Review

EASL EUROPEAN ASSOCIATION FOR THE STUDY OF THE LIVER | JOURNAL OF HEPATOLOGY

Progress in the development of preventive and therapeutic vaccines for hepatitis C virus

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Hepatitis C virus (HCV) is a blood borne disease estimated to chronically infect 3% of the worlds' population causing significant morbidity and mortality. Current medical therapy is curative in approximately 50% of patients. While recent treatment advances of genotype 1 infection using directly acting antiviral agents (DAAs) are encouraging, there is still a need to develop vaccine strategies capable of preventing infection. Moreover, vaccines may also be used in future in combination with DAAs enabling interferon-free treatment regimens.

Viral and host specific factors contribute to viral evasion and present important impediments to vaccine development. Both, innate and adaptive immune responses are of major importance for the control of HCV infection. However, HCV has evolved ways of evading the host's immune response in order to establish persistent infection. For example, HCV inhibits intracellular interferon signalling pathways, impairs the activation of dendritic cells, $CD8^+$ and $CD4^+$ T cell responses, induces a state of T-cell exhaustion and selects escape variants with mutations $CD8^+$ T cell epitopes. An effective vaccine will need to produce strong and broadly cross-reactive $CD4^+$, $CD8^+$ T cell and neutralising antibody (NAb) responses to be successful in preventing or clearing HCV.

Vaccines in clinical trials now include recombinant proteins, synthetic peptides, virosome based vaccines, tarmogens, modified vaccinia Ankara based vaccines, and DNA based vaccines. Several preclinical vaccine strategies are also under development and include recombinant adenoviral vaccines, virus like particles, and synthetic peptide vaccines. This paper will review the vaccines strategies employed, their success to date and future directions of vaccine design.

Keywords: Hepatitis C; Vaccines; Therapeutic vaccines; Preventative vaccines; Antivirals; Cellular immunity; Neutralising antibody.

Received 31 May 2010; received in revised form 27 August 2010; accepted 7 September 2010

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Introduction

HCV infects 3% of the world's population (~130 million individuals) and causes an estimated 476,000 deaths per year as a result of HCV-associated end-stage liver disease and its complications [1,26,131]. Significant advances have been made in the treatment of both acute [65,153] and chronic hepatitis C infection [46,55,92,96,158]. With the recent development of directly acting antiviral agents (DAAs) for HCV, significant improvements in sustained virological response rates are now possible for patients infected with HCV genotype 1 [94,141]. However, even during the next many years treatment will still be based on the administration of interferon alpha and ribavirin [98] which is not only expensive but also associated with a substantial number of side effects. Overall, only a small minority of patients with chronic hepatitis C can currently be cured in most real-world settings with interferon-based treatments [40].

An effective preventive vaccine would considerably reduce the number of new infections and thereby reduce the burden on health care systems. However, there are many impediments to the development of a vaccine for HCV including the existence of multiple HCV genotypes, limited availability of animal models and the complex nature of the immunological response to HCV. Clearance of hepatitis C infection requires strong and broadly cross-reactive $CD4^+$, $CD8^+$ T cell [80,130,132] and neutralising antibody (NAb) responses [114].

The development of a multi-specific T cell response during acute HCV infection is associated with the spontaneous clearance of infection [133] and may provide a level of protection against reinfection [51]. It is also apparent that neutralising antibody is protective and associated with the rapid clearance of hepatitis C viraemia [83,114]. Reinfection among individuals who are repeatedly exposed to HCV, such as injecting drug users (IDU), however, raises concerns that the development of long-term protective immunity for HCV may not be possible [147]. However, cohort studies in IDUs are not able to completely assess the number of episodes of viral clearance compared to persistence [52]. We know that spontaneous recovery does occur in the setting of a successful immune response against HCV [80,87,138] and although the correlates of protective immunity are not completely understood the development of an effective vaccine for HCV should be achievable, as supported by vaccination studies performed in chimpanzees [61].

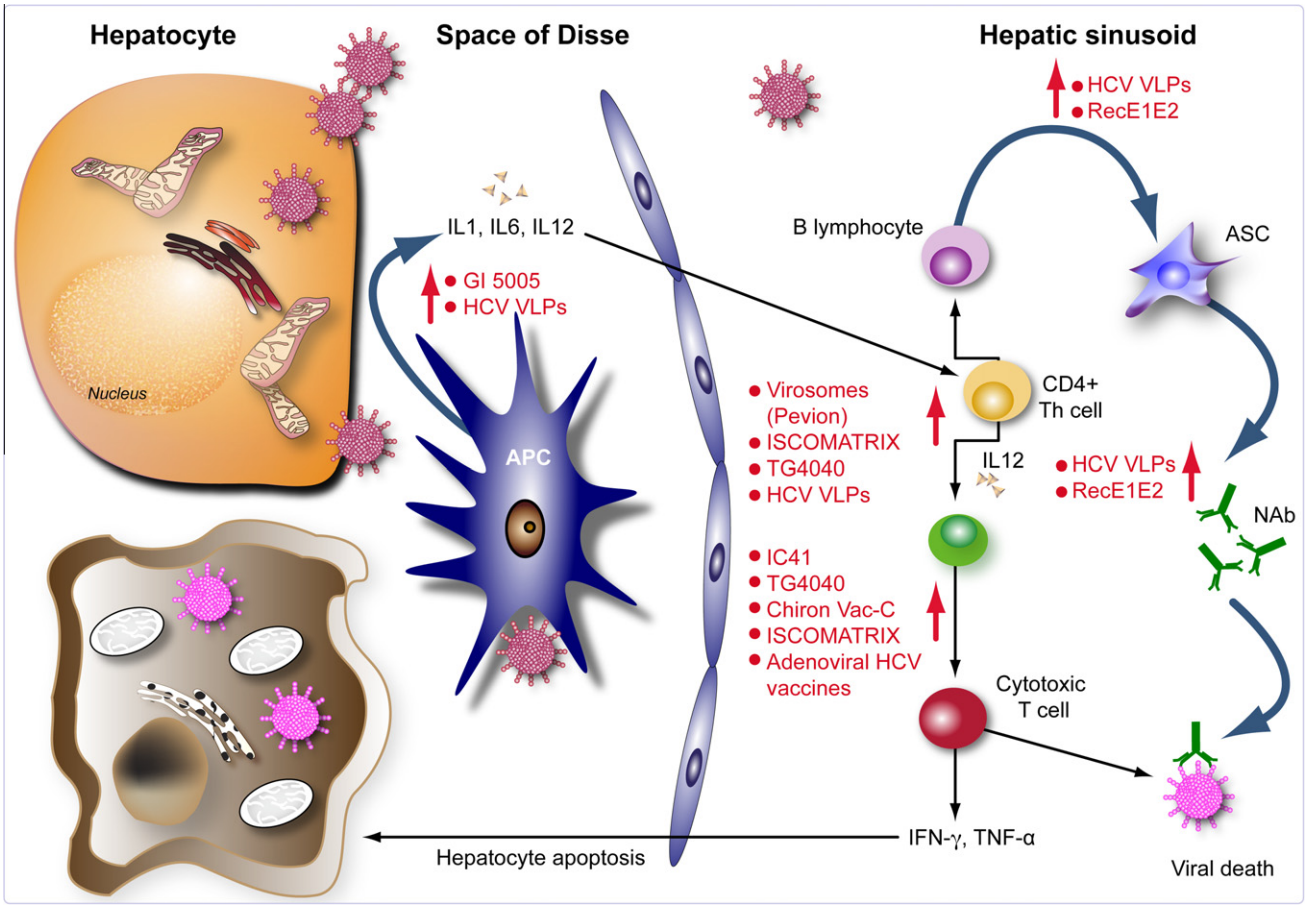

Fig. 1. Summary of the immune responses required to clear HCV and the major sites of action of different HCV preventive and therapeutic vaccines.

Several approaches to HCV vaccine development have now been studied and include recombinant E1 and E2 proteins [22], synthetic peptides [38,72,151], DNA [119], and prime-boost strategies [43] (Fig. 1). Success of these approaches has been limited for a number of reasons including: the delivery of a limited number of protective viral epitopes, the inclusion of incorrectly folded recombinant proteins, the limited humoral and cell mediated responses that are associated with DNA vaccines, and the use of adjuvants with relatively poor potency. It is also now apparent that vaccine inducing strong T cell responses alone may not be sufficient to prevent hepatitis C infection [119]. An effective preventive vaccine against HCV will, therefore, need to induce strong neutralising and cellular immune responses.

Correlates of hepatitis C cell mediated immunity

It is apparent that clearance of hepatitis C infection requires early and multi-specific class 1 restricted $CD8^+$ T cell [28,87,104,132,133,138,142] and class II restricted $CD4^+$ T cell [31,50,77,130] responses to both structural and non-structural HCV proteins (Table 1)(Fig. 1). Clearance of HCV and protection from reinfection is determined not only by the magnitude and/or breadth of multifunctional $CD8^+$ T cells but also by the quality, functional potency, and cytotoxic potential of HCV-specific $CD8^+$ T cells [14,80–82] and the selection of high-avidity $CD8^+$ T cells [110] which respond to a diverse array of HLA-class I restricted HCV-core, E1, NS3, NS4, and NS5 epitopes [14,20,21,80–82,127]. Studies in chimpanzees have also shown that it is possible to induce memory responses that are protective against reinfection [30]. The ability to produce strong HCV specific $CD8^+$ and $CD4^+$ T cell responses are important considerations for effective HCV vaccine design. It may also be important to consider the differences that exist in $CD8^+$ T cell specificities in peripheral compared to intrahepatic $CD8^+$ T cells [45,74,108,154].

The relative contributions of individual viral proteins to the total magnitude of the HCV-specific $CD4^+$ and $CD8^+$ T cell responses also play an important role in determining the outcome of infection. Using various approaches it has been possible to determine a hierarchy of $CD4^+$ and $CD8^+$ T cell responses, particularly to the NS3 protein [80,130,137,152]. It is also possible to elicit strong HCV core specific $CD4^+$ and $CD8^+$ T cell responses in naïve human lymphocytes [89]. These studies argue that the inclusion of $CD8^+$ T cell epitopes representing key viral proteins, like core and NS3, will be essential for the development of a cell mediated vaccine for HCV. However, a recent meta-analysis of the efficacy of HCV vaccines in chimpanzees has shown that the inclusion of structural proteins in vaccines was more significantly associated with protective immune responses compared to vaccines based on non-structural proteins of HCV [30].

Table 1. Summary of the correlates of protective HCV immunity and the mechanisms of viral evasion of immune responses.

Correlates of protective immunity	Mechanisms of viral immune evasion
Neutralising antibodies to linear epitopes E1 and E2 glycoproteins	**Viral factors**
Neutralising antibodies to conformational epitopes in E2 (AR3)	Existence of viral quasispecies
Broad and strong CD8+ T cell responses to NS3>core>NS4>NS5	Impairment of DC maturation
Strong cytotoxic T cell response	Inhibition of interferon-γ response
Strong CD4+ T cell responses	Viral CD4+ escape mutants
	Viral CD8+ escape mutants
	T cell exhaustion (e.g. upregulation of PD1 and other molecules)
	Humoral antibody escape:
	HDL/SR-B1 interactions
	Glycan interference
	Interfering antibody epitopes
	Host factors
	MHC Class I restriction
	Limited TCR repertoire
	IL28B gene polymorphisms

Neutralising antibody (NAb) responses to HCV and neutralising epitope domains

A strong line of evidence now exists demonstrating that NAb responses to epitopes in the viral E1 and E2 glycoproteins can be protective [155] and is associated with resolution of hepatitis C infection [114]. Vaccination of chimpanzees with mammalian cell, but not yeast cell-derived recombinant HCV E1 and E2 glycoproteins has been shown to prevent the development of chronic infection with both homologous and heterologous viruses [22,25,60,73,124]. Similarly, neutralisation of a viral inoculum with either rabbit hyperimmune serum directed to homologous virus [37] or human anti-HCV sera [38] and immunoglobulin [155] protected chimpanzees against viral challenge, thus providing strong evidence that NAb contributes to protection against HCV.

E2 neutralising epitopes

Distinct neutralizing antibody epitopes have been identified in hypervariable region 1 (HVR1) of the E2 protein [37,38,62,88,124] and in the region downstream of HVR1, including the binding sites for the putative HCV receptor, CD81 [8,53,62,68,84,143,144]. Also, epitopes in the amino-terminus of HVR1 may induce neutralising antibody responses that are associated with self-limiting hepatitis C infection [160]. In contrast, antibodies to the carboxy-terminal end of HVR1 may not be neutralising and may represent epitopes that interfere with the optimal development of neutralising antibody response [36].

It is now apparent that epitopes within E2 can be broadly cross neutralising [136], some of which do not overlap with the CD81 binding domain of the E2 protein [53,143]. Also, monoclonal antibodies directed to a conserved conformational region of the E2 protein that is known to contain a major neutralising antigenic region referred to as antigenic region 3 (AR3) [84] of HCV genotype 1a virus are able to cross neutralise HCV of different genotypes. AR3 is located on the viral envelope and is formed by three discontinuous segments situated between amino acids 396–434, 436–447, and 523–540 of the E2 protein (Fig. 2). Importantly, passive immunization of human liver chimeric Alb-uPA/SCID mice with monoclonal antibodies directed to AR3 protect these mice against challenge with human serum derived HCV, further highlighting the importance of effective NAb and the significance of delivering HCV neutralising epitopes in the correct conformation [84]. It should, therefore, be possible to develop an effective vaccine strategy that will include conserved neutralising epitopes outside of HVR1, and a HCV VLP based vaccine has the very real potential to achieve this goal.

E1 neutralising epitopes

Less is known about neutralising immune responses to the E1 glycoprotein of HCV and until recently there have been relatively few reports describing neutralising epitopes contained within E1 [53,101,143]. However, a broadly cross-neutralising epitope has been identified between amino acids 313–327 of a highly conserved region of the E1 protein [101,143] (Fig. 2). Monoclonal antibodies directed to this epitope strongly neutralise HCV/HIV pseudotypic particles bearing the envelope glycoproteins of HCV genotypes 1a, 1b, 4a, 5a, and 6a and less so against 2a and 2b. These monoclonal antibodies also neutralised cell culture derived HCV of genotypes 1a and 2a. In addition, it has also been shown that cross-neutralizing E1-specific antibodies can be produced following vaccination of mice with a retrovirus-based HCV virus like particle vaccine [33,116] highlighting the importance of including E1 in a preventive HCV vaccine.

Viral entry

The entry of HCV into hepatocytes is dependent on a complex series of interactions between the virus and a number of cell surface proteins including the LDL receptor [9,34,57], the tetraspanin CD81 [97,99], SR-B1 [33,57] and the tight junction proteins Claudin 1 and Occludin [115,117]. Antibodies that block

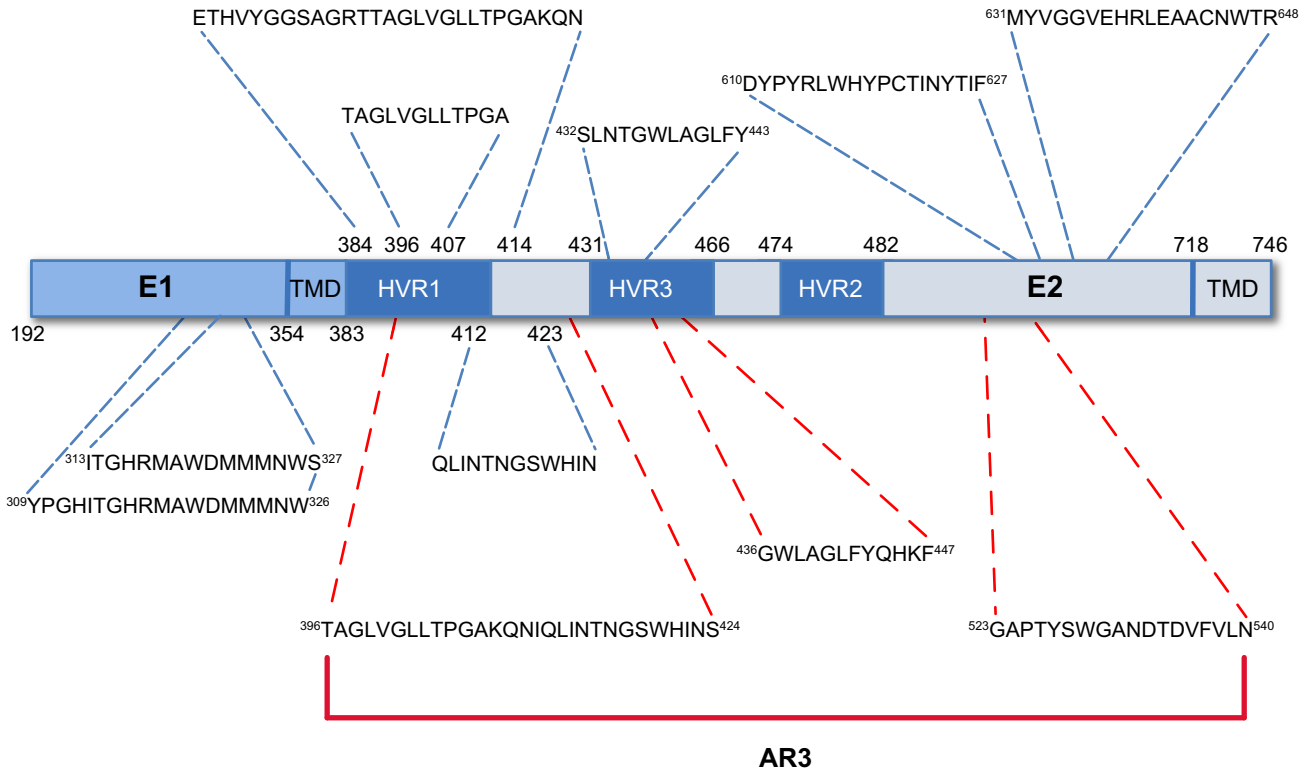

Fig. 2. Summary of neutralizing domains described in both the E1 and E2 proteins. AR3 is formed by three discontinuous epitopes on the viral envelope.

the binding of HCV to CD81, SR-B1, or Claudin 1 reduce the entry of cell culture derived HCV into hepatocytes. However, by combining antibodies specific for Claudin 1, SR-B1, and CD81 almost completely abolished viral entry [75]. These findings demonstrate the complex nature of HCV neutralization and serve to highlight that for a preventive neutralizing strategy to be effective it will need to target several key domains on the viral envelope.

Box 1.

An effective preventative HCV vaccine will need to produce strong multifunctional HCV specific CD4+ and CD8+ T cell responses together with cross reactive neutralizing antibodies that will provide broad protection against the various genotypes and quasispecies of HCV. However, the ability to design a vaccine to incorporate specific potentially protective HCV specific CD8+ T cell epitopes has been hampered by the problem of HLA class I restriction and the natural hierarchy of T cell response that will vary from one individual to another. The induction of cross-protective HCV specific neutralizing antibody responses to epitopes in both the E1 and E2 glycoproteins highlights the importance of including epitopes from both envelope proteins for a vaccine strategy to be effective. The inclusion of neutralizing E2 epitopes into a vaccine also requires the presentation of these epitopes in the correct conformation, as highlighted by the protective role of antibodies directed against the major neutralising antigenic region AR3 on the viral envelope. Few vaccine strategies other than a live attenuated vaccine or HCV VLPs are likely to be able to fulfil all of these criteria.

Evasion of the immune responses by HCV

Hepatitis C virus has evolved several ways of evading the host's immune response in order to establish persistent infection [15–17,47,50,103,113,149] (Table 1). HCV exists as a population of closely related viral quasi-species that are sufficiently distinct immunologically to enable emerging HCV variants to elude the host humoral and cellular immune responses [17]. The virus is also able to modulate the host antiviral cytokine response by inhibiting intracellular antiviral interferon signalling pathways [15,103].

HCV also impairs the activation of dendritic cells (DC) although this has not been a consistent finding [4,5,18,24,66,126]. DC isolated from patients chronically infected with HCV are able to mature normally in response to HCV lipopeptides [23] and DC from chimpanzees chronically infected with HCV are not functionally impaired [79]. In contrast, both $CD8^+$ and $CD4^+$ T cell responses are impaired by HCV. Hepatitis C virus-specific $CD8^+$ T cells from patients with chronic hepatitis C have impaired effector function [149]. In addition, HCV upregulates the expression of PD-1 on peripheral and intrahepatic $CD8^+$ T cells resulting in T-cell exhaustion [106,113,120] while blockade of PD-1 is associated with a functional restoration of $CD8^+$ T cell function in HCV [106]. Differential expression of several other co-stimulatory and co-inhibitory receptors on virus-specific $CD8^+$ T cells may also further influence T cell responses. Examples include CTL-A4 [106] 2B4 (CD244) [129] or CD86 [121]. These may all have important implications for the design of an effective therapeutic HCV vaccine as the primary vaccine recipients will be patients with chronic infection in whom significant abnormalities of T cell function already exist.

Table 2. Summary clinical trials with preventive and therapeutic vaccines for HCV.

Prophylactic vaccine	Immunogenicity	Challenge inoculum	Outcome	Phase I - IV	Registration number*
Chiron Corp HCV E1/E2 Vaccine		Recombinant E1 and E2 proteins	Trial completed data not yet published	Phase I randomized, observer - blinded, placebo - controlled study	NCT00500747
Therapeutic vaccines	**Immunogenicity**	**Challenge inoculum**	**Outcome**	**Phase I - IV**	**Registration number***
Pevion Biotech Ltd		Virosome-formulated peptides (CD4 and CD8 components)	Ongoing- not recruiting,data not published	Phase I	NCT00445419
Intercell AG	Induce HCV-specific T cell responses Weak reduction in viral load	IC41 HCV peptide vaccine with polyarginine	Completed	Phase II	NCT00602784
Intercell AG	HCV-specific T cell responses only one patient experiencing a one-log_{10} reduction in viral load	IC41 HCV peptide vaccine with polyarginine	Trial ongoing not recruiting	Phase II	NCT00601770
Innogenetics / GenImmune	Induce both humoral and cellular immune responses against HCV E1	135aa C-terminally truncated recombinant form of E1 protein, formulated on alum	2 placebo controlled trials completed; program stopped as no effect on fibrosis progression was detected	Phase IIb	
GlobeImmune	Reduction in ALT compared with placebo and reduction in viral load to -1.4 log	GI-5005; an inactivated recombinant *Saccharomyces cerevisiae* expressing a hepatitis C virus NS3-Core fusion protein	ongoing	Phase I	NCT00124215
GlobeImmune	ETR 74% vs 59% for standard of care therapy. SVR 58% vs 48% for standard of care therapy	GI-5005 combined with pegylated Interferon plus Ribavirin	ongoing	Phase II	NCT00606086
Tripep	Safe, immunogenic and transient effects on viral load	DNA based vaccine CHRONVAC-C® in combination with electroporation	Recruiting patients	Phase I/IIa	NCT00563173
Transgene	T cell responses (detected by ELISpot IFN-γ) and viral load reduction up to 1.5 log observed	TG4040 MVA virus carrying and expressing non-structural proteins (NS3, NS4 and NS5B)	Recruiting patients	Phase I	NCT00529321

*Prophylactic and therapeutic trials registered with clinicaltrials.gov.

Further mechanisms to evade the host immune response include the selection of escape variants that carry mutations in key $CD8^+$ T cell epitopes [16,107,145] or through the restriction of T-cell receptor repertoires [102] thereby impairing viral specific CD8 responses.

In addition to escaping cellular immune responses HCV has also evolved several mechanisms to evade humoral responses. These include the association of HCV with high-density lipoprotein [33,148] leading to the interference of binding of neutralising antibody to HCV. The HDL mediated interference of neutralisation

results from a complex interplay with the cell surface protein Scavenger Receptor-B1 (SR-B1) and enhanced viral entry into hepatocytes [10]. The viral envelope is also heavily glycosylated and the presence of these glycans protects the virus against the development and binding of neutralising antibodies [58,91]. Finally, it has also been reported that some sequences in the C-terminal region of HVR1, (amino acids 434–446) may interfere with protective neutralizing antibody responses, providing the virus with another mechanism to evade protective antibody responses [159].

Finally, the ability to clear hepatitis C infection spontaneously and following antiviral treatment may be genetically predetermined, as shown by the high rate of spontaneous clearance of acute hepatitis C infection and the high sustained virological response rates following treatment with pegylated interferon plus ribavirin in association with specific polymorphisms of the *IL28B* gene [48,122,135,139]. The presence of such genetically predetermined antiviral responses may also have a significant impact on the host's ability to develop protective immune responses following vaccination, adding yet another layer of complexity to the design of an effective vaccine for HCV.

HCV vaccines in clinical trials

Preventive HCV vaccines

Recombinant proteins

Recombinant envelope glycoprotein's were amongst the earliest preventive vaccine candidates for HCV but relatively few have entered into clinical trials. One such vaccine candidate includes the envelope gpE1/gpE2 proteins in an oil water adjuvant MF59 together with a CpG oligonucleotide (Chiron Corp) [61,146]. This vaccine has entered into a Phase I randomized, observer-blinded, placebo-controlled study and was registered with clinicaltrials.gov on July 12, 2007. This trial will evaluate the safety, tolerability and immunogenicity of the HCV E1E2/MF59 vaccine in healthy HCV-negative adults. The study has been completed but not reported to date (Table 2).

In another approach a yeast derived recombinant HCV core protein adjuvanted with ISCOMATRIX has been studied in a Phase I placebo controlled, dose escalation clinical study of the safety of the vaccine in 30 human participants. Antibody responses were detected in all but one of the participants. In contrast, $CD8^+$ T cell responses were only detected in two of the thirty participants and T cell cytokines were detected in 7 of the 8 participants in the highest dose group [32].

Therapeutic HCV vaccines

Approaches that aim to increase HCV specific cell-mediated responses may improve the likelihood of achieving a sustained virological response and therapeutic vaccines may provide a means of achieving this goal. The concepts underlying therapeutic vaccines can be divided into three major strategies; (i) combining a therapeutic vaccine with antiviral therapies to enhancing anti-HCV immunity with the major aim of preventing viral relapse after stopping antiviral therapy, (ii) treating first with a therapeutic vaccine to induce HCV-specific immune responses followed by antiviral treatment in order to maximise early viral suppression and thereby increase sustained virological response rates, (iii) using therapeutic vaccination to produce partial control of HCV infection without inducing HCV clearance. Several therapeutic vaccine strategies have been explored and although these have provided promising results to date they have had limited success in clearing infection. Consequently, studies to investigate their role as an adjunct to pegylated interferon/ribavirin therapy have now commenced.

Synthetic peptide vaccines

IC41 (Intercell)

The IC41 vaccine (Intercell AG, Vienna, Austria) contains five synthetic peptides encoding for four HCV specific HLA-A2 restricted CTL epitopes (core35–44 and 132–140, NS3 1073–1081, NS4 1764–1772) and three highly promiscuous $CD4^+$ T cell epitopes (core23–44, NS3 1248–1261, NS4 1767–1786) that have been adjuvanted with poly-L-arginine to augment Th1/Tc1 (IFN-gamma) responses (Fig. 1). The sequences contained within these epitopes are highly conserved in the most prevalent HCV genotypes 1a, 1b, and 2.

The immunogenicity of IC41 with or without poly-L-arginine was initially investigated in a randomized, placebo controlled trial [41]. Although half of the recipients experienced local injection site reactions the vaccine was well tolerated. A dose dependent proliferative $CD4^+$ and $CD8^+$ T cell response was induced in the majority of vaccine recipients. Furthermore, the inclusion of poly-L-arginine was shown to be important for the production of functional interferon-γ secreting T cells [41].

A subsequent randomized double-blind phase II study was performed in sixty HLA-A2-positive chronic HCV patients who had either relapsed or failed to respond to previous pegylated interferon/ribavirin therapy [71]. The vaccine was well tolerated with the most common adverse events in the vaccine recipients including local reactions and influenza-like illness. Over two thirds of patients developed HCV-specific Th1/Tc1 responses and one third developed sustained T cell responses lasting up to six months after the last vaccination. However, responses were generally weak with viremia persisting in all recipients and only one patient experiencing a one-$\log_{10}$ reduction in viral load (Table 2)[71].

Based on the premise that IC41 is able to induce HCV specific T cell responses, the role of IC41 as an adjunctive add-on immunotherapy to treatment with pegylated interferon/ribavirin has also recently been investigated in a phase II trial [151]. Thirty-five HLA A2 positive patients infected with HCV genotype 1 were given six doses of IC41 from weeks 24 to 48 of pegylated interferon/ribavirin treatment and followed for a further six months. Vaccination did not decrease the virological relapse rate although those who developed sustained virological responses also developed strong HCV-specific responses (Table 2) [151].

An optimized vaccine schedule was subsequently tested in healthy volunteers. Of note, bi-weekly intradermal injections of IC41 induced much stronger T cell responses than the initial monthly subcutaneous vaccination [42]. This improved vaccine schedule was tested in 50 patients with chronic hepatitis C and resulted in a significant decline in viral load after 4 months of biweekly therapeutic vaccination [70]. This study provided proof of concept that therapeutic vaccination aimed at inducing HCV specific T cell responses is able contribute to the control of HCV infection.

Recently, a group of investigators from Japan reported phase I dose-escalation study to assess the safety and immune responses

to the HLA-A2-restricted HCV core peptide YLLPRRGPRL in 25 HCV-positive patients [156]. The vaccine was well tolerated in all subjects and produced HCV core-specific CTL responses in peripheral blood mononuclear cells from 15 of 25 patients. Phase II studies to determine vaccine efficacy are expected to commence in the near future.

Virosome-based HCV vaccines

Pevion Biotech have announced the start of phase I clinical testing of its virosome-based hepatitis C virus (HCV) vaccine in December 2006. The vaccine is based on a combination of the PeviPRO and PeviTER platforms that utilize synthetic HCV peptide antigens. A Phase I single-blinded, randomised, placebo controlled, dose escalating study of one virosome formulated CD4 and two virosomes formulated CD8 HCV vaccine components (PEV2A and PEV2B) administered to healthy adult volunteers have been registered with clinicaltrials.gov in March 2007 (Table 2). However, to date the results of this trial have not yet been published.

Tarmogens: globeImmune GI-5005

Tarmogens are a novel development in immunotherapeutic vaccines that consist of whole heat-killed recombinant *Saccharomyces cerevisiae* yeast that have been genetically modified to express one or more protein targets, including viral proteins. Tarmogens are avidly taken up by dendritic cells and stimulate both innate and specific cellular immune responses [13]. This technology has now been applied to HCV with the development of a vaccine candidate (GI-5005a) that encodes a core-NS3 fusion [56](Fig. 1). This vaccine induces potent HCV NS3 and core specific T cell responses in vaccinated mice [56].

A Phase 1b double-blind, placebo-controlled, dose-escalation therapeutic trial study of GI-5005-01 evaluated the subcutaneous administration of 7 doses of GI-5005 as a monotherapy. There were no dose limiting serious adverse events (SAEs) reported. Patients receiving GI-5005 had viral load reductions of up to 1.4 $\log_{10}$ while subjects in the placebo group had HCV RNA reductions <0.75 $\log_{10}$. Normalisation of the ALT was observed in up to 50% of patients receiving the highest vaccine dose compared to only 11% in the lowest dosing group and 0% in the placebo group. HCV specific $CD8^+$ T cell responses were also observed in GI-5005 treated subjects, but not placebo subjects [54,128].

A Phase 2 trial comparing the virological response rates of pegylated interferon/ribavirin (SOC) with and without G1-5005a in patients infected with HCV genotype 1 has commenced. Patients in the triple therapy arms were up to 12% more likely to achieve an early virological response (EVR) than those receiving SOC alone [85]. .A subsequent Phase 2b study in genotype 1 interferon-naïve patients GI-5005 administered with SOC increased the end of treatment response to 74% from 59% and SVR from 58% from 48% compared with SOC alone [63,95].

Vaccination with HCV E1 protein (innogenetics/genImmune)

In 2003, a pilot trial from Belgium was published suggesting that therapeutic vaccination of chronic hepatitis C patients with a 135aa C-terminally truncated recombinant form of the E1 protein, formulated on alum may slow down fibrosis progression although no changes in HCV RNA levels were seen in that trial [109]. Subsequently, two placebo controlled multicentre trials were initiated in Europe exploring the effects of two different doses of HCV E1 on fibrosis progression. In the first study patients received 20 μg E1 and were biopsied after 15 months. The second trial (T2S-918 study) was a 3-year study in which 122 patients received 4 courses of 6 injections of 50 μg E1. In both trials, humoral and cellular immune responses against E1 were induced, however, the T2S-918 study failed to achieve its primary endpoint of an improvement in fibrosis scores [150]. The programme was stopped in 2007.

Vaccination HCV E1E2/MF59 (Chiron Corp)

The HCV E1E2/MF59 vaccine has undergone further development in a phase Ib therapeutic trial in conjunction with pegylated interferon and ribavirin in patients who have previously not responded to standard therapy. The vaccine was shown to be safe and in those who developed a first phase antiviral effect the addition of the vaccine enhanced the second phase viral clearance [27]. This further highlights the potential utility of protein-based vaccines as adjunctive therapeutic agents.

Modified vaccinia Ankara virus (MVA)-based HCV vaccines: TG4040

TG4040 is a recombinant poly-antigenic T cell vaccine based on a modified vaccinia Ankara virus (MVA) that encodes for the HCV NS3, NS4, and NS5B proteins [44]. The safety and biological activity of TG4040 in 15 treatment-naïve HCV subjects have been evaluated in a phase I open-label, multicentre, dose escalation study (Table 2)[59]. Six of 15 patients received 3 weekly injections of the vaccine while the remaining 9 patients received a 4th injection at 6 months. HCV-specific T cell responses were detected in all patients as early as one week after the first vaccination and were maintained during the 6-month follow-up. Vaccination reduced HCV viral loads by up to 1.5 $\log_{10}$ and the strongest vaccine specific T cell responses were observed in patients who achieved the greatest viral load reductions [59].

DNA based vaccines: ChronVac-C®, Tripep AB, Sweden

A further approach to producing a T cell based vaccine has been the development of DNA vaccines. A T cell vaccine based on a codon optimized HCV non-structural (NS) 3/4A DNA-gene expressed under the control of the cytomegalovirus immediate-early promoter (ChronVac-C®) has recently been developed by Tripep AB (Sweden)(Fig. 1). The vaccine has now entered into a phase I/IIa clinical trial in HCV genotype 1 infected patients viral loads <800,000 IU/ml to assess safety and immunogenicity (Table 2)[125]. No severe adverse reactions were observed in any of the dosing arms. Two patients had viral load reductions of up to 1.2 and 2.4 $\log_{10}$ and the development of HCV-specific T cell responses coincided with the time of the viral load reductions [125]. This trial has provided proof-of-concept for a therapeutic DNA-based vaccine and has paved the way for the further clinical development of ChronVac-C® as an additional therapeutic agent to pegylated interferon and ribavirin for the treatment of HCV.

Box 2.

Therapeutic vaccines provide novel additions to treatment strategies aimed at increasing HCV specific cell-mediated responses to increase the likelihood of achieving a sustained virological responses. This would be particularly useful in the difficult to treat patient populations such as those infected with HCV genotype 1, HCV/HIV coinfection, relapsers, and nonresponders to prior pegylated interferon/ribavirin and those with the unfavourable T/T homozygote or C/T heterozygote IL28B polymorphisms. Synthetic peptides representing $CD8^+$ and $CD4^+$ T cell epitopes of the core and non-structural proteins combined with poly-L-arginine as adjuvant, recombinant E1 and E1/E2 proteins, DNA and modified vaccinia Ankara virus encoding non-structural HCV proteins have provided encouraging results in so far as producing HCV specific T cell responses and reductions in viral load in vaccinated individuals. A particularly encouraging result has recently been reported with a vaccine candidate based on recombinant *S. cerevisiae* encoding a HCV core-NS3 fusion protein (GI-5005-01). This vaccine produces strong HCV specific $CD8^+$ T cell responses following immunization of chronically infected patients. Furthermore, when administrated in combination with pegylated interferon/ribavirin, the SVR was significantly improved compared to patients treated with pegylated interferon/ribavirin alone. Several approaches combining therapeutic vaccines with pegylated interferon/ribavirin are now under evaluation in clinical trials and the future inclusion of the newly developed directly acting antiviral drugs may provide opportunities to further improve the overall treatment outcomes.

In a different approach, Alvarez-Lajonchere and coworkers vaccinated HCV-chronically infected individuals in a Phase I study with a therapeutic vaccine (CIGB-230) containing a combination of a DNA plasmid expressing HCV structural antigens and recombinant HCV core protein. The individuals, all non-responders to previous interferon plus ribavirin treatment, received 6 doses of vaccine by intramuscular injection at 4-week intervals. Neutralizing antibody and HCV core specific T cell responses developed in the majority of patients and although viremia persisted, almost half of the vaccinated individuals developed an improvement in liver histology with a reduction in fibrosis [2]. The CIGB-230 vaccine, therefore, may hold promise of a potential therapeutic vaccine for patients chronically infected with HCV.

Preclinical vaccine strategies

Recombinant adenoviral HCV vaccines

Recombinant adenoviruses expressing structural and non-structural proteins of HCV have proven to be valuable tools to help define the hierarchy of HCV specific $CD8^+$ T cell responses [14,137](Fig. 1). Unlike synthetic peptides, which are able to deliver a limited number of epitopes adenoviral vectors contain complete viral genes, thereby delivering intact HCV proteins and are not limited by HLA class restriction. Adenoviral vectors encoding HCV core and NS3 proteins are able to stimulate protective HCV specific cellular immune responses [3,93] and when co-administered with an IL12 cytokine expression plasmid produces highly efficient cytotoxic $CD8^+$ T cell responses [93]. These features make adenoviral-HCV vectors attractive T-cell based vaccine candidates.

Recently, investigators have used an adenoviral serotype 6 vector to construct a recombinant adenoviral HCV vaccine expressing the viral NS3 to 5B proteins [19,39]. This vaccine induced broad, cross reactive HCV specific $CD8^+$ T cell responses in mice and rhesus macaques that are not suppressed by pre-existing adenovirus 5 immunity. In an extension of these initial studies, Folgori and coworkers investigated a prime boost strategy in five chimpanzees that were immunized with two doses, four weeks apart of a replication-deficient serotype 6 adenovirus encoding HCV NS3-NS5B. At week 25 the chimpanzees were vaccinated with a replication-deficient serotype 24 adenovirus encoding the same HCV antigens. This was followed by three intramuscular doses of a recombinant NS3-NS5B DNA vaccine before challenging the chimpanzees with a heterologous HCV inoculum. Potent HCV specific peripheral and intrahepatic $CD8^+$ T cell responses were induced by vaccination and these were accompanied by the clearance of HCV in 4 of the 5 challenged chimpanzees. Vaccination also resulted in multi-specific T cell responses against several viral proteins, with the strongest responses directed against NS3 [43]. The success of these recombinant adenovirus vaccines provides promise for the development of effective therapeutic vaccines for HCV. The immunogenicity of the vaccine is now being tested further in healthy volunteers [6].

Modified vaccinia Ankara virus (MVA)-based HCV vaccines

Another approach to the development of HCV therapeutic vaccines has been the use of modified vaccinia Ankara (MVA) viruses engineered to encode HCV specific genes. One such vaccine encoding the HCV NS3-4-5B genes has been tested in HLA-class I transgenic mice and shown to produce strong long lasting cross-reactive HCV specific $CD8^+$ and $CD4^+$ T cells. These responses could be readily boosted by an additional dose of vaccine given after 6 months, suggesting that the vaccine is able to induce effective memory T cell responses [44].

In a separate study chimpanzees were vaccinated with DNA plasmids encoding HCV core-E1-E2 and NS3 followed by booster immunizations with recombinant MVA encoding core-E1-E2 and NS3 genes [123]. This DNA prime-MVA boost immunization induced strong Th1- and Th2-cytokine responses together with strong HCV specific $CD8^+$ T cell responses. In addition, all animals achieved high HCV-specific antibody tires after the prime-boost combination, but not with the DNA prime alone. A subsequent challenge with a chimpanzee adapted heterologous virus demonstrated that the DNA prime-MVA boost was associated with the control of HCV viremia in the acute stage but unfortunately did not protect against chronic infection [123]. This highlights the induction of T-cell responses alone may be insufficient for the prevention of chronic HCV persistence.

HCV virus-like particles (VLP's)

A HCV VLP based vaccine would make it possible to deliver important neutralising antibody and core specific T cell epitopes in a single vaccine construct that will most closely resemble

mature virions antigenically (Fig. 1). The development of an HCV VLP-based vaccine strategy is also supported by other examples of VLP based vaccines that have now been successfully licensed for persistent viral infections, such as hepatitis B virus [67,112] and human papilloma virus [49]. Although both vaccines induce cell-mediated immune responses the protective efficacy of these vaccines relies on their ability to induce long lasting neutralising antibody.

Insect cell-derived HCV VLPs have produced encouraging results for HCV vaccine design [11,12,105,134]. However, insect cell-derived E2 antigens are not able to stimulate a protective antibody response in primates [22,124] and methods to improve their immunogenicity will be necessary. HCV VLPs contain potentially neutralising epitopes that are present in the native protein sequence of both the E1 and E2 proteins [53,84,101,143,144]. An important feature of HCV VLPs is that cross protective neutralising epitopes will be present on the surface of the VLPs as part of AR3 [84]. Immunisation with heterodimers of recombinant E1 and E2 proteins has been shown to protect chimpanzees against challenge with homologous virus [22]. However, it is unknown whether heterodimers of recombinant E1 and E2 will successfully reproduce important cross-neutralising regions like AR3. Unlike linear recombinant E1 and E2 proteins and synthetic peptides, HCV VLPs are the most likely vaccine candidate to faithfully reproduce this important antigenic region. Also, in both mice and non-human primates HCV VLPs are superior in immunogenicity compared to DNA or recombinant envelope protein vaccines [12]. A HCV VLP vaccine based on a single genotype has been shown to induce cross-protective neutralisation of HCV [100,105]. It may, therefore, be expected that a vaccine that includes VLPs of genotypes 1a, 1b, and 3a should induce broad cross protective immune responses.

HCV VLPs provide a promising vaccine candidate because they induce humoral and cellular responses against HCV structural proteins [12,105]; bind NAb against HCV [134]; stimulate the maturation of human dendritic cells [7]; elicit protective CTL responses in mice against recombinant vaccinia viruses encoding structural proteins of HCV [7,105]; elicit protective CTL responses against HCV in chimpanzees [35] and have superior immunogenicity compared to recombinant proteins and DNA vaccines [64,86,105]. With so many favourable immunological characteristics further development of a preventive HCV VLP based vaccine appears warranted.

Recombinant proteins

Recombinant envelope glycoproteins had shown early promise as vaccine candidates because of their ability to induce protective neutralising antibody responses in chimpanzees, although this was only directed against the homologous virus [22]. In addition, E1 and E2 glycoproteins expressed in insect, yeast, and bacteria are less effective at inducing protective antibody responses than glycoproteins produced in mammalian cells [124]. Attempts to improve the immunogenicity of a HCV envelope gpE1/gpE2 vaccine have included the addition of an oil water adjuvant MF59 together with a CpG oligonucleotide (Chiron Corp). The vaccine prevented the development of chronic infection in chimpanzees after heterologous viral challenges [61,146].

A recombinant core polyprotein vaccine produced in yeast formulated in ISCOMATRIX adjuvant [118] has recently been tested in rhesus macaques and shown to induce strong long-lived $CD4^+$ and $CD8^+$ T-cell responses [118]. A second vaccine consisting of a recombinant HCV core-NS3-NS4-NS5a-NS5b polyprotein adjuvanted in MF59 has also been shown to induce broad proliferative $CD4^+$ and $CD8^+$ T cell responses against the core and NS proteins in mice [146].

In an attempt to improve the strength and breadth of antibody and cellular immune responses against HCV a recent study investigated a prime-boost vaccination strategy [90]. Mice immunised with recombinant E1E2 glycoproteins adjuvanted with MF59 containing the CpG oligonucleotide 7909 or a recombinant NS3-4-5 polyprotein adjuvanted with ISCOMATRIX developed strong $CD4^+$ T but not $CD8^+$ T-cell responses. In contrast immunization of mice with defective chimeric Venezuelan equine encephalitis and Sindbis (VEE/SIN) viruses encoding HCV gpE1/gpE2 or NS3–NS4–NS5 produced strong $CD8^+$ T-cell but low $CD4^+$ T helper responses. However, by first priming with adjuvanted viral proteins followed by boosting with the chimeric alphavirus-HCV vaccines it was possible to induce strong $CD8^+$ and $CD4^+$ T cell responses against the respective viral proteins. First priming mice with MF59-CpG-adjuvanted E1E2 followed by a VEE/SIN-E1E2 boost induced strong cross-neutralising antibodies [90]. These prime-boost vaccines may hold promise for the development of a preventive HCV vaccine.

Synthetic peptide vaccines

Studies using synthetic peptides have provided important insights into important protective epitopes. Both humoral and cellular immune responses to HCV have been produced with peptide vaccines [38,53,69,78,111,143,144]. An approach aimed at delivering multiple neutralising epitopes involved the polymerisation of single vaccine peptides representing potentially cross-neutralising epitopes into synthetic polymer vaccines that also included a promiscuous T-helper epitope and a lipid moiety as an adjuvant [144]. This self-adjuvanting vaccine induced cross-neutralising antibodies in mice and although promising, the titres of neutralising antibody to individual viral epitopes were relatively low.

Synthetic lipopeptides representing HCV specific MHC class I epitopes have also been shown to induce strong $CD8^+$ T cell response in rodents, although this response is dependent on the presence of covalently linked helper-T cell epitopes [111]. The increased immunogenicity of lipopeptides containing both T helper and $CD8^+$ epitopes may be related to their increased uptake by antigen presenting cells [157]. However, peptide strategies based on delivering a limited number of epitopes are unlikely to be broadly cross protective for a virus like HCV.

Conclusions

HCV has a propensity to cause chronic infection, however, it is possible to develop robust cellular and humoral immune responses capable of resolving infection. An effective vaccine will need to reproduce such broad immune responses in order to ensure that viral clearance will occur. By better understanding the correlates of an effective immune response it will be possible to develop effective preventive and therapeutic vaccine strategies. However, HCV has evolved several mechanisms to evade the host immune response in order to sustain its own persistence. In addition to these well-developed viral escape adaptations, a

vaccine that delivers relatively limited humoral and cellular immune responses could select neutralization escape variants or result in skewing T cell repertoires leading to viral escape, particularly following challenge with heterologous virus [29].

In addition to these concerns to date only a few vaccine candidates have progressed to phase I/II trials. Published data on both the efficacy and safety of these vaccines is limited. However, with several different vaccine approaches in various stages of development it appears inevitable that the most promising of these vaccines candidates will enter into clinical trials. For therapeutic vaccines novel approaches including combination therapy with pegylated interferon and ribavirin [63] or possibly with the newly developed DAAs [76,94,140] may prove to be more effective approaches for the treatment of patients who fail to respond to currently available treatments. With the enormous global burden of disease posed by hepatitis C and the continuing transmission of HCV there is a genuine need for an effective vaccine for this disease. Success in developing effective vaccines for HCV will require a strongly committed effort by both research laboratories and industry partners alike.

Conflict of interest

The authors declared that they do not have anything to disclose regarding funding or conflict of interest with respect to this manuscript.

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