B can down-regulate TRIF through a caspase-8–mediated degradation pathway, inhibiting TLR3-mediated interferon signaling and broadening the spectrum of TRIF suppression beyond proteolytic dissociation only [23]. The non-structural protein 4B (NS4B) of the flaviviruses, such as hepatitis C virus and West Nile virus, emerged as a key mediator for immune evasion strategies by inhibiting the Toll-like receptor 3 signaling through degradation of TIR-domain containing adapter inducing-interferon-β [24,25]. This tactical manipulation by viral proteins, including the NS4B, efficiently represses the host's innate immune system to enhance viral multiplication and release [26].

NS4B protein also inhibits the autophosphorylation of TBK1, which results in a block of phosphorylation of IRF3 [27]. This inhibition sabotages the downstream signaling that is necessary for interferon-β production, an essential antiviral cytokine [37]. In addition, the caspase-8 dependent cleavage of TRIF is promoted by NS4B, which mediates a direct decrease in TRIF protein abundance resulting in inhibition of TLR3-triggered IFN signaling [24, 28]. In addition, to direct cleavage, NS4B of some flaviviruses, including HCV suppresses TLR3-mediated interferon pathways by promoting the degradation of TRIF [28, 29]. This TRIF degradation strategy is a complex viral countermeasure to evade host innate immune surveillance, as some viruses have evolved virus-encoded proteins toward Toll-like receptors in order to inhibit IFN production or proinflammatory cytokine release [40]. For example, dengue virus NS2B3 cuts MITA/STING and Influenza A virus NS1 imitates a TRAF3-binding motif to the RNA signaling pathways subsequently [25, 31].

Interfering with IFN signaling and antiviral effector function

NS5A and PKR: "reprogramming" antiviral effectors

PKR is a classic, interferon-induced antiviral IFN-stimulated gene (ISG); HCV can also evade or antagonize PKR-associated pathways. NS5A has long been linked to PKR inhibition [32], and more recent data indicate that NS5A domain I associate with PKR to counteract an antiviral pathway that would otherwise restrict formation of infectious particles through IRF1-dependent mechanisms [33]. This highlights that HCV not just inhibits IFN induction, but also manipulates downstream effector networks to maintain a productive infection.

The hepatitis C virus (HCV) nonstructural protein 5A (NS5A) is a key player in viral replication and assembly, known to associate with several cellular proteins including the interferon-inducible, double-stranded RNA-activated protein kinase [34,35]. This interaction is important as PKR serves a dual role: it is both a sensor of viral infection, through its association with viral dsRNA, and an effector of the innate immune response that controls viral replication by inhibiting protein synthesis and promoting apoptosis [35, 36]. In this scenario of complex host-pathogen interaction, HCV NS5A plays an active role in undermining the virus-permissive antiviral effects exerted by PKR, through down-regulation of its activity and more particularly by dysregulating one of its downstream targets, eukaryotic initiation factor 2 alpha (eIF2α) phosphorylation event also involved in the cellular antiviral response [37, 38].

In addition, it has been demonstrated that NS5A can associate with PKR via a complex including cyclophilin A and thus inhibiting the dimerization of PKR which is required for its activation [36]. Furthermore, it has been reported that NS5A possesses a PKR-binding domain raising the possibility that direct interaction establishes PKR kinase activity inhibition of eIF2α phosphorylation and thus bypasses infection an essential host antiviral response [38]. This evasion strategy ensures the virus has full translational capacity for its own replication, indicating a highly sophisticated hijacking of host cellular processes [39].

Systems-level suppression of innate pathways

The integrated analysis of HCV ISGs identifies a large and diverse network of viral-host interactions with the innate response, both at sensing, signaling and effector levels, supporting that HCV chronicity is presided over by a number of, rather than one and only "master switch", partially redundant antagonists [2].

Inhibition of early antiviral cells: Blocking NK cells in virus-nonspecific adaptive immunity-NK cell suppression With E2–CD81

NK cells aid in the early containment by cytolytic activity and cytokine secretion. The binding of CD81 on NK cells by HCV E2 may impede NK activation, cytokine production and degranulation which could attenuate early antiviral pressure and have the potential to influence adaptive responses [40]. Natural killer (NK) cells are critical components of the innate immune system and they coordinate early antiviral defense [41]. These cells are important for the recognition of virally infected target cells and can contribute to the early containment and control of viral pathogens via cytokine production as well as direct cytolytic activity [41–43]. Their capacity to detect pathogen-associated molecular patterns and inflammatory mediators on one side, as well as to mediate antibody-dependent cellular cytotoxicity through CD16 on the other side, highlight their versatility in both innate and adaptive immunity [44].

However, viruses, in their perpetual evolutionary struggle against the host immune system, have evolved complex mechanisms to evade detection by NK cells by targeting frequently modulate key activating pathways or manipulate the expression of ligands that are recognized by NK cell receptors [44, 45]. One such avoidance is via the modulation of expression of major histocompatibility complex class I molecules, which when down-regulated may inadvertently activate NK cells or when up-regulated inhibit them [46]. Apart from that, pathogens have also evolved strategies for interfering with NK cell function themselves by influencing cytokine production or through the induction of inhibitory signaling pathways thus thwarting the immune response and promoting viral persistence [47]. One, for example Epstein-Barr Virus, uses a variety of strategies to modulate NK cell function and tip the balance from an antiviral state towards immune tolerance or evasion [48].

Evasion of adaptive cellular immunity

T-cell exhaustion and inhibitory receptor pathways (PD-1 axis)

Chronic HCV infection is characterized by sustained antigen stimulation and T-cell dysfunction. Both intrahepatic and peripheral HCV-specific $CD8^+$ T cells can be PD-1 positive, suggestive of exhausted component with functional impairment at effector level and failed control of the virus [49]. This mechanism has been invoked to explain why strong priming can be initiated but the infection not cleared in many hosts. Chronic antigenic stimulation, such as in the case of chronic infections and cancer, leads to T-cell dysfunction that is characterized by a phenomenon referred to as T-cell exhaustion [50]. This hypofunctional profile is associated with a gradual decay of effector functions, and metabolic dysfunction in combination to high and sustained expression of inhibitory receptors [51]. This unique T cell subset is referred to as exhausted T cells and they are characterized by loss of ability to produce cytokines, diminished proliferative capacity and unable to recall memory [51,52]. Importantly, this state is not simply anergy or activation, but has a specific transcriptional/metabolic/epigenetic signature [53]. Such gradual loss of T-cell function is conducive to immune evasion, resulting in tumor growth and maintenance of chronic infections [54,44]. These findings reveal that it is important to decipher the sophisticated regulatory network of T-cell exhaustion, which will be helpful for more rational clinical applications [56].

In particular, T cell exhaustion is a diverse cellular state predominantly found in the CD8+ T cell population and is caused by chronic antigenic stimulation [56, 57]. The chronic stimulation hijacks T-cell normal differentiation, which results in a particular hyporesponsive stage that differs from functional effector or memory T cells [58]. This state features reduced cytolytic activity, dampened cytokine release and metabolic re-programming collectively resulting in the incapacity of the immune system to efficiently eliminate pathogens or malignant cells [54, 59]. Thus, terminally exhausted CD8+ T cells have also been frequently associated with failure of immune checkpoint blockade, emphasizing the importance of unveiling their specific molecular and cellular traits [60].

Regulatory networks and tolerogenic antigen presentation

HCV infection is characterized by regulatory immunity that limits antiviral effector mechanisms. Dendritic cells of the myeloid subset isolated from chronically HCV-infected patients can suppress CD4+ T-cell proliferation in an IL-2/IL-10–dependent manner and induce FoxP3+ regulatory T cells expansion, promoting a tolerogenic milieu favoring persistence [61]. Conceptual connections between HCV immune escape and Treg activation have been discussed drawing both on the point that immune regulation can be both a consequence of and cause of chronicity [62].

The intricate network of immune regulation and the concept of tolerogenic antigen presentation are key mechanisms for cancer cells to escape recognition from the host immune system, ultimately leading to cancer immune escape [63]. This evasion is frequently associated with dysfunction of antigen presentation machineries and modulation of immune checkpoint pathways, resulting in an impaired antitumoral response [64, 65]. They accomplish this by reducing or even completely losing the expression of antigens recognized and attacked by immune cells, such as occurs with the concomitant regularities of phenomena like immune escape [65]. Further contributing to antigen inaccessibility to CTL is the often-hostile tumor microenvironment, which promotes immune subversion through soluble factors and metabolic repatterning [66]. At the same time, tumors engage in active immunosuppression by selecting for antigen-presenting cells, especially dendritic cells (DCs), that are “tolerized” through genetic reprogramming of these cells to retract immune recognition and favor tumor progression rather than rejection [61]. In particular, these tolerized DCs can also affect the expression of co-stimulatory molecules such as down-modulation of CD80 and CD86 or up-regulation of inhibitory receptors that in turn transfer inhibitory signals that counteract critical co-stimulatory signals and lead to T cell anergy or apoptosis [66].

Altered antigen presentation and DC function

HCV has the capacity to subvert antigen-presenting cell maturation and function, with negative consequences on priming quality and T-cell help. For instance, HCV can block cell surface expression of MHC class II (DR) in the context of DC-SIGN/DC-SIGNR–mediated interactions, thereby decreasing $CD4^{+}$ T-ce11 activation

and subsequent support to $CD8^+$ cells / B-cells [67]. Advanced tumors have evolved an arsenal of strategies to evade the host immune system including their interactions with dendritic cells and antigen presentation, which actively favor an immunosuppressive environment within the tumor [68]. This escape is frequently reported to rely on the fact that malignant cells educate/tolerize the immune system, and the derivation of phenotypic and functional changes in DCs by tumor microenvironment that affect their ability to present antigens or few DC subsets may exert tolerogenic properties [69]. This dysregulation inhibits efficient anti-tumor immune responses, promoting tumor growth and metastases [67]. Importantly, the functional status of DCs in the TME is one of the important determinants in disease progression as activated and fully functional DCs are usually associated with a good clinical outcome [68]. This pivotal subset of DCs also highlights the tumors' selective exploitation of these cells to promote immune escape, frequently by either causing antigen anergy or producing immunosuppressive cytokines that can keep DCs in an immature state [69]. This process may present as down-regulation (or loss) of antigen expression by cancer cells, which limits their recognition by immune cells and allows for immune-escape [70].

In addition, tumors change the pre-metastatic niche to promote an immune-permissive setting and serially adjusting to immune pressure upon dissemination, thus DCs are preferred targets of the suppressive mechanisms that help for both local and metastatic evolution [71]. In this context, the tumor microenvironment (TME) may be able to promote different programs in DCs, including the secretion of high levels of IL-10 which can antagonize their antigen presentation function and desensitize them to subsequent stimulation of other immune cell types [73]. Such IL-10 mediated suppression not only results in the inhibition of DC maturation and ability to secrete IL-12, but also leads to a differentiation of immunogenic DCs into tolerogenic DCs as well, promoting immune escape [75]. Likewise, accumulation of IL-6 inside the tumor microenvironment can as well disrupt DC function and further impede anti-tumor immunity [76].

Evasion of humoral immunity (neutralizing antibodies)

Glycan shielding, epitope masking, and structural plasticity of E2

The HCV E1/E2 complex is highly glycosylated; conserved N-glycans can shield out the conserved neutralizing (n) epitopes and restrict binding of the antibodies to such epitopes [1]. Glycan shielding and "glycan shifts" are dynamic escape routes where mutation changes glycosylation patterns to cause a shift in epitope exposure [77]. Structural studies present mechanisms whereby some broadly neutralizing antibodies can pierce E2's glycan shield, thereby providing a foundation for rational immunogen design [78]. More generalizable structural syntheses underscore that E2's genetic diversity and its conformational flexibility open multiple bottleneck-off escape pathways such as point mutation, allostery and glycan reshuffling [79].

These are important immune evasion strategies utilized by a number of viruses, including HCV, but make the development of vaccines and new treatments more difficult [80, 81]. In the case of the HCV, the E1E2 glycoproteins, particularly E2, are structurally highly plastic and extensively glycosylated providing to hide viral epitopes and evade neutralizing antibodies [81, 82]. In particular, E2 glycoprotein has multiple N-glycosylation sites and a significant fraction of these glycans constitute a thick armor that hides well characterized neutralizing epitopes from antibody recognition [83, 84]. Cis-limited glycosylation, including conserved and genotype-specific N-glycosylated sites is used to hide host and humoral self-NPAs [83,84]. This glycan shield is a common immune evasion strategy seen with other viruses, like HIV and influenza virus, which strongly reduces the efficiency of neutralizing antibodies due to steric constrains that prevent antibody access to antigenic sites [85].

This mode of glycan presentation also enables fast mutational escape, with minor shifts in glycosylation sites such as the N417S or N417T substitutions within antigenic site 412 resulting in steric hindrance of antibody binding and neutralization [86]. In addition, the hypervariable region 1 (HVR1) within E2 has a profound influence on antibody sensitivity and functions to shield from neutralizing antibodies epitopes located outside HVR1; such protection is modulated by polymorphisms in aa positions 400–404 [87]. The glycan shield physically prevents antibody binding and functionally contributes to the structural plasticity of E2, thus enabling conformational changes that mask an epitope from extensive antibody interactions [88]

Lipoviroparticles and apolipoprotein-mediated antibody evasion

HCV circulates in patients as a hybrid particle, the LVP form, associated with host lipoproteins and apolipoproteins L; largely ApoE. This association may inhibit both effective neutralization by steric hindrance of viral epitopes and modify usage of entry factors [89]. Experiments demonstrate that virion bound ApoE is involved in evasion from neutralizing antibodies which are present in the plasma of patients, and this represents a major challenge for any sterilizing immunity that may be vaccine induced [90]. HCV is a useful model to understand how viruses hijack host lipid metabolism to promote immune evasion especially given the establishment of lipoviroparticles [91, 02]. The lipid-enriched nature of these special, subviral particles and the interaction with host apolipoproteins render them analogs of native lipoprotein particles, which in turn promotes viral entry and systemic spread [91, 93]. Concretely, the hepatitis C virus (HCV) incorporates host apolipoproteins (apo), including apoE, into its virion structure during assembly and egress resulting in major changes of its antigenic profile with a significant benefit for immune evasion from neutralizing antibodies [92, 94]. This strategy allows HCV to sally forth in the bloodstream with impunity, because it is sensed as lipoprotein by the immune system and uses host lipid pathways not just for assembly but also for successful spread within the host and establishment of a persistent infection [93, 95, 96].

Generation of these lipoviroparticles is closely associated with host VLDL synthesis and secretion pathways, and viral components are incorporated into the VLDL-precursor particles in the endoplasmic reticulum [97]. The co-option process consists in the viral exploitation of cellular processes that apolipoproteins including ApoB-100 (this was one of happening to it) are lipidated by microsomal triglyceride transfer protein, essential for the generation of very-low-density lipoprotein precursors which over same created were used by HCV as a support for its morphogenesis [82]. Furthermore, during assembly, the virus previously promotes host lipid metabolism to accumulate triglycerides in the liver and upregulates enzymes that participate in the biosynthesis of cholesterol and fatty acid, therefore guaranteeing a constant supply of lipids for its replication [97, 98]. This complex cross-talk illustrates the virus's clever approach in modulating host lipid metabolism to establish a generalized environment favoring viral replication and immune escape [99]. Complicating immune recognition,

respectively, the hypervariable regions and the large numbers of glycans on E1 and E2 envelope proteins produce a glycan shield, which covers conserved epitopes, thereby preventing neutralizing antibody binding [100].

Cell-to-cell spread: escaping antibody pressure at the transmission level

Thus, HCV has the potential to infect via cell-to-cell transmission that is somewhat resistant to neutralization by a variety of nAbs and could account for its ability to persist even in the presence of circulating nAbs [101]. This pathway may be especially important within the liver microenvironment where cellular contact and receptor utilization can facilitate "subtle" dissemination.

Direct cell-to-cell spread also serve as an important mechanism of escaping humoral challenge, as viruses can move between neighboring cells without encountering the extracellular environment [102]. This mode of cell-to-cell transmission enables viruses to spread in the face of strong neutralizing antibody responses that would otherwise effectively prevent infection by cell-free virions [103]. This tactic is especially important for viruses that use cell-to-cell spread as a major means of dissemination since trans proliferation makes transmission almost independent of the presence of antibodies against free viral particles [104]. For example, herpes simplex virus 1 viruses use cell-to-cell spread to propagate infection while protected from neutralizing antibodies and innate antiviral responses [105].

In addition, this direct cell to-cell transmission reduces exposure of viral particles to the extracellular environment in which they would be potentially neutralized by antibodies [106]. Moreover, this process is linked to viral pathogenicity and the correlation between increased cell-to-cell fusion and diseases severity were shown in SARS-CoV-2 variants [107, 108]. For instance, alphaherpesviruses are by nature cell-associated, making use of different intercellular transmission routes for infectious spread, which allows them to escape immune responses including opsonizing antibodies, complement factors and immune cells [109]. This complex viral spread strategy underlies the inherent challenge in developing vaccines against such pathogens, because most traditional vaccines are designed to elicit neutralizing antibodies that inhibit extracellular virions [110]. Certainly, some viruses independently resistant to antibody neutralization based on pentamer-dependent cell-to-cell spread

have been found when compared to cell-free spread [110], emphasizing the adaptive value of this mode of transmission. This is seen in clinical strains of human cytomegalovirus that are predominantly shed as cell-associated virus and enter cells via one of the several routes at over a log10-level of resistance to neutralizing antibody, interferon and cellular restriction factors [112].

Antibody–virus coevolution and exploitation of vulnerable escape pathways

Recent longitudinal studies of reinfection/clearance underscore how NAb can go on to direct viral evolution and in some cases preempt constrained viral escape pathways: early escape substitutions will often diminish receptor binding or fitness, later rendering viruses susceptible to neutralization by matured broadly NAb that mediate clearance [113]. These results are consistent with vaccine approaches aimed at inducing bNAb lineages targeting immunotypes of the virus that include functionally constrained sites.

Integration: HCV evasion succeeds

The best explanation of HCV persistence lies in the concept of conjoined evasion:

Early phase: quick inhibition of NFN induction (NS3/4A, NS4B) and reduction of antiviral state generation [114,115,116,117,118].

Early-mid phase: transcriptomic remodeling of IFN-effector pathways (NS5A-PKR axis) 'shelters' replication and the assembly steps even under sub-optimal immune-triggered activation [119,120].

Adaptive phase: NK/DC dysfunction and exhaustion/regulation undermine durable clearance of replication [121.122,123,124,125].

Humoral selection pressure: Calibanovirus® These humoral CRMs include envelope glycan masking, LVP mimicry, high sequence variation and antibody-protected transmission pathways that counteract neutralization to allow sustained dissemination [127,128,129,130].

These layers are also redundant and temporally ordered, so single-pathway interventions will be insufficient.

Implications for vaccines and immunotherapy

To overcome this challenge, immunogen design should take into consideration glycan shielding of the target sites on E2 and conformational diversity of E2 but relate to induce bNAbs that can shape conserved functional sites as well as tolerate glycan variability [127,128], An LVP/ApoE cloak indicates that vaccines might have to elicit antibodies that cross-neutralize across lipoprotein-associated states or exert Fc-mediated functions other than classic neutralization [129,130]. Cell-to-cell spread may call for such combination approaches: antibodies plus targeting entry co-factors or boosting intrahepatic T-cell/NK surveillance. Reversal of exhaustion/regulation (e.g., check-point modulation) is conceptually attractive but must be balanced against risk of hepatic immunopathology; mechanistic human data on PD-1–linked dysfunction support consideration of targeted immunomodulation [124,125].

Hepatitis C virus (HCV) remains a formidable global health threat, with an estimated 50 million people infected worldwide despite the emergence of direct-acting antiviral agents [112]. This wide distribution highlights the imperative of an effective prophylactic vaccine to block new infections and to achieve the ambitious goals of the World Health Organization (WHO) for HCV elimination by 2030 that are likely out of reach for many countries [113,114]. The potential role of a vaccine in limiting virus spread is inhibited by the extraordinary capacity for immune avoidance that coronavirus has and its broad heterogeneity [115]. In particular, the well-documented error-prone RNA-dependent RNA polymerase of HCV gives rise to an array of quasispecies which are sufficiently heterogeneous to evade host immune surveillance or reduce vaccine effectiveness [116, 117].

The genetic diversity poses a significant obstacle to T-and B-cell based immunity and hence the vaccine developers [115]. For example, HCV has a high frequency of mutation which causes genetic heterogeneity throughout the viral genome and seven genotypes with more than 65 subtypes allow HCV to escape from the selective pressures exerted by neutralizing antibodies as well CD8+ T cell responses [117]. Such broad genetic diversity —25–35% difference in nucleotide sequence among genotypes and 15–25% between subtypes—results in the constant emergence of escape mutations capable of abrogating both natural and vaccine-induced immunity [118, 119]. The molecular flexibility paired with structural diversity of

immunodominant regions, however, offers HCV an advantage for escaping the innate and adaptive immune system to prevent viral clearance and vaccine development [120]. One of the major obstacles in HCV vaccine design and development is to break away from this enormous genetic diversity to elicit broad-based immune responses that can recognize multiple viral isolates [121, 122]. In addition, the ability of the virus to establish chronic infections in a large proportion of acutely infected individuals demonstrates even more clearly its complex immune escape properties: indeed, in most cases an efficient and prolonged antiviral response is not induced by the host immune system [118]. The high genetic variation of HCV, with multiple genotypes and subtypes generating significant amino acid diversity, is a substantial barrier to the development of a vaccine that will confer universal protection across genotypes [123, 124].

To achieve this, it is important to identify conserved epitopes that can induce cross-neutralizing antibodies with a wide specificity against all allele groups of all major HCV types even if the virus is capable of developing resistance-associated substitutions [125, 126]. This genetic flexibility allows HCV to rapidly escape both humoral and cellular immune responses resulting in persistence of infection and complicates vaccine development [127, 128]. The great genetic diversity between the eight main HCV genotypes up to 30% makes it even more complex for a pan-genotypic vaccine [129]. Such extensive diversity is exacerbated by substantial intra-genotypic heterogeneity, a feature that can be attributed to the elevated mutation rate of the viral polymerase [130], particularly within envelope glycoproteins targeted by host antibodies.

Conclusion

Such immune evasion by HCV leads to a systems-level program of ransom designed at the levels of innate antiviral countermeasures within infected cells, suppression and redirections of IFN responses, qualitative perturbations in antigen presentation capabilities and effector lymphocytes mobilization, complex humoral escape afforded by E1E2 structure, glycosylation and lipoprotein envelopment. It is still paramount to comprehend these mechanisms working together, rather than

individually, particularly in the context of next-generation vaccines and immunotherapy.

HCV represents a best model of pathogen that does not persist accepting immune silence, but with multiple levels of host immunity subversion strategy working together. Instead of one primary mechanism of escape, HCV exploits a coordinated network immune evasion programme at the systems-level spanning innate sensing-intracellular signaling, interferon signaling, cellular effector function antigen presentation and humoral recognition. Viral NSPs, such as NS3/4A, NS4B and NS5A impair major signaling hubs (MAVS, TRIF, STING, TBK1 and PKR), limiting IFN induction and remodelling the antiviral effector pathways. Together with the defect in natural killer cell activity, dendritic cell maturation and antigen presentation, early immune responses are directed toward tolerance rather than viral elimination.

During infection, persistent antigen exposure results in functionally exhausted virus-specific T cells and consolidates regulatory networks which further inhibit antiviral immunity. Concurrently, HCV creates a formidable humoral barrier consisting of glycan shielding and conformational variability of its envelope glycoproteins, binding to host lipoproteins as lipoviroparticles, and use of antibody-refractory cell-to-cell transmission pathways. These mechanisms act in cooperation and redundancy, such that incomplete immune control is seldom followed by sterilizing immunity. Altogether, this adaptive evasion network accounts for both the high frequency of chronicity after natural exposure and the longstanding inability to devise successful preventive vaccination strategies.

Of note, the success of direct-acting antiviral treatments in achieving virologic cure does not diminish the worldwide requirement for preventive and immune-based strategies. The continuous transmission and risk of reinfection, and the restricted availability of therapy in most areas, underline the significance of further insight into HCV immune evasion. Exploring how these mechanisms dynamically interact in space and time are central for driving the development of next generation vaccine and immunotherapeutic strategies.

Recommendations and Future Directions

Strategies for vaccine development need to focus on functionally restricted viral regions.:

1. Any theoretical vaccine must take into consideration the high degree of genotypic diversity, glycan shielding, and conformational flexibility in HCV envelope. Immunogens that direct immune responses toward conserved, functionally constrained epitopes—resilient to glycan variability and amino acid sequence diversity—are more likely to induce broadly neutralizing antibodies with cross-genotypic activity.
2. To counterbalance humoral immunity, cellular immune targeting should be pursued: Given the antibody-resistant of lipoviroparticles and cell-to-cell transmission, vaccines and immunotherapies would benefit from vigorous $CD4^+$ and $CD8^+$ T cell responses as well as neutralizing antibodies. Such tactics to boost intrahepatic T cell surveillance and memory longevity might be critical for long-term protection.
3. Therapeutic modulation Points of therapeutic intervention have been identified in innate immune pathways.: Inhibition of viral antagonists of innate immunity--including interactions between NS3/4A, NS4B and MAVS, TRIF and STING may provide a rational for strategies aimed at restoring endogenous antiviral responses. Immunotherapeutic strategies involving small-molecule or biologic agents to reconstitute interferon signaling could work in synergy with existing antivirals or vaccines.
4. Immune checkpoint manipulation deserves careful investigation.: Reversal of T cell exhaustion via immune checkpoint blockade or related immunomodulatory strategies is conceptually attractive but would need to be balanced with the potential risk of hepatic immunopathology. Instead, we are likely to need mechanistically led targeted interventions—rather than good old systemic immune activation.
5. Integrated, systems-level approaches are essential.: Further analysis should focus on holistic studies describing the interplay between innate, cellular and humoral immune compartments rather than evasion mechanisms alone. Longitudinal human studies, matched with structural, immunological and

systems biology analyses, will be necessary for defining correlates of protection and susceptibility.

Future Research Directions

Although significant progress has been made in characterizing individual mechanisms of hepatitis C virus immune evasion, critical knowledge gaps exist with regard to how these interventions are orchestrated in vivo and how they might be combated. It will be important to close these gaps in order for mechanistic findings to be translated into long-lasting (preventive or immunotherapeutic) strategies.

1. **Spatiotemporal immune escape in the liver microenvironment:** The majority of mechanistic studies on HCV immune evasion are performed using reductionist in vitro systems. Future investigations need to focus on in vivo and ex vivo studies that determine when and where viral antagonists exert their effects in the hepatic niche. High-resolution single-cell and spatial transcriptomic profiling of infected human liver tissue may answer questions on how innate sensing, interferon responses, NK cell function and antigen presentation are coordinated during acute infection, chronic persistence and post-treatment cure.
2. **Cross-network integration of innate immune antagonism:** Although isolated interactions of HCV proteins and host signaling components like MAVS, TRIF, STING, TBK1 or PKR are well described, the manner in which these pathways connect at the systems level is not fully appreciated. The network-level effects of combined pathway blockade, including host compensatory approaches and viral trade-offs, are to be addressed in further studies. Systems biology and computational modeling methods could be useful in identifying nodes of vulnerability that are unfounded through single-pathway analyses.
3. **HCV genotype and quasispecies-specific mechanisms of action:** HCV is a highly heterogeneous virus and the majority of immune evasion studies are conducted with two to three lab-adapted strains. Comparative studies among genotypes and clinically relevant quasispecies should be performed to further the understanding on which escape mechanisms are common or context-

specific. That kind of work will be essential for the development of pan-genotypic vaccines and therapeutics.

4. **Functional consequences of lipoviroparticle heterogeneity:** Lipoviroparticle composition differs in apolipoprotein content, lipid profile and infectivity on per cell basis, but the immunological relevance of this diversity remains largely unknown. Moreover, it becomes necessary to determine how variation at the lipid-lipoprotein interface affects epitope accessibility or Fc-mediated functions of antibodies, receptor utilization and virus cell-to-cell transmission in vivo.
5. **Antibody–virus coevolution and maturation pathways:** Longitudinal natural infection, reinfection, and spontaneous clearance studies present an unprecedented opportunity for dissecting how neutralizing antibodies drive viral evolution (and vice versa). Future research will be required to delineate such paths of antibody maturation that effectively utilize limited viral escape so as to inform the design of immunogens capable of leading bnAb lineages through vaccination.
6. **Reversibility and plasticity of exhausted T cells:** While T cell exhaustion is a defining feature of chronic HCV infection, the extent to which exhausted T cells can be functionally reprogrammed after viral clearance with DAAs remains unclear. Future investigations need to address the epigenetic and metabolic resilience of exhaustion state, and if targeted immunomodulation is able to recover protective immunity without protection off an immunopathology.
7. **viral clearance Interactions, memory of immune, and reinfection risk:** DAA-induced cure provides a distinct human model for investigation of immune memory in the absence of antigen persistence. The reasons that cured individuals are still susceptible to reinfection and how immune memory following cure differs from that seen with spontaneous clearance will be important to understand in defining correlates of protective immunity, and for guiding vaccine design.
8. **Human - experimental interchange translational models:** More robust experimental models that better replicate human liver immunobiology (e.g.,

humanized mice and organoid systems) will be required to validate immune evasion mechanisms and for testing of interventions. Such hybrid models will, if matched to clinic and immunological data from the infected, provide more translational relevance.

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