

Influence of host resistance on viral adaptation: hepatitis C virus as a case study

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Abstract: Genetic and cellular studies have shown that the host's innate and adaptive immune responses are an important correlate of viral infection outcome. The features of the host's immune response (host resistance) reflect the coevolution between hosts and pathogens that has occurred over millennia, and that has also resulted in a number of strategies developed by viruses to improve fitness and survival within the host (viral adaptation). In this review, we discuss viral adaptation to host immune pressure via protein-protein interactions and sequence-specific mutations. Specifically, we will present the "state of play" on viral escape mutations to host T-cell responses in the context of the hepatitis C virus, and their influence on infection outcome.

Keywords: hepatitis C virus, viral adaptation, immune escape, adaptive immune response

Hepatitis C virus: an overview

Hepatitis C affects 3% of the human population, with an estimated 170 million people worldwide infected with the hepatitis C virus (HCV).¹⁻³ Hepatitis C constitutes a global health problem, given the progression to cirrhosis (20%–35%) in a significant proportion of individuals with chronic HCV infection, and it is a significant risk factor for hepatocellular carcinoma (increased rate of 3%–6% per year) and liver transplantation.^{4,5} The impact of HCV infection on the population is further exacerbated by the often asymptomatic nature of the disease for years after infection, such that many individuals infected with HCV are unaware of their condition and unknowingly transmit the virus, leading to a "silent epidemic".⁶

HCV is a blood-borne virus. Before the discovery of HCV by Choo et al in 1989 and the subsequent implementation of efficient blood-screening methods, blood transfusion was one of the main routes of HCV transmission.⁷ In developed countries, transmission via mucosal exposure to infected blood or blood products is commonly due to unsafe drug-injecting practices (intravenous drug use [IDU]), with 60%–80% of newly identified cases attributed to IDU,⁸⁻¹⁰ while in developing countries transmission occurs via contaminated medical equipment/contaminated blood and IDU.^{11,12} Transmission via sexual activity is less common,^{13,14} as is mother-to-child transmission.^{15,16}

HCV is a positive strand-enveloped ribonucleic acid (RNA) virus of approximately 9,600 nucleotides in length, belonging to the *Hepacivirus* genus of the *Flaviviridae* family. The single-stranded RNA molecule consists of a large open reading frame that encodes a polyprotein precursor of approximately 3,000 residues, which is processed during and after translation into three structural proteins (core protein and the envelope

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proteins E1 and E2), a small viroporin protein p7, and six nonstructural proteins (NS2, NS3, NS4A, NS4B, NS5A, and NS5B).

The NS5B protein is an RNA-dependent RNA polymerase that lacks 3'-5' exonuclease proofreading activity, resulting in a high rate of mutation that has been estimated to be around 1.2×10^{-4} substitutions per nucleotide per cell infection (greater than the estimated mutation rate for HIV at 2.4×10^{-5}).^{17,18} Evolutionary forces, such as random genetic drift and selection, affect the frequency of these mutations in the circulating HCV strains. These changes may affect the virus at different levels, including replicative fitness, pathogenicity, immune escape, and response to viral treatment.¹⁹

Hepatitis C virus: diverse RNA virus

Due to a high mutation rate, many different strains of HCV exist worldwide. Based on genetic heterogeneity, seven major genotypes and more than 70 subtypes have been described.^{20,21} These genotypes differ in their amino acid sequences by more than 20%, while their subtypes differ from each other by 10%–20% (Table 1). However, for the most part, the natural history of the infection is similar for genotypes and subtypes, with the exception of HCV genotype 3, which presents a higher prevalence of steatosis in comparison to other genotypes.²² Importantly, genotype is a significant predictor of therapy outcome with the immunomodulatory IFN α /ribavirin (RBV)-based therapy;⁶ potentially reflecting different IFN-responsiveness.

The HCV infecting a host circulates in the blood and liver as a heterogeneous population. This population consists of a pool of closely related viral strains referred to as quasispecies (Table 1).

The observed diversity across the HCV genome is not uniform, and is mainly due to differences in functional and structural constraints for the different proteins. Therefore, some regions that code for important proteins for the virus's life cycle present a restricted number of changes. In contrast, the envelope region presents a high degree of amino acid variation, especially in the N-terminus of the E2 protein, designated as hypervariable regions 1 and 2 (HVR1 and

HVR2). The amino acid sequence within these HVRs can vary up to 80% between HCV genotypes.²³ The envelope region is a known immunogenic region for neutralizing antibodies, and it is likely that the immune pressure exerted on this region is the main driving force of the observed envelope-sequence variation resulting in the emergence of escape mutants.^{24,25–27}

Factors that influence HCV-infection outcome

Following infection with HCV, about 25%–30% of individuals can spontaneously resolve infection, but in the majority of cases the infection persists to establish chronic infection. The interplay of several viral and host factors largely influences HCV-infection outcome. From the host's perspective, age, sex, liver function, and the immune response are all important components that influence infection outcome. A strong and sustained HCV-specific T-cell response is associated with spontaneous clearance; however cells, and molecules in the innate arm of the immune response have also been implicated in determining outcome following HCV infection.

Host-resistance factors that influence HCV-infection outcome: immune response

The host's immune response is an important correlate of infection outcome, and several studies have examined variations in candidate genes involved in the immune system and HCV-infection outcome (Table 2).²⁸ These association studies, either candidate-gene studies or genome-wide association studies, underline the crucial role played by the host's immune responses in the control of HCV infection.

Table 2 Genetic variants of genes involved in the host immune response associated with HCV infection outcome^a

Family of molecules	Gene
IFN (and targets)	<i>IFNλ3</i>
	ISGs
Cytokines	<i>IL-10</i>
	<i>IL-12β</i>
	<i>TNFα</i>
	<i>KIR2DL3</i>
NK receptors	<i>HLA-B27</i>
	<i>HLA-B57</i>
	<i>HLA-DRB1*01</i>
HLA class I	<i>HLA-DRB1*04</i>
	<i>HLA-DRB1*11</i>
HLA class II	<i>HLA-DQB1*03</i>
	<i>HLA-DQB1*05</i>

Note: ^aGenes included only if reported in multiple studies.

Abbreviation: ISGs, IFN-stimulated genes.

Table 1 Genomic heterogeneity of hepatitis C virus (HCV)

Classification	Amino acid diversity across HCV genome (%)
Genotype	20–35
Subtype	10–20
Quasispecies	<5

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Non-pathogen-specific immunity

The host's innate immune response constitutes the first line of defense against an infection. This primary response relies on two strategies: "microbial-nonsel" and "missing-self" identification. The missing-self strategy is based on the recognition of molecules expressed exclusively on normal healthy cells of the host.²⁹ These molecules are not produced by microorganisms, and their expression can be lost when the host's cell is infected with a pathogen. Following viral infection, the absence of such molecules thus constitutes a missing self that will then be identified by natural killer (NK) cells (through their receptors). On the other hand, microbial nonself identification is the first pattern-recognition strategy. This process is based on the recognition of molecular structures that are not produced by the host, but are common within microorganisms.^{30,31} Such viruses as HCV produce viral pathogen-associated molecular patterns (PAMPs). These PAMPs act as a "molecular signature" of the infecting microbial organism, and are able to initiate a cascade of events leading to innate intracellular immunity. Cells recognize these PAMPs via pattern-recognition receptors, such as Toll-like receptors (TLRs); an evolutionarily conserved gene family.²² In the case of HCV infection, the virus engages TLR3 and RIG-1, which are receptors that specifically recognize double-stranded RNA (dsRNA),³² a product that is produced by most viruses during their life cycle. This recognition subsequently results in the activation of signaling pathways (or "proinflammatory" signaling pathways), leading to the production of antimicrobial effectors and proinflammatory and antiviral cytokines (eg, IFN, TNF, IL-1), as well as gene products that prime the adaptive immune responses.³² Accordingly, candidate-gene studies have shown that genetic variations in the TLR genes *RIG-I* and *TNF*, and several interleukins in the innate immune-response pathway have been associated with HCV-infection outcome.²⁸

HCV RNA triggers expression of IFN type I (α/β) and III (λ), which play a role in the defense against viral infections, due to their antiviral properties inducing a state of viral resistance that inhibits viral replication via several IFN-stimulated genes (ISGs).^{32,33,34-37} These ISGs include double-stranded PKR, and Mx protein.^{22,38,39} PKR is an intracellular sensor of viral infection that is activated in the presence of dsRNA produced by many viruses, including HCV. The activation of PKR results in the block of viral protein synthesis via eIF2 α phosphorylation.^{22,40,41} Similarly to PKR, OAS is a family of IFN-inducible enzymes that are activated by dsRNA and result in the activation of RNase L, which degrades viral RNA.^{42,43} Finally, Mx proteins belong

to the class of dynamin-like large guanosine triphosphatases, whose expression is regulated by type I and type III IFNs.⁴⁴ Their activation blocks viral transport inside the cell.²²

In 2009, several genome-wide association studies identified single-nucleotide polymorphisms (SNPs) present near the *IL28B* gene (rs12979860 and rs8099917), encoding type III IFN λ 3, that were associated with treatment response to pegylated (peg)-IFN α /RBV therapy⁴⁵⁻⁴⁸ and spontaneous clearance^{48,49} in HCV-infected individuals. Subsequently, Thompson et al observed the rates of SVR among different ethnic groups (ie, Caucasian, African-American, and Hispanic populations), and correlated these values with carriage of the "good" CC genotype for the SNP rs12979860.⁵⁰ Similarly, IFN λ 4, a recently discovered additional member of the IFN λ family, shows similar antiviral activity to IFN λ 3, as well as a strong genetic association with spontaneous and treatment-induced HCV clearance.⁵¹ At present, the IFN λ genotype is the strongest host pre-IFN α -based treatment predictor.⁵² It is still unclear how variants of these genes affect HCV-infection outcome, but recent evidence suggests that variations in the promoter and other untranslated regions of the gene are associated with differential binding of transcription factors and transcript stability.^{53,54}

It is now well established that NK cells constitute a crucial role in the fight against viral infections as part of the innate immune system. Target cells, such as virally infected cells, typically have low or no expression of human leukocyte antigen (HLA) class I molecules on their surface (to indicate "normal self" to the immune system), and are recognized and lysed by NK cells in a process referred to as missing-self recognition.⁵⁵ Downregulation of HLA class I molecules on virally infected cells is thought to be an immune evasion strategy utilized by viruses against cytotoxic CD8⁺ T cells, which are reliant on the recognition of viral peptides in complex with HLA class I, and are therefore susceptible to missing-self recognition by NK cells. Furthermore, it has recently been shown in viral infections that NK cells may modulate the host's adaptive immune response by directly deleting activated CD4⁺ and CD8⁺ T cells.^{56,57}

The cytotoxic capacity of NK cells is regulated via signals derived from a complex array of inhibitory and activating receptors. NK cell receptors, such as KIRs and CD94/NKG2A, allow NK cells to sample cells for the presence of HLA class I molecules (ligands) that are typically expressed on healthy cells.^{58,59} While the NKG2 family is relatively conserved through evolution, KIRs on the contrary are diverse and polymorphic.⁶⁰ Due to the diversity of KIRs (including

a total of 14 genes), they are more likely to generate inter-individual variation in immune responses to pathogens than the less polymorphic NKG2 family.

KIRs are located within the leukocyte-receptor complex on chromosome 19 (19q13.4), encoding for inhibitory and activating receptors that recognize the HLA class I molecules HLA-A, -B, and -C.^{61,62} The presence of particular combinations of HLA Class I alleles and KIRs within a host will determine the activation threshold of NK cells and modulate NK cell-mediated responses. The KIR–ligand interaction is highly variable, and this evolution is driven by selective pressure from exposure to pathogens.^{63–66} Furthermore, KIRs are expressed on NK cells in a stochastic manner, increasing the complexity of the receptor–ligand interaction.

Due to linkage disequilibrium, KIR haplotypes are defined by a combination of specific genes present at the centromeric and telomeric ends of the complex. The KIR A haplotype is relatively fixed in terms of gene content, while the B haplotype is characterized by variable KIR gene numbers and comprises more activating KIR genes. Differences in infection outcome have been associated with different genes within a specific KIR haplotype, including in the study by Dring et al,⁶⁷ which found that the B haplotype-associated genes *KIR2DS3* and *KIR2DL5* appear to be more frequent in chronic HCV-infected subjects in comparison to responders.

Genetic association studies examining single KIR genes with a particular phenotype can be limiting, given the coordinated interaction between multiple KIRs and the hierarchy of interactions with their HLA class I ligands. Accordingly, studies that examine KIR haplotypes and/or KIR–HLA epistatic interactions can help clarify the overall influence of KIR on infection outcome. One such study by Martin et al showed that when single KIR genes were correlated with human immunodeficiency virus (HIV)-infection outcome, *KIR3DS1* was associated with faster progression to disease, but this effect was reversed in the presence of a subset of HLA-Bw4 alleles with isoleucine at position 80.⁶⁸ Khakoo et al then showed, in the context of HCV infection, that homozygosity for the NK cell-inhibitory receptor *KIR2DL3* when in the presence of its ligand, HLA-C1, results in a higher probability of infection resolution.⁶⁹ This combined genotype results in relatively weak inhibition of NK cells, and thus likely protects against HCV by rendering NK cells more easily activated than in other subjects. While both HIV and HCV studies have suggested that a diminished inhibitory response can be associated with good outcome following infection, human papillomavirus-induced cervical cancer appears more likely, with HLA–KIR interactions that

favor NK-cell activation.⁷⁰ Therefore, the role of NK cells in contributing to such viral infections as hepatitis C and disease progression (particularly to hepatocellular carcinoma) requires further investigation.

Pathogen-specific immunity

Activation of the innate immune system is typically followed by stimulation of adaptive immune responses to viral pathogens. The antigen-specific nature of the adaptive immune response is due to rearrangements of genes (and additional somatic mutations) within T cells and B cells that encode for specific antigen receptors (T-cell receptors and immunoglobulin, respectively).²² Clearance of HCV seems to require a rapid and effective adaptive immune response targeting multiple antigenic targets of the virus polyprotein.^{22,71} Following priming of naïve T cells and B cells with viral antigens, memory cells are formed with the aim of preventing reinfection due to a stronger and higher avidity response upon reexposure. However, in the case of HCV, reinfection can be observed, particularly with a different strain of HCV, most likely due to the genetic heterogeneity observed for HCV genotypes and subtypes.

Studies have shown that a vigorous, broad, and sustained CD4⁺ and CD8⁺ T-cell response directed against HCV is generally observed within an individual experiencing a self-limited course of HCV infection.^{72–81} Sequential liver biopsies performed on infected chimpanzees revealed that viral clearance coincided with the accumulation of HCV-specific CD4⁺ and CD8⁺ T cells in the liver at 8–14 weeks after exposure to HCV.^{78,81} In addition, studies have shown a decrease in viremia that temporarily correlates with the emergence of cellular immunity within an individual mounting a brief HCV-specific T-cell response followed by a viral rebound after the loss of these T-cell responses.^{73,77} Finally, T cell-depletion studies in chimpanzees have confirmed the crucial role of cellular immune responses (CD4⁺ and CD8⁺ T cells) in the control of viral infection.^{81,82} On the contrary, chronic HCV infection is associated with an impaired or stunted immunological profile.^{72–75,80,83–91}

Impairment of cellular immune responses is a recurrent factor among chronic HCV-infected individuals. It is assumed that progression to chronicity among infected individuals is due to either weak or stunted T-cell responses developed by the host and/or immune escape (a strategy developed by the virus; discussed in Sequence-specific viral adaptations).

For both CD4⁺ and CD8⁺ T cells, the T-cell receptor is responsible for recognizing antigens bound to HLA molecules. This peptide–HLA complex is formed, processed,

and presented on the surface of an antigen-presenting cell (APC).²² The HLA molecules are highly polymorphic, and can be separated into two classes. HLA class I molecules (HLA-A, -B, and -C) are expressed on the surface of all nucleated cells, and are recognized by “cytotoxic” CD8⁺ T cells,⁹² while HLA class II molecules (HLA-DR, -DQ) present on the surface of professional APCs (eg, dendritic cells, macrophages, B cells) and present antigens that are recognized by CD4⁺ T cells. The HLA repertoire of the host determines the set of viral peptides or targets presented to the T cells. Not surprisingly, a number of HLA class I and II alleles have been associated with HCV-infection outcome.²⁸ Only a few of these associations are replicated, due to lack of power (partly as a result of the high heterogeneity of the HLA loci), unknown infecting virus, and the linkage disequilibrium between HLA class I (and to a lesser extent with the more distant class II loci-forming haplotypes, similar to what is observed for the KIR loci), which complicates the ability to identify associations between specific HLA alleles and infection outcome.

Although these host-“resistance” molecules are effective against pathogens, HCV (and other viruses) has developed several strategies to combat the host’s immune response. These strategies involve protein–protein interactions and specific mutations within the virus to escape recognition by the immune system.

Viral adaptation: relevance to infection outcome

Protein–protein interactions

To counteract the actions of the host’s immune response, HCV has developed several mechanisms to subvert the endogenous IFN response, including blocking the induction of IFNs, interference with signals triggered by IFNs, or the inhibition of ISGs.³⁹ As such, several studies have demonstrated that HCV NS3/4A affects the induction of IFN β , as well as subsequent ISGs,^{93,94} by interfering with the activation of IRF-3,⁹⁵ by disrupting RIG-I signaling^{94,96–98} and inhibiting TLR3 signaling,^{93,99} which are all essential steps of the IFN response. In parallel, NS5A and the envelope protein E2 are also assumed to block activity of PKR by binding to PKR and inhibiting formation of the PKR dimer.^{100–102} Additionally, the HCV core protein has been found to interfere with the Jak-STAT pathway that is involved in the expression of numerous ISGs.^{103–107} Overall, HCV uses numerous mechanisms to disrupt the IFN system, which can affect the action of endogenous IFN to control the HCV infection and the efficacy of IFN α -based treatment.

HCV has also been shown to affect HLA class I and II expression on the surface of APCs. The proteins core¹⁰⁸ and NS3¹⁰⁹ have been shown to affect the function of the proteasome complex¹¹⁰ and potentially HLA class I presentation. The core protein has also been implicated in reducing HLA class I expression via the transporter TAP1,¹¹¹ although in this case the change in HLA class I expression on target cells did not appear to affect CD8⁺ T-cell recognition, and may instead have been related to NK-cell cytotoxicity. Downregulation of HLA class I molecules has important consequences in relation to CD8⁺ T-cell immunity, but also NK-cell cytotoxicity via missing self, particularly for HLA-B and -C. However, it is still unclear which of the HLA class I loci are affected, as many of these studies have used pan-HLA class I antibodies. For HIV, the virus can downregulate specific HLA class I loci, but retain others as a strategy to subvert both T-cell and NK cell-mediated immune responses.¹¹²

The HCV proteins core and NS5A are also thought to be involved in the downregulation of HLA class II expression on APCs.¹¹³ Again, this is an important mechanism of viral escape from the immune system, as CD4⁺ T cells that engage HLA class II antigen presentation are critical in HCV-infection outcome, given that lack of CD4⁺ T-cell help and the loss of HCV-specific CD4⁺ T-cell responses following the acute phase of infection is strongly associated with viral persistence.^{114,115}

Sequence-specific viral adaptations

The viral population in an infected subject consists of closely related but nonidentical genomes, referred to as viral quasispecies. The presence of such viral diversity is mainly due to the error-prone RNA-dependent RNA polymerase that does not have a 3′-5′ exonuclease proofreading activity. Random genetic drift and selection pressures on the virus can change the composition of the quasispecies over time. The host immune responses as well as antiviral drugs have been identified as the main selection pressures shaping viral quasispecies.

In the context of the host’s T-cell immune response, this selection pressure can drive the evolution of HCV populations during the course of infection within a single host and circulating within the human population.¹¹⁶ As the host’s T cells see HCV in the context of the HLA molecules present on the surface of APCs, these viral mutations or viral adaptations are specific for HLA alleles. In population-based genetic studies that sampled the HLA type and viral sequence of a large number of virus-infected individuals, HLA-associated viral polymorphisms marked true *in vivo* targets of the host’s

T-cell immune response,^{117,118} as was originally shown for HIV.¹¹⁹ Such studies on viral adaptation at the population level have tended to examine chronic HCV-infected subjects, given the difficulty in accessing acute HCV-infection cohorts and also the ability to detect HLA “footprints” that are fixed (or within the major circulating viruses using bulk Sanger-based sequencing approaches) in the individual. However, there are data to suggest that bottlenecks in HCV infection occur during the acute phase of infection and emerging viral strains are likely to exhibit viral adaptations to the host’s HCV-specific immune response.¹²⁰ Accordingly, analysis of viral evolution during the acute phase of infection is important to better understand host–virus interplay and its effect on infection outcome.

Mutational escape can impact the presentation process and subsequently alter or impair the immune responses initiated by HLA molecules. These mutations provide a short-term benefit for the virus, in order to cope with immune responses. Nevertheless, absence of reversion after transmission can be observed for some mutant viruses, indicating minimal “fitness cost”.¹²¹ As a consequence, such escape mutants may spread through the population, regardless of HLA type, and ultimately the “wild-type” strain circulating in a population becomes the adapted form.¹¹⁹

Studies on single-source outbreaks in Germany and Ireland overcame some of the issues of studying viral adaptation, as the source strain was known and all exposed individuals could be assessed irrespective of the clinical nature of the acute infection (which is often asymptomatic in HCV infection). In both cases, the adaptation potential of the source virus was likely to have influenced the specific HLA alleles associated with infection outcome.^{122,123} For example, in the study of Irish women who were infected through the administration of anti-D immunoglobulin contaminated with an HCV genotype 1b strain, HLA-B8 was associated with poor outcome, and sequencing the source strain revealed existing viral adaptation within an immunodominant NS3 HLA-B8-restricted T-cell epitope,^{122,124} which is likely to have been disadvantageous for new hosts expressing HLA-B8. Similarly, in the same cohort, Fitzmaurice et al¹²⁵ found a novel T-cell epitope restricted by HLA-A3 in the NS3 protease (TVYH-GAGTK position 1080–1088), which was targeted by 60% of women in this cohort and associated with a strong “HLA footprint”, in that women who carried HLA-A3 and became chronically infected with HCV tended to exhibit a mutation within this target (K1088R). The HLA-A3 allele was found to be protective, which could be explained by the requirement of a second substitution (T1087A) to offset the significant

loss of fitness associated with the K1088R change that was also found in HLA-A3 chronic HCV-infected women in this cohort. In the cohort of German women who had received HCV-contaminated anti-D immunoglobulin, spontaneous HCV clearance was associated with HLA-B-27,¹²³ and the source sequence showed no evidence of viral adaptation in an immunodominant HLA-B27 T-cell epitope. Mutations in this epitope are commonly observed in chronic HCV-infected subjects with HLA-B27. Similarly, preservation of targeted epitopes for the HLA-B57 allele is also associated with viral clearance.¹²⁶

But what is the contribution of viral adaptation to HLA-specific T-cell responses to overall viral diversity? HIV and simian immunodeficiency virus studies have demonstrated the role of CD8⁺ T-cell selective pressure in shaping viral evolution, showing that more than 50% of mutations developed after acute infection are associated with CD8⁺ T-cell responses,¹²⁷ including the reversion of CD8⁺ T-cell escape mutations, likely due to viral fitness cost.^{128–130} Similar observations were made with HCV studies,^{124,131} however, these viral adaptations were observed to a lesser extent, as shown by Kuntzen et al,¹³² with up to 11% of mutations occurring within regions targeted by detectable CD8⁺ T-cell responses (in nonenvelope regions). Interestingly, of the de novo mutations observed in the Kuntzen et al study, 19% of these changes were reversions to wild type, with the majority (80%) of these reversions occurring upon transmission or during the acute stage of the infection. We have also previously shown less extent of HCV variation during the acute phase of infection compared to HIV and a high number of synonymous changes relative to nonsynonymous changes observed between longitudinal sequences in subjects, reflecting greater constraint along the HCV genome than for HIV.¹³³ These HCV studies suggest that reversion is likely to happen upon transmission if immune pressure is no longer present, as escape mutations are generally associated with a fitness cost and overall HCV may not be as flexible as HIV. It should be noted that the number of known T-cell epitopes for the two viruses is heavily biased toward HIV, and this may account for some of the discrepancy reported in the proportion of viral adaptations of total viral diversity observed during the natural course of early infection.

More recently, there has been some evidence to suggest that NK cells can directly influence viral evolution. Alter et al¹³⁴ looked at NK cell-mediated immune pressure on HIV evolution via KIR-associated amino acid polymorphisms along the HIV-1 sequence of chronic HIV-infected individuals. Their study revealed polymorphisms associated

with the presence of inhibitory receptor KIR2DL2 in Vpu and Env, showing that these polymorphisms allow binding of KIR2DL2 upon HLA-C presentation, whereas the HIV wild-type sequences do not. These KIR-associated polymorphisms can modulate the interaction of KIR⁺ NK cells with HIV-1-infected CD4⁺ T cells, thus demonstrating that the viral variants presenting these “KIR footprints” seem to impact on NK-cell recognition and/or antiviral activity and constitute another mechanism by which HIV can escape NK cell-mediated immunity, a sequence-specific manner akin to what has been observed for viral immune escape from T cells.

Today, the impact of NK cells on HCV infection has only been investigated with respect to the presence of a specific KIR and outcome of infection. However, given the numerous features shared by HIV and HCV, specifically their high mutation rates and genetic association studies showing an association between infection outcome and specific KIRs and NK-cell activation, HCV evolution could also be subject to NK cell-mediated immune pressure. At present, such an analysis has not been done with HCV-infected subjects, and further research is necessary to understand the role and impact of NK cells on HCV-infection outcome.

Overlap between selection pressures: relevance to treatment outcome

In addition to immune selective pressure, antiviral treatment is another factor that can influence within-host viral diversity. The use of IFN-based treatment could be taken as an indicator of IFN-based immune pressure on the virus. However, data on the impact of IFN-based treatment on viral diversity are limited and contradictory.^{135–138} No specific resistance mutations have been associated with impairment of IFN therapy, which can be explained by the indirect action of IFNs on viral infections.¹³⁹ Rather than specific mutations, there is evidence to suggest that sequence heterogeneity within areas of the HCV genome may affect IFN-based treatment outcome. For example, Enomoto et al^{140,141} demonstrated in Japanese subjects infected with HCV genotype 1b strains that greater sequence heterogeneity within the IFN sensitivity-determining region of the NS5A protein was associated with IFN-treatment outcome. However, overall there are limited data to suggest specific viral variants are selected by IFN-based therapy (and likely IFN responses in general).

Unlike IFN, the new direct-acting antiviral (DAA) drugs used for hepatitis C do not enhance existing immune responses, but directly target the action of viral proteins involved in the virus's life cycle. First-generation inhibitors

of NS3/4a serine protease, telaprevir, and boceprevir, in conjunction with peg-IFN α /RBV, were approved for clinical use over 12 months ago, and more recently the second-generation NS3/4a protease inhibitor simeprevir and the NS5B inhibitor sofosbuvir have been approved for clinical use in combination with peg-IFN α /RBV. These new DAA drugs have a >90% efficacy rate against HCV genotype 1. However, HCV is a rapidly evolving virus with a high mutation rate, and the rapid emergence of drug-resistance mutations can jeopardize treatment outcome for these drugs that directly target the virus (unlike IFN-based therapies).^{142–144} Furthermore, reports have shown evidence of drug-resistant variants in treatment-naïve subjects, but typically at low frequencies.¹⁴⁵

Of interest are those sites along the viral genome that are likely to be under two selection pressures: immune response and antiviral treatment. HIV studies have previously shown the influence of immunological host factors on the emergence of drug-resistance variants, demonstrating the increased risk for the subject to develop mutations at sites at which the two selective forces intersect. John et al¹⁴⁶ examined HIV sequences and HLA data from a cohort of 487 HIV-1-infected individuals, and identified an increased incidence of drug-resistance mutations among individuals that expressed an HLA that targeted the same region. In another cohort of 94 HIV-1-positive subjects, Mueller et al¹⁴⁷ demonstrated that several drug resistance-associated mutations along the protease acted as CD8⁺ T-cell escape mutants. Few HCV studies have shown similar results, given the recent introduction of the new DAAs. However, Salloum et al looked at substitutions R155K and A156T, which constitute key positions for resistance to DAAs, such as telaprevir and boceprevir, and the overlap with the HLA-A68-restricted epitope (HAVGI-FRAAV) from position 1175–1184.¹⁴⁸ They investigated the impact of these protease inhibitor-resistant mutants at the replication level, as well their influence on the HCV-specific antiviral CD8⁺ T-cell response. Their replication assay showed a low and intermediate fitness cost associated with the R155K and A156T changes, respectively, and that these variants enabled immune escape from HLA-A68-restricted CD8⁺ T cells. HLA-A68 is a common allele in Native American populations, but less so in Caucasian and Asian populations (<http://www.allelefrequencies.net>). We also analyzed HCV sequences from over 400 subjects infected with HCV genotypes 1a, 1b, and 3a, and evaluated the prevalence of drug-resistance mutations within sites along the HCV NS3 protease and NS5B polymerase genes for which drug and immune selective pressures are likely to intersect. We identified

sites within immunodominant T-cell epitopes in which host immune pressure may affect the frequency of drug-resistant variations in subjects undergoing specific DAA treatment and the site and variation of interest is a known immune escape mutation that is often offset with surrounding compensatory mutations.¹⁴⁹

In parallel to DAAs, agents targeting host proteins or nucleic acids are being developed. These compounds target cellular proteins and microRNAs that are essential for the viral life cycle, and thus inhibit the ability of HCV to use host-cell components for continued infection. These pharmaceutical agents include cyclophilin inhibitors, which aim to disrupt the interaction of NS5a and cyclophilin A,¹⁵⁰ and an antagonist of microRNA 122 that inhibits its binding to the 5' untranslated region of the HCV genome, an essential step for HCV replication.^{151,152} These agents could be an important addition to DAAs in the treatment of HCV, as they have a high genetic barrier to resistance, with no cross-resistance with DAAs and present pan-HCV genotypic activity.¹⁵³

Conclusion

The host's immune response is an important correlate of infection outcome. Accordingly, coevolution between host and pathogen has resulted in a number of strategies for both organisms to improve fitness. In the context of human pathogens, such as HCV, the life cycle and mutation rate between host and pathogen are different, and reflect the strategies undertaken by each to improve survival. For the host, the innate and adaptive immune responses combine to combat the pathogen using nonspecific (but nonself) and specific means to rid the pathogen or at least minimize damage. The evolution of these molecules, such as IFN, TLRs, KIR, and HLA, has resulted in multicopy, polymorphic systems that can identify and combat a large number of pathogens. However, such pathogens as HCV can subvert a number of the host's immune mechanisms via protein-protein interactions, such as downregulation of HLA molecules on the surface of the APC and interfering with the IFN signaling pathway and via a rapid mutation rate. Viral adaptation via sequence-specific changes is associated with T-cell and B-cell escape. In the case of T cells, these viral adaptations are recognizable at the population level and within a single infected individual.

In this review, we have focused on viral adaptation to HCV-specific T-cell responses, but clearly antibodies directed against specific HCV antigens generated from B cells also influence viral diversity, particularly in the envelope proteins. Importantly, knowledge of viral adaptation to the different arms of the host's immune response will be useful in the

design of a preventative vaccine for both HIV and HCV, as well as in identifying new treatment strategies.

Disclosure

The authors report no conflicts of interest in this work.

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