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# Development of Preventive Vaccines for Hepatitis C Virus E1/E2 Protein

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**ABSTRACT**

Hepatitis C virus (HCV) is responsible for a vast majority of liver failure cases. HCV is a kind of blood disease estimated to chronically infect 3% of the worlds population , causing significant morbidity and mortality. Therefore, a complete knowledge of humoral responses against HCV, resulting antibodies, and virus-receptor and virus-antibody interactions, are essential to design a vaccine. HCV epitopes or full sequence of HCV proteins can induce HCV specific immune responses. In fact, structural proteins are usually the main target of humoral responses and non-structural proteins are usually the main target of cellular responses. Hence, various vaccines based on distinct antigenic combinations are developed to prevent HCV infection and the current study tried to summarize them.

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## Introduction

Hepatitis C virus (HCV) is an enveloped, single stranded RNA virus belonging to Flaviviridae family and hepacivirus genus (1,2). It is estimated that 3% of the world's population have HCV infection. Approximately, 75% of acute HCV infections develop to chronic HCV out of which 3%-11% cause liver cirrhosis, liver failure, and hepatocellular carcinoma (HCC) (3-6). Although HCV was discovered in 1988, an effective vaccine is not available yet. Therefore, several studies sought to produce an effective vaccine against HCV infection. Facts that hamper the vaccine production include lack of an appropriate disease host model, moderate titer of produced antibodies against the envelope glycoproteins and their short half-life, and inability to produce high amounts of virus in tissue culture (7).

Despite extensive research, an effective vaccine is not available yet. A vaccine should be able to stimulate neutralizing antibodies in order to be efficient. Recent treatment of HCV used directly acting antiviral agents (DAAs), but there is an important demand to study the new vaccine to prevent infection. It is reported that annual health care costs of patients with mild chronic HCV infection, HCC, and cirrhosis are about €2,756, €11,437 , and €6,258, respectively (7).

Furthermore, other obstacles are also on the way of HCV vaccine development. For instance, different HCVs with distinct divergent sequences in specific regions of the genome are identified that suggest various mutations in viral genome during the infection. Mutations especially involve the N-terminal region of E2 glycoprotein; i e, hypervariable region 1 (HVR1) and this region has the highest variability amongst the known isolates (8). This genetic heterogenicity helps the virus escape host's immune system. HCV treatment conventionally depends on interferon alpha (INF-α) and ribavirin associated with adverse side

effects. Recently, a new class of drugs, called direct acting antivirals (DAA), were developed to be used in combination with INF-α and ribavirin in order to increase their effectiveness, however, the setbacks are high cost and increased side effects (9). Keeping in mind the high expenses and side effects of the current treatments of hepatitis C and considering the fact that only a small percent of patients can be completely cured (10), an effective HCV vaccine is apparently needed.

Developments are made in the field of producing model systems to study the virus-receptor and virus-antibody interactions. Although the exact relationship between HCV and neutralizing antibodies is unknown, these model systems improved the knowledge about the nature of antibodies responding against HCV and the complexity of host-virus relationships.

Infection with HCV induces weak immune response, thus 75% to 85% of infected population develop chronic infections (11); however, 20% of infected individuals can resolve the infection by their natural immune responses. Thus, an effective vaccine can be achieved. Moreover, considering the high cost of HCV treatment and its deteriorating effect on human health, producing a beneficial HCV vaccine seems to be a logical answer.

HCV epitopes or full sequence of HCV proteins can induce HCV specific immune responses. In fact, structural proteins are the main target of humoral responses and non-structural proteins are the main target of cellular responses. Hence, various vaccines, based on distinct antigenic combinations, are developed to prevent HCV infection that the current study tried to summarize them.

## Effects of neutralizing antibodies on chronic HCV infection

Immunization against HCV should stimulate the production of neutralizing antibodies and its efficacy is assessed based on the amount of neutralizing antibodies it produces (12). Analysis of structural sequences in chronic infection indicated that neutralizing antibody response is correlated with the sequence evolution and is likely the result of immune escape (13). In 2012 Raghuraman et al., evaluated the neutralizing antibody and cellular immunity responses in a patient with chronic hepatitis C and cleared the infection after 62 weeks. Viral clearance was associated with neutralizing antibody emergence in the week 48 of infection (14).

Neutralizing antibodies play important roles in patients with former HCV infection that cleared the virus and are infected again. A study was conducted on injecting drug abusers infected with HCV that were able to clear the virus. These subjects were infected again with HCV and 83% of them were able to clear the infection again, considering that 25% of individuals infected with HCV cannot clear the virus. Moreover, the duration and viremia were significantly lower in reinfected subjects (15).

These results suggest that neutralizing antibody production in the initial phase of infection is correlated with viral clearance rate. Passive delivery of neutralizing antibody to model animals protects them from viral infection. In the study by Morin et al., 250 mg/kg of HCV1 human monoclonal antibodies, which identify a linear and conserved epitope in E2 glycoprotein, was administered to chimpanzees 30 minutes after injecting HCV genotype 1aH77. The animals that received 250 mg/kg were completely protected against the infection whereas the ones that received 50 mg/kg were susceptible to infection. Infection was reduced to an undetectable level after administration of 250 mg/kg of HCV1 antibodies to a chimpanzee 42 days after infection; however, infection rate increased again after 14 days. These viruses had N415D mutation in E2 glycoprotein that granted them immunity to HCV1 antibodies; three other chimpanzees with chronic HCV infection were administered with 40 mg/kg of HCV1 antibodies, viral load was reduced to an undetectable level for 21 days in one of them, but after that viruses with N417S1 mutation emerged again. The other two chimpanzees did not show virus return. Therefore, application of sterilizing doses of antibodies before infection is preventing, though they are not protective after getting infected and are only able to reduce the viral load (16).

## E1 and E2 glycoproteins

The envelope proteins are located on the surface of the virion and are the major viral antigens that evoke protective immune responses. E1 and E2 are two envelope proteins of HCV. The E1 glycoprotein contains 192 amino acids ranging from position 192 in the polyprotein to 38, while E2 glycoprotein is a 363-amino-acid-long protein ranging from position 384 to 746 in the polyprotein (17).

Recently, the crystal structure of the core of E2 ectodomain was determined (18,19). Unlike previous considerations (20), E2 glycoprotein does not represent the structure shared by class II viral fusion proteins, but rather it has a globular structure with many regions without any regular secondary structure (18, 19). These new findings suggest that E1 alone or in association with E2 may be responsible for the fusion step, since E2 lacks the structural hallmarks of fusion proteins (18, 19, 21, 22). However, this hypothesis is not proven yet, because the structural data concerning the E1 ectodomain are inadequate.

E1 and E2 interact with each other to form a non-covalent heterodimer; finally, disulfide bonds stabilize E1 and E2 to appear as large covalent complexes on the surface of the virion (23). Moreover, the N-linked glycosylation of the E1 and E2 is also essential to form these multi-subunit complexes that facilitate the entry of the virus into host cells (23-25).

RNA-dependent RNA polymerase of HCV lacks the repair activity; therefore, HCV genome shows extensive intergenotypic and intragenotypic variabilities (26). The genes encoding E1 and E2 glycoproteins display the highest degree of genetic heterogeneity, and hypervariable region 1 (HVR1) of E2 is the most variable of all regions of the HCV genome (27, 28). Cheng et al., showed that E1 and E2 of HCV subtype 1b that is the most variable among different subtypes, and E1 is more variable than E2 in subtype 1b. Furthermore, they found more N-glycosylation sites in E2 than E1 in all subtypes. E1 of subtype 1b had the most N-glycosylation sites compared with other E1 proteins (29). N-glycosylation plays an important role in the folding and immunogenicity of HCV glycoproteins (41). The glycan shield masks neutralizing epitopes on the virion surface reducing the immunogenicity of the envelope proteins (38). According to Cheng's study, E1 glycoprotein may play an important role in provoking neutralizing antibodies and should be further studied, however, few neutralizing antibodies are identified against E1 glycoprotein, which indicate its low immunogenicity (30).

A physical interaction between E1-E2 heterodimer and host cell surface receptors is needed for the entry of viruses into cells. Glycosaminoglycans and low-density lipoproteins (LDLs) are thought to be vital for initial attachment of the virion and the host cell (31). Additionally, four other receptors to function in viral entry include CD81 (32), occludin (33), scavenger receptor class B type 1 (SR-BI) (34), and claudin-1 (35).

## Antibodies that target E1

Neutralizing antibodies that target E1 are few since E1 is covered by E2 in the E1-E2 heterodimer. In a study on H-111 antibody against E1 glycoprotein, it was observed that this ab is exclusive for YEVRN-VSGVYH sequence near the N-terminal of E1 and forms immune complex with E1E2. Notably, this antibody is capable of connecting to E1 of genotypes 1a, 2b, and 3a, which suggests the fact that H-111 epitope is hugely conserved. An effective vaccine should include epitopes that are conserved between various genotypes; therefore, the vaccine can prevent cell binding and entry of the virus. Therefore, ---RN-SG-Y sequence is a suitable candidate for the development of HCV vaccine (36).

Meunier et al., evaluated the human monoclonal antibodies against E1 glycoprotein that had the potential to control HCV infection. IGH526 and IGH505 were recognized as antibodies that could identify different, but overlapping epitopes. These antibodies strongly neutralized HCVpp covered by genotype 1a, 1b, 4a, 5a, and 6a glycoproteins and to lesser extent genotype 2a glycoproteins; however, they had no effect on genotype 3a glycoproteins. Topography of epitopes of the two antibodies revealed a region between amino acids 313 and 327 of E1 glycoprotein (30).

In 2011, Kachko et al., assessed the profile of antibodies induced by immunization of mice and chimpanzees with recombinant E1-E2 glycoproteins. In their study, the quality of antibodies was evaluated using peptide scan technique and in vitro neutralization test for three different HCV subtypes including 1a, 2a, and 1b. Interestingly, the results suggested that epitopes with most immunogenicity induced antibodies that had the least neutralizing capacity. Moreover, three important regions for neutralization were confirmed in this study including a region with high conservation of 85% to 95% and a potential of cross-neutralization. Therefore, it was suggested that deletion of some immunogen regions from the coating glycoproteins results in the production of more efficient neutralizing antibodies (37).

Grollo et al., used human serum containing anti-HCV IgG to isolate E1 and E2 epitopes of HCV genotype 1aH77. Bound epitopes were identified using mass spectroscopy; three different antigenic regions in E1E2 glycoprotein were specified using anti-HCV IgGs. At least four of these epitopes were conserved in three HCV genotypes and bound to serum antibodies found in patients with chronic HCV or the ones in convalescence period. Synthetic vaccines based on these epitopes trapped virions in patients with viremia, they also prevented entry of HCVpp to Huh7 cells (38).

## Antibodies that target E2

In 1995, Zibert et al., introduced the hypothesis that neutralizing antibodies target HVR1 region of E2 glycoprotein. To test their hypothesis they used serum of patients infected with HCV and concluded that HVR1 N-terminal was immunodominant in natural infections (39).

Immune serums produced against HVR1 sequence were capable of protecting chimpanzees against the same strain of virus. These antibodies were specialized for each genotype and had limited ability to neutralize variants that escaped immune system by mutations in HVR1 region (40).

In 1998, Lechner et al., sought to identify the E1 and E2 regions responsible for the production of neutralizing antibodies. They transcribed pE1 and ΔpE2 proteins that included amino acids 174 to 337 of E1 and 411 to 688 of E2 glycoproteins of HCV-AD78 isolate, respectively. The ΔpE2 protein lacked the HVR1 region. The pG-HVR1 protein that represented the amino acids 384 to 410 of HVR1 region of HCV-AD78 isolate was used as the control. These three proteins were tested by an immunoprecipitation assay in order to identify antibodies in the sera of individuals infected with HCV-AD78. The sera were obtained four to eight months after the infection and patients either cleared the infection or developed chronic liver disease. Results showed that both groups had a high level of anti-pE1 and -pE2 antibodies. Therefore, this part of envelope proteins constitutes B-cell epitopes. Interestingly, antibody response against pE1 and ΔpE2 was not significantly different between the two groups; however, anti-pGHVR1 antibody correlated with infection clearance. Rabbit antibodies against pE1 and ΔpE2 were used to assess the ability of HCV binding to susceptible cells in tissue culture. Results indicated that despite the production of antibodies against epitopes outside HVR1, these antibodies were not capable of viral clearance (41).

Most neutralizing identified antibodies target the entry domain of CD81; HCV also evolved various ways to cover this region of E2. E2 glycoprotein has 11 N-glycosylation domains and these domains participate in HCV folding and entry (42). It seems that these regions limit immunogenicity of E2 and prevent action of neutralizing antibodies, similar to gp120 in HIV (43,44).

In 2010, Francois Helle et al., concluded that glycosylation domains in E1 had no part in hiding potential epitopes of this protein. However, at least three glycans in E2, named EN11, EN5, and EN1 support binding of HCVpp to neutralizing antibodies and create an immune escape mechanism for the virus (45). It seems that antibodies act as a stimulus for escaping the immune system (46).

Studying the serologic responses to synthetic oligonucleotides derived from HVR1 showed several serologic cross-reactions to non-HVR1 peptides.

Variability of HVR1 sequence was significantly correlated with severity of humeral response cross-reaction. Thus, HVR1 is stimulated by the host's immune response (47). HCV sequence evolution analysis in infected individuals showed that in the absence of neutralizing antibodies HVR1 sequence remains constant, although as soon as the production of neutralizing antibodies begins, HVR1 sequence starts evolving (13).

Analysis of the responses to HVR1-specific antibodies in patients infected with HCV suggested that individuals that cleared the infection rapidly, had detectable anti-HVR1 antibodies in their serum in the first six months of their infection (48). Therefore, anti-HVR1 antibodies help the viral clearance under some circumstances.

Although anti-HVR1 antibodies act primarily on a specific genotype, evidence suggests that anti-HVR1 antibodies that have reactivity with a wide spectrum of non-related HVR1s can be produced. In 1999, Shang et al., successfully created antibodies capable of reacting with 16 out of 17 non-related sequences of HVR1. In their study rabbits were immunized with a series of synthetic HVR1 peptides, and then the produced anti-HVR1 antibodies were purified. These antibodies were capable of trapping HCV in the plasma of 22 out of 23 patients; moreover, they prevented the binding of HCV to MOL14 cells (49).

As mentioned before, CD81-binding domain in E2 glycoprotein is a target of neutralizing antibodies. This sequence is on the surface of E2 and antibodies against it are highly preserved, thus have cross-neutralizing abilities (50-52).

Residuals involved in binding to CD81 were identified using targeted mutagenesis in this motif. Studies that investigated mapping of the residuals and their results are summarized in Table 1.

An astonishing feature was that most of these residues were aromatic and hydrophobic and strictly conserved between genotypes. For example, in the case of Trp437, phenyl alanine was the dominant amino acid. Several neutralizing antibodies against E2 block the binging of CD81 and have amino acids that pass through 411 to 428, 429 to 448, and 523 to 549 regions; therefore, they overlap in the area involved in the binding of CD81.

A portion of antibodies targeting E2 can bind to their epitopes in synthetic sequences that are the analogues of residues 411 to 428 (epitope 1) and 429 to 448 (epitope 2). Antibodies that bind to the epitope inside 523 to 549 residue (epitope 3) include 1:7 and A8; however, these antibodies are conformation-dependent (50, 53, 54). Another portion of antibodies identify detached epitopes and bind to E2 only when it is folded. These antibodies include CDH5, CBH7, and AR3A. Antibodies against E1 and E2 glycoprotein are summarized in Table 2.

**Table 1.** Residues Involved in CD81 Binding

| Residue[1] | Epitope[2] | Substitute Amino Acid[3] | Reference |
|---|---|---|---|
| Trp 420 | I | - | (59) |
| His 421 | I | - | (60) |
| Trp 437 | II | Phe | (61) |
| Leu 438 | II | Ile,Val, Met | (61) |
| Leu 441 | II | - | (61) |
| Phe 442 | II | Leu, Met, Ile | (61) |
| Tyr 527 | III | - | (59) |
| Trp 529 | III | Phe | (59) |
| Gly 530 | III | - | (59) |
| Asp 535 | | | (59) |
| $Y^{613}$RLWHY[d] | III | - | (62) |

1. Analysis was done on genotype 1a
2. Amino acid location in antigen region
3. Substitute amino acids seen in other HCV isolates

**Table 2.** Antibodies Targeting HCV Envelope Glycoproteins; Amino Acids Reported by H77 Subtypes

| Antibody | Target Epitope (Amino Acid) | Epitope Type | Target Reaction | Antibody Source | Reference |
|---|---|---|---|---|---|
| **H-111** | 192-211 | Linear | Unknown | Human | (52) |
| **IGH505** | 312-327 (E1) | Linear | Unknown | Human | (30) |
| **IGH526** | 312-327 (E1) | Linear | Unknown | Human | (30) |
| **9/27** | 396-407 (HVR1) | Linear | SR-BI | Rat | (63) |
| **7/59** | 384-391 (HVR1) | Linear | SR-BI | Rat | (63) |
| **H77.39** | 415 and 417 | Linear | CD81/SR-B1 | Mouse | (64) |
| **3/11** | 412-423 | Linear | CD81 | Rat | (65) |
| **HCV1** | 412-423 | Linear | CD81 | Human | (66) |
| **95-2** | 412-423 | Linear | CD81 | Human | (66) |
| **AP33** | 412-423 | Linear | CD81 | Mouse | (67, 68) |
| **2/69a** | 436-443 | Linear | Unknown | Rat | (63-65) |
| **1/39** | 432-443 | Linear | CD81 | Rat | (65) |
| **AP320** | 444-471 | Linear | Unknown | Mouse | (69) |
| **6/41a** | 480-492 | Linear | Unknown | Rat | (65) |
| **11/20** | 436-447 | Linear | CD81 | Rat | (63) |
| **2/64a** | 524-531 | Linear | CD81 | Rat | (63) |
| **H53** | 540-550 | Conformation-dependent | Unknown | Mouse | (59,70) |
| **H35** | 523 and 530 | Conformation-dependent | CD81 | Mouse | (59,70) |
| **H48** | 530 | Conformation-dependent | CD81 | Mouse | (59,70) |
| **Fab e137** | 416, 420, 529, 530 and 535 | Conformation-dependent | CD81 | Human | (71) |
| **Fab e20** | 529, 530 and 535 | Conformation-dependent | CD81 | Human | (71) |
| **AR3A** | 436, 424-447, 523, 530, 535, 538 and 540 | Conformation-dependent | CD81 | Human | (51) |
| **AR3B** | 436, 424-447, 530, 535 and 540 | Conformation-dependent | CD81 | Human | (51) |
| **AR3C** | 424, 530, 535, 538 and 540 | Conformation-dependent | CD81 | Human | (51) |
| **AR3D** | 424, 447, 436-530 and 535 | Conformation-dependent | CD81 | Human | (51) |
| **7 : 1** | 523, 529, 530 and 535 | Conformation-dependent | CD81 | Human | (51, 53) |
| **A8** | 523, 529, 530 and 535 | Conformation-dependent | CD81 | Human | (51, 53) |
| **L1** | Unknown | Conformation-dependent | Unknown | Human | (51, 53) |
| **CBH4B** | Unknown | Conformation-dependent | Unknown | Human | (36, 72) |
| **CBH4D** | Unknown | Conformation-dependent | Unknown | Human | (36, 72) |
| **CBH4G** | Unknown | Conformation-dependent | Unknown | Human | (36, 72) |
| **CBH5** | 523, 525, 530, 535 and 540 | Conformation-dependent | CD81 | Human | (72, 73) |
| **CBH7** | 540 and 549 | Conformation-dependent | CD81 | Human | (72, 73) |
| **ALP98** | 644-651 | Linear | Unknown | Mouse | (69, 74) |
| **ALP1** | 647-658 | Linear | Unknown | Mouse | (69, 74) |

## Prospects for HCV vaccine production

Several vaccines were designed based on the ability of viral subunits to stimulate host's immune system. The first promising results in this field were obtained by Cho et al., in 1994. They successfully immunized chimpanzees against HCV using the purified E1E2 glycoprotein. In their study seven chimpanzees were injected with purified E1E2 glycoprotein; five of them showed complete resistance against homolog HCV challenge, whereas two chimpanzees developed the infection; however, the severity of infection was lower compared with that of the control group (55).

Frey et al., evaluated the efficacy of vaccination with E1E2 glycoproteins derived from HCV genotype 1a in 60 healthy volunteers. Vaccine was safe and well-tolerated. By the enzyme-linked immunosorbent assay (ELISA) and neutralization tests with vesicular stomatitis virus /HCVpp, measurable amounts of

E1E2 neutralizing antibodies together with CD4+ cell responses were recorded (56).

Only one of the neutralizing antibody producing vaccines was tested on humans. Chiron Corporation created this recombinant E1/E2 vaccine. In preliminary studies on chimpanzees, vaccine could produce significant number of antibodies against E1 and E2; five chimpanzees were immunized against homologous virus and the antiviral effect was correlated with the level of anti-E2 antibodies (55).

Recombinant E1/E2 vaccine with MF59 adjuvant was tested on 41 healthy volunteers. Initial results showed that 15 individuals had antibodies against 313 to 327 region, 21 against HVR1, 23 against epitope 1, and 13 against epitope 2 (57).

In 2013, Law et al., immunized healthy volunteers with recombinant E1/E2 glycoproteins derived from HCV1a subtype. Cross-neutralization was tested using Huh7.5 cells and HCV cell culture (HCVcc). Vaccination induced neutralizing Abs against heterologous HCV1a. In order to evaluate cross-neutralizing responses, three people were selected based on their capacity to neutralize HCV1a virus. At least one of them exhibited broad cross-neutralizing responses against all HCV genotypes (58).

Results of this study and other similar studies increased the hope to produce a vaccine with the ability to produce broad cross-neutralizing. Moreover, using recombinant E1/E2 glycoproteins opened new horizons for researchers to achieve an effective HCV vaccine. One of the most important problems in the field of HCV is drug resistance, genotypes and different genotypes, extremely high cost of drugs, HCV carrier population, lack of an animal model for the survey immune response of the vaccine, etc. For these reasons, there is an urgent need to identify a prophylactic HCV vaccine.

## Conclusion

Results of the current study and other similar studies increased the hope to produce a vaccine with the ability to produce broad cross-neutralizing. Moreover, using recombinant E1/E2 glycoproteins will open new horizons for researchers to achieve an effective HCV vaccine.

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## Conflicts of interest

The author declared no conflict of interest.

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