

Production and immunogenicity of different prophylactic vaccines for hepatitis C virus (Review)

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Abstract. Hepatitis C virus (HCV) infection is a global health challenge, and prophylactic vaccines are the most effective way to eliminate the infection. To date, numerous forms of preventive vaccines have entered the clinical trial stage, including the virus-like particle (VLP) vaccine, recombinant subunit vaccine, peptide vaccine and nucleic acid vaccine. The rational design makes it easier to obtain specific vaccine structures with a broad spectrum and strong immunogenicity. Different vaccine antigens can evoke different immune responses, including humoral and T-cell immune responses, and can be produced using different expression systems, such as bacteria, yeast, mammals, plants, insects or parasites. Intracellular and insoluble production and a narrow immune spectrum are two difficulties that limit the application of vaccines. The present study summarizes the immunogenicity of different preventive vaccines, evaluates the characteristics of different expression systems used for vaccine production, and analyzes the strategies to enhance the secretion and immune spectrum of vaccine proteins.

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1. Introduction

Hepatitis C virus (HCV) infection is a global health problem that human beings have struggled with for ~30 years. Individuals infected with HCV develop chronic hepatitis, and a proportion might develop cirrhosis and liver carcinoma. The World Health Organization has set a goal to eliminate the public health burden of HCV by 2030 (1). A platform in the use of medicines to treat chronic hepatitis C was constructed in Australia for the collection and management of treatment, virological outcome and other relevant clinical data of patients with HCV to better inform the limitations of HCV infection (2). Although the development of direct-acting antiviral agents (DAAs) for chronic HCV has resulted in a 95% cure rate for patients infected with HCV genotypes (3), there are still millions of new infections and tens of millions of re-infections worldwide every year. In such cases, an effective prophylactic vaccine is necessary to control HCV infection. Significant progress has been made in the development of vaccines against HCV in the past 10 years despite no commercial vaccine having appeared so far. The characteristics and properties of different forms of vaccines and the advantages and disadvantages of various vaccine expression systems are summarized in the present study, to provide new insights into the research and development of HCV preventive vaccines.

In the present review, a literature search was performed using the PubMed, Elsevier Science Direct and China National Knowledge Infrastructure databases with 'Hepatitis C virus' and 'vaccine' as the primary key words. On this basis, 'immunogenicity', 'immune response', 'production', 'expression' and 'rational design' were used as key words for the secondary search. Studies related to therapeutic vaccines were excluded, unless they described the immune protection on HCV genotypes different from the infected one. Studies not written in English or Chinese were excluded.

2. Different forms of preventive vaccines and their immunogenicity

HCV is a positive-sense, single-stranded RNA virus that belongs to the Flaviviridae family. The HCV genome contains two untranslated regions and an open reading frame encoding

structural proteins [core, envelope protein 1 (E1) and envelope protein 2 (E2)] and non-structural proteins (p7, NS2, NS3, NS4A, NS4B, NS5A and NS5B) (Fig. 1) (4). The complete or partial genome has been used for vaccine preparation. Compared with the attenuated vaccine or inactivated vaccine prepared using live viruses, the preparation of vaccine using genetic engineering technology has further improved its safety and immunogenicity (5). Genetically engineered vaccines are widely used to prevent infection by various viruses (6). Different forms of genetically engineered vaccines have been developed, and their properties are summarized in Fig. 2.

VLP vaccine. The structural proteins of HCV can self-assemble into viral particles without infection *in vitro*. VLP vaccines can not only induce T-cell (CD4⁺ and CD8⁺) immune responses, but also stimulate the formation of protective neutralizing antibodies (7,8). Research has found that different assembly forms lead to different particle sizes (diameter ranging from 30-80 nm), in which only particles of a specific size (55-60 nm in diameter) are infectious and can cause specific antibody responses (9). VLPs with the same gene sequence can be assembled into different structures in different expression systems. For example, VLP vaccines containing the same structural protein sequences have distinct immunogenicity due to the different glycosylation mechanisms in insect and mammalian cells (10,11). This suggests that the composition of the sugar chain could affect the conformation of the whole virus particle. In addition, some chimeric VLPs have been widely used. To date, the hepatitis B virus (HBV) core protein (12), the small (S) envelope protein of HBV (13), and papaya mosaic virus coat protein (PapMVCP) (14) have been fused with partial or total sequences of HCV envelope protein to prepare VLP vaccines. However, the immunogenicity of chimeric VLPs is lower than that of vaccines prepared using HCV self-proteins.

VLPs have been proven to induce protective immune responses against viruses without adjuvants, and some studies have shown that the use of adjuvants can significantly enhance the immunogenicity of VLP vaccines (15-17). Use of an anionic self-adjuncting lipopeptide containing the Toll-like receptor 2 agonist Pam2Cys (E8Pam2Cys) enhanced the immunogenicity of VLPs composed of HCV structural proteins (core, E1 and E2) (15). The improvement in VLP and E2-specific antibody responses in VLP + E8Pam2Cys vaccinated mice required up to three doses of non-adjuncted VLPs to match the antibody titers obtained with a single dose of VLPs formulated with this lipopeptide. Further research found that co-formulation of this lipopeptide with VLPs could improve dendritic cell uptake and maturation, and could also induce better VLP-specific interferon (IFN)- γ -mediated responses.

Recombinant subunit vaccine. The recombinant subunit vaccine is mainly concentrated in the envelope proteins E1 and E2 of the HCV, as the epitopes on the envelope proteins are important for virus invasion and neutralizing antibody identification (18,19). Recombinant subunit vaccines based on envelope proteins can inhibit viral infections by stimulating protective human neutralizing antibodies (HMABs). Topological analysis showed that E1 is a multi-pass transmembrane protein (crosses the membrane three times) with

most domains inside, and E2 is a single-pass transmembrane protein with most domains outside (Fig. 3A). Antigenic epitopes are concentrated in the E2 ectodomain due to the existence of binding sites for cellular factors such as CD81, scavenger receptor class B type I and claudin-1 (20-22). Antigen epitopes on the surface of the envelope protein seriously affect a broad spectrum of vaccines. HMABs generated by conservative antigen epitopes (divided into linear and conformational epitopes) have a wide range of neutralization characteristics and can cross-neutralize HCVs of different genotypes. HMABs (AP33, 3/11, 95-2 and HCV-1) corresponding to the 412-423 amino acid (aa) linear epitope show extensive neutralizing activity against HCV pseudoparticles (HCVpp) of genotype 1-6 (Fig. 3B and C) (23); HMABs (CBH2, HC11 and HC1) or antigen region 3 (AR3) corresponding to the conformational epitopes of two discontinuous sequences, 424-443/523-540 aa, or three discontinuous sequences, 394-424/437-447/523-540 aa, can also widely neutralize HCVpp of genotypes 1-6 (Fig. 3B and C) (24). Further experiments on virus-escaping mutants showed that no virus escape body was found in antibody HC1 at different experimental concentrations of 0.05-100 $\mu\text{g/ml}$ (25). This indicated that the influence of the conformational epitope on the broad spectrum of the vaccine was greater than that of the linear epitope. The discovery that epitopes determine the broad spectrum of neutralizing antibodies will help in artificially designing specific vaccine structures to stimulate broadly neutralizing antibodies (26,27). Notably, not all epitopes could induce the production of protective neutralizing antibodies. The interfering antibody induced by the E2 region 434-446 aa does not have neutralizing activity; however, its binding to E2 can mask the binding of other neutralizing antibodies to the adjacent antigen epitope region 412-426 aa, thereby reducing the effective neutralizing activity corresponding to this epitope (28,29). Thus, elicitation of antibodies with interfering capacity should be avoided when producing an effective cross-neutralizing vaccine.

The epitopes of E2 are mostly concentrated in the ectodomain; therefore, the removal of the C-terminal transmembrane domain (TMD) has no significant effect on its immunogenicity (30). In addition, the truncated soluble E2, after removing hypervariable (HVR)1, HVR2 and intergenotypic variable region, can still be folded correctly, but the immunogenicity is reduced (31-33). Changing the glycosylation modification mode of the envelope protein also causes changes in its antigenicity and immunogenicity. A comparison of the two glycosylation modification modes in insect and mammalian cells showed that the sugar chain was essential for the immunogenicity of the E2 vaccine, but the complex sugar chain was not conducive to immunogenicity (34). Further research showed that insect E2 induced stronger neutralizing antibody responses against the homologous isolate used in the vaccine, but the two proteins elicited comparable neutralization titers against heterologous isolates (35). Adjuvants are required to exert the immunogenicity of recombinant subunit vaccines (36,37). Co-immunization with recombinant E2 vaccine and the saponin adjuvant QuilA, prepared by mammalian cells, can produce anti-E2 antibody titer much higher than that found using Freund's, monophosphoryl lipid A, cytosine

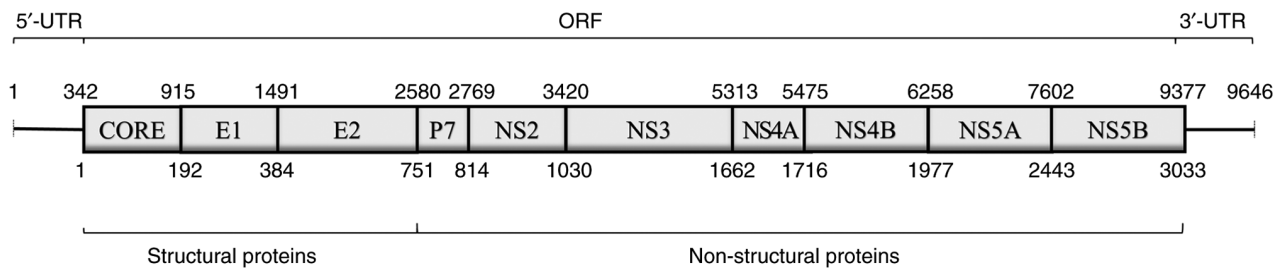


Figure 1. Structure and organization of hepatitis C virus genome and proteins using the NCBI reference sequence (NC 004102.1). The number above the sequence is the base position of all genes and the number below is the amino acid position of the corresponding proteins. 5'-UTR, 5' untranslated region; ORF, open reading frame; 3'-UTR, 3' untranslated region; NS 2, 3, 4A, 4B, 5A and 5B, non-structural proteins 2, 3, 4A, 4B, 5A and 5B; p7, hepatitis C virus non-structural protein p7; E1, hepatitis C virus envelope protein 1; E2, hepatitis C virus envelope protein 2; CORE, core protein.

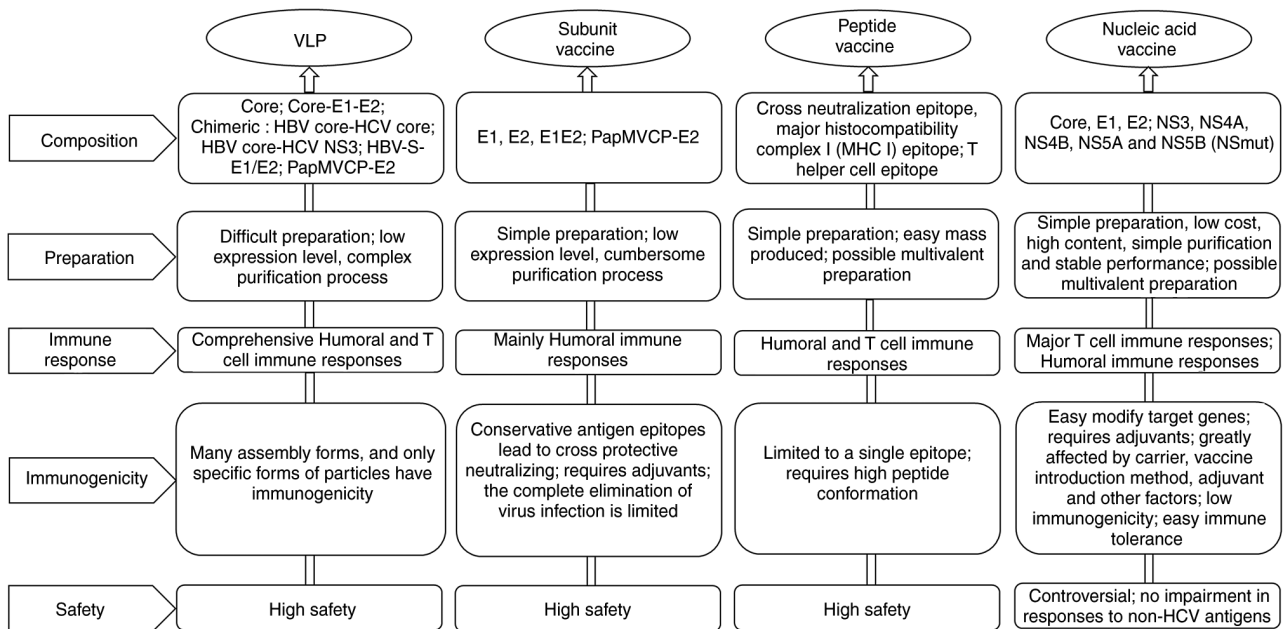


Figure 2. Comparison diagram of four different forms of HCV vaccine. The term composition indicates the key components from HCV or other viruses. Preparation refers to the evaluation of the expression level and the purification process. Immune response refers to humoral T-cell immune responses that are induced by the vaccine. Immunogenicity refers to all factors that contribute to the immunogenicity. Safety indicates the effects on the human body. HCV, hepatitis C virus; VLP, virus-like particle; PapMVCP-E2, papaya mosaic virus coat protein fused to HCV envelope protein 2; NS 3, 4A, 4B, 5A and 5B, non-structural proteins 2, 3, 4A, 4B, 5A and 5B; NSmut, non-structural protein mutation; HBV, hepatitis B virus; HBsAg-S-E1/E2, HBV surface antigen fused to HCV envelope protein 1/2; core, HCV core protein; E1, HCV envelope protein 1; E2, HCV envelope protein 2.

phosphorothioate guanine oligodeoxynucleotide (CpG ODN) or α -galactosylceramide derivatives, and the effect of two adjuvant combinations (QuilA and CpG ODN) is better than that of a single adjuvant (36).

Peptide vaccine. Peptide vaccines with immunogenicity can be screened by constructing a phage expression peptide library or directly synthesized using cross-neutralization epitopes, specific major histocompatibility complex class I epitopes and T helper cell epitopes. Such vaccines contain only limited epitopes and cannot stimulate a wide range of immune responses. In patients, after standard antiviral treatment, peptide vaccines can induce HCV-specific T-cell responses to enhance the sustained virological response and reduce relapse rates (38). However, the immunogenicity of vaccines can be enhanced by modification. For example, in one study, the affinity and immunogenicity of peptide vaccines were improved after the leucine at position 8 of

the cytotoxic T lymphocyte epitope of HCV core antigen (132-140 aa) was mutated to alanine (39). A nano-poly-peptide vaccine based on an NS3 polypeptide mixture can stimulate stronger CD4⁺ T-cell responses and induce stronger CD8⁺ T-cell immune responses than the NS3 polypeptide vaccine (40). In addition, the latest research showed that an overlapping peptide nanoparticle vaccine prepared based on the p7 protein successfully stimulated CD4⁺ and CD8⁺ T-cell responses (41). This was the first study to demonstrate the immunogenicity of p7 as a vaccine target and provides a new idea for the preparation of peptide vaccines. Different application schedules and injection routes may also influence the immunogenicity of HCV peptide vaccines. In a phase I clinical trial for the dose and injection route of the HCV peptide vaccine IC41, when increasing the frequency of vaccinations from 4 or 6 to 8 or 16 times per cycle (16 weeks), and decreasing the time window from 4 weeks to 1 or 2 weeks, the T-cell response rates, in particular the rates

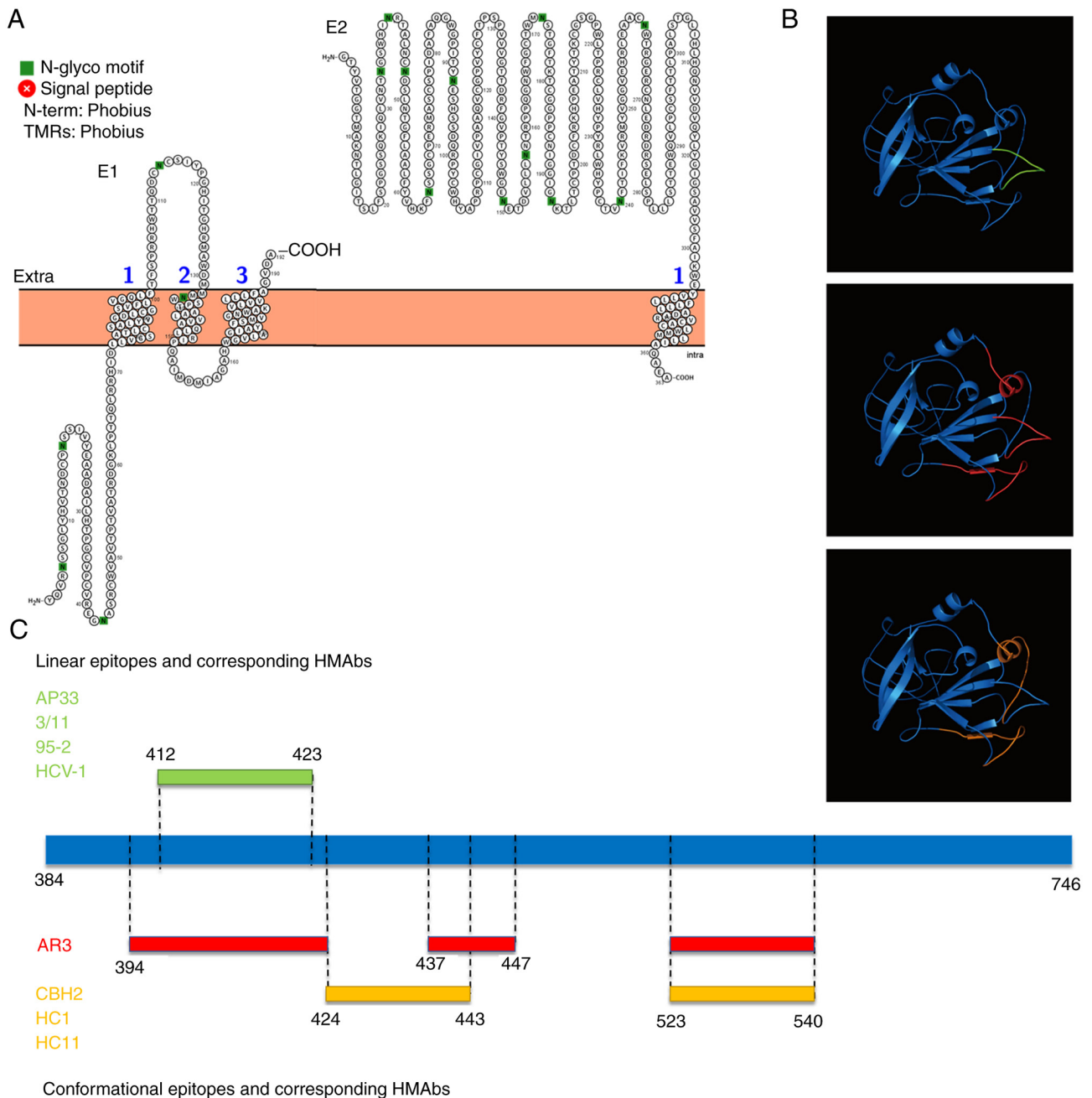


Figure 3. Epitope distribution of envelope protein. (A) Topology analysis of envelope proteins E1 and E2. (B) Conserved epitope sites in the tertiary structure of the E2 ectodomain. (C) Conserved epitope sites and the corresponding HMABs in the primary structure of E2. (B and C) Linear epitopes are highlighted in green, and two different conformational epitopes are highlighted in red and yellow, respectively. Other regions of E2 are presented in blue. Topology analysis using Protter online software (<http://wlab.ethz.ch/protter/start/>). Tertiary structure analysis using ExPasy online software (<https://swissmodel.expasy.org/interactive>). Intra, intracellular domain; extra, extracellular domain; N-glyco motif, N-glycosylation motif; N-term, N-terminal; TMRs, transmembrane regions; HMABs, human neutralizing antibodies; HCV-1, hepatitis C virus-1.

measuring CD8⁺ T-cell function, were enhanced up to 2-fold compared with previous studies using the same formulation of the IC41 vaccine (42-44).

Nucleic acid vaccine. DNA vaccines are prepared based on the coding sequence of HCV structural proteins or non-structural proteins such as E1, E2, NS3, NS4A, NS4B, NS5A or inactivated NS5B (NSmut) gene. Viruses (adenovirus or vaccinia virus) and eukaryotic cytoplasmic particles are usually used as vaccine vectors. DNA vaccines made of structural protein-coding sequences usually stimulate humoral

immune responses and produce protective neutralizing antibodies (45), whereas vaccines derived from non-structural protein sequences mainly sustain cellular immune responses. A prime-boost vaccine with chimpanzee-derived adenovirus-3 NSmut and modified vaccinia Ankara NSmut could successfully induce CD4⁺ and CD8⁺ T-cell responses to all candidate HCV antigen epitopes, but its phase I clinical trial results showed that the candidate vaccine did not provide better protection against chronic HCV infection than the placebo (clinicaltrials.gov identifier: NCT01436357) (46). It was found that the relative frequency of CD4⁺, CD25⁺

and Foxp3⁺ regulatory T cells (Tregs) was increased in the blood and liver in patients with chronic persistent HCV infection even after successful DAA treatment (47,48). Tregs were demonstrated to attenuate vaccine-induced protective CD4⁺ and CD8⁺ T-cell immune responses (49). Therefore, a novel HCV DNA vaccine, GLS-6150, consisting of the DNA plasmid encoding adjuvant IFNL3 with DNA plasmids encoding the HCV non-structural proteins (NS3/NS4A, NS4B and NS5A genes), reduced the frequency of Tregs and increased HCV-specific T-cell responses in a phase I clinical trial (NCT02027116) (50). Another study reported that the immunogenicity of a DNA vaccine mixture (vaccine cocktail) composed of conserved sequences of coding proteins of multiple HCV genotypes was much greater than that of DNA vaccines from a single genotype (51). The breadth and intensity of the T-cell response have been improved, although clinical experiments have not yet been performed. Experiments on safety showed that the injection of DNA vaccines does not impair the ability of the body to respond to non-HCV antigens (52).

Rationally designed vaccine. In the early stages of vaccine rational design research, neutralizing antibodies associated with specific virus clearance can be found by analyzing the antibody spectrum of the virus, and then specific immunogens can be rationally designed to facilitate the production of such antibodies. For example, reasonable design of a specific epitope in E2 can produce HC33.1 antibody with strong antiviral effect (53). A recent study successfully constructed an AP33 epitope structure simulant using an anti-idiotypic method. In a mouse model, an antibody with the same recognition site and residue as AP33 was induced as an immunogen, and the antibody had the ability to resist HCV infection (54). This research opens up a new method for the rational design of vaccines. Limited by technology, the rational design thus far is restricted to linear epitopes and the analog design of conformational epitopes cannot be realized.

The rational design of conformational epitope vaccines must be based on an in-depth study of protein structure and immune recognition information. Envelope glycoprotein E2 has a highly conserved neutralization surface that is not covered by the N-linked glycans and has three main overlapping neutralization sites: Antigen site 412, 434 and AR3. Although it is composed of highly conserved amino acid residues, its conformation is flexible (21). It is difficult to obtain ideal immune effects for peptides designed according to epitope sequences, possibly since they do not have the correct conformation. Analysis of the preferred conformation of the neutralization site provides a basis for the design of a conformational vaccine.

3. Different expression systems for vaccine preparation

To date, candidate vaccine proteins composed of partial or complete protein sequences of HCV have been successfully produced in a variety of expression systems, such as mammalian and insect cells, *Pichia pastoris*, *Escherichia coli* expression systems, plant leaf expression systems, and even the parasitic host *Leishmania tarentolae*. The hosts and expression

vectors used in different expression systems are summarized in Table I.

Eukaryotic expression system. A number of in-depth studies have focused on the preparation of HCV vaccines using eukaryotic expression system, as the recombinant vaccine protein is closest to the natural state of the vaccine and has post-translational protein modifications such as glycosylation. Mammalian cell-derived recombinant envelope proteins have been reported to have higher immunogenicity and human cell binding ability than those produced in yeast or insect cells (55,56). Different vaccine candidates have been successfully produced in mammalian cells, including Chinese hamster ovary (CHO) (57), COS-7 (57,58), Huh-7 (59,60) and human embryonic kidney 293T (13,61) cells. A comparison of envelope protein expression in COS-7 and CHO cells showed two different expression patterns: The former prepared full-length protein with transient expression form, while the latter produced truncated target protein in a stable manner (58). This suggests that transient expression is more suitable for maintaining protein integrity than transient expression. The *Pichia pastoris* expression system has the advantages of low cost and simple operation for recombinant protein preparation. The structural proteins of the core, E1 and E2, have been prepared as subunits or VLP vaccines in *Pichia pastoris* under methanol induction, and the target proteins have antigenicity. However, size-exclusion chromatography and SDS-PAGE experiments have suggested that E2 is mainly produced in a dimer or polymer form (62). The fragment from 612 to 620 aa has been reported to be a dimerization sequence (63). The tendency to aggregate is probably an intrinsic property of HCV glycoproteins, which leads to low protein synthesis when using a non-viral vector (64). In fact, the maximum yield of HCV glycoproteins prepared using yeast cells was 35 mg/l. The yield of vaccine protein prepared by different hosts ranged from 1-10 mg, and the highest yield was 100 mg/l from *Drosophila* S2 cells with an expression cycle of up to 9 days. In recent years, some new expression systems have been attempted for simpler genetic manipulation, higher production levels and lower-cost production. The HVR1/cholera toxin B subunit chimeric protein was expressed in *Nicotiana benthamiana* plants with a production of 6-80 µg/g of leaf tissue (65). Core-E1-E2 VLPs were successfully generated by the *Leishmania* expression system (66). These vaccines will not be suitable for use in clinics until their safety and efficacy is confirmed.

Prokaryotic expression system. Owing to the lack of protein modification by prokaryotic cells, the immunogenicity of the vaccine in a prokaryotic expression system is relatively lower than that developed using eukaryotic cells. Most HCV vaccine proteins prepared using the *E. coli* expression system are in the form of inclusion bodies, and a few truncated envelope proteins can be released into the periplasmic space of host cells using signal peptides. The expression of the target protein accounts for 40-50% of the total bacterial protein (58). It has been found that the expressed core protein can also be assembled into particles *in vitro* (with a diameter of 60 nm) (67). In addition, due to the influence of bacterial and toxin proteins, the purification cost is relatively high.

Table I. Summary of different hepatitis C virus vaccine candidate expression systems.

Expression system	Host cell line	Vector	Vaccine type	Vaccine antigen	Expression position	Max. expression level	(Refs.)	
Mammalian cells	COS-7	Plasmid	Subunit vaccine	E2	<i>In vivo</i> /soluble	Not shown	(57,58)	
	CHO	Plasmid	Subunit vaccine	E2	<i>In vivo</i> /soluble	Not shown	(57)	
	Hun7	Adenovirus	Homozygous VLPs	Core-E1-E2	<i>In vivo</i> /soluble	4.75-8 mg/l	(59,60)	
	293T	Plasmid	Chimeric VLPs	HBV S-E1 HBV S-E2	<i>In vitro</i> /soluble	5.51 mg/l	(13,61)	
Insect cells	<i>Spodoptera frugiperda</i> Sf9	Adenovirus	VLPs	Core-E1-E2	<i>In vivo</i> /soluble	Not shown	(11)	
	<i>Drosophila</i> S2 cell	Plasmid	Subunit vaccine	E2 E1-E2	<i>In vitro</i> /soluble	100 mg/l Not shown	(34,68) (69)	
Yeast cells	<i>Pichia pastoris</i>	Plasmid	VLPs	Core-E1-E2	<i>In vivo</i> /insoluble	Not shown	(70)	
				Core	<i>In vitro</i> /soluble	1.61 mg/ml	(71)	
				Subunit vaccine	E1-E2	<i>In vivo</i> /insoluble	35 mg/l	(72)
				core	<i>In vivo</i> /soluble	Not shown	(73)	
				core-E1	<i>In vivo</i> /soluble	Not shown	(73)	
				core-E1-E2	<i>In vivo</i> /soluble	Not shown	(73)	
				E2 (384-650aa)	<i>In vitro</i> /soluble	Not shown	(73)	
				E2 (384-746aa)	<i>In vivo</i> /insoluble	Not shown	(73)	
				E2 (384-680aa)	<i>In vitro</i> /soluble	Not shown	(62)	
Bacterial cells	<i>Escherichia coli</i>	Plasmid	Subunit vaccine	E2	<i>In vivo</i> /inclusion body	41.6-50%/ total somatic protein	(58,74)	
				PapMVCP ₂₇₋₂₁₅ -E2	<i>In vivo</i> /soluble	Not shown	(14)	
				Homozygous VLPs	core VLPs	<i>In vivo</i> /inclusion body	10 mg/l	(67)
				Chimeric VLPs	PapMVCP-E2	<i>In vivo</i> /soluble	Not shown	(14)
				Peptide vaccine	NS3	<i>In vivo</i> /inclusion body	Not shown	(40)
					p7	<i>In vivo</i> /inclusion body	Not shown	(41)
Plant cells	<i>Nicotiana benthamiana</i> plants	Tobacco mosaic virus	Subunit vaccine	Cholera toxin B subunit (CTB)-E2 HVR1	<i>In vivo</i> /soluble	6-80 µg/g of leaf tissue	(65)	
Parasite cells	<i>Leishmania tarentolae</i>	Plasmid	VLPs	Core-E1-E2	<i>In vivo</i> /insoluble	4-6 mg/l	(66)	

HBV S-E1, hepatitis B virus small envelope protein fused to hepatitis C virus envelope protein 1; PapMVCP-E2, papaya mosaic virus coat protein fused to hepatitis C virus envelope protein 2; NS3, non-structural proteins 3; p7, hepatitis C virus non-structural protein p7; E1, hepatitis C virus envelope protein 1; E2, hepatitis C virus envelope protein 2; HVR1, hypervariable 1; VLP, virus-like particle.

4. Difficulties in vaccine preparation

How to prepare HCV vaccine protein in a soluble secretory form. The vaccine protein, especially the envelope glycoprotein, is mainly expressed intracellularly and is insoluble through the addition of the signal peptide sequence during recombinant expression (71). It is generally believed that the strong hydrophobicity of the C-terminus of the E2 protein is the main reason for this (75,76). Therefore, several strategies have been developed to enhance protein secretion: i) Fusion preparation

with proteins having strong secretory ability: The wild-type HBV S subviral particles used in current HBV vaccines can be efficiently secreted into the cell supernatant and are easily purified. Replacing the N-terminal TMD of the HBV S protein with the TMD of HCV E1 or E2, the chimeric HBV-HCV envelope proteins (E1-S or/and E2-S) can be effectively secreted, co-expressed and assembled into VLPs [S+E1-S, S+E2-S and S+(E1-S+E2-S)] with the wild-type HBV S protein (13). Unlike HCV VLPs, the chimeric HBV-HCV VLPs could only induce a humoral immune response but not a T-cell immune

response. ii) Optimization of the structure of the target protein: Following removal of the partial domain of the C-terminal to prepare a truncated envelope protein, the truncated E2 protein (384-521 or 605-680 aa) could be effectively secreted by *Pichia pastoris* cells, while intact E2 (384-746 aa) was mainly located in the insoluble part of ruptured cells (73). This is in concordance with another study that found that C-terminal truncated enveloped proteins could be efficiently secreted to the culture medium by mammalian cells (77). Further research demonstrated that the C-terminus of E2 that began with aa 718 contained an endoplasmic reticulum retention signal (75,76), and topological analysis also showed that aa 718 was the beginning of the TMD (Fig. 3A). iii) Optimization of the signal peptide structure: When the α -factor signal peptide of the *Pichia pastoris* expression system was changed to the leader sequence of sucrose invertase 2, extracellular expression of E2 could be realized (62,72). However, the underlying mechanism remains unknown. In addition, using the 374-383 aa of envelope protein E1 as a signal sequence, the E2 protein could be expressed and secreted by mammalian cells (78).

How to widen the immune spectrum of a vaccine. Due to a number of HCV genotypes, the vaccine that is usually effective for one genotype is ineffective for other genotypes. Therefore, broadening the spectrum of vaccines has become a research challenge. Based on the published literature, there are the following strategies to broaden the spectrum of vaccines: i) Selection of antigen epitopes with strong conservation. The appearance of broadly neutralizing antibodies (bNAbs) is important for the body to remove HCV infection (79). Analyzing the distribution characteristics of antigen epitopes in the tertiary structure of HCV E2 protein, it was found that the antigen epitopes near the receptor-binding amino acids were conserved and could easily produce extensive neutralizing antibodies. HMABs represented by AP33, 3/11, 95-2 and HCV-1 bind to the 412-423 aa linear epitope (23), which can show extensive neutralizing activity against HCVpp of genotype 1-6. CBH2, HC11, HC1 and other monoclonal antibodies bind to the conformational epitope composed of 424-443 aa and 524-540 aa sequences, which can widely neutralize the HCV 1-6 genotype (24). The molecular mechanism of the exact cell entry process for HCV remains undefined, however, and only limited antigen epitopes have been identified to this day. ii) The antigen sequences of multiple genotypes are combined to prepare a multi-antigen vaccine cocktail: A multi-antigen cocktail regimen created by comparing a DNA vaccine cocktail encoding genotype (Gt)1b and Gt3a NS3, NS4 and NS5B proteins elicited significantly higher T-cell responses to Gt1b and Gt3a NS5B proteins than single-genotype NS3/4/5B DNA vaccine (51). Obviously, the multi-antigen vaccine cocktail method is not suitable for VLPs and subunit vaccines, for which the correct conformation of the protein is necessary. iii) Rational designing of a conservative structure suitable for multiple genotypes: Structure-based vaccine designs have been successfully used in influenza virus, human immunodeficiency virus and other variable viruses for the purpose of optimizing the presentation of key conserved epitopes, masking sites using N-glycans or stabilizing the conformations of the envelope glycoproteins (80,81). Similarly, the E2 antigen, in which a mutation H449P was designed to stabilize

the conformation of a conservative immunogen domain D in the internal fluidity neutralization surface, successfully induced bNAbs with cross-neutralizing activities against HCVpp of the 1b, 2a, and 4a genotypes (27). Analyzing the sequence or structure of cross-neutralizing antibodies, the corresponding epitope structure simulant was designed using anti-idiotypic technology. The immunogenic effectiveness of AP33 linear epitope mimics has been confirmed in mouse models (54) and the design of structural epitope mimics is the development direction of this strategy.

5. Conclusions

An effective vaccine for HCV is, essentially, an antigen that elicits immune responses to key conserved epitopes. In the present review, the composition, immunogenicity, advantages and disadvantages of all different types of vaccines were summarized and compared, and it was found that the source of antigen determines the immunogenicity of vaccine. Structural proteins are involved in the invasion of the virus into host cells and are used in preference to stimulate the humoral immune response of the host. Non-structural proteins are mainly involved in virus replication and cause specific T-cell immune responses. HCV can be divided into a number of genotypes due to its genetic variability. The conserved key epitopes form flexible conformation. Vaccines using the wild-type and full-length antigen cannot stimulate an ideal immune effect. Future research will still focus on improving the immunogenicity and broadening the spectrum of vaccines. Analysis and optimization of the epitope structure corresponding to some extremely effective neutralizing antibodies has significant guidance value for the correct conformational expression of vaccines. Therefore, the rational design, with structure as the purpose and function as the starting point, is the most effective way to obtain broad-spectrum vaccines. In addition, the vaccine cocktail model can stimulate stronger and broader spectrum T-cell responses and has higher immunogenicity and a broader spectrum than vaccines from a single source.

Compared with other mature preparation technologies of viral vaccines, improving the preparation level of an HCV vaccine is also an important research direction in the future. By summarizing the preparation methods and levels using different expression hosts, it is indicated that deleting adverse protein domains, sampling the sugar chains, or constructing multivalent vaccines with proteins from other viruses, can effectively neutralize the insoluble characteristics of HCV self-proteins. Moreover, with the in-depth study of rational design, a new soluble protein structure may be obtained.

In conclusion, all these successful advances indicate that the high-level preparation of new vaccines with high immunogenicity and a broad spectrum is possible. Rational design will become the main focus in the future. At the same time, the present review indicates an urgent need for in-depth research on the structure and function of HCV proteins. A new vaccine will be expected to completely eliminate new cases of HCV infection.

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Availability of data and materials

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Authors' contributions

QZ conceived the idea of this study and was a major contributor in writing the manuscript. KH, XHZ and MX assisted in writing the manuscript. XPZ and HL reviewed the literature. All the authors have read and approved the final manuscript. Data authentication is not applicable.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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