REVIEW

Status of hepatitis C virus vaccination: Recent update

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Abstract

Hepatitis C virus (HCV) infection is still a major public health problem worldwide since its first identification in 1989. At the start, HCV infection was post-transfusion viral infection, particularly in developing countries. Recently, due to iv drug abuse, HCV infection became number one health problem in well-developed countries as well. Following acute HCV infection, the innate immune response is triggered in the form of activated coordinated interaction of NK cells, dendritic cells and interferon α. The acquired immune response is then developed in the form of the antibody-mediated immune response (ABIR) and the cell-mediated immune response (CMIR). Both are responsible for clearance of HCV infection in about 15% of infected patients. However, HCV has several mechanisms to evade these antivirus immune reactions. The current review gives an overview of HCV structure, immune response and viral evasion mechanisms. It also evaluates the available preventive and therapeutic vaccines that induce innate, ABIR, CMIR. Moreover, this review highlights the progress in recent HCV vaccination studies either in preclinical or clinical phases. The unsatisfactory identification of HCV infection by the current screening system and the limitations of currently available treatments, including the ineligibility of some chronic HCV patients to such antiviral agents, mandate the development of an effective HCV vaccine.

Key words: Hepatitis C; Viral envelope glycoproteins; Immune response; Hepatitis C virus vaccine; Clinical trials

Core tip: Hepatitis C virus (HCV) is a major global public health problem. Chronic HCV infection affected around 130-150 million of the world's population. The current treatment is expensive, showed some restrictions and is not efficient for all patients. No licensed vaccine is available for clinical use, however, researches on developing a preventive and therapeutic safe vaccine, are evolving. This review gives an overview of HCV structure, immune response and viral evasion mechanisms. It also evaluates the available preventive and therapeutic vaccines that induce innate, antibody-mediated immune response, cell-mediated immune response and interferon, and highlights the progress in recent HCV vaccination studies either in preclinical or

clinical phases.

INTRODUCTION

Hepatitis C virus (HCV) is an enveloped icosahedral virus of 55-65 nm size. The virus is distantly related genetically to both the pestiviruses and flaviviruses. Within the family Flaviviridae, HCV is the only known member of genus Hepacivirus that affects man as the primary host[1]. HCV is enveloped and has a positive sense, single-stranded RNA (+ ssRNA) genome[2]. The genome is of 9.600 kb length that is divided into four regions. It is flanked at its 5' and 3' ends by two highly conserved untranslated regions involved in the translation and replication process of the virus[1]. HCV RNA encodes a unique polyprotein of about 3000 amino acids. The polyprotein encodes at least 10 proteins that are cleaved by host and viral proteases to produce three structural proteins: core protein (core) and two envelope glycoproteins (E1 and E2). E1 and E2 were found to be responsible for fusion and virus entry by receptor-mediated endocytosis into hepatocytes and are the primary targets of the humoral immune response[3,4]. The seven nonstructural (NS) proteins include p7 ion channel, NS2-3protease, NS3 serine protease and RNA helicase, NS4A polypeptide, NS4BandNS5A proteins and NS5B RNA-dependent RNA polymerase[5-7].

HCV replication is highly dynamic with viral half life of 2-3 h[8] with production and clearance of 10^{12} virions particles per day[9]. Like RNA viruses, there is lack of proof reading function of the NS5B viral polymerase (used by HCV during replication) which contributes to the high genetic variability[10] that generates an estimated 10^{-5}-10^{-4} errors/nucleotide/replication cycle[11,12], which also creates diverse population of viral variants or quasispecies within the infected subject overtime[13]. There are seven major HCV genotypes that differ from each other by 30%-35%, and also over 100 subtypes[10,14]. The hepatitis disease severity and the response to antiviral drugs are related to HCV genotypes. Infection with one genotype does not prevent infection with the other types. Concurrent infection with two strains is possible. The envelope glycoprotein genes display some of the highest levels of genetic heterogenicity within E2 (named the first hypervariable region-HVR1)[13] that show greater variability than E1. HVR1 is highly immunogenic, but

is not essential for viral entry/infection[15]; however, it has been implicated in virus neutralization and persistence[16]. HCV attachment then entry into hepatocytes is a complex multistep process which involves interaction with CD81, scavenger receptor class B type I (SR-B1)[17] that has prominent role in cell-to-cell transmission of the virus[18]. Furthermore, tight junction proteins claudin-1 and occludin[19], low density lipoprotein receptor, EGFR, EphA2[20], fusins, and transferrin receptor 1[21] have also pivotal roles in the lateral transmission of HCV[13].

HCV is mainly transmitted by parenteral routes through blood transfusions, drug abuse, unsafe injections, and healthcare-related procedures[22]. The virus replication in liver cells is not cytolytic, but it causes hepatitis secondary to antiviral immunopathology and inflammation of hepatocytes. Acute HCV infections self-resolve in 15%[23] and progress to chronic infection with persistent viremia in 85% of HCV infected subjects with consequent development of liver fibrosis, cirrhosis, liver cell failure and eventually hepatocellular carcinoma (HCC) in about 30% of patients[24]. Acute HCV infection often goes unrecognized and the infected subject becomes a rich source for HCV transmission *via* high-risk behavior, IV drug abuse, and blood transfusion or organ donations. Chronic HCV infections affect 170 million people globally[10,25]. The World Health Organization (WHO) announced in 2014 that chronic HCV infection affected around 130-150 million of the world's population[26]. Center for disease control and prevention (CDC) has estimated that in United States 18000 new HCV infections occur yearly corresponding to one new case every 30 min[6]. Decompensating chronic HCV infection is considered as the most common indication for liver transplantation, accounting for 40%-50% of liver transplants[27].

Several antiviral drugs were developed for treatment of HCV infection and showed high cure rate especially in combination[22]. Introduction of directly acting antivirals (DAA) overcame the problems of low efficacy and side effects demonstrated for the current standard of care therapy (SOC), *i.e.*, interferon (IFN)-α and ribavirin. Eradication of infection and decreasing the impact of chronic HCV-related diseases at affordable costs are currently an area of great hope.

It is well recognized that a strong and broad cytotoxic T lymphocyte (CTL) response is important for HCV clearance[28]. Currently, there is no available vaccine for the prevention of chronic HCV; however, several vaccines are being developed for prophylaxis and therapy. An effective affordable preventive vaccine should be able to induce strong neutralizing antibodies as well as powerful cellular immune responses to provide the best long-term goal for controlling the HCV infection globally[29]. In addition, therapeutic vaccines may be used with DAAs and thus providing IFN-free

treatment protocols[30].

IMMUNE RESPONSE TO HCV

Since its discovery, HCV has shown a very restricted host range, limited to humans and non-human primates. The most physiological suitable model for studying both the innate and adaptive immune responses following HCV infection is chimpanzee[31], but, its use is restricted and has ethical constrains[2]. Acute HCV infection is mainly asymptomatic, making it difficult to identify and study patient's immune response in the early phase of infection[32]. Both, innate and adaptive immune responses are essential for the control of HCV infection[30]. It is well recognized that the virus itself does not kill hepatocytes. Secondary inflammatory immune responses to the virus antigens expressed on the surface of HCV-infected hepatocytes and to the presence of virus core proteins cause hepatocytes damage.

Following HCV infection, an early antibody-mediated immune response (ABIR) with neutralizing activity acts by binding to circulating HCV particles followed by cascade of events that destroy the virus. Simultaneously, the coordinated interaction of CD4+ and CD8+ cell-mediated immune response (CMIR), natural killer (NK) cells, dendritic cells and INF γ sterilizes the body from HCV and virus-infected hepatocytes. However, HCV shows several mechanisms to evade these antivirus immune reactions. There is interplay between viral strategies directed to delay the onset of antiviral immune responses and host strategies that limit or even eradicate infection[33]. The virus envelope proteins E1 and E2 are the virus tools. The virus - hepatocyte interaction begins at the step of virus attachment to hepatocyte receptors, where E2 acts as the primary mediator for virus attachment. Several molecules then join, including E1 and E2 heterodimer that mediates viral cell entry[13]. The major receptor for viral entry into hepatocytes is CD81 molecule on the hepatocyte surface. Mature dendritic cells have an essential role in presenting viral antigen thus activates T-cells to initiate anti-viral cell-mediated immune response. Basically, NK cells is the first line of host defense against invading viruses by recruiting virus-specific T-cells and inducing circulating antiviral immunity in liver[34]. The relative T-cell populations evolved with consequent release of different cytokines ultimately determine the HCV clearance or persistence in infected patient. Spontaneous HCV clearance is associated with potent, broadly directed, multispecific, and long-lasting anti-HCV T cell responses[35-37]. T cell depletion experiments in chimpanzees confirmed the essential role of cellular immunity in controlling HCV infection[38]. In hepatocytes, both CD4+ and CD8+ T-cell responses have an active role in the outcome of HCV infection, where, CD8+ T-cells limit viral replication by cytolytic and non-cytolytic immune mechanisms that are greatly dependent on CD4+ T-cell function[13].

It was well recognized that this initial vigorous T-cell responses decrease and disappear in patients who progress to chronicity. There is loss of CD4+ T-cell help, a switch to a regulatory T cell profile, ongoing viral replication with antigenemia, viral epitope escape, with chronic circulating antigen stimulation resulting in T-cell exhaustion[39].

Emerging evidence supports the protective role of virus neutralizing antibodies, and the ability of the B cell response to modify the course of infection[4]. B cells and rapid induction of cross-reactive neutralizing antibodies (nAbs) responses play an active role in the spontaneous recovery of HCV infection[19,40-42], where nAbs target epitopes within the HCV envelope glycoproteins E1 and E2, or the E1E2 heterodimer. Studies demonstrated that most of the identified nAbs target regions were found within E2[13], including the "hyper-variable region 1"[4]. It was shown that the HVR1 region (amino acids 384-410)[43] encodes immunodominant epitopes that elicit neutralizing antibodies, which have a role in recovery from acute infection. However, these epitopes tend to be isolate-specific and therefore are of limited utility in vaccine development[4]. Using an infectious retroviral HCV pseudoparticle model system, antibodies targeting the HCV envelope glycoproteins have been shown to neutralize infection *in vitro*[44]. It was recognized that host neutralizing responses in HCV-infected patients target viral entry after HCV binding most likely related to HCV-CD81, and HCV-SR-BI interactions, as well as membrane fusion[45,46]. *In vivo*, transfusion of human monoclonal antibody (HCV1) mapping to E2 amino acids 412-423 protected a naïve chimpanzee from HCV challenge and reduced viral RNA levels in an acutely infected animal[47]. In humans, it was shown that control of HCV infection is associated with more rapid development of a broad nAb response, independent of the infection viral genotype[42]. The majority of these antibodies with broad neutralizing activities recognized conformational epitopes on E2[48] Moreover, it was demonstrated that spontaneous clearance of chronic HCV infection is associated with the appearance of neutralizing antibodies and a reversal of T-cell exhaustion[49]. However, it was previously suggested that neutralizing antibodies identified in chronic HCV patients are not able to control chronic HCV infection, where, the neutralizing antibody response of the host lags behind the fast emerging HCV envelope glycoprotein sequences of the quasispecies population[50].

On the other hand, HCV has several viral strategies directed to delay the onset of immune responses. The virus develops different mechanisms to evade antibody-mediated clearance, mutational escape is the most widely used mechanism of viral evasion[32]; Other mechanisms include: (1) viral decoy epitopes; (2) epitope masking, by shielding of neutralizing epitopes by glycosylation of defined amino acids of envelope glycoproteins[51]; furthermore, (3) lipid shielding,

where HCV RNA circulates in the blood of persistently infected patients in lipoviroparticles (LVPs), which are heterogeneous in density and associated with host lipoproteins and antibodies[52]; as well as (4) induction of antibodies interfering with neutralizing antibodies[53]; (5) the interplay of HCV glycoproteins and SR-BI with human serum factors of which high-density lipoprotein (HDL) is a major component[54]; and (6) the ability of HCV particles to move from one cell to another in a neutralization-resistant fashion[1,13]. *In vitro* and *in vivo* data suggest that HCV can spread by cell-to-cell transmission[55,56], enabling HCV to bypass extracellular fluids, thereby denying nAbs access to viral particles[18]. Initial studies demonstrated that neutralizing antibodies do not inhibit cell-to-cell spread; however, it was shown that antibodies to SR-B1 and to HVR1 do inhibit this route of transmission[18,55]. It has been recently reported that a nanobody targeting the E2-CD81 binding site efficiently inhibits cell-to-cell transmission, suggesting that transmitting virus is not located in a synapse that is inaccessible to antibodies and that the lower molecular weight of the nanobody may promote access to cell-tethered virus[57]. Furthermore, it was demonstrated that viruses lacking HVR1 are more susceptible to neutralization by a panel of human mAbs and patient sera, suggesting that the HVR1 masks the E2-CD81 binding site[16]. HCV also proved to inhibit intracellular IFN signaling pathways, impairs the activation of dendritic cells, CD8^{+} and CD4^{+} T cell responses, elicits a state of T-cell exhaustion and selects escape variants with mutations of CD8^{+} T cell epitopes[30]. Also, it was shown that HCV suppresses early innate immune responses by multiple mechanisms, most notably, evasion of complement mediated lysis, *via* down-regulation of complement factors[58,59], and incorporation of host CD55 and CD59 into virus particles[32,60], by alteration of the downstream effects of IFNα expression or by blocking its production, and by down-regulation of NK activity[61].

HCV VACCINES

Currently, no available vaccines for HCV are licensed or in use; however research in this area is ongoing. The main goal of developing a prophylactic or therapeutic HCV vaccine depends on the development of an efficient cell culture system that can support HCV replication[62] and can lead to production of commercially available vaccine containing all the major HCV antigenic epitopes.

The development of an effective HCV vaccine is challenged by several factors including: (1) the characteristics of the virus itself, since several viral genotypes with sequence dissimilarity of 30%-50% are recognized[63]. Hypervariability of HCV proteins is an important constraint, however multi-epitope type vaccine production is a promising strategy in vaccine development for HCV[5]. Genetic diversity poses threats

for vaccine development from the perspective of target antigens and the potential for escape from vaccine-induced immune responses[7]. Immune escape has been demonstrated directly and indirectly for natural infections in both T cell[64,65] and B cell[50] epitopes, thus a successful vaccine should contain virus epitopes with minimum risk of immune escape; (2) Designing a vaccine targeted at inducing broad T cell responses in spite of the fact that the quality of a successful T cell response is not completely understood[1]; (3) The long term maintenance of long-lived memory CD4^{+} and CD8^{+} T cells responses generated by a successful HCV vaccine[1]. Experimentally, an essential role for memory CD8^{+} T cells in long-term protection from chronic hepatitis C was previously demonstrated in chimpanzees[38]. Multi-epitope vaccines containing conserved epitopes of the virus are considered a promising tool to overcome this obstacle; and (4) Narrow species tropism for HCV, as no widely accepted small animal model is available. Most HCV vaccine trials have been conducted in chimpanzees, being the best model permitting challenge with infectious HCV[13]. Meta-analysis of HCV vaccine efficacy in chimpanzees has shown that an effective vaccine contained part or all of the HCV envelope region inducing nAb responses, or both humoral and cellular immune responses[7].

HCV vaccine should induce a multi-specific and vigorous cellular immune response, including both CD4^{+} and CD8^{+} T cells, and a strong and cross-neutralizing antibody response against the HCV envelope[1,30]. The immunogenicity and potency of multi-epitope DNA- or peptide-based vaccines in HCV infection in mice models has proven the capability of these vaccines to elicit strong cellular immune responses[66].

An ideal HCV vaccine should induce potent antibody and cellular immune responses, recognizing diverse HCV genotypes and inhibit cell-to-cell viral transmission[25]. For an effective preventive vaccine, it should be able to elicit nAbs to block viral access to target cells, and to stimulate the T-cell responses targeting infected cells[67]. Thus it is suggested to include epitopes from HCV structural proteins (core, E1, E2) in their correct three-dimensional conformations, to elicit the production of high titers of broad nAbs, together with HCV-specific T-cell epitopes from HCV nonstructural proteins (NS3, NS4, NS5), to induce strong cellular responses[68]. Over the last few years, numerous HCV vaccine approaches have been assessed in mice and primates, but only few vaccines have progressed to human trials[22]. Several problems have been encountered: restricted humoral and cell-mediated responses, the low delivery of potentially protective viral epitopes, and the low effectiveness of the adjuvants used in the different protocols[69].

TECHNOLOGIES OF HCV VACCINE PRODUCTION

The potential risks associated with using attenuated HCV

viral particles for vaccine production have resulted in the use of HCV genes and proteins formulated in novel vaccine modalities[70]. Neutralizing antibodies to highly conserved conformational and linear epitopes have been identified[4]. E2 crystal structure[3] has provided an essential framework to delineate the molecular interactions with CD81 and broadly neutralizing antibodies, confirming many of the salient points concluded from earlier receptor and antibody epitope mapping experiments[30]. Different strategies have been developed in order to enhance the immunogenicity of multi-epitope DNA and peptide vaccines. Among these strategies: (1) using the proper adjuvant, where combining vectored vaccines with protein antigen as adjuvant represent a valuable vaccine platform for infectious diseases where both T and B cells are crucial for protection[25,71,72]; (2) improvement of delivery, where *in vivo* electroporation (EP), induce an efficient uptake of DNA by cells, significantly enhance T-cell responses, and increase expression of desirable gene[73]; and (3) application of different vaccination regimens as DNA prime-peptide boost immunization regimen[5]. Current approaches for the vaccine against HCV include the use of recombinant E1 and E2 proteins, synthetic peptides, virus-like particles (VLPs), recombinant nonpathogenic live vectors , DNA vaccines, dendritic cells, and prime-boost strategies[69,74].

Recombinant protein vaccines

Innate and adaptive immunity have been shown to be induced by HCV protein(s) expressed in bacteria, yeast or mammalian cells[63]. Simply, the genes encoding HCV viral proteins have been cloned into yeast and the recombinant protein expressed is then purified for use in HCV vaccine production. The advantage of recombinant protein vaccines is that they do not contain the pathogen or its genetic material and they do not require cultivation of the organism[61]. Recombinant HCV E1E2 glycoproteins have been shown to be capable of eliciting cross-neutralizing antibody responses against heterologous HCV genotypes in guinea pigs, rodents, chimpanzees, and healthy human volunteers[75-78]. Also, recombinant HCV E1E2 protein formulated with adjuvant MF59 was shown to induce protective antibody responses in chimpanzees and was immunogenic in humans[25,76].

Cell culture based vaccines

The development of an infectious cell culture system for HCV in 2005[62,79] aided much in HCV vaccine research[63]. The choice of the cell culture is controlled by its permissiveness for HCV replication and its compliance with WHO criteria of cell cultures to be used by manufacturers of human vaccines. The choice of virus strains for this purpose should consider the use of multiple strains with conserved epitopes of part or all of the HCV envelope region that induce both neutralizing Ab and multispecific cellular immune responses implicating both CD4+ and CD8+ cells. A system for cell culture of infectious HCV particles (HCVcc) has recently been established. Retroviral HCV pseudotypes (HCVpp) and recombinant cell culture-derived HCV (HCVcc) have been successfully used to study viral entry and antibody-mediated neutralization[46]. *In vitro* HCV was proven to be controlled by antibody-mediated neutralization targeting viral envelope[20]. Recently, experimental studies in mice demonstrated that immunization with cell-culture-derived HCV elicit HCV neutralizing antibodies and thus providing new insight for HCV vaccine production[80,81].

Synthetic peptides vaccines

These are viral peptides coupled with adjuvants that are able to induce humoral as well as cellular immunity. In mice, potential HCV multi-epitope peptide vaccine was evaluated, where vaccination with VAL-44 synthesized from nonstructural proteins of the HCV NS5A, NS4B and core proteins induced strong cellular immune responses[66]. Hepatitis B virus (HBV) sub-viral particles have been used as carriers for various transmembrane proteins and produced using the same industrial procedures that are established for the HBV vaccine. The N-terminal transmembrane domain (TMD) of HBV S protein can be replaced by the TMDs of HCV envelope proteins E1 and E2, to generate fusion proteins containing the entire HCV E1 or E2 sequence that are efficiently coassembled with the HBV S into particles[82]. Recently, a recombinant E1E2 vaccine (derived from HCV 1a) induced protective humoral immune responses in chimpanzees challenged with homologous or heterologous HCV 1a strains[76].

DNA vaccines

DNA vaccines are naked plasmid DNA molecules that express HCV proteins capable to induce a viral-specific immune response. DNA vaccines are able to induce strong CTL responses. Moreover, their production is feasible, inexpensive, and they are safe in animals and humans[28,83]. Plasmids encoding HCV NS3/4a (ChronVac-c) have been recognized as potential therapeutic vaccines for HCV in mice[84].

Virus vector vaccines

Viral vectors able to express foreign antigens are an effective tool to induce T cell immunity and are promising for the induction of strong humoral responses against infectious diseases. Adenovirus, MVA, alphavirus or paramyxovirus vectors are examples of vectors for developing HCV vaccine[63]. Adenovirus (Ad) is considered to be one of the most potent vectors for eliciting CD8+ T cell and antibody responses in humans[85]. It was well recognized that combining adenovirus vector with protein antigen can induce in experimental animals strong antibody and T cell responses that surpass immune responses achieved by either vaccine alone[25]. Furthermore, the

authors showed that recombinant antigen can induce polyclonal responses that partially limit cell-to-cell HCV transmission[25]. However, adenovirus immunogenicity may be unachievable due to anti-Ad preexisting immunity, since preexisting high-titer anti-vector NAbs may interfere with the immunological potency of such vaccines[86]. Chimpanzee adenoviruses were shown to be safe, highly immunogenic in humans, and insensitive to human adenovirus (HAdV) preexisting immunity[87]. A recent study demonstrated that adenoviral vectors expressing HCV nonstructural proteins induced protective T cell responses in chimpanzees and were immunogenic in healthy volunteers[25]. A study conducted by Garrone *et al*[88] reported a weaker induction of immune responses by a measles virus vector encoding HCV E1 compared to adenovirus vector encoding E1E2[88]. Furthermore, replication-defective, recombinant Sindbis virus vector expressing the gene for HCV glycoproteins E2 and E1 in combination with Freund's incomplete adjuvant was shown to induce effective humoral and cellular responses against HCV in vaccinated mice[71]. On the other hand, virus-like particles (VLPs) are attractive vectors for gene delivery as they mimic the properties of native virions, being safe, and easily manufactured[61].

Dendritic cell-based vaccination strategies

Recently, dendritic cell (DC)-based vaccines against HCV have been developed by a lot of researchers. Infusion of *ex vivo* stimulated DCs loaded with HCV antigens has been reported[64]. Induction of T cell-mediated immune responses by DC vaccination is highly dependent on efficient antigen loading of the DCs[89]. An experimental study was conducted in mice to evaluate the efficacy of immunization with the NS5a-loaded DCs in comparison to plasmid encoding NS5a and NS5a protein. Vaccination with NS5a mRNA-transfected DCs or NS5a protein-pulsed DCs, induced significantly stronger $CD4^+$ and $CD8^+$ T-cell responses and protection from challenge with vaccinia virus expressing NS3/NS4/NS5, in comparison to vaccination with NS5a DNA-transfected DCs, plasmid encoding NS5 or rNS5a protein formulated with alum[89]. Also, a recent experimental study in mice showed that multi-epitope-based HCV vaccine that targets DCs offers an effective approach to inducing a broad immune response and viral clearance in chronic, HCV-infected patients[90]. No active human clinical trials regarding this type of vaccines have been reported[63].

PRECLINICAL STUDIES

Several preclinical vaccines, including recombinant adenoviral vaccines, virus like particles, and synthetic peptide vaccines are being developed[30]. It was shown that HCV carrier rate in chimpanzees that have been vaccinated with E1E2 and challenged with heterologous virus was significantly lower than in unimmunized control ones[1]. Even more, vaccinated chimpanzees produced high titers of nAbs against HCV genotype 1a that can neutralize the *in vitro* infectivity of HCVpp and HCVcc containing E1 and E2 envelope proteins derived from various genotypes[88]. This high-titer anti-E2 and/or anti-E1 antibodies, was also demonstrated in both mouse and macaque[88].

Meta-analysis of the efficiency of HCV vaccines in chimpanzees showed that the inclusion of all or part of the HCV envelope glycoproteins in vaccines leads to significantly more protective immune responses than are obtained with vaccines based on nonstructural proteins[7]. Trials to develop an HCV vaccine capable of inducing both T cell and antibody responses showed that rodents primed with the adenovirus anti-HCV vaccine targeting the E1E2 viral envelope glycoproteins and boosted with the adjuvanted protein were able to develop cross-neutralizing antibodies and potent T cell responses that surpassed immune responses achieved with either vaccine component alone[25].

Passive administration of neutralizing monoclonal antibodies as therapeutic or prophylactic approaches in mouse models have been previously evaluated, where polyclonal anti-HCV Ig[91] and anti-E2 neutralizing monoclonal antibodies[48] were capable of preventing HCV infection. Recently, a study was conducted in BALB/c mice to evaluate cellular and humoral immune responses against multi-epitope DNA and peptide, it was found that immunization of mice with multi-epitope peptide formulated with M720 as an adjuvant developed higher HCV-specific levels of total IgG, IgG1 and IgG2a than those immunized with multi-epitope DNA vaccine[5]. Rhesus macaques were used to study the immunogenicity of DNA plasmids encoding consensus sequences of HCV genotypes 1a and 1b non-structural proteins NS3/4a, NS4b, NS5a, and NS5b. Broad immune responses to multiple HCV nonstructural antigens were detected 2 wk following immunization[28]. Beaumont *et al*[92] described chimeric HBV-HCV subviral envelope particles produced by industrial procedures adapted from those established for the HBV vaccine and showed that pre-existing immunity acquired through HBV vaccination does not influence the immunogenicity of the HCV E2 protein presented by these chimeric particles in an animal model.

CLINICAL STUDIES

Only a few HCV vaccines have progressed to the clinical phase trials, yet the published data on both the efficacy and safety of these vaccines is still scarce, due to many scientific, logistic and bioethics challenges[86]. Recently, several human monoclonal neutralizing antibodies were identified[63]. Vaccines in clinical trials include recombinant proteins, synthetic peptides, virosome based vaccines, tarmogens, modified vaccinia Ankara based vaccines, and DNA based

Table 1 Hepatitis C virus proteins, their function, role in immune response and position in different hepatitis C virus vaccines

HCV proteins	Genetic stability	Function	Role in immune responses	HCV vaccines
Structural proteins Core (C): P22	Stable	Major component of viral nucleocapsid	Target for neutralizing Ab and CMI	Synthetic peptides vaccines; *e.g.*, VAL-44 in mice[66]
		Binds viral RNA during assembly	Block the innate immune response	Recombinant yeast cells (GI-5005) that produce an HCV NS3-Core fusion protein in mice[70]
				DNA vaccine: CIGB-230 in humans[100]
E1: gp 35 envelope glycoproteins	High degree of genetic diversity	Receptor binding and HCV entry into target cells	Target for nAbs	The primary immunogen for vaccinology[8,78]
E2: gp 70 envelope glycoproteins			Continuous variation allowing viral escape of HCV specific CD8 T cells and nAb and exhaustion of CD8 cell in chronic HCV infection[101]	Recombinant HCV E1E2 glycoproteins in chimpanzees/humans -core/E1/E2 (CIGB-230) in human[13,96,100]
			Target for pan-genotypic drug therapy	Fusion proteins containing the entire HCV E1 or E2 sequence that is efficiently co-assembled with the HBV S into particles[82]
				E1E2MF59 HCV DNA vaccine in human[8]
Non-structural proteins NS1: p7 small polypeptide	Stable	Ion channel localized to plasma membrane	No assigned role	-
NS2: p23		Protease transmembrane protein Function	NS2/3 cleavage is required for persistent viral infection in chimpanzees	Full-length HCV NS2 protein DNA vaccine in mice[102]
HCV Proteins	Genetic stability	Function	Role in immune responses	HCV vaccines
NS3: p70		Bifunctional serine protease and RNA helicase	Act as a critical immune regulator during HCV infection[102]	Recombinant yeast cells (GI-5005) that produce an HCV NS3-Core fusion protein in mice[70]
NS4A: p8	Stable	Protease cofactor	Block the innate immune response[103]	Ad vector vaccine (NS3-NS5B in chimpanzees/humans[25,87]
				DNA vaccine: *e.g.*, Chron Vac-c in mice with multiple epitopes; NS3/4a, NS4b, NS5a, and NS5b in Rhesus macaques[84]
				ChronVac-C- NS3/4A-expressing DNA vaccine in human in human[73]
				DNA plasmid vaccine encoding NS3/4a, NS4b, NS5a, and NS5b in Rhesus macaques[28]
NS4B: p27	Stable	Proteins Components of the viral replicase complex	Block the innate immune response[103]	Synthetic peptides vaccines; *e.g.*, VAL-44[66]
				DNA vaccine with multiple epitopes; NS3/4a, NS4b, NS5a, and NS5b in Rhesus macaques[28]
NS5A:p56/58	Stable	Cofactor for NS5B Regulate response to INF-α treatment	Block the innate immune response[103] Modulate the cellular environment to favor virus replication and persistence[104] Protects cells from TNF-α-mediated apoptotic death[104]	In mice: Synthetic peptides vaccines; *e.g.*, VAL-44[67] -NS5a-loaded DCs[89] in Rhesus macaques: DNA vaccine with multiple epitopes; NS3/4a, NS4b, NS5a, and NS5b[28]
NS5B: p68	Stable	RNA dependent polymerase	No assigned role	DNA vaccine with multiple epitopes; NS3/4a, NS4b, NS5a, and NS5b in Rhesus macaques[28] Ad vector vaccine (NS3-NS5B in chimpanzees/humans)[97]

HCV: Hepatitis C virus.

vaccines[30]. Prophylactic HCV vaccines are approaching phase Ⅲ clinical trial readiness[93].

It has been shown that the synthetic peptide vaccine IC41 [containing 7 relevant HCV T-cell epitopes and the T helper cell (Th) 1/Tc1 adjuvant poly-L-arginine] can induce HCV-specific IFNγ secreting CD4$^+$ and CD8$^+$ T cells in healthy volunteers and in subset of difficult to treat chronic HCV infected patients[94]. Moreover, it enhances sustained virologic response rates obtained with SOC treatment of chronic HCV infected patients[95]. Also, recombinant E1E2 vaccine (derived from HCV 1a) was approved for phase Ⅰ clinical trial in human beings[13]. It was found to induce antibody and cellular immune responses in healthy volunteers[96] as well as in combination with new antiviral treatment for chronic hepatitis C patients[8].

Phase Ⅰ human clinical trials in healthy volunteers also include usage of Ad vectors based on human adeno virus rare serotype 6 (HADV6) and chimpanzee Ad 3 (ChAd3) expressing the HCV nonstructural proteins, where it was found to induce potent, durable,

and protective T cell responses in chimpanzees. T cell responses targeted multiple proteins and were capable of recognizing heterologous strains. HCV-specific T cells consisted of both CD4^{+} and CD8^{+} T cell subsets; secreted interleukin-2, IFN-γ, and tumor necrosis factor-α could be sustained for at least a year after boosting with the heterologous adenoviral vector[97]. Also, vaccination of healthy volunteers with HCV-1 E1E2 structural glycoproteins from a single strain of HCV elicited broad cross-neutralizing serum antibodies against all known major genotypes of HCV[77,98].

For better management of chronic HCV infection, combination approach of vaccination or immune modulation together with antiviral therapy is currently evaluated by a lot of researchers using structural or nonstructural HCV proteins. Cohort and vaccine-based preclinical studies have indicated the importance of T cell-based immunity in controlling viral infection[99]. Therapeutic vaccines like viral-vector-based vaccine TG4040 proved to induce HCV-specific cellular immune responses, and reduced viral load in chronic HCV infected patients in phase I clinical trial[99]. Therapeutic vaccination in combination with SOC treatment was recently evaluated in Swedish study. ChronVac-C [plasmid DNA encoding NS3/NS4A under the control of cytomegalovirus (CMV) immediate-early promoter] was analyzed in a clinical Phase I/IIa trial for treatment of naive HCV genotype 1 patients. Patients had received four monthly vaccinations in the deltoid muscles with 167, 500, or 1500 μg codon-optimized HCV NS3/4A-expressing DNA vaccine. The vaccine was delivered by *in vivo* electroporation (EP). Treatment was proved to be safe and well tolerated and vaccinations significantly improved IFN-γ-producing responses to HCV NS3[73]. CIGB-230 DNA vaccine containing core/E1/E2 is one of the DNA vaccines that reached the clinical evaluation. Its immunogenicity as a therapeutic vaccine candidate has been evaluated in non-responders to treatment with IFN plus ribavirin in phase one clinical trial. The vaccine was proved to enhance the immune response in such patients[100]. Also, E1E2MF59 HCV DNA therapeutic vaccine was analyzed in phase 1b study in 100 μg/0.5 mL IM in eight doses at 4-wk interval in chronic HCV infected patients who were under SOC therapy. The vaccine was proved to be safe and a SVR was achieved in the form of E1E2 neutralizing antibodies and specific CD4^{+} cell proliferation[8]. ChronVac-C, TG4040 (MVA-based), and GI-5005 (whole yeast-based) are the most promising HCV vaccines to be approved in combination with SOC therapy in the near future to inhibit or treat the chronic HCV infection[70]. Table 1 summarizes the structure, function, role in immune response and position of all HCV proteins in available HCV vaccines.

CONCLUSION

HCV acute infection resolves spontaneously in 15% of infected patients. In the majority of patients, it progresses to immune mediated hepatocyte damage, resulting in fibrosis, chronic liver disease, cirrhosis, or hepatocellular carcinoma. The virus develops different strategies to evade the immune responses and the host has different immune responses to stop the acute infection or to tolerate chronic infection to be overtaken by virus induced hepatocyte damage. Vaccination is the best policy in combating virus infections. Development of prophylactic or therapeutic vaccine for HCV is of great medical priority. Several HCV vaccine approaches were developed including recombinant protein, synthetic peptide, plasmid DNA, vector-based vaccines and dendritic cell-based vaccination. Phase I /II human clinical trials, for most of them showed promising and encouraging data on the different types of these vaccines are continuously evolving. Different types of HCV vaccines are currently evaluated and proposals to improve each regarding the design, the selection of immunogens, safety, tolerability and the proper administration regimens are in progress.

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