Will There Be a Vaccine to Prevent HCV Infection?

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Abstract
Prevention of hepatitis C virus (HCV) infection by vaccination has been a priority since discovery of the virus and the need has not diminished over the past 25 years. Infection rates are increasing in developed countries because of intravenous drug use. Reducing transmission will be difficult without a vaccine to prevent persistence of primary infections, and also secondary infections that may occur after cure of chronic hepatitis C with increasingly effective direct-acting antiviral (DAA) regimens. Vaccine need is also acute in resource poor countries where most new infections occur and DAAs may be unaffordable. Spontaneous resolution of HCV infection confers durable protection, but mechanisms of immunity remain obscure and contested in the context of vaccine design. A vaccine must elicit a CD4+ helper T cell response that does not fail during acute infection. The need for neutralizing antibodies versus cytotoxic CD8+ T cells is unsettled and reflected in the design of two very different vaccines evaluated in humans for safety and immunogenicity. Here we review the status of vaccine development and the scientific and practical challenges that must be met if the burden of liver disease caused by HCV is to be reduced or eliminated.

Keywords
► hepatitis C virus
► vaccine
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► persistent infection

The Need for a Vaccine 25 Years after the Discovery of HCV

The hepatitis C virus (HCV) was identified in 1988 as the major cause of transfusion and community acquired non-A non-B hepatitis.1 The enormous scope of the global public health problem was rapidly defined, and it was determined that approximately three-quarters of all infections persist for life and substantially increase the risk of progressive liver diseases. Twenty-five years later, in 2013, the first all-oral antiviral regimen to replace type I interferon for treatment of some HCV infections was approved in the United States, a historic advance against this virus that has infected over 2% of the world’s population.2 A rich pipeline of direct-acting antiviral (DAA) drugs in phase II and phase III trials promises to yield additional regimens with markedly improved efficacy, safety, and convenience compared with traditional interferon-based therapies.3 There is hope that widespread use of these potent agents will fundamentally alter the HCV epidemic, stemming the surge of HCV-related cirrhosis and hepatocellular carcinoma that is expected to peak in the coming decades,4,5 and preventing new HCV infections by shrinking the pool of infectious persons.6 With development of DAAs active against all HCV genotypes, even global eradication of HCV is a theoretical possibility.5,7

Thus, the question arises, is a vaccine to prevent HCV infection still needed and, if so, who would it protect? Answers to these important questions depend on the feasibility of achieving widespread DAA treatment uptake and the ultimate goals of HCV control efforts. Potential roles for an HCV vaccine range from protection of select populations with identifiable risk of infection to implementation of universal vaccination programs. Two populations that could be reasonably targeted for vaccination include health care workers with...
occupational risk of blood exposure and persons who are cured by DAA therapy but remain at risk of re-exposure to the virus through injection drug use (IDU). Because antiviral cures may not restore or impart protective immunity, an effective vaccine administered after costly DAA-mediated cure could provide relatively inexpensive insurance against a second persistent HCV infection. This approach to vaccination could conceivably tip the balance in favor of treating subjects who carry this risk.

A broad vaccine strategy to disrupt HCV transmission would likely be necessary to realize the more ambitious goal of a major reduction in the global HCV burden. Injection drug use is the primary route of transmission in industrialized nations where DAA therapy will be most widely deployed. Despite years of prevention efforts, IDU associated acute HCV diagnoses are increasing in certain populations such as young adults in the United States. Sobering reports from numerous state health departments and correctional facilities document a doubling of HCV diagnoses among adolescents and young adults over the past decade due to escalating epidemics of IDU and needle sharing. Although modeling studies of urban IDU cohorts suggest that aggressive HCV screening and DAA treatment of active users could substantially reduce HCV prevalence and transmission, an increasing proportion of IDU associated HCV infections occur in rural parts of the United States where fewer public health resources exist for intensive case finding and treatment. DAA regimens will be priced close to the cost of interferon-based regimens that they will replace, likely exceeding $100,000 in the United States. These prices are considered cost effective relative to the expense of HCV complications, but could render widespread uptake of DAA treatment unaffordable even in high-income nations. An effective vaccine provided to uninfected IDUs could over a decade or more substantially reduce incidence of new HCV infections in IDU cohorts. Given the inherent difficulties of identifying active IDU for treatment, comprehensive strategies incorporating both DAA treatment with prophylactic and posttreatment vaccination may have the best chances of substantially reducing the HCV burden in IDU cohorts in the developed world.

It is notable that the HAV and HBV vaccines were initially deployed selectively to prevent infection in those with identifiable risk, but their full impact on global liver disease was not realized until implementation of universal vaccination in regions of the world where the viruses cause greatest harm. Most of the 185 million HCV seropositive individuals live in developing countries where iatrogenic transmissions via contaminated injections and transfusions remain major portals of infection. The silent nature of the HCV pandemic and anticipated costs of DAA therapies present even more formidable obstacles to the “treatment as prevention” approach to HCV infection control in these settings. It is estimated that fewer than one in six are aware of their infection, and massive HCV screening programs will be required to significantly scale-up treatment to the point where new infections would be prevented. This will almost certainly be problematic in settings where the health care infrastructure lacks the capacity to provide safe infection control practices. DAA costs might be sharply discounted for lower-income countries, as for HIV antiretrovirals, if similar public pressure, licensing policies, and private/public funding are applied. Nevertheless, even if pricing were reduced 1,000-fold in low-income nations, costs of large-scale HCV screening and DAA treatment could far exceed those of a program to prevent infection by vaccination. Specific targeting of an HCV vaccine to those at greatest risk in developed countries (IDU) or developing countries (iatrogenic and IDU) will be difficult and so development of a vaccine that is compatible with universal distribution will be important if preventing transmission is the goal.

Here we review what is known about correlates of protective immunity against HCV, current approaches and status of HCV vaccine development, and the scientific challenges that must still be overcome for success.

Is It Possible to Vaccinate against HCV?

In the modern era of vaccine development there has been remarkable progress in preventing infection with hepatotropic viruses. The current hepatitis A (HAV) and B (HBV) vaccines, licensed in the United States in 1986 and 1995, respectively, have substantially reduced the burden of liver disease over the past two decades as universal immunization programs have become more common in many developed and developing countries. A vaccine that prevents hepatitis E virus (HEV) infection has potential to further reduce liver disease if deployed in regions of the world where endemic and epidemic spread is common. The HAV, HBV, and HEV vaccines share three important characteristics. First, they are comprised of recombinant subunit proteins (HBV surface protein and HEV capsid) or whole inactivated virus (HAV) and are straightforward to produce from a technical and cost perspective. Second, these immunogens elicit durable antibody responses that are strongly correlated with apparent protection from infection, defined as sterilizing immunity. Third, the HAV, HBV, and HEV vaccines are monovalent, but effectively prevent infection in all regions of the world regardless of local diversity in viral genotype or serotype.

HCV presents a very different and much more difficult target for vaccination. At least seven HCV genotypes that vary in nucleotide sequence by ~30% have been described globally. Considerable variation is localized to domains of the HCV envelope glycoproteins targeted by neutralizing antibodies. The HCV genome is also highly mutable because of an error prone RNA polymerase that facilitates evasion of host immune responses, perhaps including those generated by vaccination. Most importantly, unlike the other hepatatitis viruses, resolution of acute HCV infection does not necessarily confer sterilizing immunity upon re-exposure to the virus. Early experiments documented virus replication in chimpanzees that were rechallenged after complete control of an earlier HCV infection. In some cases, sequential infections were caused by the identical strain of HCV.
The absence of sterilizing immunity was initially interpreted as worrisome for development of a protective HCV vaccine. With the development of more robust assays to quantify HCV replication, it became apparent that although immune chimpanzees were susceptible to reinfection, the duration and magnitude of viremia was often substantially reduced, even when a different genotype of the virus was used for challenge. Attenuated HCV replication has also been described in humans who successfully controlled an earlier primary infection. Most importantly, secondary infections in chimpanzees and humans are much more likely to resolve than primary infections. In humans, it is estimated that 80% of secondary infections resolve spontaneously compared with 30% of primary infections.

Symptoms of acute primary and secondary hepatitis C are typically mild or even inapparent so vaccination to generate sterilizing immunity is not an imperative. There is no long-term or irreversible damage to the liver when the infection resolves spontaneously. Moreover, HCV replication does not persist. A persistent HCV infection and the uncertain influence of direct-acting antiviral (DAA) therapy on immunity and secondary infection are unknown. Left panel. Approximately 70% of primary HCV infections persist. CD4+ T cell responses fail before the virus is cleared. CD8+ T cells become exhausted or select for escape mutations in class I epitopes. Serocconversion to HCV proteins occurs after several weeks to months of infection. Neutralization of contemporaneous viruses is uncommon. The status of CD4+ T cells, CD8+ T cells and neutralizing antibodies after DAA-mediated cure is unknown. Right panel. The fraction of secondary infections that resolve or persist after DAA cure is unknown (blue shaded area). The rate of resolution will be considerably less than the 80% observed after spontaneous clearance of a first infection, if HCV-specific adaptive immune responses are permanently impaired or fail to generate memory as expected. A second goal of preventive vaccination is to repair any long-lasting damage to HCV-specific humoral and/or cellular immune responses after DAA cure so that the rate of persistence upon reinfection is reduced (blue shaded area). Vaccination after cure will be a critical adjunct to DAA therapy if future research demonstrates that CD4+ T helper cells do not respond to secondary infection and the repertoire of CD8+ T cells against intact, previously escaped, and/or new epitopes unique to the reinfecting virus is restricted.

**Fig. 1** (A) Immunity during and after resolution of primary hepatitis C virus (HCV) infection. Left panel. Approximately 30% of acute primary HCV infections resolve spontaneously. Viremia typically peaks at ~8 to 12 weeks after infection and drops precipitously with expansion of circulating CD4+ and CD8+ T cells. Transaminases (yellow shaded area) usually peak coincident with the onset of HCV-specific T-cell immunity. Importantly, CD4+ and CD8+ T-cell immunity is sustained until well after apparent resolution of infection and long-lived memory populations persist in blood and liver for years to decades. Serocconversion to HCV proteins occurs as acute infection is controlled and neutralization of contemporaneous viruses is common. Anti-HCV antibody titers can decline after resolution of infection. Right panels. Viremia is observed after secondary HCV infection, but ~80% resolve spontaneously and only 20% persist. Secondary infections can resolve within days, viremia and liver transaminases are substantially reduced compared with primary infection, and control of HCV replication is associated with rapid recall of T-cell immunity and possibly neutralizing antibodies. One goal of HCV vaccination is to generate in unexposed humans the long-lived immunity induced by spontaneous control of infection. (B) Persistent HCV infection and the uncertain influence of direct-acting antiviral (DAA) therapy on immunity and secondary infection. Left panel. Approximately 70% of primary HCV infections persist. CD4+ T cell responses fail before the virus is cleared. CD8+ T cells become exhausted or select for escape mutations in class I epitopes. Serocconversion to HCV proteins occurs after several weeks to months of infection. Neutralization of contemporaneous viruses is uncommon. The status of CD4+ T cells, CD8+ T cells and neutralizing antibodies after DAA-mediated cure is unknown. Right panel. The fraction of secondary infections that resolve or persist after DAA cure is unknown (blue shaded area). The rate of resolution will be considerably less than the 80% observed after spontaneous clearance of a first infection, if HCV-specific adaptive immune responses are permanently impaired or fail to generate memory as expected. A second goal of preventive vaccination is to repair any long-lasting damage to HCV-specific humoral and/or cellular immune responses after DAA cure so that the rate of persistence upon reinfection is reduced (blue shaded area). Vaccination after cure will be a critical adjunct to DAA therapy if future research demonstrates that CD4+ T helper cells do not respond to secondary infection and the repertoire of CD8+ T cells against intact, previously escaped, and/or new epitopes unique to the reinfecting virus is restricted.
relapse once the acute infection resolves spontaneously, and only rarely in patients who clear the virus after type I interferon and ribavirin treatment,34,35 so there is limited risk of a long-lived cellular reservoir of virus genomes to reinitiate infection if immunity weakens. Collectively, these observations provide a compelling argument that it is possible to vaccinate against HCV, but with a very different objective when compared with the other major hepatitis viruses. For HCV, the most realistic goal of vaccination is not to induce sterilizing immunity, but instead to skew acute HCV infection toward resolution and away from persistence.

**Defining Protective Immunity against HCV Infection**

A detailed understanding of immune mechanisms that contribute to rapid control of an acute HCV infection would aid development of a vaccine to prevent persistence. However, correlates of protective immunity are still not completely defined after 25 years of study in humans and chimpanzees. There is still considerable uncertainty about the relative contribution of humoral versus cellular immune responses to resolution of primary and secondary HCV infection.

**Humoral Immunity**

A key unresolved question is whether antibodies facilitate resolution of acute infections. Evidence supporting this possibility is mixed. HCV infections do resolve in some humans with primary hypogammaglobulinemia36 and in some chimpanzees that do not develop antibodies against the HCV envelope glycoproteins. It is also notable that passive transfer of hepatitis C immunoglobulin37 or a neutralizing anti-E2 monoclonal antibody38 to chimpanzees immediately after virus challenge delayed the onset of viremia and acute hepatitis, but had no obvious impact on the course of infection. Collectively, these observations argue against an absolute requirement for antibodies in control of an established acute infection.

Humans do generate an antibody response to envelope glycoproteins E1 and E2 regardless of whether the infection ultimately persists or resolves. Early broad neutralizing activity could conceivably reduce the risk of HCV persistence in the context of a broader adaptive immune response. Functional antibody responses in patients with resolved and chronic infections have been compared using HCV pseudoparticles (designated HCVpp) that display HCV envelope glycoproteins on a VSV or retroviral capsid. Early studies using HCVpp bearing envelope glycoproteins of standard HCV laboratory strains found no association between infection outcome and the titer of serum neutralizing antibodies. A more accurate picture of neutralization was obtained when antibodies and envelope glycoproteins used to construct HCVpp were derived from the same patient or relevant donor. In one of the first studies of this design, Lavillette and colleagues measured neutralizing antibody responses against a virus transmitted by hemodialysis.39 Among the 17 patients studied, those with the lowest levels of viremia had the highest serum neutralizing antibody titers against the transmitted (donor) virus. A subsequent study of pregnant women accidentally infected with HCV after treatment with a common lot of contaminated immunoglobulin documented higher rates of virus clearance in those with acute phase antibodies that blocked HCVpp entry into cultured hepatocytes.40 Other studies documented that neutralization is usually weak in primary infections that persist and is associated with emergence of immune escape variants.41,42 Recent case reports have also documented an association between the onset of neutralizing antibody responses and spontaneous control of HCV replication anywhere from a few weeks to more than a year after infection.43 Whether immune humans develop neutralizing antibodies after reinfection with the virus has not been widely studied and so their role in rapid termination of a second infection remains uncertain. In one study, 10 of 12 secondary infections in humans resolved spontaneously.32 A broad neutralizing antibody response was observed in a subset of these cases, including the two that resulted in persistent replication of the virus.32

It is important to emphasize that most studies of serum antibody neutralization were retrospective in nature and so T-cell immunity was not measured contemporaneously in the same subjects. It is somewhat difficult to disentangle the relative contribution of humoral versus cellular immunity to resolution of infection in the few studies where both responses were measured.32,43

**Cellular Immunity**

Evidence from human and chimpanzee studies supports a role for HCV-specific CD4+ helper and CD8+ cytotoxic T cells in control of primary and secondary HCV infections. A temporal kinetic association between the appearance of HCV-specific T cells in blood and an initial sharp drop in viremia, usually at ∼8 to 12 weeks after primary infection, provided the first evidence that cellular immunity might be important to control of acute hepatitis C (Fig. 1A).44,45 Follow-up studies revealed that T-cell responses are often qualitatively different in resolving and persisting infections. The frequency of HCV-specific T cells in blood is generally greater in subjects who clear the infection when quantified by direct visualization with MHC tetramers or production of antiviral cytokines.44,45 Successful primary responses also tend to be broader, targeting more HCV proteins or epitopes.44,45 More recent studies in chimpanzees suggest that phenotypic differences might distinguish sustained T-cell responses from those that fail before virus is cleared. CD8+ T cells from animals that controlled HCV replication were more likely to express CD127, the interleukin-7 receptor α subunit that is marker of memory precursors, during the early phase of infection when they were still viremic.46 CD8+ T cells from animals that clear infection are also more likely to have a phenotype associated with proliferation (Ki67 positive/Bcl-2 low) and activation (HLA-DR positive).47 Programmed cell death 1 (PD-1), which is expressed on CD8+ T cells in chronic hepatitis C and may contribute to the exhausted phenotype, was not predictive of HCV persistence at the early stages of infection in these chimpanzees.46
It is important to emphasize that chronic hepatitis C sometimes develops despite early robust expansion of CD4+ and CD8+ T cells that target multiple class II and I epitopes.43,44 These individuals can exhibit a substantial drop in viremia and prolonged period of partial control of virus replication before persistence is established (Fig. 1B). To date the best predictor of a persistent course is premature failure of the CD4+ T helper cell response.43,44,48 Loss of CD4+ T cells that produce antiviral cytokines and/or proliferate in response to HCV antigen stimulation has been associated with resurgent virus replication.49 Once lost, the helper response is rarely re-established and persistent infection ensues. The absence of CD4+ T cell help is thought to impair CD8+ T cells immunity, resulting in functional exhaustion and selection of HCV variants that carry escape mutations in class I epitopes.43,44

HCV-specific T-cell memory generated after resolution of primary infection is thought to contribute to rapid control of a second infection in humans and chimpanzees. In representative studies, re-infection of chimpanzees with HCV ~ 7 years after resolution of a first infection resulted in substantial acceleration of the T-cell response.28,29 Virus-specific memory T cells reached peak frequency within 7 to 10 days compared with 10 to 12 weeks after the first infection. HCV replication was controlled within 14 days and viremia was several orders of magnitude lower than in the first infection.28,29 Antibody-mediated depletion of CD8+ T cells29 immediately before a third infection of these animals prolonged viremia while depletion of CD4+ T cells28 resulted in persistence instead of rapid resolution. These observations provided direct evidence that memory T cells are an important component of protection in immune individuals who are re-exposed to the virus. It is important to emphasize that some secondary infections in humans and chimpanzees30,50 do persist despite memory T-cell immunity and so protection is not absolute. In one remarkable example, an immune chimpanzee developed a persistent infection after multiple sequential challenges with different strains and genotypes of HCV.50

Models for Preventive Vaccination

Studies of immunity and HCV replication during the acute and chronic phases of infection suggest models for preventive vaccination of humans. A successful vaccine would ideally reduce the magnitude and duration of primary viremia and skew infection outcome strongly toward resolution (Fig. 1A). Induction of protective immunity may be relatively straightforward for HCV-naïve vaccinees. There is considerable uncertainty around the status of immunity in those who are cured using interferon-free DAA regimens and remain at risk for re-infection (Fig. 1B). The permanency of immune system dysfunction after cure has not been explored. It is possible that some CD8+ T cells, especially those that are less exhausted because cognate class I epitopes escaped early in infection,51–53 would respond upon re-exposure to virus. Whether CD8+ T cells could respond to epitopes that remained intact in the first infection, or recognize a new repertoire of class I epitopes, is much more difficult to predict. Protection will depend on whether the CD4+ T cell compartment is restored after DAA cure. It is unlikely that effective memory CD4+ T cell populations would emerge under this circumstance (Fig. 1B). A new CD4+ T cell response that resembles the one generated after primary infection may be the best case scenario. It is likely that the level of protection in those cured of persistent infection would be no better, and perhaps worse, than the 30% rate of spontaneous resolution observed in HCV naïve subjects. Preventive vaccination after DAA-mediated cure may therefore be required to provide a consistently high level of protection from reinfection for those at risk (Fig. 1B).

Finally, studies of HCV infection and immunity have not yet provided clear correlates of protective immunity. The possibility that an early humoral response that broadly neutralizes contemporaneous viruses can contribute to termination of infection cannot be excluded. Strong cellular immunity is a hallmark of infections that resolve, but only when sustained well past the point of apparent clearance of the virus. Whether a vaccine must prime antibodies or CD8+ T cells is a matter of considerable debate and reflected in divergent approaches to vaccination reviewed below. There is a consensus that strong CD4+ T cell immunity that does not fail late in the course of acute hepatitis C is essential. An absence of hypotheses to explain premature loss of HCV-specific CD4+ T cells, or how it might be prevented by vaccination, remains a very significant blind spot for HCV vaccine development. There are as yet no reliable surrogate markers to predict whether a CD4+ T cell response generated by infection or vaccination is destined to succeed or fail.

Current Status of HCV Vaccines

Traditional approaches involving attenuation or inactivation of whole virus have received little consideration for vaccination against HCV. Generation of a live-attenuated HCV vaccine with no ability to persist, subvert immunity, or cause progressive liver disease is for now a conceptual and practical impossibility. A classical whole-killed vaccine could not be considered until the relatively recent development of cell culture systems that facilitate replication of HCV. One genotype 2 virus produced in the HCV cell culture system was inactivated and used to immunize mice.54 Sera from these animals inhibited infectivity of genotype 1 and 2 viruses in a hepatocyte cell culture model and the genotype 2 virus in immunodeficient mice with humanized livers.54

The discovery of HCV coincided with a revolution in recombinant DNA technology to produce virus proteins and vectors and knowledge of how antigens are processed for CD8+ T cell priming. These advances provided new options for the design of vaccines to elicit humoral and cellular immune responses.

Vaccines to Generate Neutralizing Antibodies

The subunit HBV surface antigen, licensed as a vaccine 2 years before the discovery of HCV, was the first to be produced using recombinant DNA technology. This advance provided the initial impetus for development of recombinant subunit E1 and E2 envelope glycoproteins as a vaccine by Chiron

Seminars in Liver Disease Vol. 34 No. 1/2014

Will There Be a Vaccine to Prevent HCV Infection? Honegger et al. 83

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Corporation (now Novartis, Basel, Switzerland), with the goal of generating protective neutralizing antibodies. Heterodimeric vaccines containing HCV genotype 1 E1 and E2 glycoproteins, when combined with an adjuvant, elicited strong humoral immune responses in nonhuman primates. Although some of these infections persisted, a comparison of controls and vaccinated animals from multiple studies revealed that resolution was much more common in the latter group. Antibodies alone are almost certainly not sufficient to terminate HCV infection once it is established, and so an important contribution of CD4+ T cells primed by the vaccines can’t be excluded. The possibility that the vaccine-primed E1/E2-specific CD4+ T cells are resistant to inactivation during acute HCV infection, and facilitate priming of antibodies and CD8+ T cells that contribute to clearance, merits further study.

The recombinant E1/E2 heterodimer combined with an oil–water emulsion adjuvant developed by Chiron/Novartis has been assessed for immunogenicity in human volunteers not at risk for infection. Strong CD4+ T cell responses were detected in lymphoproliferation assays and serum antibodies inhibited entry of genotype 1 HCV into hepatocytes. A recent reanalysis of serum from a subset of patients documented the potential for very broad pan-genotypic neutralization of HCV after vaccination with E1/E2 derived from a single genotype 1 isolate of the virus. No further progress in clinical development of this vaccine has been reported and so its fate is uncertain.

Other approaches to elicit anti-HCV antibodies have been evaluated in animal models. Expression of HCV envelope glycoproteins from plasmid DNA, and recombinant virus vectors have been used to induce antibodies in chimpanzees. Several of the virus vectors incorporated HCV E1 and/or E2 and nonstructural genes to induce CD8+ T cells and are reviewed below. Finally, prime-boost strategies long favored for vaccination against HIV may also have utility for HCV. One recent study documented that recombinant viral vectors and synthetic virus like particle (VLP) triggers incorporating HCV E1 and E2 elicited broadly neutralizing antibodies in macaques. Design of advanced synthetic immunogens may also be facilitated by crystallographic analysis of the interaction between broadly neutralizing monoclonal antibodies and the HCV glycoproteins.

**Vaccines to Generate HCV-Specific T Cells**

At the time HCV was discovered, key steps were taken toward understanding how antigens are processed for priming of cytotoxic CD8+ T lymphocytes. Townsend, McMichael, and colleagues documented in 1986 that CD8+ T cells recognize short viral peptides presented on the cell surface by class I MHC molecules. Subsequent studies rapidly defined the pathway for processing antigenic peptides from viral proteins produced in the cytoplasm. These advances sparked tremendous innovation in vaccine technologies to prime CD8+ T cells. The newly discovered HCV provided a potentially relevant target for proof of concept studies. A wide array of replicating and nonreplicating virus vectors, plasmid DNA, and adjuvants that facilitate antigen delivery to the cytosol for class I antigen processing have been adapted for use as HCV vaccines. Genetic vaccines, including plasmid DNA or recombinant virus vectors, have been most commonly used. They typically express nonstructural proteins like NS3, NS4, and/or NS5 because they are the dominant target of CD8+ T cells and well-conserved across HCV genotypes when compared with the envelope glycoproteins. VLP are the exception; they are comprised of the HCV envelope glycoproteins and core protein. All of these concept vaccines primed CD8+ T cells in rodents, a relatively low hurdle for immunogenicity. A very small number of vaccines were shown to elicit robust CD8+ T cell immunity in the blood and/or liver of macaques and baboons. Fewer have been assessed for immunogenicity and protection of chimpanzees from HCV challenge. They included a VLP comprised of the HCV E1, E2, and core proteins and recombinant nonstructural proteins formulated with the iscomatrix adjuvant, and genetic vaccines that encoded nonstructural proteins. Collectively these vaccines reduced primary viremia after challenge with HCV, and some by several orders of magnitude as summarized in a meta-analysis of all chimpanzee vaccine studies. Suppression of acute-phase virus replication was associated with recall of vaccine-primed T cells. The outcome of infection in vaccinated chimpanzees was, however, highly variable in these studies. In general, individual vaccine studies included too few animals, and approaches to analysis of cellular immunity were too varied, to draw conclusions about the vectors, antigens, and nature of T-cell responses that provided protection. That vaccine design might have a profound influence on infection outcome even when the same nonstructural proteins are used to prime T cells is illustrated by two studies. In the first example, acute-phase HCV replication was substantially suppressed in five chimpanzees vaccinated with recombinant NS3, NS4, and NS5 proteins formulated with the iscomatrix adjuvant when compared with the mock-vaccinated controls. However, all vaccinated animals, but none of the controls developed a chronic infection. In the second example, priming and boosting of chimpanzees with recombinant adenoviruses and plasmid DNA encoding the NS3, NS4, and NS5 proteins provided a very different result. Sharp suppression of acute-phase viremia was also observed in all five animals that received this vaccine. However, the infection was ultimately controlled in four individuals after several weeks of very low level, intermittent viremia. A follow-up study revealed that after challenge, CD8+ T cells induced by vaccination with the recombinant adenovirus and plasmid DNA had sustained expression of CD127, lower levels of PD-1,
and enhanced effector functions when compared with primary T cells from the mock-vaccinated controls that developed persistent infections.\textsuperscript{75} This result obtained with the iscomatrix-based vaccine raised the troubling possibility that some antigen and adjuvant combinations have the potential to unpredictably enhance persistent infection. The reason for very different infection outcomes with these vaccines despite suppression of acute phase viremia is not known. The frequency of circulating HCV-specific T cells that produced interferon-γ after vaccination and virus challenge was determined in all vaccine studies, but this measure alone appears to be insufficient to predict infection outcome.\textsuperscript{58} More detailed phenotypic and functional analyses will be needed to gain insight into factors that determine if a vaccine will reduce or possibly even increase the rate of persistent infection in humans.

Phase I safety and immunogenicity trials of two vaccines designed to prevent infection by eliciting T-cell immunity have been completed. Immunogenicity of an HCV core protein formulated with the iscomatrix adjuvant was determined in human volunteers not at risk for HCV infection. T cell responses were detected in only a subset of vaccinees. Phase I testing of a prime-boost regimen with the recombinant adenovirus vectors encoding NS3, NS4, and NS5 developed by Okairos (now Glaxo Smith Kline) was also undertaken in humans, supported by the promising outcome of the chimpanzee studies.\textsuperscript{80} Vaccination resulted in CD4\textsuperscript{+} and CD8\textsuperscript{+} T cell immunity with attributes generally associated with control of virus infection.\textsuperscript{80} The T cells displayed multiple effector functions. Memory CD8\textsuperscript{+} T cells that expressed CD127 but not PD-1 were sustained in circulation.\textsuperscript{80} Importantly, epitopes conserved in HCV genotype 1 and 3 viruses were recognized, indicating the potential for cross-genotypic protection. A version of this vaccine involving a prime with recombinant adenovirus vector and boosting with modified vaccinia virus Ankara is now being evaluated in a staged phase I/II study in Baltimore and San Francisco (see ClinicalTrials.gov NCT01436357). The goal of this study is to prevent HCV persistence in HCV-naïve IDU populations at high risk for infection.

**Will There Be a Vaccine to Prevent HCV Infection?**

Uncertainty about correlates of protective immunity is illustrated by very different approaches to the design of two vaccines that have been evaluated in humans. The Chiron/Novartis vaccine comprised of E1 and E2 envelope glycoproteins in an oil-water emulsion adjuvant was designed to elicit neutralizing antibodies and helper CD4\textsuperscript{+} T cells required to generate a humoral immune response. Although immunogenicity data appeared promising, no plan for an efficacy trial in the foreseeable future is apparent. Alternative vaccines to assess role of antibodies and T-helper cells in protection from HCV infection and persistence are not on the horizon. The second vaccine developed by Okairos/GSK will test the hypothesis that a T-cell response primed with nonstructural HCV proteins is sufficient to reduce the incidence of chronic hepatitis C by preventing persistence of the virus. It will also provide critical proof that large vaccine efficacy trials can be conducted in IDU, a largely marginalized population where basic needs like stable housing are often unmet and the risk of incarceration and even death is increased.\textsuperscript{81,82} Because of these unique challenges, it is estimated that the phase I/II trial will be ~ 4 years in duration. Whether this vaccine prevents persistence will not be known until early 2016 (ClinicalTrials.gov NCT01436357). Exactly how efficacious a vaccine like the one developed by Okairos/GlaxoSmithKline must be to have a material impact on liver disease in rural and urban IDU populations has not been widely studied. Modeling suggests that a vaccine targeted to IDU in San Francisco that is even 50\% efficacious would substantially reduce the incidence of chronic liver disease over a period of years or decades, but with the assumption of high vaccine uptake among those at risk of infection.\textsuperscript{15} Predictive modeling of how a combined program of vaccination and DAA treatment to reduce the virus donor pool would interrupt HCV transmission in rural and urban IDU populations is needed.

Progress toward a preventive HCV vaccine could be considered slow when judged by the limited number of candidates that have entered clinical testing. It is important to weigh progress against the considerable scientific and practical challenges that must be overcome to develop an HCV vaccine. As noted above, the timeframe from initiation to completion of HCV vaccine efficacy trials is measured in years and there is a paucity of at risk human cohorts and institutional infrastructure for this purpose.\textsuperscript{83} At the same time, animal models to validate vaccine concepts before human testing are limiting. New regulations in the United States define a highly restrictive set of conditions for use of chimpanzees in biomedical research.\textsuperscript{84} A variety of new infection models involving rodents and even lower nonhuman primates are under development and have been reviewed elsewhere.\textsuperscript{85} The pace of progress is rapid, but as yet no model recapitulates key features of HCV infection and immunity in humans. Advancing new vaccine concepts to phase I, and particularly phase II, human trials may be difficult without evidence that infection outcome is improved and not worsened in animals. Perhaps more importantly, iterative improvements to vaccine design may be required if protection in humans is less than optimal in initial efficacy trials. An empirical approach to vaccine development that involves cycles of evaluation in human subjects and improvements to increase efficacy will almost certainly require refinement of clinical trial design to shorten duration and/or a more tractable animal model with the same fidelity to human HCV infection and immunity provided by the chimpanzee. Vaccine trials may well provide the best insight into correlates of protective immunity, but will require phenotypic and functional analyses of adaptive immune responses in human vaccinees that are technically challenging, more similar to current vaccine development programs for HIV than past successful efforts for the other hepatitis viruses. It is likely that the imperative to develop HCV vaccines, and increase the pace of progress, will become more apparent as the cost of reinfection after DAA therapy is better defined in developed
countries. Ideally, the outcome of this process will be a preventive, pan-genotypic HCV vaccine that is similar to other hepatitis virus vaccines in cost and ease of production for use in developed countries, but especially in developing regions of the world where most new HCV infections occur and DAA therapies may well be unaffordable.

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