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Hepatitis C virus vaccine design: focus on the humoral immune response

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Abstract

Despite the recent development of safe and highly effective direct-acting antivirals, hepatitis C virus (HCV) infection remains a significant health problem. In 2016, the World Health Organization set out to reduce the rate of new HCV infections by 90% by 2030. Still, global control of the virus does not seem to be achievable in the absence of an effective vaccine. Current approaches to the development of a vaccine against HCV include the production of recombinant proteins, synthetic peptides, DNA vaccines, virus-like particles, and viral vectors expressing various antigens. In this review, we focus on the development of vaccines targeting the humoral immune response against HCV based on the cumulative evidence supporting the important role of neutralizing antibodies in protection against HCV infection. The main targets of HCV-specific neutralizing antibodies are the glycoproteins E1 and E2. Recent advances in the knowledge of HCV glycoprotein structure and their epitopes, as well as the possibility of getting detailed information on the human antibody repertoire generated by the infection, will allow rational structure-based antigen design to target specific germline antibodies. Although obtaining a vaccine capable of inducing sterilizing immunity will be a difficult task, a vaccine that prevents chronic hepatitis C infections, a more realistic goal in the short term, would have a considerable health impact.

Keywords: HCV, Antibody, Vaccine, Humoral immune response, Glycoprotein E1, Glycoprotein E2, Virus neutralization

Background

The hepatitis C virus

Hepatitis C virus (HCV) is an enveloped, positive-sense single-stranded RNA virus that belongs to the *Hepacivirus* genus within the *Flaviviridae* family. Its genome of 9.6 kb is translated into a single large polyprotein, which is processed by cellular and viral proteases into ten mature proteins, comprised of three structural (core, E1, E2) and seven non-structural (NS) proteins (p7, NS2, NS3, NS4A, NS4B, NS5A, and NS5B) [1]. HCV has high genetic diversity with seven main genotypes and more than 60 subtypes, of which genotype 1 is the most prevalent [2]. The difference at the nucleotide level is approximately 30% between genotypes and 15% between subtypes of the same genotype. Additionally, HCV shows enormous genetic diversity within an infected individual, where it exists in the form of quasispecies generated by the high error rate of the HCV polymerase and the elevated replication rate of the virus. These quasispecies can differ by up to 10% in their nucleotide sequence [2–4].

The natural history of hepatitis C infection

HCV is an important health problem that affects approximately 1% of the global population [5]. Blood transfusions, nosocomial transmission, sharing equipment between injecting drug users (IDU), and tattoos are recognized as common modes of HCV transmission. There

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is also evidence that HCV may be transmitted sexually among men who have sex with men (MSM) [6]. Following the initial HCV infection, a variable incubation period follows, after which approximately 25% of subjects clear the virus [7]. Fulminant hepatic failure due to acute HCV infection is rare (< 1%), but is a dramatic clinical syndrome with high mortality. The risk of chronic hepatitis C (CHC) infection is high, and around 75% of patients remain HCV RNA positive after acute hepatitis C [7]. According to the World Health Organization (WHO), 71 million people were living with CHC infections worldwide in 2015, and around 2 million new infections occur each year [5, 8]. The long-term natural history of CHC leads, after many years of fibrosis, to liver cirrhosis in approximately 10–20% of patients within 20–30 years. Once cirrhosis is established, decompensated cirrhosis, end-stage liver disease, and hepatocellular carcinoma may develop [9]. Inevitably, terminal liver disease leads to death or the necessity for liver transplantation [9].

Worldwide elimination of HCV: the need for a prophylactic vaccine

HCV treatment has changed substantially in the last decade with the appearance of direct-acting antivirals (DAAs) [6], which specifically inhibit the function of various NS proteins essential for viral replication, such as the serine protease (NS3/4a) and the RNA-dependent polymerase (NS5b) [10]. After 2014, the second generation of DAAs was available and dramatically increased the cure rate to more than 95% [11]. Moreover, DAA therapy is safer, and its duration is shorter than interferon therapy, the previous standard of care [12]. Following this therapeutic advance, in 2016 the WHO set out to reduce the rate of new HCV infections by 90% by 2030. This initiative involves the scale-up of HCV screening, risk behavior reduction, and unrestricted access to DAA treatment [13]. Based on this strategy, lowering the total number of HCV-positive people worldwide would therefore reduce de novo infections.

However, in the absence of an effective vaccine, there are some limitations to this approach [14, 15]: 1) HCV treatment itself has several unresolved problems. First, between 2 and 5% of HCV-infected patients are not cured of their HCV infection, and DAA therapy can select for resistant variants that limit the effectiveness of the treatment. Second, DAAs are still expensive and inaccessible in most developing countries. 2) Both acute hepatitis C and CHC are largely asymptomatic, and approximately 80% of people infected worldwide are not aware of their infection. Consequently, only 20% of HCV-infected patients are diagnosed, and only 15% of those have been treated [6]. All undiagnosed and untreated patients continue to develop the disease and are potential transmitters of the virus. Reaching treatment rates greater than 60–70% will be problematic, especially in underdeveloped countries. 3) Many subjects infected with HCV and new HCV infections occur in marginalized populations that are difficult to access, such as people who inject drugs (PWIDs), sex workers, MSM, and incarcerated people. These people have limited access to HCV screening and treatment. 4) HCV clearance with DAA therapy does not protect against reinfection. The immunity generated against HCV during CHC is not usually protective, and HCV reinfection after DAA therapy can hamper elimination targets [16]. 5) Finally, the diagnosis of HCV infection is usually performed at advanced stages of liver fibrosis and, although the HCV treatment is successful, severe liver damage is often not completely reversed.

All these reasons make the development of a prophylactic vaccine very likely necessary to control HCV infection worldwide. Effective vaccination strategies at the population level have been the only reliable method to control the transmission of different viral infections by providing herd immunity [17]. Furthermore, in the case of HCV, sterilizing immunity by vaccination would not be necessary to control transmission in high-risk groups. A vaccine reducing viral titers would be sufficient [18]. Furthermore, it has been modeled that a vaccine with only 30% efficacy would have substantial effects on HCV transmission when administered to a high number of high-risk uninfected PWIDs [19–21].

Current vaccine approaches

Current approaches to the development of a vaccine against HCV include the production of recombinant proteins, synthetic peptides, DNA vaccines, virus-like particles (VLPs) and viral vectors expressing various antigens [22]. These vaccines are aimed to induce either cellular, humoral, or both immune responses [23]. Interestingly, VLPs are emerging as attractive candidates in HCV vaccine design because they can induce high levels of both cellular and humoral immune responses [24–26]. Nevertheless, further studies are required to discover ways to induce long-lasting and highly protective immune responses.

Vaccines inducing T cell-mediated immunity are usually centered on relatively conserved HCV proteins, such as the NS3, NS4, NS5, and core proteins, which are targets of $CD8^+$ T cells [27]. Some preclinical assays and phase 1 vaccine trials targeting only T-cell responses have been unsuccessful [23, 28]. A promising vaccine based on priming with chimpanzee adenovirus three coding NS proteins (ChAd3-NS)

and boosting with modified vaccinia Ankara virus (MVA-NS) was tested in human volunteers. This regimen induced broad HCV-specific memory $CD4^+$ and $CD8^+$ T cells [29]. Subsequently, this vaccine was tested in a phase 1/2 trial in PWIDs (clinicaltrials.gov identifier NCT01436357), but no protection was shown in patients with CHC infection [30], highlighting the need for a vaccine that induces humoral immune responses along with cell-mediated immunity. Vaccines aimed to induce humoral immune responses are based on the HCV glycoproteins E1, E2, or the E1E2 heterodimer, which are the main targets of protective broad-spectrum neutralizing antibodies (bnAbs). The current vaccines that induce humoral responses against HCV in pre-clinical and clinical trials have been the subject of recent seminal reviews [23, 28], and are summarized in Table 1 [31–52]. Within this approach, the most effective candidate so far is a recombinant E1E2 (rE1E2) purified protein based on the HCV genotype 1a. This vaccine was protective in chimpanzees after homologous challenge [53], and reduced rates of persistence after heterologous challenge [54]. The rE1E2 protein in an oil-in-water emulsion was safe in humans [43], and induced bnAb response [44, 55], although only in three of 16 vaccinated individuals [44].

In this review, we focus on the development of vaccines targeting the humoral immune response against HCV due to the cumulative evidence supporting the important role of cross-reactive bnAbs in protection against HCV infection. Moreover, recent crucial information about the structure of HCV glycoproteins, their epitopes, and the protective antibody response in humans opens new and exciting expectations in this field. A full description of potential vaccines inducing T cell-mediated immunity and the role of T-cell responses in HCV clearance and protection from reinfection is beyond the scope of the present review. Therefore, we refer the reader to recent excellent seminal reviews for further reading [23, 56].

The humoral immune response against HCV infection: evidence supporting antibody-based vaccines

Animal models

There is ample evidence that passive immunization in animal models with HCV-specific nAbs may protect from infection by homologous and heterologous HCV strains and completely clear the acute infection [37, 57–67]. The first in vivo studies demonstrating that nAbs protect against homologous HCV were conducted in chimpanzees. Rabbit hyperimmune serum to the synthetic hypervariable region 1 (HVR1) of HCV E2 glycoprotein, as well as plasma from a CHC patient, neutralized the infectivity of homologous HCV in chimpanzees [59, 60]. The administration of the monoclonal nAb HCV1 (directed against the E2 glycoprotein) prevented the infection of a chimpanzee with HCV genotype 1a, and reduced viral load in acutely and chronically-infected animals [66]. A study conducted by Bukh et al. found a prolonged suppression of HCV replication after challenge with homologous, but not heterologous genotypes, in chimpanzees passively immunized with nAbs from an individual with chronic HCV genotype 1a infection [57]. Protection against homologous challenge was also observed in human liver chimeric mouse models after the infusion of nAbs from CHC patients [65, 67], or a pool of three monoclonal nAbs (AR3A, 3B and 4A) targeting the HCV E2 and E1E2 complex [58].

Due to the high genetic diversity across HCV genomes, the early development of bnAbs capable of blocking infection with multiple heterologous HCV strains is a challenge. Although several studies have shown that active or passive immunization protects against heterologous HCV challenge in chimpanzees [62] and humanized mice [37, 61, 63, 64], this phenomenon is not universal because not all genotypes are blocked.

The use of chimpanzees as a model for the study of humoral immune responses against HCV infection has a great advantage due to its genetic similarity to humans. However, high costs and ethical concerns limit its use [68]. Thus, alternative animal models, particularly humanized mice, are under development to create the 'ideal' model fully mimicking clinical settings. Some aspects that can be improved include the humanization levels of hepatocytes and immune cells, the elimination of host-specific factors that block HCV infection, and the humanization of the liver sinusoidal endothelium [69].

Humans

Beyond animal models, human studies are essential to understand nAbs-mediated humoral immune responses against natural HCV infection and to develop an effective vaccine. In this regard, substantial progress has been made in recent years. HCV-specific nAbs can be detected in the serum of infected people approximately 8–12 weeks after HCV infection [70, 71], although the range is flexible according to the patient's clinical history [72]. Several studies have shown that nAbs-mediated humoral immune response is long-lasting, necessary to control and clear HCV infection, and can protect from HCV reinfection [22, 28, 46, 73–83]. High-titers and rapid nAb responses have been detected in patients who have spontaneously resolved an acute HCV infection, while a

Table 1 Summary of HCV vaccine candidates based on E1/E2 glycoproteins in preclinical or clinical trials

Vaccine / HCV genotype	Target	Cross-genotype neutralization activity using HCVpp and/or HCVcc	Immunized species	Refs
rE2(**Δ**123) and rE2(**Δ**123A7) / HCV 1a (H77c)	E2	1a (H77), 2a (J6), 3a (S52), 5a (SA13)	Guinea pigs	[31]
rHCV E1/E2 with admixed sulfated S-lactosylarchaeol (SLA) archaeosome formulation as adjuvant / HCV 1a (H77)	E1E2	N/A	C57BL/6 x BALB/c F1 mice	[32]
HCVp6-MAP. Six peptides (p6) in a multiple antigenic peptide (MAP) derived from conserved epitopes in E1 (1), E2 (2), NS4B (1), NS5A (1) and NS5B (1) / HCV 4a (ED43)	E1, E2, NS4b, NS5a, NS5b	2a (JFH1) and a chimeric 2a/4a (ED43/JFH1)	BALB/c mice	[33]
DNA vaccine encoding sE1E2 into IMX313P (oligomers by fusion with the oligomerization domain of the C4b-binding protein) or sE1 and sE2 as separate immunogens / HCV 1b (HCV-N)	E1, E2, E1E2	1a (H77.20, UKN1A20.8), 1b (ukn1b5.23), 2a (ukn2a1.2, a2.4), 2b (UKN2B1.1, B2.8), 3a (UKN3A1.28, A1.9, A13.6), 4a (UKN4.11.1, 4.21.16), 5 (UKN5.14.4), 6 (UKN6.5.8, 6.5.340)	BALB/c mice	[34]
HCV-like particles bearing core, E1 and E2 from four genotypes / 1a (H77), 1b (BK), 2a (JFH1), and 3a	E1, E2, core	1a (H77), 1b (BK), 2a (JFH1/J6, JFH1), 3a (HIC-109)	BALB/c mice White Landrace pigs	[35, 36]
Chimeric HBV/HCV virus-like particles bearing three conservative linear epitopes from E1 and E2 and HVR1 mimotope / N/D	E1, E2	1a (JFH1/H77, H77C/JFH1), 1b (Hebei, J4/JFH1), 2a (JFH1/J6, JFH1)	BALB/c mice	[37]
rE2(**Δ**123) / HCV 1a (H77c)	E2	1a (H77c), 2a (J6), 3a (S52), 4a (ED43), 5a (SA13), 6a (EUHK2), 7a (QC69)	Albino Dunkin Hartley guinea pigs	[38]
rHCV E1/E2 with MF59C.1 as an adjuvant / HCV 1a	E1E2	Genotype 1a/1b patients	Humans (Phase Ib)	[39]
Chimeric HBV/HCV virus-like particles bearing E1 or E2 / HCV 1a (JFH1/H77)	E1, E2	1a (JFH1/H77; 7a), 1b (JFH1/J4; UKN5.23), 2a (JFH1 WT; UKN 2a1.2), 3 (JFH1/S52; UKN3A.1.28)	New Zealand rabbits	[40]
rHCV E1E2 / HCV 1a (HCV-1)	E1E2	1a (H77), 2a (J6), 3a (S52), 4a (ED43), 5a (SA13), 6a (HK6a)	Chimpanzees	[41]
HCV virus-like particles bearing E1E2 or E1 / HCV 1a (H77)	E1, E1E2	1a (H77), 1b (CG1b, CON1), 2a (JFH-1), 2b (UKN2B), 4c (UKN4)	Macaques (*Macaca fascicularis*) Human CD46 ± IFNαβR-/- mice	[42]
rHCV E1/E2 with MF59C.1 as an adjuvant (oil-in water emulsion) / HCV 1a	E1E2	1a (HCV-1, H77), 1b (UKN1B 12.6), 2a (J6), 3a (S52), 4a (UKN4.11.1), 5a (SA13)	C57BL/6J mice, macaques (*Macaca mulatta*), humans (Phase I, NCT00500747)	[43–46]
DNA vaccine expressing HCV Core, E1 and E2 / HCV 1b (CIGB-230)	E1, E2, core	N/A	Humans (Phase I)	[47–49]
HCV virus-like particles bearing core, E1, and E2 with AS01B as an adjuvant (a combination of monophosphoryl lipid A and QS21 saponin) / HCV 1b (CG1b)	E1, E2, core	N/A	Chimpanzees (Pan Troglodytes)	[50]
rHCV E1 with aluminum hydroxide as an adjuvant / HCV 1b	E1	N/A	Humans (Phase I)	[51]
DNA vaccine expressing HCV E2 / HCV 1a	E2	N/A	Chimpanzees (Pan Troglodytes)	[52]

Δ123: E2-receptor-binding domain lacking hypervariable region (HVR) 1 and 2, and the intergenotypic variable region (igVR) (384-408) or replaced with glutathione disulfide linkers (461-485 and570-580); **Δ123A7**: a disulfide-minimized version that contains seven cysteine to alanine mutations (A7: C452A, C486A, C569A, C581A, C585A, C597A, C652A); **HCVcc**: cell-cultured viruses; **HCVpp**: HCV pseudoparticles; **N/A**: Cross-reactive neutralizing antibodies not evaluated; **N/D**: Origin not indicated

delayed or absent nAb response is associated with HCV persistence and chronicity [75, 78, 79, 82, 84, 85]. NAbs can also be developed during CHC, although it usually takes a long time [86–89]. These nAbs from CHC patients cannot clear the CHC infection spontaneously, likely due to HCV escape mutations at nAb recognition sites [90, 91]. However, this nAb-mediated humoral immunity is associated with reduced liver fibrosis [81]. Interestingly, an exceptional case was observed in a patient with CHC infection who spontaneously cleared the HCV after a strong development of cross-reactive nAbs [80]. BnAbs against HCV were also detected in human immunodeficiency virus (HIV)/HCV-chronic co-infected patients, although they declined or disappeared in many patients after HCV clearance with therapy [92].

Several recent findings have boosted interest in the potential of protective nAbs against HCV, stressing the importance of bnAbs to protect from different HCV genotypes and to limit reinfections [93]. The existence of protective immunity against HCV reinfection with different genotypes remains controversial. Although some studies reported only limited protection against heterologous reinfection [79, 94–96], others showed an apparent cross-genotype immunity [77, 97]. In any case, it is becoming clear that a diverse bnAb response can protect from HCV infections of various genotypes and is associated with spontaneous HCV clearance [78, 79, 98, 99]. In this regard, it has been shown that the combination of distinct human nAbs had complementary and synergistic effects on the neutralization of diverse HCV strains [98, 100, 101]. Additional evidence comes from research in which nAbs targeting multiple epitopes were isolated from people who cleared HCV infection [84, 101], studies showing synergy between nAbs [102], and experiments in a mouse model where HCV infection was eliminated by using a mixture of HCV-specific nAbs [58].

The envelope glycoproteins E1 and E2

The main targets of HCV-specific nAbs are the glycoproteins E1 (aa192–383 of the polyprotein) and E2 (aa384–746). They are type-I transmembrane proteins, highly glycosylated, with an N-terminal ectodomain and a C-terminal hydrophobic domain anchoring them to the membrane (Fig. 1). They form E1E2 heterodimers that mediate the entry of HCV into the cell through a complex process involving several receptors and co-receptors, including tetraspanin CD81, the "scavenger" receptor SRB1, and the tight junction membrane proteins claudin 1 and occludin [103]. The virus is internalized by clathrin-dependent endocytosis, and the viral genome is released into the cytoplasm by fusion of the viral membrane with the endosome at low pH, a process also mediated by the E1E2 glycoproteins.

The E1 protein is smaller and less variable than E2. E1 is poorly characterized since only crystal structures for two discrete fragments containing residues 192–271

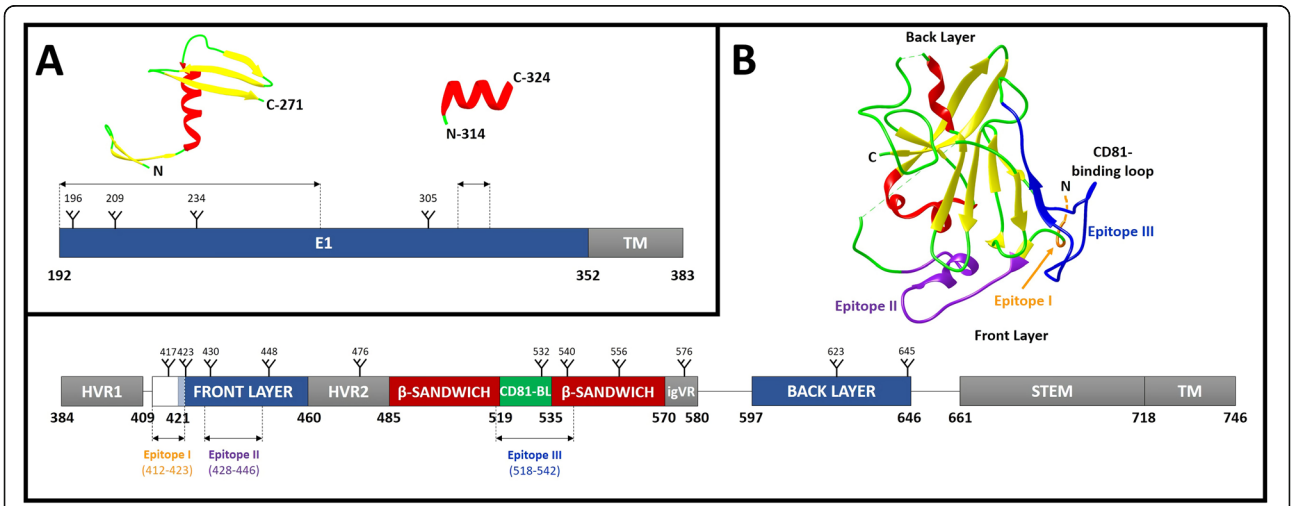

Fig. 1 Hepatitis C E1 and E2 glycoprotein structures. **a** Linear diagram of HCV E1 (aa192–383) and crystal structures of E1 segments aa192–271 (PDB: 4UOI) and aa314–324 (PDB: 4N0Y). **b** Linear diagram of HCV E2 (aa384–746) and ribbon representation of the E2 crystal structure (PDB: 4MWF). E2 is divided into the following structural components: three hypervariable regions (HVR1, HVR2 and igVR), a front layer, two β-sandwich regions, CD81 binding loop, a back layer followed by the stem region and transmembrane (TM) domain. The neutralizing face with epitopes I (orange), II (violet) and III (blue) is indicated. **a-b** N-linked glycosylation sites as tree-like representations and well-defined regions containing α-helices and β-sheets are shown in the linear diagram and X-ray crystallographic structure of both glycoproteins, respectively

[104] and 314–324 [105] have been resolved so far (Fig. 1a).

In recent years, two groups have managed to crystallize the ectodomain of E2 together with fragments of two different antibodies, which has constituted a significant advance in the knowledge of the structure of this protein [106, 107]. The two crystal structures show that the E2 ectodomain contains a central immunoglobulin-like β-sandwich highly stabilized by conserved disulfide bonds. This central region is flanked by an N-terminal "front layer" consisting of a β-strand and a short α-helix, and a C-terminal "back layer" containing antiparallel β-sheets and short α-helices [106, 107] (Fig. 1b). However, there are still many questions to be resolved in this field, such as the fact that the crystallized structures differ in the formation of disulfide bridges; important regions of E2 are missing in the structures; E2 was not entirely glycosylated; and, finally, E2 crystallization has been obtained in the absence of E1.

E1 and E2 epitopes

The elucidation of the E2 structure has led to significant progress in the identification of different antigenic domains and regions of the protein, which will undoubtedly result in the more rational design of vaccines capable of inducing nAbs [22]. E2 is the most variable protein of HCV and the main target of nAbs. Therefore, studies of B-cell based vaccines have focused on this protein. Most of the E2 variability is located in three hypervariable regions: the hypervariable region 1 (HVR1, aa384–409), the hypervariable region 2 (HVR2, aa460–485) and the intergenotypic variable region (igVR, aa570–580) [108]. HVR1 is an immunodominant motif located at the N-terminal end of the protein that mutates during infection, generating escape variants to HCV-specific nAbs.

In contrast, other regions of E2 show moderate variability or are conserved across different genotypes, including areas necessary for the interaction between HCV and cellular receptors, mainly the CD81-binding site. This site is composed of conserved residues from three different regions of E2 that define three epitopes targeted by bnAb [109–111]: Epitope I is located at the N-terminal region (aa412–423); epitope II is at the front layer (aa428–446); and epitope III at the CD81-binding loop (CD81bl) (aa518–542) (Fig. 1b). However, distinct nomenclature is used in different laboratories to describe overlapping antigenic parts of the protein, which may be confusing to the reader. Thus, in addition to the above-mentioned epitopes, five antigenic regions (ARs1–5) [112], and five domains (A-E) [113] have been described. Epitope I shares key residues with domain E; epitope II with domains B, D, and AR3; and epitope III with domain B and AR3 [108, 111]. Furthermore, contact residues on both E1 and E2 are required for some antibodies mediating broad virus neutralization [112]. These residues lie in the AR4 and AR5 regions. In individuals with acute HCV infection, nAb responses to AR3/domain B are dominant [114], and nAbs targeting this region are usually isolated from B lymphocytes of HCV-infected patients [89, 115, 116].

Less is known about the immunogenic regions on E1, but bnAbs have also been described for this protein. The N-terminus (aa192–202) [117] and the fragment encompassing residues 313–328 [105, 118] have been identified as sites inducing nAbs.

Antibody-based vaccine development

Challenges for vaccine development: viral strategies to evade antibody neutralization

HCV has evolved several mechanisms to counteract antibody neutralization. Firstly, the high mutation rate of HCV promotes the generation of many genetically and antigenically different genotypes, subtypes, and quasispecies [2–4]. Most of the variability is accumulated in the E1 and E2 glycoproteins and contributes to evade the host immune response [119]. As described before, hypervariable regions of E2 are immunodominant and induce isolate-specific or non-nAbs. These hypervariable regions sometimes mask more conserved epitopes, preventing their recognition by nAbs [120, 121]. What is more, some conserved epitopes that participate in receptor recognition show conformational flexibility, which may facilitate escaping from cross-reactive nAbs [122] (Fig. 2). Glycans also contribute to conserved epitope shielding in E2 [123]. E2 contains 11 highly conserved N-linked glycosylation sites, some of which mask the binding site to the cellular CD81 receptor [124–126]. Host-derived lipoproteins, which form part of the mature HCV virion, also hide relevant nAb epitopes [127, 128]. Furthermore, HCV-infected cells in cell culture generate lipid droplets containing the E2 glycoprotein [129]. These droplets may act as antibody decoys, lowering the number of antibodies available to neutralize the virus. Yet another mechanism of HCV to evade antibody recognition is its capacity to spread through direct cell-to-cell transmission [130]. Finally, it has been shown that the enhanced resistance to interferon-induced transmembrane proteins (IFITMs) observed in some HCV variants favors escape from nAbs [131]. IFITMs block viral entry by modulating membrane properties, which improve antibody-mediated neutralization [131].

Rational immunogen design for antibody-based vaccines

Despite encouraging results, the goal of developing an HCV vaccine remains a challenge. As stated previously,

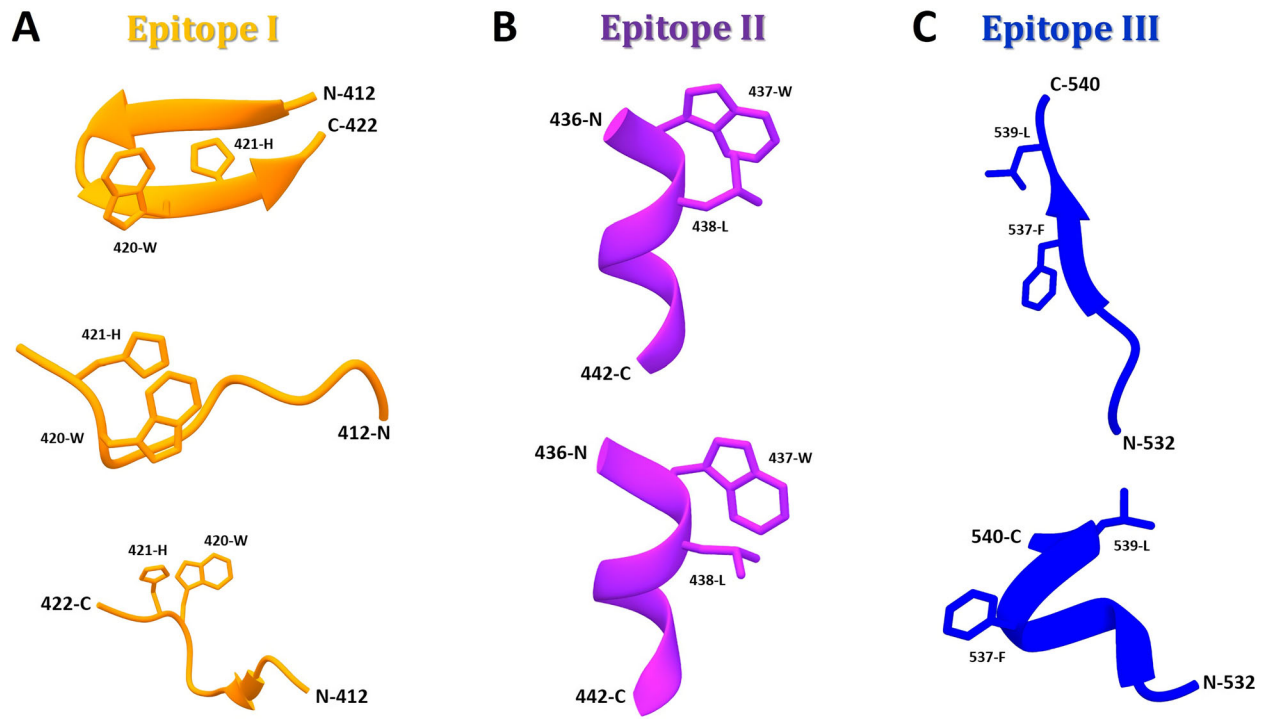

Fig. 2 E2 epitopes (I to III) adopt distinct conformations when complexed with different antibodies. **a** Conformations of epitope I: (Upper) Closed β-hairpin in complex with nAb HCV1 (PDB: 4DGY); (Middle) extended-coil conformation in complex with nAb 3/11 (PDB: 4WHY); and (Lower) intermediate-coil conformation with an anti-parallel β-sheet in complex with nAb HC33.1 (PDB:4XVJ). **b** Conformations of epitope II: (Upper) Preferred state of E2 in the viral particle containing a short 1.5-turn α-helix (aa437–442) and an extended conformation (aa443–446, not shown) in complex with nAb HC84.27 (PDB: 4JZO); and (Lower) conformational changes through short α-helix flipping out ('open state') to expose aa437(W) and aa438(L) residues for nAb mAb#8 (PDB: 4HZL). **c** Conformations of epitope III: (Upper) Open and stabilized strand in β-sheet conformation with aa537(F) and aa539(L) residues flipped out into the hydrophobic part of the Ig-like domain in complex with AR3C Fab (PDB: 4MWF); and (Lower) helical disordered conformation with aa537(F) and aa539(L) residues solvent-exposed in complex with non-nAb DAO5 (PDB: 5NPJ)

many reasons make it difficult to achieve. However, increasing knowledge about the HCV glycoprotein epitopes offers the opportunity to design immunogens to avoid the induction of isolate-specific or non-nAbs while potentiating the induction of bnAbs. In this regard, an interesting approach has been the generation of an E2 glycoprotein with deleted HVR1, HVR2 and IgVR [132, 133]. This protein elicited bnAbs after immunization of guinea pigs while inducing reduced levels of non-nAbs [38].

N-glycans in E1 and E2 mask epitopes targeted by nAbs [123]. Therefore, the deletion of these glycans may induce a more potent nAb response against HCV. Accordingly, the removal of different N-glycosylation sites both in E1 and E2 improved its immunogenicity and led to increased bnAb responses [134–137]. Interestingly, the glycosylation pattern of E2 can also affect its immunogenicity. Thus, E2 expressed in insect cells showed increased bnAbs as compared to E2 expressed in mammalian cells [64].

In recent years, conformational flexibility of some conserved broadly neutralizing epitopes in HCV E2 has become apparent [110, 138]. For example, the epitope I (AS412) can adopt at least three distinct conformations when complexed with different antibodies: extended [139], β-hairpin [140], and an intermediate conformation [141] (Fig. 2a). Epitopes II (AS434) (Fig. 2b) and III (CD81bl) (Fig. 2c) also display structural flexibility [61, 142–145]. This flexibility appears to be a mechanism to evade nAbs and has important consequences for vaccine design [146]. Thus, future vaccines could require stabilization of neutralizing epitopes, as has been proposed for other viruses such as HIV [147, 148], respiratory syncytial virus (RSV) [149–151], and severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) [152, 153]. A cyclic variant of the epitope I of E2 stabilized in the β-hairpin conformation was designed first [154]. However, this variant was unable to induce nAbs. Subsequently, another version of the epitope I in the β-hairpin conformation was generated based on the θ-defensin structure [146]. This construct induced nAbs in mice, but the response was still low. Further efforts are required to improve the immunogenicity of structure-based HCV epitopes, including incorporation to virus-like particles or nanoparticles [155].

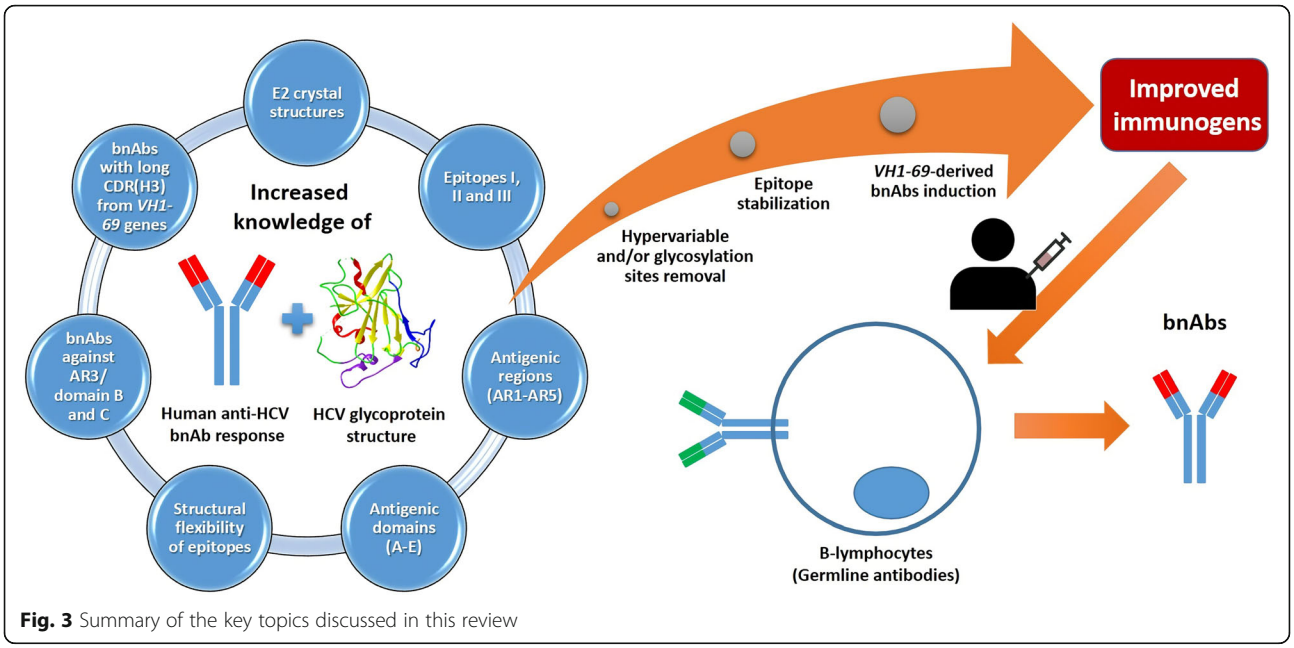

Fig. 3 Summary of the key topics discussed in this review

Another important piece of information that is becoming available is a detailed picture of the human humoral immune response against HCV [86–89]. For example, it is now known that bnAbs in HCV-infected patients are predominantly induced by AR3/domain B and domain C of E2 [155], and specific combinations of these antibodies together with antibodies targeting the E1E2 complex (AR4) are associated with natural HCV clearance [98]. Thus, antigen design focused on eliciting antibodies against these regions should be considered in a potential vaccine. Moreover, the analysis of the interactions of the natural antibody repertoire generated by the infection with the E1E2 glycoproteins will aid the design of new effective vaccines. In this regard, it has been reported recently that potent anti-HCV cross-reactive nAbs with little somatic hypermutations are derived from human *VH1–69* genes [74, 84, 156]. These germline-encoded antibodies are also precursors of a large portion of specific nAbs against other viruses, such as HIV, influenza, and RSV [157–159]. Structural has shown that the cross-neutralizing activity of those antibodies is related to their long complementarity-determining regions (CDR) H3, which contain a disulfide motif that interacts with conserved E2 epitopes [106, 160–162]. Additionally, an ultralong CDRH2 favors extensive contact with E2 [163]. These results underline the potential advantages of producing *VH1–69*-derived nAbs by vaccination.

Conclusions

Despite the impressive efficacy of DAA treatment against HCV, it is unlikely that the virus will be controlled entirely without a prophylactic vaccine. Cumulative evidence from animal models and humans strongly indicates that bnAbs can protect from HCV infection. Recent advances in the knowledge of HCV E1 and E2 glycoprotein structure, and the human antibody repertoire generated by HCV infection, will allow rational structure-based antigen design to induce bnAbs (Fig. 3). Achieving sterilizing immunity by vaccination will be a difficult task. However, a vaccine preventing CHC infections, a reasonable goal in the short term, would have a substantial health impact.

Abbreviations

bnAbs: Broad-spectrum neutralizing antibodies; CD81bl: CD81 binding loop; CHC: Chronic hepatitis C; CDR: Complementarity-determining region; DAAs: Direct-acting antivirals; HCV: Hepatitis C virus; HIV: Human immunodeficiency virus; HVR1: Hypervariable region 1; HVR2: Hypervariable region 2; IDU: Injecting drug user; IFITMs: Interferon-induced transmembrane proteins; igVR: Intergenotypic variable region; MSM: Men who have sex with men; MVA: Modified Vaccinia Virus Ankara; nAbs: Neutralizing antibodies; NS: Non-structural; PWIDs: People who inject drugs; RSV: Respiratory syncytial virus; NS5b: RNA-dependent polymerase; NS3/4A: Serine protease; SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2; VLPs: Virus-like particles

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Authors' contributions

Conceptualization: SR and IM. Data curation: DSC and IM. Funding acquisition: SR and IM. Investigation: DSC, SR, and IM. Supervision and visualization: SR and IM. Writing – original draft preparation: DSC, SR, and IM. Writing – Review & Editing: DSC, SR, and IM. All authors have read and approved the final manuscript.

Authors' information

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