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Hepatitis C virus vaccine candidates inducing protective neutralizing antibodies

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Abstract

Introduction—With more than 150 million chronically infected people, hepatitis C virus (HCV) remains a substantial global health burden. Direct-acting antivirals have dramatically improved viral cure. However, limited access to therapy, late stage detection of infection and re-infection following cure illustrate the need for a vaccine for global control of infection. Vaccines with induction of neutralizing antibodies (nAbs) have been shown to protect successfully against infections by multiple viruses and are currently developed for HCV.

Areas covered—Here we review the progress towards the development of vaccines aiming to confer protection against chronic HCV infection by inducing broadly nAbs. The understanding or viral immune evasion in infected patients, the development of novel model systems and the recent structural characterization of viral envelope glycoprotein E2 has markedly advanced our understanding of the molecular mechanisms of virus neutralization with the concomitant development of several vaccine candidates.

Expert commentary—While HCV vaccine development remains challenged by the high viral diversity and immune evasion, marked progress in HCV research has advanced vaccine

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Declaration of Interests

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development. Several vaccine candidates have shown robust induction of nAbs in animal models and humans. Randomized clinical trials are the next step to assess their clinical efficacy for protection against chronic infection.

Keywords

Hepatitis C; vaccine; immunity; antibodies

1. Introduction

With more than 150 million people infected worldwide, hepatitis C virus (HCV) infection remains a major global health concern. Following a generally asymptomatic initial infection, a large majority of patients develop chronic hepatitis and are subsequently at risk to progress to liver cirrhosis and hepatocellular carcinoma (HCC) within twenty years.[1] Various parameters may accelerate disease progression, such as alcohol consumption and human immunodeficiency virus (HIV) co-infection.[1] Twenty-five years after the discovery of HCV, the development of a prophylactic vaccine remains a major challenge.[2–7] This is largely due to the high mutation rate of HCV and numerous other strategies employed by the virus to escape host immune responses.[4]

Recent approval of direct-acting antivirals (DAAs) has greatly improved the outcome for treated patients, with cure now possible for the majority of cases.[8] Despite being highly effective, DAA treatments present important drawbacks, one being limited access due too high cost.[9] The current price of a DAA treatment course reaches thousands of dollars, while it is estimated that approximately 80% of HCV-infected individuals are living in low- and middle-income countries.[10] Even with production of generics, it remains unclear whether these will be accessible for these populations.[10] Importantly, in the absence of screening programs, a large proportion of chronic HCV carriers are unaware that they are infected and do not seek DAA treatment, thus potentially transmitting the infection.[9] Additionally, DAAs are less effective when patients are diagnosed at late stages, such as during hepatic decompensation or liver transplantation (LT).[8, 11, 12] Moreover, recent studies demonstrate that HCC risks persists following viral once liver fibrosis is established.[9, 13]. Moreover, even a DAA capable of curing 100% of HCV-infected patients will not be able to protect patients from newly acquired infection. Re-infection following HCV cure remains possible – a major challenge in injection drug users.[14] These limitations underscore the need for a protective vaccine that can be employed in parallel with new treatments.

While the high viral diversity may preclude to develop a prophylactic vaccine, protecting all vaccines against HCV infection, modeling studies estimate that even a vaccine with a lower efficacy will have a substantial impact on HCV prevalence and incidence, especially in high risk populations,[15, 16] as well as important economic benefit.[17] It is of interest to note that it has been suggested, that even a partially effective vaccine would continue to have a substantial benefit even in the setting of highly effective treatments.[16] Collectively, these modeling approaches suggest that both an effective antiviral therapy as well as an efficient vaccine will be required to fully control HCV spread and eradicate viral infection on a

global level.[18] Furthermore, a parallel approach of treatment and vaccination appears to be in particular of relevance for populations at high risk for HCV infection such as intravenous drug abusers.

While vaccines inducing protective neutralizing antibodies (nAbs) have been successfully developed for many viral infections including hepatitis B,[19] poliovirus,[20] or human papillomavirus,[21] the development of a B cell vaccine with induction of neutralizing antibodies protecting against HCV infection has been a challenge[4]. B cell vaccines exert their major protective effect through the induction of cross-neutralizing antibodies.

The choice of an appropriate HCV immunogen is critical to anticipate and prevent viral escape[4]. NAbs are essential components of host immune responses elicited following HCV infection and several studies have demonstrated that nAbs clearly play a role for protection against HCV chronic infection.[22] NAbs are considered to mainly interfere with HCV entry which is a complex multistep process consisting of interactions between the viral envelope glycoproteins (GPs) E1/E2 and host cell entry factors.[23] Thus, HCV GPs play a major role in the initiation of hepatocyte infection and are the main target of nAbs. Several vaccine candidates evaluated in clinical trials are based on eliciting strong humoral immune responses by using HCV envelope proteins as immunogens (Table 1).[4, 24] A key aspect of this approach is the inclusion of highly conserved epitopes to overcome viral diversity and prevent viral evasion. Furthermore, consideration of highly conserved conformational epitopes for vaccine design is essential to deliver an antigen truly representative of neutralizing epitopes that are recognized in infected humans.[25, 26]

Two recent studies delineating the crystallographic structure of the HCV E2 core domain in complex with antigen-binding fragments of two distinct antibodies have greatly improved the understanding of HCV envelope glycoprotein (GP) structure. These structures are a major advance toward the utilization of conserved epitopes for rational vaccine design.[27, 28] Aiming to address the progress and challenges of vaccine development, here we review the molecular mechanisms of virus neutralization, the impact of nAbs for control of viral infection in patients and provide an overview on vaccine candidates inducing nAbs which are in preclinical and clinical development.

2. Molecular mechanisms and targets of virus neutralization

E1 and E2 envelope glycoproteins are the natural targets of the protective antibody response. Since E2 interacts with the HCV co-receptors, scavenger receptor class B type 1 (SRB1)[29] and the tetraspanin CD81[30] during virus entry, the majority of nAbs are directed against E2. There is some evidence that the E1E2 heterodimer, not E2 alone, interacts with a third co-receptor, the tight junction protein claudin-1.[31] A major region mediating virus neutralization is the hypervariable region 1 (HVR1), encompassing the first 27 residues of E2.[32, 33] Antibodies to HVR1 are isolate-specific and over time will select viral quasispecies that are not recognized by these antibodies.[34] This region also blocks access of other broadly neutralizing antibodies to their cognate epitopes[35, 36] and the binding of antibodies to HVR1 was recently shown to interfere with the binding of broadly neutralizing

antibodies to E2 by steric hindrance.[37] Taken together, we believe that vaccine strategies should avoid the generation of HVR antibodies.

Antibodies with broad neutralizing activities mainly target conformational epitopes on E2 (reviewed in [22]), with some directed against the E1E2 heterodimer.[38] Based on cross-competition analyses and epitope mapping of broadly neutralizing human monoclonal antibodies (HMAbs) that were isolated from B cells of chronically infected patients, at least five clusters of overlapping conformational epitopes have been identified. Three clusters, designated as antigenic domains B, C and D, are composed of conformational epitopes on E2,[39–41] and two clusters, designated as antigenic regions (AR) 4 and 5, are composed of conformational epitopes on the E1E2 heterodimer.[38] It should be noted that the AR3 cluster substantially overlaps with antigenic domain B, as identified by cross-competition and epitope mapping studies.[42] Interestingly, AR5 overlaps with an antigenic domain C HMAb, CBH-7, although the latter antibody binds to E2 and does not require E1.[38, 39] A sixth cluster of broadly nAbs to overlapping linear epitopes are located immediately downstream of HVR1, designated as antigenic domain E, encompassing amino acids 412-423, that have been defined mainly by murine MAbs.[43–46]. It should be noted that neutralizing murine MAbs, typically generated via immunization with recombinant E2 protein, have also been mapped to regions encompassing domains B, C and D but they tend to be antibodies to linear epitopes[22, 47] with decreased breadth of neutralization against different HCV genotypes[44]. However, some neutralizing murine MAbs that recognize conformational epitopes on E2 have been described.[44, 48]

Epitope mapping by alanine substitution studies identified two E2 residues, Gly530 and Asp535, that are required for binding by the majority of currently known domain B HMAbs.[49] Residues Gly523 and Trp529 are also required for some, but not all, of these antibodies. Importantly, Trp529, Gly530 and Asp535 participate in the interaction of E2 with CD81.[50] Thus, antigenic domain B HMAbs exert broad neutralizing activities by competing with CD81 for binding to conserved residues on E2 that are necessary for this step in virus entry. Similarly, three critical residues form a core region for antigenic domain D epitopes at Leu441, Phe442 and Tyr443.[41] Leu441 and Tyr443 are absolutely conserved among all known HCV isolates.[51] Phe442, however, is only 84% conserved (LANL database). The domain D region is also involved in interaction with CD81. For AR4- and AR5-specific epitopes that require the E1E2 heterodimer, their associated antibodies do not mediate neutralization by inhibiting E2 interaction with CD81, but instead potentially block E1E2 engagement with a different co-receptor or conformational changes in the E1E2 structure during virus entry.[38] With the elucidation of the E2 core structure,[27, 28] it became clear that the vast majority of nAbs, encompassing antigenic domain B and D, and AR3, engage the neutralizing face of E2 (Fig. 1A). However, critical residues for antigenic domain C, which mediates virus neutralization, are located on the “non-neutralizing” face, along with a cluster of conformational epitopes that do not mediate virus neutralization, designated as antigenic domain A (Fig. 1B).

Not all broadly neutralizing antibodies are created equally. In virus co-culture studies, some antigenic domain B antibodies are associated with viral escape (with and without compromising viral fitness), whereas others are not associated with escape. At a critical

antibody concentration of HC-1, a domain B HMAb, a complete elimination of infectious virions will occur.[52] These studies point to two discontinuous regions that participate in domain B epitopes, aa 425-443 and aa 529-535. The variable region aa 425-443 is responsible for escape and the conserved region aa 529-525 is responsible for virus interaction with CD81. Antibodies to antigenic domain E (aa 412-423) are also associated with different patterns of viral escape. Escape from virus neutralization with AP33 and HCV1 nAbs occurs when there is an N-glycan shift from Asn417 to Asn415 or residue substitutions at Asn415.[53–55] However, glycan shift-mediated escape is not associated with other antigenic domain E HMAbs, such as HC33.1.[56] A particular concern for antigenic domain E in vaccine design is that this region is not highly immunogenic in humans. The rate of HCV infected subjects with antibodies to this region ranges from 2–15%[57, 58], while antibodies to antigenic domain B are frequently observed. Escape from antigenic domain D HMAbs (a subset of the antigenic domain B-D/AR3 supersite in Fig 1A) has not been observed, despite some variability at critical binding residue Phe442, as noted above. Structural studies provided an explanation for the lack of viral escape in virus co-culture studies.[51] Three E2 residues, located at 441-443, form a hydrophobic protrusion that serves as the core binding site for domain D HMAbs. When Phe442Ile or Phe442Leu mutations are present, the interaction with the paratope formed by the heavy chain CDRs leads to a decrease in binding energy of the complex that can be compensated by increasing the antibody concentrations.

In summary, vaccine development should focus on specific epitopes within antigenic domains B and D that are not associated with viral escape and less on domain E that is of poor immunogenicity and possibly elicits neutralizing antibodies that are associated with viral escape without compromised viral fitness. Moreover, these studies illustrate the importance of functional and biochemical characterization of broadly reactive nAbs to create a high-resolution, functional map of neutralizing and non-neutralizing epitopes that can be employed for rational design of a B cell vaccine.

3. Viral evasion from neutralizing antibodies

The ability of HCV to adapt to its host is a major limitation for the development of a prophylactic vaccine. In patients, HCV circulates as a pool of quasispecies, viral variants which are genetically distinct and continually evolving due to a high viral mutation rate.[59] HCV variants reinfecting the liver graft are characterized by both enhanced entry in hepatocytes and escape from nAbs.[60] HCV has developed numerous strategies to avoid recognition by host humoral responses. HCV envelope glycoproteins (GPs) are the principal HCV proteins exposed during viral infection and the first target of humoral responses.[61] One study of over 26 years of HCV GP E1E2 sequences in a chronically infected patient demonstrated that humoral immunity applies a selective pressure to HCV, resulting in the continuous generation of variants with E1E2 mutations that are not neutralized by circulating nAbs.[34] Similarly, Dowd et al. observed that during the acute phase of HCV infection, the evolution of HCV GP sequences is driven by nAbs.[62] Nevertheless, the exact determinants of HCV genetic evolution and diversity are only partially understood and it is likely that although host selective pressure is a major determinant of viral quasispecies evolution, it is not the only one.

Many mutations occur in specific regions called hypervariable regions (HVR) of HCV GPs. Farci et al. demonstrated a correlation between HCV diversity and viral clearance. Patients infected with a relatively stable HCV population are more susceptible to viral clearance, whereas chronicity correlates with genetic variability and evolution, especially in HVR1 of HCV GP E2.[63] The importance of this region for HCV persistence has been further confirmed by several studies demonstrating that HVR1 deletion increases the exposure of conserved epitopes facilitating virus neutralization by monoclonal antibodies and patient sera *in vitro* and *in vivo*.[35, 36, 64] In addition to a direct shielding role of HVR1, it has recently been proposed that nAbs binding poorly to HVR1 may interfere with the binding of broadly nAbs to other antigenic domains, due to steric hindrance which ultimately favors viral persistence.[37]

Although HCV variability already represents a strong obstacle for the design of a B cell vaccine, HCV has also developed other mechanisms to avoid nAbs recognition. The N-glycosylation of HCV E2 has been described as an important mechanism for viral escape from nAbs, by masking epitopes to which these antibodies bind.[65–67] More precisely, five E2 glycans have been associated with a decreased sensitivity of HCV to nAbs, with four of them shielding CD81 binding sites that are highly targeted by nAbs.[66] Furthermore, under selection pressure, a shift in the glycan attachment site within the CD81 binding epitope on E2 can also occur, which decreases the efficacy of nAbs.[54] The capacity of HCV to disseminate using cell-to cell transmission has also been described as an efficient strategy to avoid the extracellular compartments where nAbs circulate.[68, 69] Interestingly, host targeting agents such as anti-receptor antibodies are able to prevent HCV cell-to-cell transmission, as well as DAA resistance related to this process.[70–73] Other studies also demonstrated that mutations in HCV GP E2 may alter the use of host-receptors CD81 and scavenger receptor B1, thus conferring resistance to nAbs.[74, 75] In patients, circulating HCV is associated with lipoproteins and forms hybrid lipo-viral particles (LVP). These associations have been shown to reduce the access of nAbs to their epitopes, facilitating HCV persistence.[75, 76] A recent study further demonstrated that host apolipoprotein E present at the surface of the LVP mediates evasion from nAbs.[77]

4. Impact of neutralizing antibodies for control of HCV infection: lessons from clinical cohorts

Since the discovery of HCV in 1989, many studies have focused on host immune responses following viral infection to elucidate the contributions of both cellular and humoral immunity for control and protection against HCV infection. The development and improvement of *in vitro* models for HCV has provided essential tools to elucidate the exact roles played by nAbs during the early phase of HCV infection.[78] Pseudotyped retroviral particles expressing HCV E1E2 glycoproteins (HCVpp) have been extensively used to evaluate the neutralization capacities of HCV-infected patient sera. Pestka et al. used sera from a cohort of pregnant women accidentally infected with a single HCV strain and followed for over 17 years.[79] Generation of HCVpp bearing the GPs of this specific strain, followed by neutralization experiments, revealed that viral clearance is associated with early appearance of nAbs during the early phase of HCV infection. The titer of nAbs then

diminished following viral clearance. In contrast, women who developed chronic hepatitis only presented an absent or low titer of nAbs during the initial phase of HCV infection, and the titer increased only during the late phase of infection.[79] Similarly, Osburn et al. evaluated the breadth of nAbs produced during the acute phase of HCV infection and observed that spontaneous control of viral infection is associated with a rapid production of broad nAbs. In contrast, patients who developed persistent infection had a delayed appearance of broad nAbs.[80] Importantly, the study of a patient who spontaneously recovered from HCV chronic infection highlighted that the appearance of nAbs at week 48 was followed by viral clearance at 65 weeks post-infection, confirming their key role for the control of HCV infection.[81]

The importance of nAbs for protection against HCV recurrence has been further demonstrated in a retrospective study of patients with advanced hepatitis B virus (HBV) or HCV infection undergoing LT and who were treated with HBV immunoglobulins (HBIG). These HBIG were potentially contaminated with anti-HCV nAbs, due to the absence of HCV serological screening before 1990.[82] Interestingly, patients infected with HCV before LT and treated with HBIG had a lower incidence of HCV viremia after transplantation, compared to patients who did not receive HBIG or received HBIG after March 1990.[82] Moreover, patients who were HCV-negative before LT and received HBIG were less susceptible to acquire HCV infection than non-treated patients.[82]

Because they are frequently exposed to HCV, people who inject drugs have been included in many cohorts to study HCV recurrence. Osburn et al. observed that while only 25% of HCV-infected people are able to spontaneously clear a primary infection, a clearance rate of 83% is observed after HCV re-exposure.[83] Additionally, patients clearing the secondary infection generate broad humoral immune responses, whereas these cross-nAbs are rarely detected in patients developing chronic infection.[83] These studies clearly demonstrate the crucial role played by nAbs to protect against HCV infection. It is important to note that neutralizing antibodies are considered to exert their function in concert with T cell responses which have been shown to play a key role for HCV control and clearance.[81, 84–88] Furthermore, recent data from clinical cohorts with acute HCV infection demonstrate an important role for T cell responses in the induction of B cell responses.[89] Indeed, in HCV-infected patients, the presence of HCV-specific CD4+ T cells expressing markers of follicular T-helper (Tfh) cells and secreting interleukin 21 following viral exposure were observed.[89] Furthermore, expression of inducible T-cell co-stimulator was associated with induction of HCV-specific antibodies in patients with acute HCV infection.[89]

5. B cell vaccine candidates in preclinical and clinical development

One of the first approaches in vaccine development was the concept of a subunit vaccine based on recombinant envelope glycoproteins. Early studies of the Houghton group at Chiron showed that immunization of seven chimpanzees with recombinant E1/E2 glycoproteins derived from genotype 1a induced nAbs that appeared to protected five of the seven animals from homologous HCV challenge.[90, 91] Furthermore, the vaccine reduced rates of chronic infection following homologous and heterologous HCV challenge,[92] an important finding given that most HCV-associated disease occurs during chronic infection.

The recombinant E1E2 vaccine combined with MF59 adjuvant was then evaluated in a phase I clinical trial with 60 healthy volunteers.[93] The vaccine induced strong humoral and CD4+ T cell responses, and was well tolerated.[93] Studies with HCVpp demonstrated cross-neutralizing activity of the volunteer antisera against heterologous 1a, 1b and 2a HCV genotypes.[94] Using chimeric cell culture-derived HCV assays, Law *et al.* demonstrated that the recombinant E1E2 vaccine elicited broadly cross-nAbs in the volunteers, suggesting the presence of epitopes conserved among all major genotypes of HCV.[95] Indeed, peptide mapping and competition studies demonstrated that immunization with recombinant E1E2 elicits antibodies targeting multiple cross-neutralizing epitopes.[96]

The recombinant E1E2-MF59 vaccine was further evaluated in a phase 1b study, either alone or in combination with pegylated-interferon (Peg-IFN) alpha2a and ribavirin for the retreatment of 78 treatment-experienced genotype 1a/1b patients.[97] The vaccine was safe and elicited E1E2 nAbs, which positively correlated with better treatment response. Although the vaccine alone did not induce significant changes in viral load, it was associated with a higher sustained virological response rate when used in combination with Peg-IFN/ribavirin (RBV) (17% combined compared to 8% Peg-IFN/ribavirin alone).[97] This study suggests a potential for using HCV vaccines in combination with DAAs for difficult to treat patients or multiresistance.

The immunogenicity of an E1 candidate vaccine with aluminum hydroxide adjuvant was evaluated in a phase I study in 2004.[98] The individuals who received this vaccine developed humoral responses against E1, although the neutralization capacity of the antibodies was not evaluated.[98] Interestingly, when chimpanzees were vaccinated with either genotype 1b E1 or E2 recombinant protein with alum adjuvant, only antibodies against E1 were shown to neutralize HCVpp and protect the chimpanzees from persistent hepatitis following homologous challenge.[99] These findings highlight the importance of E1 for candidate HCV vaccines. Further insight into the structure and function of the E1/E2 complex will likely improve our understanding of nAbs targets and contribute to further vaccine development.

HCV-like particles (LPs) derived from recombinant HCV structural proteins (core, E1 and E2) are morphologically similar to authentic HCV virions.[100] These HCV-LPs induce strong humoral and cellular immune responses in mice[101] and baboons,[102] but only limited B cell responses were observed in chimpanzees, despite strong induction of cellular immune responses.[103] Since HCV-LPs were produced in insect cells, they were likely not fully representative of authentic HCV virions, which may have limited their immunogenicity. Indeed, glycosylation of envelope glycoproteins is slightly different in insect cells,[100] which may explain the limited induction of nAbs. Pseudotyped virus-like particles expressing E1 and/or E2 envelope glycoproteins that were produced in mammalian cells induced cross-nAbs in macaques.[104] Inactivated cell culture-derived HCV virions induce cross-nAbs that confer protection from HCV infection in human liver chimeric mice.[105] These findings suggest that virus-like particles produced in mammalian cells may better reflect the immunogenicity of authentic HCV virions with superior induction of nAbs.

Vector-based immunization is also capable of inducing humoral immune responses in chimpanzees. A DNA vaccine encoding E2 prevented progression to chronicity in vaccinated chimpanzees, although did not induce sterilizing immunity.[106] In another chimpanzee study, vaccination with recombinant DNA (prime) and adenovirus (boost) vaccines expressing HCV core, E1E2 and NS3-5 genes induced long-lasting E2-specific antibody responses.[107] Although one vaccinated chimpanzee had sterilizing immunity against heterologous virus challenge, other vaccines with lower levels of E2-specific antibody developed chronic infections.[107] A therapeutic DNA vaccine expressing HCV structural proteins and NS3 was evaluated in a Phase I clinical trial of chronically infected patients who did not respond to previous IFN/RBV treatment.[108] All patients generated antibodies against core, and the majority (86.6%) of patients developed antibodies against E1. Following vaccination, 6 of the 15 patients previously lacking nAbs responses became positive for nAbs. Furthermore, over 40% of vaccinated individuals demonstrated improved liver histology (i.e. reduction in fibrosis), although this was attributed to cellular immune responses as opposed to nAbs.[108]

Interestingly, chimeric HBV/HCV vaccines have also been explored. These vaccines are based on the fact that the hepatitis B envelope protein (S) self-assembles into subviral particles, which constitutes the HBV vaccine in current use. Foreign epitopes, such as HCV envelope E1 and E2 protein sequences, can be inserted into the S protein to generate chimeric subviral particles.[109] One such vaccine replaced the N-terminal transmembrane domain of the HBV surface antigen with the transmembrane domain of genotype 1a HCV E1 and E2.[110] Vaccination of rabbits induced a strong humoral response to both HBV and HCV envelope proteins. These antibodies demonstrated cross-neutralizing activity against heterologous genotype 1a, 1b, 2a and 3 HCVpp, while HBV responses showed no loss of activity.[110] Further evaluation in other animal models and humans is warranted.

Collectively, these studies from the past decades have indicated the potential for a protective B cell-based vaccine against HCV. Although humoral responses generated by vaccines will likely be able to play a key role for prevention of infection, induction of cellular immune responses will likely be required for efficient protection against chronic infection.[2–7, 9] Interestingly, for several of the above reviewed approaches, concomitant induction of T cell responses has been demonstrated.[93, 104, 111] Ultimately, immunogens inducing both humoral and cell-mediated immune responses may be the optimal approach to provide full protection against HCV infection and liver disease.

6. Expert commentary

Despite hopes raised following the approval of new highly effective therapies able to cure the majority of patients, in the absence of a vaccine, it is probable that treatment-induced HCV eradication will remain a dream. Indeed, limitations of these treatments, especially in term of costs and access, restrain their use in countries with limited resources, where HCV incidence is high. Furthermore, an important proportion of patients are unaware of being infected with HCV and may further transmit the disease, thus favouring HCV spread and persistence in the population. Finally, re-infection following treatment-induced cure limits

this approach in particular in injection drug users. Thus, an effective vaccine may be needed to control and eliminate HCV infection on a global level.

The development of B cell-based vaccines has been hampered by the high viral heterogeneity and efficient escape to host immune responses. The recent understanding of the molecular mechanisms of viral neutralization including the elucidation of the crystal structure of HCV envelope glycoprotein E2 has contributed to the identification of epitopes for cross-neutralizing antibodies.[27, 28] Detailed studies in patient cohorts have shown that neutralizing antibody responses are associated with control of infection, and B cell-based vaccine candidates show promise in preclinical animal models and clinical trials. Induction of T cell responses may further enhance the efficacy of vaccine candidates.[2, 4] Future preclinical and clinical studies will provide further insights to bring a protective vaccine closer to reality.

7. Five-year view

Vaccine development is a long and complex process characterized by decades between preclinical studies and licensure and large-scale production. Current HCV vaccine candidates in development are still at the beginning of this process and the full impact of various mechanisms of viral escape to host immune responses still remains to be seen. Recent clinical trials studies have shown promise to induce cross-neutralizing antibodies although their clinical proof-of-concept for protection against infection remains to be shown. The recent advances in understanding the structure of the viral envelope combined with novel animal models assessing protective immune responses[112] will accelerate the development of improved vaccine candidates which ultimately may protect against HCV infection. A continuing effort is needed to reach that goal which appears to be possible to achieve within the next decade.

Abbreviations

DAA	direct acting antivirals
GP	envelope glycoprotein
HCC	hepatocellular carcinoma
HBV	hepatitis B virus
HBIG	hepatitis B immunoglobulins
HCV	hepatitis C virus
HCVpp	hepatitis C virus pseudoparticles
HIV	human immunodeficiency virus
HVR	hypervariable region
pegIFN/RBV	pegylated interferon/ribavirin

LT	liver transplantation
LVP	lipoviroparticle
nAbs	neutralizing antibodies

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Reference annotations

* Of interest

** Of considerable interest

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8. Key issues

- Hepatitis C virus (HCV) infection is a major global health concern, with approximately 170 million people infected worldwide at risk for life-threatening liver disease and cancer.
- While recently licensed DAAs cure the majority of HCV-infected patients, high costs and limited access are major challenges for global control and eradication.
- Key challenges for the development of B cell vaccines are viral heterogeneity and evasion
- Studies in clinical cohorts demonstrated that neutralizing antibodies play a crucial role in viral clearance among individuals who spontaneously resolve HCV infection as well as protection against HCV re-infection.
- B cell vaccine candidates have been shown to induce cross-neutralizing antibodies in clinical trials, however proof-of-concept for protection against HCV infection remains to be shown.
- Ultimately, applying immunogens inducing both humoral and cell-mediated immune responses may offer the best approach for full protection against HCV infection and virus-induced liver disease.
- The recent advances in understanding the structure of the viral envelope combined with novel animal models assessing protective immune responses will accelerate the development of improved vaccine candidates
- A continuing effort is needed to develop a protective vaccine – a goal which appears to be possible to achieve within the next decade.

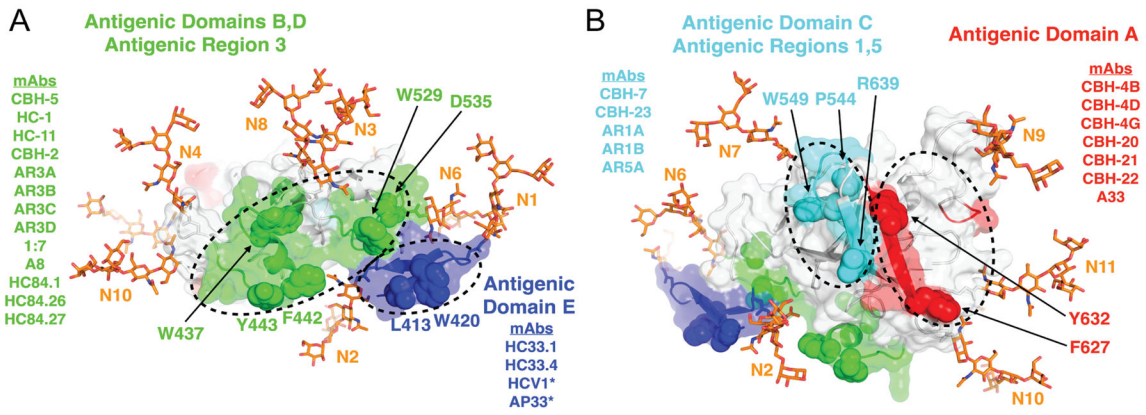

Figure 1. Antibody binding supersites on the E2 surface

Antigenic domains and antigenic regions are grouped into four supersites (colored green, blue, cyan and red, circled by dashed lines), based on previous binding competition and alanine scanning studies, shown on A) the neutralizing antibody face (CD81-binding region) and B) the non-neutralizing face of E2. Key binding residues shared by multiple antibodies are labeled and shown in space fill, and representative mAbs are shown for each supersite. The E2 structure is from the isolate H77 E2 core crystal structure[27, 28]. N-terminal residues (aa 412-420) missing from the crystal structure were modeled using Rosetta[113] and the crystal structure of HCV1 bound to aa 412-423.[114] Glycan structures were added using the Glyprot web server[115] and are shown as orange sticks and labeled according to the order of their bound asparagine residues in the E2 sequence (N1-N11; N5 not visible). All antibodies shown are human, except for those denoted by asterisks, which are mouse-derived (HCV1 is from human antibody transgenic mice).

Table 1

B cell vaccine candidates in preclinical studies in chimpanzees or clinical trials in humans

Summary of the different HCV vaccine candidates in preclinical or clinical development with description of the strategy employed and the main results obtained.

Strategy employed	Animal model/Patient	Results	References
Genotype 1a Recombinant E1E2 proteins	Chimpanzees	• Protection from homologous challenge in 5/7 animals • Ameliorated infection and disease in remaining infected animals compared to control chimpanzees • Presence of cross-nAbs in serum of protected animals	[90] [91]
Genotype 1a DNA encoding E2 protein	Chimpanzees	• Prevention of progression to chronicity • No sterilizing immunity	[106]
HCV-like particles derived from recombinant HCV structural proteins (core, E1 and E2) *+/− AS01B adjuvant*	Chimpanzees	• Limited B cell response • Strong induction of cellular immune responses	[111]
E1 recombinant protein *aluminum hydroxide adjuvant*	Phase I Healthy volunteers	• Well tolerated • Induction of specific humoral and cellular responses • No neutralization experiments	[98]
Genotype 1a Recombinant E1E2 proteins *MF59 adjuvant*	Phase I Healthy volunteers	• Well tolerated • Development of humoral and CD4+ T cell responses • Presence of cross-nAbs in serum of vaccinees	[93] [94] [95]
DNA encoding core E1E2 proteins (CIGB-230)	Phase I Treatment-experienced genotype 1b infected patients	• Development of anti-core and E1 antibodies • Improved liver histology for 40% of patients, possibly attributed to cellular immune responses	[108]
Genotype 1a Recombinant E1E2 proteins *MF59 adjuvant +/− pegIFN/RBV*	Phase Ib Treatment-experienced genotype 1a/1b infected patients	• Safe • Development of nAbs • Better treatment response in association with pegIFN/RBV • Higher SVR rate when used in combination with pegIFN/RBV	[97]

nAbs, neutralizing antibodies; pegIFN/RBV, pegylated interferon ribavirin; SVR, sustained virological response