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Viral Evasion and Challenges of Hepatitis C Virus Vaccine Development

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Abstract

Hepatitis C virus (HCV) is a major global disease burden, often leading to chronic liver diseases, cirrhosis, cancer, and death in those infected. Despite the recent approval of antiviral therapeutics, a preventative vaccine is recognized as the most effective means to control HCV globally, particularly in at-risk and developing country populations. Here we describe the efforts and challenges related to the development of an HCV vaccine, which after decades of research have not been successful. Viral sequence variability poses a major challenge, yet recent research has provided unprecedented views of the atomic structure of HCV epitopes and immune recognition by antibodies and T cell receptors. This, coupled with insights from deep sequencing, robust neutralization assays, and other technological advances, is spurring research toward rationally HCV designed vaccines that preferentially elicit responses toward conserved epitopes of interest that are associated with viral neutralization and clearance.

Introduction

Hepatitis C virus (HCV) is estimated to infect approximately 185 million individuals, or 3% of the global population [1], with chronic infection that can lead to liver failure and hepatocellular carcinoma. While more prevalent in developing countries, HCV remains a significant problem in Europe and the United States, where in the latter it infects 1% of the population and is responsible for more deaths than all other infectious diseases combined [2].

HCV is a small, blood-borne, positive stranded RNA virus in the flaviviridae family that has been a target of vaccine and therapeutic research efforts since its discovery in 1989 [3]. However, clinical trials of several vaccines over the past decades (reviewed in [4,5]) have failed to produce an approved vaccine. Direct-acting antiviral (DAA) therapeutic drugs that target HCV nonstructural proteins have been approved for HCV, with cure rates of over

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90%. But the emergence of DAA resistance variants [6], high treatment cost, drug safety issues associated with advanced cirrhosis and treatment [7], and importantly, the observation that many infected individuals are unaware of their infections and thus unlikely to seek treatment (estimated to be 95% of those infected) [8] make a vaccine a high global health priority. Here we will address the challenges associated with viral evasion and recent advances toward a HCV vaccine, focused on mapping, characterizing and inducing potent broadly neutralizing antibodies.

HCV immune evasion and sequence variability

A number of mechanisms have been identified through which HCV evades the immune response, as reviewed by others [9,10]. These include:

1. Viral sequence variability, leading to mutated proteins that lose binding to adaptive immune receptors.
2. Immunogenic decoy epitopes that focus the immune response away from broad neutralization-associated epitopes.
3. Epitope shielding by glycans, mobile protein regions, non-neutralizing antibodies, and lipoproteins.
4. Direct cell-to-cell transmission.
5. Downregulation of major histocompatibility complex (MHC) expression [11,12].

Of note, points 1–4 apply to the antibody response, while points 1 and 5 are relevant to the cellular immune response. The concept of antibody interference, where neutralizing antibodies are out-competed by non-neutralizing antibodies for viral envelope binding (noted in (3)), has been proposed in humans that were vaccinated with E1E2 protein [13]; but studies using oligoclonal and monoclonal antibodies from HCV-infected individuals have not supported interference at the identified sites [14,15]. Interestingly, antibodies to hypervariable region 1 (HVR1) at the N-terminus of the E2 protein has recently been associated with interference against broadly neutralizing antibodies [16].

High sequence variability is a hallmark of HCV, with seven major genotypes that vary in prevalence between countries, continents, and socioeconomic groups [17,18]. Notably, within infected individuals, the virus actively evades the immune system [19] and evolves into a large number of quasispecies through error-prone replication [20]. This presents a major challenge for vaccine design, requiring the identification of functionally important, conserved portions of the virus to target immunologically.

The sequence variability of HCV is not uniform within protein coding regions. Figure 1 shows the sequence conservation and amino acid preferences at all positions of the E2 envelope glycoprotein, which is the primary target of the host antibody response. E2 is punctuated by several regions with variable sequences, named HVR1 (aa 384–410), HVR2 (aa 460–485), and igVR (aa 570–580), while several other regions exhibit moderate to complete sequence conservation, for example residues 412–423 which is a linear epitope

targeted by well-characterized broadly neutralizing antibodies [15,21,22]. Several other regions and residues of E2 are nearly 100% conserved, such as residues 502–520 which were noted to be important for recognition of host entry receptors and antibodies [23], as well as certain cysteine residues that participate in disulfide bonds, and proline residues (e.g. P525, P544, P545, P567, P568) that are likely functionally or structurally important in light of their absolute or near-absolute conservation.

Mapping HCV envelope receptor and antibody recognition

While early work found that HVR1, which is associated with non-neutralizing antibodies and escape via mutation, is an immunodominant site for antibody recognition [24], later studies have identified and characterized sets of neutralizing monoclonal antibodies that target other sites on the viral envelope (reviewed in [25]). Many target the E2 glycoprotein, and were cloned from infected human sera [15,26–29], alternatively from mice [22,30–32] or an alpaca [33] immunized with E2 protein (the alpaca immune receptor was a single chain nanobody). Additionally, a subset of human neutralizing antibodies specifically recognizes the E1E2 heterodimer [34] or E1 alone [35]. Based on monoclonal antibody (MAb) binding competition, linear peptide binding, and alanine scanning epitope mapping studies, studies have identified several sites on E2 that antibodies preferentially target, designated by varying nomenclatures with overlapping definitions (Table 1). For this review, antigenic domain nomenclature will be used. Figure 2 shows mapping and structural data for antigenic domain E and the combination of antigenic domains B and D, referred to as the B–D supersite. The latter occupies a contiguous face on the E2 protein and overlaps with antigenic region 3 based on independent mapping work [28]. As shown in Figure 2, these sites include key binding residues of the CD81 receptor, which is a primary entry receptor for HCV [36,37]; antibodies targeting these sites compete directly with virus binding to CD81. It should be noted that in some instances alanine scanning mutagenesis does not identify antigenic side chains that contact the antibody. Residue D535, for example, does not contact the AR3C antibody (approximately 11 Å from the antibody), rather it appears to stabilize a key loop, which is likely why it is critical for binding based on alanine scanning (Figure 2). Other E2 sites include antigenic domain A, which is associated with non-neutralizing antibodies [38] and includes residue Y632 [29], and antigenic domain C, which is associated with neutralizing antibodies [39] and includes residue W549 [40]. A prototypical antigenic domain C antibody is CBH-7, which neutralizes HCV, competes with E1E2-specific MAbs for E1E2 binding [34] (suggesting proximity of antigenic domain C to the E1 interface) and prevents CD81 engagement, though interestingly it can bind the CD81-E2 complex [26]. Some of these envelope regions, such as antigenic domain B, are relatively immunogenic [41], while others, such as antigenic domain E, are of low immunogenicity [14,42].

Experimental structural characterization studies have revealed the molecular basis of antibody targeting of several key epitopes, as shown in Figure 3, and provide a framework for rational vaccine design. These include structures of antibodies targeting linear epitopes of E1 [43], E2 [16,44–51], and two structures of antibody-bound E2 “core” proteins [52,53]. Collectively, these highlight the structural dynamics of certain key regions of HCV, for instance at residues 412–423 which exhibits a β hairpin [44,48,54] or extended conformations [46,47] when engaged by antibodies, in addition to likely dynamics within

the 430–446 region [51]. Notably, despite high overall structural agreement between two solved E2 core structures (0.8 Å α -carbon atom root-mean-square distance), there is a discrepancy in disulfide bonding patterns, as noted by others [55,56]; it is not clear whether this is due to the different engineered core constructs, expression systems, distinct genotypes, or natural E2 heterogeneity.

Mapping determinants of viral neutralization and escape

Investigation of patient-derived HCV isolates in vitro [57–59], cell culture viral evolution in the presence of antibodies [29,60–62], and MAb therapy in humans [63,64] and chimpanzees [65] have identified a number of residues and mutants that mediate escape from broadly neutralizing antibodies; several are highlighted in Table 1. These include the notable example of antigenic domain E sites N415 and N417, where a glycan shift to N415 (via N417S mutation) or certain mutations at N415 were selected under immune pressure in phase II clinical trials of the HCV1 MAb [63], later confirmed in more detail by deep sequencing analysis [64]. However N415 is not a major binding determinant for HC33.1 (Figure 3A), which binds antigenic domain E in an extended conformation, as opposed to the β hairpin conformation engaged by HCV1 and AP33 (Figure 2). The role of conserved glycans at other sites on E2 in attenuating antibody neutralization has also been noted [66,67]. Interestingly, escape-associated E2 residues in Table 1 have been identified in multiple independent studies, including residues Q444 and K446. As the latter residue, along with residue F442, is a contact residue for antigenic domain D antibodies, it is possible that such mutants directly impact recognition of, thereby mediating escape, from antibodies targeting this site, despite evidence of escape resistance in vitro.

Such limited, but observed escape mutants in antigenic domain E and the B–D supersite led some to suggest a combination of antibodies targeting these sites would lead to improved protection and eliminate escape [68]; supporting this observation, a neutralizing antibody combination was used to eradicate established HCV in humanized mouse models [69].

The development of in vitro assays to measure HCV neutralization has provided the tools to characterize the breadth, overlap, and determinants of antibody-mediated neutralization, as seen in two recent studies that employed large panels of HCV pseudoparticles (HCVpp) representing diverse isolates to assess breadth of neutralization for panels of MAbs [70,71]. One of these studies, using genotype 1 HCVpp only, found evidence for context-dependent effects of E2 mutants on antibody binding and neutralization, in addition to effects from mutants distal from the putative antibody binding site [70]. The other study tested HCVpp representing genotypes 1–6 and found that different isolates displayed different levels of overall neutralization sensitivity across the MAb panel [71]. This supports results from other studies where broadly neutralizing human MAbs targeting a linear epitope exhibited varying neutralization efficiencies against different isolates, despite identical epitope sequences [15]. Recent in vivo work suggests that HVR1, which includes three functional subdomains [72] and is the target of early infection strain-specific responses [19], is also responsible for varying antibody neutralization across genotypes for due to shielding of multiple conserved epitopes [73]. These studies collectively highlight the structural and dynamic complexity of

the E2 glycoprotein, for which further research is needed to uncover the molecular basis of escape and resistance.

Previous B cell and T cell vaccine studies

Several HCV envelope vaccine candidates have been tested in humans, recently reviewed in [5], and include E1 protein [74], core-E1E2 DNA [75], and E1E2 protein [76,77], with the latter having been tested in healthy and chronically infected individuals (phase I and phase Ib, respectively), with epitopes and neutralization breadth determined for immunized human and goat sera [78]. The primary T cell-based vaccine candidate for HCV, developed by Okairos and currently in phase I/II studies, contains its nonstructural proteins (NS3–NS5b) in recombinant viral vectors [79]. A version of this vaccine failed to induce a sufficient response in chronically infected patients in the context of therapeutic vaccination trial [80], with T cell exhaustion noted as a likely cause. Though it is still unclear whether antibody, CD4+ or CD8+ T cell response, or a combination thereof, is necessary for an effective vaccine in humans [4], E1E2 and virus-like particle (core-E1–E2) protein vaccines were found to produce strong B and T cell responses [77,81]. Thus a designed vaccine including the envelope glycoproteins can serve as the framework, or at minimum a component, of an effective HCV vaccine.

Rational design of an HCV vaccine

The correlation of host antibody response to HCV with viral clearance, coupled with recent successes in structure-based vaccine design for other viruses such as HIV [82,83], RSV [84] and influenza [85], suggests that a rationally designed vaccine that elicits neutralizing antibody responses to conserved epitopes is a viable route to an effective HCV vaccine. Several engineered E1, E2 and E1E2 antigens have been recently described [32,86,87], including structure-based epitope designs [88,89], the majority of which were tested for immunogenicity in mice, though varying conditions (e.g. protein versus DNA, adjuvant, vaccine dosage and schedule) and immunological readouts prevent a direct comparison of results from these designed antigens. Further studies in this regard can define correlates of robust response and neutralization breadth for epitope-based and protein-based antigens, with designs explicitly accounting for escape variants, fully conserved envelope residues, as well as structural data from experiments and simulations.

Recent work has indicated that the E1 protein assembles into homotrimeric arrangements on the viral surface, mediated by a GxxxG motif in its transmembrane domain [90]. Defining this crystallographically, in addition to the E1–E2 interface, would provide the basis for structure-based design of stabilized E1E2 trimers, analogous to the HIV gp140 trimers currently being investigated as immunogens [91,92]. Furthermore, high resolution structural characterization of additional antibody-epitope complexes to further elucidate the structural repertoire of CD81 binding site targeting (as was performed for HIV CD4 binding site antibodies [93]), in addition to the complexes of E2 with entry receptors CD81 and SR-B1, would complement epitope mapping studies and would be highly informative in defining critical residues, regions, and structural features for vaccine design.

Conclusions

Insights from recent structural, sequencing and immunogenicity studies provide the basis for novel vaccine designs for this challenging viral target. While accounting for the primary mechanisms of viral escape, namely sequence variability and HVR1, immunogens can be tuned using rational protein design to optimize their capacity to induce broadly reactive neutralizing antibodies that target critical sites on the viral envelope.

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Highlights

- HCV is a global health problem and a challenging vaccine target
- HCV actively evades the immune response through high sequence variability
- Structural and amino acid determinants of antibody recognition have been identified
- Designed HCV vaccines can present key conserved epitopes and minimize viral escape

Figure 1.

Amino acid sequence variability of HCV envelope glycoprotein E2, the primary target of the antibody response. A sequence logo [94] was generated using a multiple sequence alignment of over 600 E2 sequences downloaded from the Los Alamos HCV database [95]. This gives amino acid propensities at each E2 position (numbering based on the H77 HCV isolate), with total height at each position representing sequence conservation (more variable positions have lower height). Hypervariable regions HVR1, HVR2 and igVR are shown by dotted red boxes, with HVR1 and epitope region 412–423 (antigenic domain E) highlighted.

Figure 2. Mapping neutralizing antibody and CD81 receptor binding determinants on the E2 glycoprotein. A) E2 alanine scanning for domain E MAbs (HC33.1, HC33.4, H77.39, AP33), domain B MAb HC-11, and CD81 receptor at E2 positions 412–423 [16,32]. Values represent percent antibody or receptor binding compared to wild-type E2; N = not reported. Cell colors for binding percentiles are: 0–20% red, 21–40% orange, 41–60% yellow, 61–90% white, > 90% green. B) E2 alanine scanning for AR3 MAbs AR3A, AR3C, domain B MAbs HC-1 and HC-11, domain C MAb CBH-7, domain D MAbs HC84.24 and HC84.26, and CD81 receptor at select E2 binding positions in the domain B–D supersite [28,29,32,61]. Cell colors are as in (B). C) Positions of key neutralizing antibody binding residues for domain E (blue spheres) and domain B–D (green spheres) colocalized on the E2 structure. The E2 protein (tan) and bound AR3C MAb (green cartoon) are from the E2 core crystal structure [52] with N-terminal E2 residues 412–423 modeled using Rosetta [96] (residues 412–420 are disordered in the crystal structure), and HCV1 MAb superposed onto residues 412–423 for reference, based on the HCV1-E2 412–423 complex crystal structure [44].

Figure 3.

Mapping the structural landscape of neutralizing antibody recognition of E1 and E2 envelope glycoproteins. Antibody-bound peptide epitopes of E1 and E2, as well as the E2 core protein (aa 421–645, with HVR2 removed), are shown in magenta. Antibody heavy and light chains are green and cyan, respectively. Corresponding PDB codes are 4N0Y (IGH526/E1 314–324), 4DGY (HCV1/E2 412–423), 4XVJ (HC33.1/E2 412–423), 4HZL (mAb#8/E2 430–442), 4JZN (HC84.1/E2 434–446), 4MWF (AR3C/E2 core).

Table 1

Antibody binding sites on E2, key residues, and nomenclature.

Residues	Key Residues	AD ¹	AR ²	Epitope ³
412–423	L413, W420	E	1	I
434–446	W437, L441, F442	D (B)	3	II
529–535	W529, G530, D535	B	3	III
544–549	W549	C (A)	1	III
627–637	F627, Y632	A (C)	-	-

¹ Antigenic domain [15,29,38]. Parentheses indicate antigenic domains with a subset of antibodies found to bind that site, based on epitope mapping.

² Antigenic region [28].

³ Described in a review by Drummer [97].

Table 2

Selected escape mutants identified from in vivo HCV isolates, HCV cell culture (HCVcc), and antibody efficacy studies.

Position	Original AA	Mutant AA	Context	Comment/Association	Reference
413	L	I	cell culture with HC33.1 HMAB	not seen outside of cell culture	[62]
415	N	D/K/S	HCV1 MAb clinical trial		[63]
415	N	Y	cell culture with AP33 MAb		[60]
417	N	S	HCV1 MAb clinical trial		[63]
419	S	N	cell culture with HC33.1 HMAB	loss of viral fitness	[62]
424	R	K/S	genotype 1 neutralization panel	broad resistance	[70,98]
431	D	G	cell culture with CBH-2 HMAB		[61]
438	L	F	cell culture with HC-11 HMAB		[61]
439	A	E	cell culture with CBH-2 HMAB		[61]
442	F	I/L	genotype 1 neutralization panel		[70]
444	Q	R	HCV1 MAb chimp efficacy	restores infectivity of N417S	[65]
444	Q	Y	chronic infection (26 year)		[57]
446	K	H/E/N	genotype 1 neutralization panel		[70]
446	K	R	chronic infection (26 year)		[57]
447	F	L	acute infection clone VL	infectivity, antibody resistance	[59]
458	S	G	acute infection clone VL	infectivity, antibody resistance	[59]
478	R	C	acute infection clone VL	infectivity, antibody resistance	[59]
501	S	N	chronic infection (26 year)		[57]
506	V	A	chronic infection (26 year)		[57]
655	E	G	cell culture with AP33 MAb	resistance with N415Y mutant	[60]