Current Status of a Hepatitis C Vaccine: Encouraging Results but Significant Challenges Ahead

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Persistent hepatitis C virus (HCV) infection affects 170 million people worldwide. Acute HCV infection is often asymptomatic, but many infected individuals develop persistent infections that may lead to development of end-stage liver diseases, including liver cirrhosis and hepatocellular carcinoma. Thus, an HCV vaccine that could significantly lower the chronicity rate would have a major impact on the disease burden. Unfortunately, HCV is a highly mutable virus, and escape mutations can undermine vaccine-induced virus-specific immunity. Also, HCV exists as multiple genotypes, and so genotype-specific vaccines might be required to achieve broad protection. Finally, vaccine development has been hampered by the lack of a small animal model and cell culture systems, but these are currently being established. Despite these obstacles, several vaccine candidates tested in the chimpanzee HCV model have shown some encouraging results.

Introduction

Hepatitis C virus (HCV) is a positive single-stranded RNA virus belonging to the *Flaviviridae* family. HCV exists globally as six different genotypes and more than 50 subtypes. The HCV genome encodes a single polyprotein that is cleaved into structural (core protein and envelope glycoproteins E1 and E2), p7, and nonstructural (NS2-NS5B) proteins. The structure and function of the HCV proteins have been reviewed elsewhere [1]. HCV is parentally transmitted through blood but can also be transmitted sexually and perinatally from mother to child. Post-transfusion HCV infection has been markedly reduced in the Western world due to the routine screening of blood products. In this region, the most frequent source of new infections is syringe-sharing among drug addicts. In developing countries, unsafe medical practices such as nonsterile therapeutic injections, use of unscreened blood products, or other unsafe practices in the community are common reasons for HCV transmission. HCV infection still represents a global health problem, with a prevalence of 3% or 170 million people [2]. Acute HCV infection is often unrecognized, because symptoms are mild or absent, but the majority of infected individuals become chronic carriers. Chronic HCV infection increases the risk of developing end-stage liver diseases (liver cirrhoses and liver cancer) 10 to 30 years postinfection, and HCV has become the most common cause of liver transplantation in adults.

In general, HCV infection is left untreated until the disease reaches a stage where liver enzyme levels are elevated or liver biopsies reveal evidence of fibrosis. Current treatment consists of combination therapy with pegylated interferon alpha and the nucleoside analogue ribavirin but only 40% to 50% of patients who can complete the 6- to 12-month treatment have a sustained virologic response [3,4]. The majority of nonresponders is infected with HCV genotype 1, which is the most prevalent in Western countries. Moreover, treatment is expensive and associated with severe adverse effects together with a long list of contraindications including decompensated liver cirrhosis and psychiatric disorders. Also, only a minority of HCV-infected individuals in developed countries is cured, and the current drugs are too expensive for use in most developing countries. Thus, development of a vaccine to prevent HCV infection or its progression to chronicity is highly desirable. Furthermore, a therapeutic vaccine could have a beneficial effect either alone or combined with current treatment modalities.

Host Immune Responses During Acute and Chronic HCV Infection: A Predictor of How to Make a Vaccine?

Adaptive immune responses in acute and chronic HCV infection have recently been reviewed by Bowen and

Walker [5]. In brief, broad, vigorous, and sustained CD4+ T-cell and CD8+ T-cell responses against epitopes within both structural and nonstructural HCV proteins early in infection have been associated with viral clearance. An efficient CD4+ T-cell activation is responsible for both induction of a strong interferon (IFN)-γ response and induction of CD8+ memory T cells permitting rapid control of HCV. In addition, the importance of CD4+ T cells is underlined by studies of experimentally infected chimpanzees, which have resolved HCV infection, showing that CD4+ T-cell depletion immediately before reinfection results in incomplete HCV resolution, even though HCV-specific CD8+ T cells have been developed during the primary infection. Thus, establishment and maintenance of memory CD4+ T cells appears to be important for the maintenance of memory CD8+ T cells.

Neutralizing antibodies seem to play a minor role in determining the initial outcome of an acute HCV infection. Experimentally infected chimpanzees and most patients who manage to resolve their HCV infection have undetectable levels of neutralizing antibodies, whereas neutralizing antibody titers are relatively high in chronic infections [6,7].

Thus, studies of immune responses in natural HCV clearance have taught us that a successful HCV vaccine most likely should elicit strong, early, and broad CD4+ and CD8+ T-cell responses. In addition, even though neutralizing antibodies seem to play a minor role in natural protection, it has been demonstrated that they have the potential to prevent HCV infection [8,9]. Therefore, induction of neutralizing antibodies might still play a critical role in development of an effective vaccine.

HCV Vaccine Design Challenges: Similar to those Faced in HIV Vaccine Development?

Parallels can be drawn between HCV and HIV. Both viruses establish persistent infections; they have developed mechanisms to evade antiviral defenses [10,11] and have high mutational rates resulting in the emergence of many circulating virus variants with ample possibility of viral escape. Therefore, the HCV and HIV vaccine research fields are faced with similar challenges. The possibility for immune escape could influence antibody-induced virus neutralization. Several neutralizing epitopes have been identified within the HCV envelope proteins [12,13] with at least one epitope situated in the hypervariable region 1 (HVR1) of E2 [14]. Increased diversity in HVR1 correlates with disease progression, and decreased diversity correlates with resolving disease [15] supporting an immune escape mechanism for viral persistence. HIV research shows that the virus envelope glycoproteins conceal conserved receptor and coreceptor binding sites in crypts that are masked by hypervariable loops and by glycosylation (shielding), making it hard to generate antibodies against the more conserved regions and thereby cross-reactive antibodies [11]. Whether this is a mechanism employed by HCV has not been clarified.

Similarly, viral evolution over the course of infection might facilitate HCV persistence through mutation of key epitopes targeted by T lymphocytes [16], and a strong association between viral persistence and the development of T-cell escape mutations has been demonstrated in the HCV chimpanzee model [17]. Also, prospective studies on acutely infected patients have revealed that cytotoxic T lymphocyte (CTL)-mediated immune responses were followed by development of HCV escape mutations in those individuals who eventually developed persistent infections [18,19]. Actually, CTL-mediated immunologic pressure also seems to affect viral evolution, since the relative number of amino acid changes are higher in HLA-I class epitopes [20]. It is, however, still not understood why some individuals manage to clear an infection and others do not.

In contrast to HIV, the HCV field does not face the issue of viral integration, and HCV can be cleared from some infected individuals. However, the antiviral defense mechanisms developed by HCV will be an important challenge in relation to development of vaccines that cannot provide sterilizing immunity but rather aim to lower the chronicity rate of acute HCV.

HCV Vaccine Candidates Tested in Chimpanzees: The Only Animal Model that Permits Virus Challenge

Figure 1 shows different vaccine strategies explored for HCV. They include recombinant proteins, DNA vaccines, recombinant viruses, or combinations of these, as well as synthetic peptides and viral-like particles (reviewed in [21•]). We have focused on describing some of the HCV vaccine studies performed in the chimpanzee model and discussing how studies of these candidates have highlighted the challenges in HCV vaccine design. To date, the chimpanzee is the only available animal model that permits HCV challenge after vaccination [22].

The first promising HCV vaccine candidate aimed to prevent infection by inducing neutralizing antibodies [23]. Chimpanzees were immunized with recombinant E1/E2 heterodimers produced in mammalian cells. Both homologous and heterologous HCV challenge studies were performed. A summary of the experimental details and outcome is given in Table 1. After homologous challenge, only two of 12 vaccinated chimpanzees became persistently infected, whereas seven of 10 control chimpanzees became persistently infected. A high titer of "neutralization of binding" was directly correlated with protection in five animals with sterilizing immunity. After heterologous challenge all animals became infected. However, the majority of

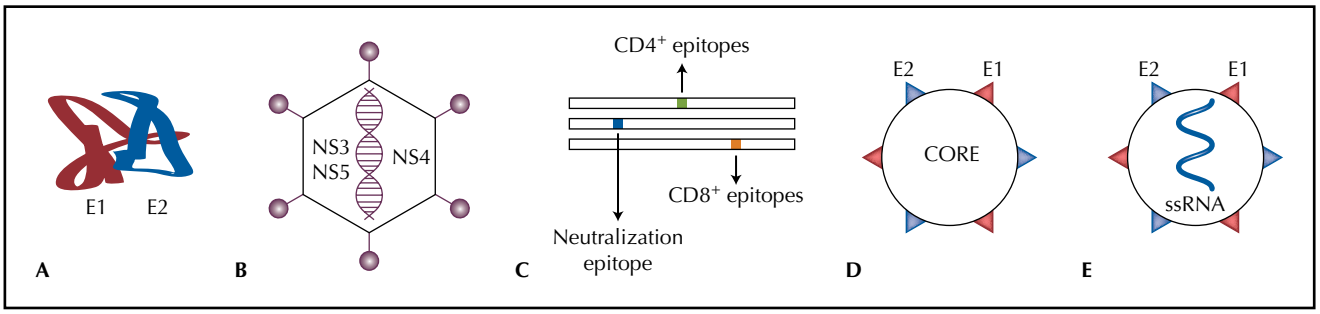

Figure 1. Examples of different hepatitis C virus (HCV) vaccine design strategies. **A**, Recombinant E1/E2 heterodimer protein produced in mammalian cells, currently in phase 1 trial [23]. **B**, A prime-boost regimen with adenoviral vector and plasmid DNA encoding NS3-NS5B antigens, in preclinical development [24••]. **C**, Multiepitope long peptide vaccine consisting of peptides specific for NS3, NS4, and NS5B, in preclinical development [33]. **D**, Virions built of HCV structural proteins, Core, E1, and E2 produced in baculovirus, in preclinical development [30,31]. **E**, Production of HCV in cell culture followed by inactivation. This approach has not yet been developed, but the new HCV cell culture systems could make it feasible.

Table 1. Outcome of experimental challenges of chimpanzees immunized with recombinant gpE1/gpE2 of HCV

Group	*n*	Vaccine components	Vaccination/ boosts (months)	HCV challenge	Infection		
					No	Acute	Chronic
Vaccine	12	Oil/water adjuvanted HCV-1 gpE1/gpE2	0,1, and 7	Homologous HCV-1 (at month 8)	5	7	2
Control	10	Unimmunized		HCV-1	0	10	7
Vaccine	9	Oil/water adjuvanted HCV-1 gpE1/gpE2	0,1, and 7	Heterologous HCV-H (at month 8)	0	9	1
Control	14	Unimmunized		HCV-H	0	14	8

HCV—hepatitis C virus.
Data from Houghton and Abrignani [23].

the immunized animals resolved the acute infection, whereas most control animals progressed to chronicity. Thus, the recombinant E1/E2 vaccine protected against low-dose homologous HCV challenge, and lowered carrier rate after homologous and heterologous challenges. The recombinant E1/E2 vaccine is currently in a phase 1 clinical trial.

Other studies testing envelope-based vaccine candidates have been conducted on smaller groups of chimpanzees (reviewed in [22]). One study investigated the effect of a DNA vaccine encoding truncated E2 protein. After homologous challenge (100 CID_{50}) both chimpanzees developed an acute resolving infection, whereas one control animal became persistently infected. Another research group showed that a vaccine consisting of combinations of plasmid DNA expressing E1/E2 and purified E1/E2 proteins could not protect against challenge with 100 CID_{50} of a homologous virus, but viremia was significantly delayed compared to the control chimpanzee inoculated with the same dose.

The envelope-based vaccines mentioned above have in common that they induced significant anti-envelope antibody levels, which might have lowered chronicity rate after low-dose HCV challenge. Several T-cell epitopes are located in both E1 and E2, and it is therefore possible that cellular immune responses influenced the outcome.

Recently, the use of viral vectors has emerged within the HCV vaccine field. Viral vector vaccines have the potential to effectively introduce immunogenic proteins into mammalian cells and induce both cellular and humoral immune responses to the inserted gene and to the vector, which functions as adjuvant. In the HIV field, this approach is already established with several ongoing phase 1 and 2 trials using adenovirus as viral vector [11].

Folgori et al. [24••] recently published an encouraging study using a prime-boost regimen to deliver a T-cell HCV genetic vaccine encoding parts of the nonstructural region (NS3-NS5B) of an HCV genotype 1b strain. The experimental details and outcome is summarized in Table 2. The final outcome of the vaccinated animals did not differ significantly from the control group, because four of five animals receiving the vaccine and three of five controls cleared the infection. However, both the virologic and clinical courses of infection were markedly different between the groups. The vaccinated animals controlled the virus infection earlier, as evident by lower HCV RNA plasma levels (average about 100 times lower) and lower liver enzyme levels. The most encouraging observation was the development of a potent and cross-reactive T-cell response in the vaccinated group evident by HCV-specific IFN-γ–producing CD8+ T cells, including intrahepatic memory CD8+ T cells in all four

Table 2. Outcome of experimental challenges of chimpanzees immunized with adenovirus encoding NS3-NS5B of HCV

Group	*n*	Vaccine components	Vaccination/boosts (weeks)	HCV challenge (at week 49)	Infection		
					No	Acute	Chronic
Vaccine	5	Adenovirus and DNA plasmid encoding NS3-5B from HCV-1b	Adenovirus 0, 1, and 25; DNA 35, 37, and 39	Heterologous HCV-1a	0	5	1
Control	5	Adenovirus and DNA plasmid encoding HIV-1 gag antigen	Adenovirus 0, 1, and 25; DNA 35, 37, and 39	HCV-1a	0	5	2

HCV—hepatitis C virus.
Data from Folgori et al. [24••].

animals with viral clearance. Possible viral escape was investigated in the vaccinated animal that developed a persistent infection. Sequence analysis of the dominant CD8+ T-cell epitope showed a novel major variant in a sample obtained at week 86. IFN-γ production from peripheral blood mononuclear cells (PBMCs) was evaluated after stimulation with peptides spanning either the wild-type or the mutated sequence. The T cells only reacted to the wild-type peptides and not the new major variant, suggesting viral escape at the CD8+ T-cell level [24••]. Sequence analysis of the control animals that developed a persistent infection was not performed.

Immune escape was also observed in another recent HCV vaccine study [25]. In brief, a prime-boost vaccine strategy was applied using plasmid DNA coding for the HCV proteins NS3, NS5A, and NS5B as priming, and recombinant vaccinia virus expressing the same antigens as boosts in two chimpanzees. Despite an initial induction of a broad multispecific T-cell response, immune escape from CD4+ T cells occurred in one of the two vaccinated animals following homologous challenge.

The new generation of vaccines combining plasmid DNA and viral vector boosts are encouraging for future vaccine development, as they induce broad and strong immunologic responses in the vaccinated chimpanzees. Other viral vectors such as vesicular stomatitis virus (VSV) and Semliki Forest virus (SFV) are available, but in the HCV context, these have only been tested in murine models for immunogenicity [26,27]. Despite the encouraging results seen in the study by Folgori et al. [24••], future vaccine candidates will need to address the frequent immune escape by HCV. Also, in most studies performed in chimpanzees, focus has been on demonstrating that induced immune responses have the potential of influencing HCV infection; therefore, challenge was performed shortly after the last vaccination boost (4–10 weeks). However, it would be interesting to wait even longer (1–2 years) before the animals are challenged to gain knowledge on the durability of the T-cell responses.

The effects of neutralizing antibodies have been described as an interplay between antibodies and T cells, where the antibodies are thought to blunt viral infection, allowing CD4+ and CD8+ T cells to clear infected cells [28]. As E1/E2 protein immunizations elicit antibody responses that might have neutralizing effect in chimpanzees, it may be beneficial to combine T-cell–based vaccines with vaccines targeting neutralizing epitopes. Rollier et al. [29] performed a study in chimpanzees targeting both the cellular and humoral immune response by immunizing with plasmid DNA encoding four HCV genes: Core, NS3, and truncated forms of E1 and E2 from an HCV genotype 1a strain. This was done at week 0, 8, and 16, followed by recombinant protein boost with the same antigens at week 24, 29, 33, and 45. Subsequently, the chimpanzees were challenged with a heterologous HCV genotype 1b strain (week 49). One of two immunized chimpanzees resolved the heterologous HCV challenge infection. Only the animal with resolved infection had a broad cellular response against NS3 skewed towards a T-helper 1 response, whereas both vaccinated animals developed antienvelope antibodies. Neutralization capacity of the antibodies was not determined.

HCV Vaccine Studies in Rodents and Other Nonsusceptible Animals: Does Observed Immune Response Predict Responses in Humans?

Only a few HCV vaccine candidates have been tested in the chimpanzee model. Here, we review some of the more innovative HCV vaccine approaches, which to date, only have been studied in rodents or monkeys as a first evaluation of their capacity to induce humoral and cellular immune responses. Murine models have been used extensively to characterize immunologic responses induced by HCV vaccine candidates. In addition, baboons and rhesus monkeys have been used, as they are, next to the great apes, the closest in evolutionary distance to humans. HCV-like particles (HCV-LPs) are noninfectious and exhibit biophysical, morphologic, and antigenic properties similar to the putative HCV virions. They are synthesized using a recombinant baculovirus that contains the cDNA of the HCV structural proteins (Core, E1, and E2). So far, the HCV-LPs have been tested in mice and baboons and elicit broad, strong, and long-lasting cellular and humoral responses against Core, E1, and E2. Despite the fact that HCV-LPs are exogenous particles, they induce CD8+ T-cell

responses. The authors indicate that this is a result of cross presentation of antigens by dendritic cells [30–32].

Peptide-based vaccines make it possible to direct the immune response against known immunogenic epitopes or epitopes predicted by bioinformatics technologies and to combine different epitopes selected from a wide range of antigens as a strategy to induce multispecific T-cell responses. Fournillier et al. [33] used a multi-epitope long HCV peptide, consisting of epitopes from nonstructural proteins NS3, NS4B, and NS5B as a vaccine containing both HLA-I and HLA-II restricted epitopes. The vaccine was delivered together with a T-helper peptide from hepatitis B virus in incomplete Freud adjuvant. The immunogenicity of the vaccine was evaluated in vivo in HLA-A2 and HLA-A2, HLA-DR transgenic mice and in vitro using PBMCs from HCV-infected individuals and showed induction of both CD4+ and CD8+ T-cell responses. Similar studies have been conducted using peptide-based vaccines targeting other HCV antigens and using different peptide delivery systems. One study used liposomes with peptides specific for Core, E2, and NS4B, and another study used virosomes from influenza bearing Core peptides [34,35]. In both vaccine studies, strong CTL responses were induced. However, it is important to notice that peptide-based vaccines have higher susceptibility to the occurrence of viral escape, as only a defined number of epitopes are targeted.

In attempts to expand the possibilities of HCV vaccine testing in rodents, surrogate HCV challenge models have recently been developed. Surrogate HCV models are produced as HCV-recombinant vaccinia viruses (HCV-rVV). Different versions of HCV-rVV exist expressing either the whole HCV polyprotein [36] or selected HCV genes [32,34,37,38]. These models have been applied to evaluate protection by HCV vaccine candidates such as HCV-LPs [32], liposomal peptides [34], and adenoviral vectors [36]. In these studies, the vaccinated animals had reduced viral load compared to controls upon challenge with the surrogate HCV. Another research group produced a panel of HCV-rVVs expressing NS3 of HCV genotypes 1a, 1b, 2, 3, and 4 with the purpose of analyzing cross-genotype immunity induced by vaccination [38]. Mice were immunized twice with recombinant genotype 1b NS3 protein. Twenty-eight days after the first immunization, the mice were challenged with HCV-rVVs expressing NS3 of the four other HCV genotypes. HCV-rVV titers decreased up to 54-fold after 1b challenge and up to 8.5-fold after 1a challenge. No reduction was observed when challenge was performed with rVV expressing NS3 from genotype 2, 3, or 4. Thus, the cross-genotype protection was limited.

It is important to keep in mind that even though vaccine candidates elicit encouraging immune responses in mice, this does not necessarily indicate similar responses in higher primates. Therefore, these murine models should only be considered as first step in evaluation of vaccine candidates prior to chimpanzee studies.

New Experimental Systems to Accelerate HCV Vaccine Development

HCV pseudo-particle cell culture system

Previously, it has been difficult to correlate the presence of HCV-specific antibodies with neutralizing capacity. However, recent progress has made it possible to detect and quantify the neutralizing capacity of HCV-specific antibodies in vitro. Bartosch et al. [39] developed infectious, genetically tagged HCV pseudo-particles (HCVpp) harboring unmodified E1 and E2 glycoproteins by pseudo-typing murine leukemia virus with HCV E1 and E2 glycoproteins and using GFP as reporter molecule. Hsu et al. [13] developed a similar system by pseudo-typing HIV with HCV envelope glycoproteins and with luciferase as reporter molecule. The HCVpp systems have been employed to define HCV receptor sites on cells, mechanism of virus cell fusion, and identification of neutralizing antibodies [12,13,39–41]. Neutralizing antibodies have been detected predominately in chronic infections in experimentally infected chimpanzees and in humans. The titers increased over time, indicating that the presence of neutralizing antibodies correlates partly with progression to chronicity and not with resolution of infection [6,7]. In contrast, others have reported a correlation between neutralizing antibodies and control of viral replication in HCV patients [42]. The HCVpp system has been expanded to all six HCV genotypes, and has permitted analysis of cross-genotype neutralization of, for example, the monoclonal antibody AP33 [43] and sera from a well-characterized patient infected with HCV genotype 1a [7]. In both studies, a broad neutralizing capacity was found. Induction of antibodies recognizing conserved conformational epitopes might be of significant benefit to future vaccine and therapeutic antibody development.

HCV cell culture system

The identification of HCV cDNA, from strain JFH1 (or J6/JFH1), which replicate and produce infectious virus in cell culture, has finally made it possible to study HCV lifecycle in vitro [44,45]. The new HCV cell culture systems also have the potential to further improve HCV vaccine development, because they can be used to identify antibodies that neutralize the authentic HCV virus. Also, they can be used in testing new antiviral drugs.

In general, viral vaccines currently in use are made from live-attenuated or inactivated viruses. Because HCV induces chronic disease in the majority of infected individuals, the use of live-attenuated HCV as a prophylactic vaccine is not an option due to the risk of developing compensating mutations that could result in re-emergence of a pathogenic virus strain. Inacti-

vated HCV virus applied as a vaccine, on the other hand, might be a feasible approach in the future. It has not been possible to produce HCV virus in vitro, but the recent development of a true HCV cell culture system might make development of an inactivated HCV vaccine approach possible, in particular if the system can be improved to yield higher HCV titers.

Development of a small animal model for HCV infection
The severe combined immunodeficiency urokinase plasminogen activator (SCID-uPA) mouse engrafted with human livers is an exciting small animal model for studies of HCV infectivity [46,47]. In this model high titers of HCV are obtained 2 weeks after inoculation with a relative low dose of HCV. Recently, it was demonstrated that HCV strain J6/JFH1 virus, produced in the HCV cell culture systems, is fully viable in vivo as it could infect and replicate in the SCID-uPA mouse model, as well as in chimpanzees [44,47]. In HCV vaccine development, the SCID-uPA mouse model might be valuable in testing the capacity of vaccine-induced antibodies to inhibit HCV infection in vivo.

Therapeutic HCV Vaccine Approaches: An Uphill Battle

Either alone or in combination with current therapeutic drugs, a therapeutic HCV vaccine might influence the course of chronic HCV disease. Therapeutic HCV vaccine candidates that have been evaluated in human trials are described here. However, it must be recognized that research in therapeutic vaccines against other chronic viral infections (eg, hepatitis B, human papillomavirus, and HIV) has been performed for many years, but no therapeutic vaccine candidate exists to date. Thus, the development of therapeutic vaccine candidates is still an uphill battle.

Belgium-based Innogenetics has developed a vaccine candidate that consists of a truncated form of the E1 protein from an HCV genotype 1b strain, which was previously reported to prevent progression to chronic HCV infection in two chimpanzees after heterologous HCV challenge [48]. A pilot therapeutic vaccination study of 35 chronically infected patients suggested that the E1 protein vaccinations had a stabilizing effect on fibrosis as assessed by the Ishak and Metavir scoring systems. Unfortunately, the vaccine had no effect on HCV RNA levels [49]. Following these studies, a placebo-controlled, double-blind clinical trial was conducted, which showed vigorous humoral and cellular anti-E1 responses, no changes in HCV RNA levels, and only stabilization of the progression of histologic liver disease. Unfortunately, remarkably little liver disease was found in the placebo group; therefore, no significant difference between placebo and treatment was observed [21•].

Another therapeutic vaccine candidate is the peptide-based vaccine IC41. It consists of five peptides harboring several HLA-A2 CTL epitopes together with T-helper cell epitopes of HCV [50]. IC41 has been tested in a phase 2 study of 66 chronic HCV patients who were either non-responders to therapy or had experienced relapse after therapy. The vaccine was distributed six times over a 20-week period using poly-L-arginine as adjuvant and was found capable of inducing IFN-γ production in both CTLs and T-helper cells. Among the 36 patients vaccinated with active IC41, 21 patients had a T-cell response, and six of those showed transient reduction of HCV RNA. Safety and immunologic response to IC41 was also conducted in healthy HLA-A2–positive volunteers [51]. PBMCs from vaccinated individuals were restimulated with peptide, and T-cell proliferation and IFN-γ production were seen. IC41 was found to be safe and well tolerated.

Conclusions

Today, 17 years after the discovery of HCV, no prophylactic vaccine exists. The main obstacle seems to be the ability of HCV to suppress and escape host immune responses, a feature it shares with HIV. A strategy to induce immune responses that would overcome this problem has not yet emerged. Only few HCV vaccine candidates have been tested on larger groups of chimpanzees. However, it is encouraging that the E1/E2 protein-based vaccine candidate developed by Houghton and Abrignani [23] could lower the carrier rate after a heterologous challenge. Also, the T-cell–based vaccine candidate from Folgori et al. [24••] adds to the optimism of developing a HCV vaccine in the future, as this vaccine could induce strong, broad, and long-lasting immune responses that efficiently controlled virus load in the majority of vaccinated chimpanzees, even though no significant difference in chronicity rate was observed between vaccine and control groups. Learning the lesson from the HIV field, a future approach would be to hit the virus hard (eg, try to cripple the mutagenic potential and thus the evasiveness of HCV by targeting multiple regions of the HCV). Thus, a strategy might be to combine effective cellular responses towards nonstructural proteins with development of neutralizing antienvelope antibodies.

Initial testing of humoral and cellular responses induced by a vaccine candidate can be performed in rodents and other nonsusceptible animals. The new HCV cell culture systems will permit detailed analysis of vaccine-induced antibodies for neutralizing capacity in vitro, and the SCID-uPA mouse with engrafted human liver will permit further analysis of such antibodies in vivo. However, the chimpanzee challenge model will continue to be the gold standard and serve as the final challenge test prior to human trials. In addition, we believe that it is important to test prophylactic vaccine

candidates in chimpanzees prior to human trials to rule out the possibility of a vaccine actually increasing the chronicity rate by its influence on the host immune responses. Because persistent HCV infection affects millions of people globally, development of vaccines, specific inhibitor drugs, or therapeutic antibodies should definitely be encouraged in the future. Development of a prophylactic cross-reactive HCV vaccine will be of high priority, as it is the only effective means of limiting the spread of HCV infection on a global scale.

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