Prospects for Prophylactic and Therapeutic Vaccines Against Hepatitis C Virus

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Natural cross-protective immunity is induced after spontaneous clearance of primary hepatitis C virus (HCV) infection. Although this suggests that effective prophylactic vaccines against HCV are possible, there are still several areas that require further study. Current data indicate that, at best, vaccine-induced immunity may not completely prevent HCV infection but rather prevent persistence of the virus. However, this may be an acceptable goal, because chronic persistence of the virus is the main cause of pathogenesis and the development of serious liver conditions. Therapeutic vaccine development is also highly challenging; however, strategies have been pursued in combination with current or new treatments in an effort to reduce the costs and adverse effects associated with antiviral therapy. This review summarizes the current state of HCV vaccines and the challenges faced for future development and clinical trial design.

Hepatitis C virus (HCV) is a major public health problem in the United States and worldwide, with the majority of acute infections progressing to chronicity in the absence of therapy [1]. HCV is associated with 40%–60% of chronic liver disease cases in the United States, and approximately 30% of these patients develop progressive fibrosis and cirrhosis [2], making hepatitis C the major disease leading to liver transplantation [3]. Although current therapies can clear HCV infection, treatment success can be limited by a range of factors, including awareness of infection status, access to care, cost of therapy, patient adherence, relative efficacy of different regimens, adverse effects, viral genotype, and host factors. For these reasons, the development of safe, effective, and affordable vaccines against HCV remains the best long-term hope for bringing the global epidemic under control, which was emphasized in 2011 when the US Department of Health and Human Services issued the Viral Hepatitis Action Plan [4]. An immunological approach to treatment is also sought to replace or enhance treatment. Major benefits in expenses and logistics would be gained if patients could be treated with 2–3 doses of a therapeutic vaccine, as opposed to several months of combination drug therapy.

PROPHYLACTIC VACCINES

In primary HCV infections, viral replication kinetics are characterized by logarithmic increases in RNA during the first 1–2 weeks (Figure 1A). Concomitant with serum transaminase level elevations, CD4+ and CD8+ T-cell responses and increased intrahepatic interferon (IFN) γ expression viral titers rapidly decrease. A subset of infected individuals (~25%) spontaneously clear primary infections, and studies have shown that, after reinfection with HCV, secondary viral kinetics are generally shorter in duration than the primary infection, with reduced viral titers and reduced hepatic inflammation (Figure 1B) [5, 6]. It has been shown that reinfected patients display a significantly increased rate of spontaneous viral clearance (83%, compared with 25% in primary infections) and

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significantly broader T-cell responses [5]. These differences in viral kinetics between primary and secondary infections, combined with increased rates of clearance [5, 6], have provided strong support for the induction of memory immune responses through natural infection and encouraged the development of vaccines.

Clinical trials for HCV prophylactic vaccines can be divided into those designed to induce T-cell responses, targeting non-structural proteins, or those primarily designed to induce neutralizing antibodies, targeting the envelope glycoproteins E1E2 (Table 1). To date, the only efficacy data for prophylactic vaccines have been obtained from the chimpanzee model. These animal studies have shown that every prophylactic vaccine has successfully induced HCV-specific immune responses and has led to modified HCV replication soon after challenge, indicating that the vaccines effectively primed immune responses that could inhibit viral replication [6]. However, despite this initial control, persistent infections have developed in a number of vaccinated animals and have been associated with T-cell immune escape [7, 8] and higher viral mutation rates [9], suggesting that, in cases in which the immune response cannot rapidly clear the virus, there remains an environment for selective pressure. Of interest, the vaccines that have had the greatest success at leading to resolved infections have included all or part of the HCV envelope glycoproteins [6]. These data suggest that neutralizing antibodies play a role in protection but also that this region may contain T-cell epitopes that are important for clearance.

A vaccine using recombinant E1E2 proteins adjuvanted with MF59 has been shown in healthy volunteers to be safe and immunogenic [10] and, more importantly, to induce antibodies that neutralize HCV in vitro [11]. Further preclinical development of this vaccine appears to be focusing on the induction of both neutralizing antibodies and T-cell responses by combining the recombinant E1E2 protein with adenvirus vectors expressing the envelope glycoproteins of HCV [12].

All future vaccine studies should include detailed analyses of functional markers of protection, such as antibody epitopes,

Table 1. Ongoing and Completed Clinical Trials for Prophylactic Hepatitis C Virus Vaccines

<table>
<thead>
<tr>
<th>Sponsor</th>
<th>Biological Components</th>
<th>Current Status</th>
<th>Phase</th>
<th>ID No.a</th>
<th>[Publication]</th>
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<tr>
<td>Okairos</td>
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<td>I–IV</td>
<td>NCT01070407</td>
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<td>NIAID</td>
<td>Adenovirus vectors encoding nonstructural proteins NS3-NS5B</td>
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<td>NIAID</td>
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<td>NCT00500747</td>
<td>[10, 11]</td>
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<tr>
<td>NIAID</td>
<td>Adenovirus and MVA vectors encoding nonstructural proteins NS3-NS5B</td>
<td>Complete</td>
<td>I/II</td>
<td>NCT00500747</td>
<td>[10, 11]</td>
</tr>
<tr>
<td>NIAID</td>
<td>HCV E1E2/MF59</td>
<td>Complete</td>
<td>I/II</td>
<td>NCT00500747</td>
<td>[10, 11]</td>
</tr>
</tbody>
</table>

Abbreviations: HCV, hepatitis C virus; ID, identification; NIAID, National Institute of Allergy and Infectious Diseases.

* For data obtained from the ClinicalTrials.gov registry (http://www.clinicaltrials.gov).

Figure 1. Schematic representation of primary (A) and secondary (B) infections with hepatitis C virus (HCV). Viral RNA titers in the serum are shown in orange, serum alanine aminotransferase (ALT) levels indicative of hepatitis are shown as a black solid line, intrahepatic interferon γ and intrahepatic T-cell infiltration are shown as dotted black lines, and seroconversion to anti-HCV antibodies as assessed by commercial assays is shown in red.
T-cell phenotypes, homing profiles, central and effector memory T-cell phenotypes, T-helper function, and proliferation. It is possible that, in cases in which T-cell vaccines fail to clear the virus, the induced HCV-specific cells are secreting only single cytokines, such as IFN-γ, whereas it is necessary to induce polyfunctional cells that secrete multiple cytokines, as has been reported with human immunodeficiency virus (HIV) vaccines [13].

**THERAPEUTIC VACCINES**

There have been tremendous advances in the development of antiviral therapy to treat chronic HCV infections, resulting in Food and Drug Administration approval in 2011 of the protease inhibitors telaprevir and boceprevir. These and other drugs may ultimately reduce the need for therapeutic vaccines but are currently approved only for the treatment of one genotype and in combination with pegylated interferon and ribavirin, although their use does lead to shorter therapy times and higher sustained response rates. However, the treatment costs are high ($26 000–$49 000 per patient, depending on the duration plus the costs for pegylated interferon and ribavirin [14]). Thus, there still remains the problem of treating chronically infected persons worldwide for whom the use of antiviral drugs is impractical because of cost and logistics. A number of diverse therapeutic vaccine trials have been performed in HCV-infected patients or healthy volunteers in clinical settings (Table 2). Because most chronically infected patients have poor HCV-specific T-cell responses but high levels of neutralizing antibodies, approaches have focused on enhancing the cellular arm of the adaptive immune response. Current therapeutic vaccine trials to date have not succeeded in clearing HCV infections or achieving sustained reductions in viral titers. However, the studies have all successfully stimulated HCV-specific immune responses with transient reductions of viral RNA in subsets of patients [15–17]. As more information is gained about the mechanism of immune dysfunction in persistently infected patients, therapeutic vaccines may be tailored to include specialized immunostimulatory molecules that can restore immune function. These molecules may consist of ligands for Toll-like receptors or vectors that secrete cytokines shown to be poorly expressed by T cells from chronically infected patients.

The enhancement of HCV-specific immune responses in chronically infected patients is encouraging, but further investigation and follow-up of existing clinical trials are still required. The rebound from vaccine-induced decrease of viral titers in patients may be attributable to immune escape or to an inability of the therapy to adequately shut off viral replication or destroy the majority of HCV-infected cells. These are unique challenges for therapeutic vaccines and may be unattainable both from scientific and safety perspectives. However, these strategies may have an important impact on patient outcome if combined with current or newly developed antiviral therapies.

**CLINICAL CHALLENGES TO VACCINE DEVELOPMENT**

**Defining the Expected Outcome of Prophylactic Vaccination**

In general, vaccine efficacy (VE) can be considered to have 3 components [18]. The first component, VEs, is the standard measure of vaccine efficacy in reducing susceptibility to establishment of infection after exposure. The other 2 components apply to vaccinated persons who become infected: VEp, vaccine efficacy in mitigating disease progression, and VEi, vaccine efficacy in reducing infectiousness. These measures of vaccine efficacy, VEp and VEi, have been described within the similar context of HIV vaccine development [19]. Ideally, the goal of prophylactic vaccination is to prevent infection after exposure. This so-called sterilizing immunity seems to be unrealistic for hepatitis C, and in fact, true sterilizing immunity is not achieved by most vaccines that are presently licensed. Efficacy trials for most vaccines use reduction of clinical disease as the end point and not elimination of all evidence of infection. Because the major problem with hepatitis C is persistent infection, which leads to chronic liver disease, eliminating persistent infections would eventually prevent the major disease burden and most transmissions [20].

**Prophylactic Vaccine Clinical Trial Design**

For successful clinical trials, it is necessary to define populations that have a sufficient attack rate, such that efficacy can be established within a reasonable period and with a reasonable number of individuals. Although hepatitis C remains a major global health problem, with the advent of sensitive screening assays, transmission by blood or blood products has been virtually eliminated in the developed countries and in many developing countries. However, injection drug users continue to have high rates of infection, especially new users [21]. In San Francisco, the rate of new cases among seronegative drug users was recently reported to be 26.7 cases per 100 person-years [22]. This rate is certainly sufficient to design clinical trials to measure HCV vaccine efficacy, and there are several centers that have extensive experience with these populations.

However, there are certain ethical and methodological issues that must be dealt with in HCV vaccine clinical trial design. If prophylactic vaccines do not prevent acute infection but decrease duration of carriage and prevent chronic infection, it is not ethical to withhold treatment to determine whether someone will become persistently infected [23].

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Hepatitis C Virus Vaccines

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<table>
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<tr>
<th>Sponsor</th>
<th>Biological Components</th>
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<th>Phase I–IV</th>
<th>ID No. [ Publication]</th>
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<td>Pevion Biotech Ltd</td>
<td>PEV2A PEV2B Virosome-formulated CD4 and CD8 synthetic peptides</td>
<td>Complete</td>
<td>Phase I</td>
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<td>Complete</td>
<td>Phase II</td>
<td>NCT00602784 [16]</td>
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<tr>
<td>Intercell AG</td>
<td>Synthetic peptide vaccine (core, NS3, NS4) with or without poly-L-arginine.</td>
<td>Complete</td>
<td>Phase II</td>
<td>NCT00601770 [unpublished]</td>
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<td>GlobelImmune</td>
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<td>Phase II</td>
<td>NTC00606086 [unpublished]</td>
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<td>ChronTech Pharma AB</td>
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<td>Ongoing</td>
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<tr>
<td>Innogenetics</td>
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<td>Completed</td>
<td>Phase I</td>
<td>[46, 47]</td>
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<td>CIGB-230</td>
<td>Plasmid DNA expressing core, E1, E2 + recombinant core protein</td>
<td>Complete</td>
<td>Phase I</td>
<td>[48]</td>
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<td>Immunitor</td>
<td>V-5 Immunitor Heat-inactivated HCV antigens from HCV-infected donors</td>
<td>Complete</td>
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<td>CSL Limited</td>
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<td>[50]</td>
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However, if treatment is initiated at the time of diagnosis, how can it ever be determined if the vaccine can prevent chronic infections? This is a serious and very difficult problem but one for which there may be solutions. At present, it is recommended to monitor patients with acute hepatitis C for 12 weeks after diagnosis before initiating treatment to determine whether the patient will spontaneously resolve infection [23]. In a clinical trial, the participants would be monitored periodically after the last dose of vaccine typically at 3–6-month intervals. A recent meta-analysis of chimpanzee vaccine studies showed that the median duration of viremia in vaccinated animals that cleared HCV was 77 days, and in naive animals, it was 101 days [6]. These data indicate that, if a vaccine has been successful, there is a high probability that individuals will have cleared HCV within 3 months of exposure and differences would be seen between vaccinee and placebo groups.

Newly developed direct-acting antiviral agents, such as telaprevir and boceprevir, have the potential to greatly increase the success rate of treatment. To date, there have been no published studies on the use of these agents in patients with acute HCV infections. The addition of highly effective direct-acting antiviral agents to the treatment of acute infections may further increase the current success rates, reduce the period of treatment, or widen the window when therapy of acute hepatitis is most effective. Therefore, the availability of these and other new drugs may require a reassessment of clinical trial design for prophylactic HCV vaccines but still do not obviate the need for immunological interventions to combat this disease.

**Increased Risk Behavior**

Another ethical challenge that has been discussed in the context of HIV vaccine development is the potential for individuals to increase their risk behavior after vaccination, believing that they are protected against infection [24]. Although the dominant mode of HCV transmission is through drug injection with contaminated equipment, an analogous increase in HIV risk behavior has been reported among men who have sex with men after the availability of highly active antiretroviral therapy [25]. Thus, vaccine-associated increases in risk behavior could paradoxically contribute to increased HCV transmission in a community.

**Additional Considerations**

As with any new vaccine, safety considerations are of primary importance, and large safety databases will need to be established. For therapeutic vaccines, additional safety issues concern the induction of HCV-specific T cells, which are intended to kill infected cells and which may lead to increased liver injury and worsening hepatitis. To date, therapeutic vaccines tested in the clinic have proved to be safe and do not appear to result in serious adverse events. However, they have not been successful at clearing the virus. The evaluation of prophylactic vaccines intended to induce primarily cellular immunity will need to be tested in a broad population to determine whether the vaccine will be generally effective or, perhaps, will be effective only in individuals who carry certain host genes, such as specific HLA alleles. There is also the question of IL28B polymorphism. This gene encodes IFN-λ3, and single nucleotide polymorphisms in and near IL28B have been associated with spontaneous clearance of HCV and sustained responses to treatment [26]. The impact of this polymorphism or IFN-λ3 on the effectiveness of adaptive immune responses in vaccinees has yet to be investigated.

### SCIENTIFIC CHALLENGES TO HCV VACCINE DEVELOPMENT

**HCV Genetic Diversity**

HCV sequences are continually evolving during infection because of the error-prone NS5B RNA-dependent RNA polymerase [27], and the resulting viral diversity poses problems for vaccine development from the perspective of target antigens and the potential for escape from vaccine-induced immune responses. Immune escape has been shown directly and indirectly for natural infections in both T-cell and B-cell epitopes [28, 29], although this is not the only mechanism for evading HCV-specific T- and B-cell responses [28, 30]. The greatest genetic variability is observed in the E1 and E2 glycoproteins and the NS5A region with higher conservation in
NS3, NSSB, core, and the 5′ UTR [31]. Thus, the envelope region potentially poses problems in the development of cross-protective vaccines designed to induce neutralizing antibodies, whereas T-cell-based vaccines have the attraction of being able to target the more conserved regions of HCV. Still, plasma samples and monoclonal antibodies have been identified that are capable of cross-neutralizing different genotypes [32–35], and rechallenge studies in convalescent chimpanzees have demonstrated protection against divergent viral genotypes [36]. Therefore, despite the challenges presented with HCV diversity, there are promising indications that cross-protective immune responses exist in natural infections and can potentially be induced through vaccination.

Immune Correlates of Viral Clearance
We still do not know which types of immune responses correlate with protection or clearance of HCV during natural infection or the types of immune responses that are present during chronic infections that seem to exert partial control of HCV. Both CD4+ and CD8+ T cells have been shown to play a major role in clearance of HCV during primary and secondary infections, with strong support for the involvement of CD4+ immune responses to HCV NS3 in acute phase clearance [28, 37]. Antibodies to the surface glycoproteins of HCV have been shown to neutralize or control HCV in a number of in vivo experiments [38–40], and in vitro studies have provided strong support for the presence of cross-neutralizing antibodies in patient samples [32]. Although high titers of these antibodies do not appear until the persistent phase, some studies suggest that neutralizing antibodies may be associated with clearance of acute infections [41, 42].

HCV Animal Models
Prophylactic vaccine development has been hampered by the fact that the only animal model for pathogenesis or immune control of viral infection is the chimpanzee [43]. This model has proved to be pivotal in identifying important aspects of HCV infections and immune-mediated clearance, especially the role of T cells in control of viral replication [28]. Primary and secondary viral kinetics and disease progression in this model are very similar to those seen in humans [5, 6], with the establishment of persistent infections despite detectable humoral and cellular immune responses. Development of small animal models has been challenging. Immunocompromised mice have been used for xenotransplantation with human hepatocytes and have been shown to support HCV replication [44]. However, these mouse models are technically difficult to work with and are immunocompromised, making immune response studies possible only if mice bearing both human immune cells and human liver cells are developed.

Recently, an immunocompetent, genetically humanized mouse model expressing the human orthologues of HCV entry factors was developed that can support HCV entry but not yet full viral replication [45]. Further development of this model will facilitate future HCV vaccine studies and advance the field enormously, although care should be taken in extrapolating data to humans, because many immune response studies in mice have not translated well to clinical studies and it is unlikely that even a fully immunocompetent mouse model will completely reproduce the disease and pathogenesis seen in humans.

CONCLUSIONS
The prospects for HCV vaccines are better today than they have ever been. This is the result of advances in vaccine technology, methods to analyze and characterize immune responses, advances in our scientific understanding of natural HCV immunity, HCV replication and kinetics, and the development of new tools to test antibody responses, such as pseudotype particles and cell culture grown virus. However, there is still a great deal of missing information, and the clinical development of hepatitis C vaccines still presents many scientific, logistical, and bioethical challenges.

Notes

Disclaimer. The findings and conclusions in this article have not been formally disseminated by the US Food and Drug Administration or the Centers for Disease Control and Prevention and should not be construed to represent any agency determination or policy.

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References


