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Designing an HCV vaccine: a unique convergence of prevention and therapy?

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Abstract

Direct acting antivirals can cure chronic hepatitis C virus (HCV) infection but whether they will reduce global liver disease burden is uncertain. Most chronic infections are undiagnosed and transmission has increased in recent years. The first vaccine trial to reduce transmission is now underway in humans at risk for HCV infection. It will test the novel hypothesis that T cell-mediated immunity alone can prevent persistent HCV infection. Another vaccine that elicits neutralizing antibodies is at an advanced stage of development. Attention is turning to the understudied question of whether DAA cure of chronic infection restores HCV immunity. If not, it will be important to determine if preventive vaccines can also act therapeutically to reverse immune dysfunction and protect from re-infection.

Introduction

The hepatitis C virus (HCV) has a remarkable ability to establish persistent infection in the human liver. Only about 30 per cent of infections resolve spontaneously[1]. The remainder persist for life and increase the risk for serious progressive liver diseases including hepatocellular carcinoma[1]. There is not yet a vaccine to prevent HCV infection. A number of challenges have slowed vaccine development for HCV when compared with other hepatitis viruses. Immune responses that prevent HCV persistence are not fully defined, and immune correlates like antibody titers that predict protection by HAV, HBV, and HEV vaccines do not exist for HCV. How HCV evades antibody[2,3] and T cell[4] responses to establish persistent infection is also not fully understood. Vaccines protect against most if not all genotypes and strains of HAV, HBV, and HEV that circulate globally. By contrast, there are at least 7 HCV genotypes that differ by up to 30% in nucleotide sequence, complicating development of a vaccine that provides pan-genotypic protection. Finally, vaccines that prevent other forms of viral hepatitis were developed with the aid of animal infection models that are no longer available for HCV.

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The need for a vaccine to interrupt HCV transmission could be questioned with the availability of antiviral regimens that safely cure almost all chronic infections[5]. At least conceptually, detection and treatment of chronic hepatitis C with these direct acting antivirals (DAA) could replace vaccination as a strategy to interrupt transmission and perhaps eliminate HCV from human populations[6]. As an example, a national program to reduce HCV transmission in the country of Georgia by diagnosis and DAA treatment of most chronic infections is now underway[7]. However, this approach has challenges and limitations that may impede translation to other regions of the world. As reviewed elsewhere[8], an estimated 95% of HCV infections globally are undiagnosed and virus spread is increasing in developed and developing countries. In the United States, for example, a sharp spike in new HCV infections has been documented over the past decade amongst people who inject drugs (PWID) [9]. The infrastructure to identify and treat a sufficient number of chronic HCV infections to interrupt transmission does not yet exist in most parts of the world. The cost could be high when compared with deployment of an effective vaccine. Infection control may be most effective when antiviral therapy and preventive vaccination are combined, a concept supported by recent mathematical modeling of virus transmission amongst PWID[10].

Goals of Vaccination against HCV

Natural history studies have established that acute HCV infection is almost always clinically mild and therefore unrecognized[1]. Moreover, spontaneous resolution of acute infection observed in about 30% of infected individuals results in a complete cure. There is no apparent reservoir of latent HCV genomes to facilitate re-initiation of virus replication. For these reasons the objective of vaccination is not to prevent HCV infection, but instead to skew outcome towards acute resolution and away from persistence.

The goal of HCV vaccine development over the past 3 decades has been prevention of virus persistence in HCV-naïve populations. There is now increasing awareness that reinfection can occur after DAA-mediated cure of chronic hepatitis C[11]*. It is not yet known if successful DAA therapy reverses defects in HCV-specific immunity or affords protection from reinfection. Here, recent advances relevant to vaccine-mediated protection against primary HCV infection and reinfection after DAA cure are considered.

Vaccines to Prevent Primary HCV Infection

Two observations suggest that vaccination to prevent persistence infection in HCV naïve humans is feasible[12,13]. First, during acute primary infection, initial control of virus replication coincides with the appearance of HCV-specific T cell and antibody responses[13]. Sustained adaptive immune responses, particularly CD4+ T cell help, is a hallmark of infections that resolve. Second, spontaneous resolution of acute hepatitis C results in long-lived immunity and a substantially reduced probability of persistent infection in humans[14,15] and chimpanzees[16] re-exposed to the virus. This naturally acquired immunity often protects against challenge with heterologous HCV genotypes[17].

There is, however, no consensus on the importance of humoral versus cell-mediated immunity in protection afforded by spontaneous resolution of acute hepatitis C. This uncertainty is reflected in the design of a large number of vaccine candidates that have been assessed for immunogenicity in small animal models [18]. Some of these vaccines were comprised of the HCV E1 and E2 envelope glycoproteins that are targeted by neutralizing antibodies[19], while others focused on expression of non-structural proteins like NS3, NS4a, NS4b, NS5a and NS5b that are dominant targets of the T cell response[18]. Structural and non-structural HCV proteins have also been combined to elicit humoral and cellular immunity using prime-boost strategies[18,20]. A very small subset of these experimental vaccines were tested for their capacity to protect chimpanzees from virus challenge[18]. Even fewer were assessed for immunogenicity in HCV naïve human volunteers. Two candidate vaccines studied in chimpanzees and humans are highlighted here. They provide insight into the history and rationale for development of vaccines designed to elicit antibodies versus T cells for protection against HCV infection.

A vaccine to elicit protective neutralizing antibodies. Successful vaccines that protect against HAV, HBV, and HEV elicit antibodies that provide apparent sterilizing immunity. For HCV, the role of antibodies in protection from virus persistence is more complex. Passive immunization with antibodies before HCV challenge has prevented infection in animal models[21–23]. However, sterilizing immunity mediated by antibodies is a challenging endpoint for preventive HCV vaccines. One recent study demonstrated that passive immunization of chimpanzees with antibodies that broadly neutralized infectivity of several HCV genotypes in cell culture only delayed infection with homologous virus and failed to protect against heterologous virus strains[24]**. Antibodies may nonetheless contribute to suppression of acute phase virus replication and perhaps resolution of infection. For instance, broadly neutralizing antibodies have been associated with lower levels of acute phase viremia [25] and develop more rapidly in patients who spontaneously resolve HCV infection[26–28]. Animals studies have also been instructive. Transfer of neutralizing antibodies to chimpanzees after challenge with HCV resulted in at least transient suppression of viremia[22,24,29]. Neutralizing antibodies expressed from the genome of a recombinant AAV vector also facilitated clearance of HCV from human liver chimeric mice[30].

A vaccine developed at Chiron Corporation by Michael Houghton and his colleagues was comprised of recombinant HCV genotype 1a envelope glycoproteins E1 and E2 to elicit neutralizing antibodies. When formulated with microfluidized oil:water emulsion adjuvants, the E1/E2 vaccine elicited serum antibodies in chimpanzees that neutralized infectivity of most HCV genotypes in cell culture models[31]. After challenge with HCV, sterilizing immunity was observed in a subset of animals[31]. Breakthrough infections were established in other animals even when challenged with viruses closely matched to the genotype 1a E1/E2 vaccine sequence. Intriguingly, breakthrough infections were often attenuated and more likely to resolve than those in mock vaccinated controls, again suggesting that antibodies do contribute to resolution of acute HCV infection[31].

Immunogenicity of the recombinant E1/E2 vaccine has been assessed in human volunteers with no known risk factors for HCV infection. Pan-genotypic neutralizing antibodies[32]*

and strong HCV-specific CD4⁺ T cell responses[33] were induced. Recent studies to better understand the physical structure of the E1/E2 heterodimer[34] [35] and refine production of these recombinant proteins[36] were recently described by Houghton and colleagues. Further testing of improved recombinant E1/E2 formulations for safety and immunogenicity in humans could advance the concept that vaccines eliciting antibodies can alter the course of HCV infection.

A vaccine to prime protective T cells. The importance of memory T cells for protection from persistence was illustrated by studies in chimpanzees that spontaneously resolved HCV infections. Antibody-mediated depletion of CD4⁺ T cells immediately before reinfection of immune animals resulted in HCV persistence[37]. Antibody-mediated depletion of CD8⁺ T cells in a second group of naturally immune animals caused prolonged infection after rechallenge[38]. These findings, and the observation that some HCV infections resolve in the absence of an antibody response[39], supported a bold hypothesis that vaccination to prime T cell immunity might be sufficient to prevent HCV persistence. This concept was tested by Folgori, Nicosia, and colleagues at Okairos Corporation (now Glaxo Smith Kline). Genetic immunization of HCV naïve chimpanzees with adenovirus and plasmid DNA vectors encoding non-structural proteins NS3 to NS5b elicited strong CD4⁺ and CD8⁺ T cell responses that transitioned to memory populations[40,41]. This vaccine-induced T cell response was rapidly recalled upon challenge with HCV and resulted in substantial suppression of acute phase viremia when compared to mock vaccinated controls[40].

Safety and immunogenicity of this vaccine approach was established in HCV naïve human subjects with low risk for exposure to the virus. A prime boost strategy with two serologically distinct adenovirus vectors elicited T cell responses against class I and II epitopes encoded by the HCV NS3-NS5b transgene[42]**. Modification of this regimen by replacing the adenovirus vector with a modified vaccinia virus Ankara (MVA) vector for the boost substantially increased the frequency and duration of multifunctional T cells that targeted epitopes conserved in many HCV genotypes[43]**. This vaccine is now being assessed for immunogenicity and protection against virus persistence in HCV-naïve individuals at risk for infection because of injection drug use, with a reported completion date of July, 2018 (Clinical Trials.gov NCT01436357).

Vaccines to Prevent Reinfection with HCV

Whether DAA-mediated cure of chronic hepatitis C provides the same protection against persistence as spontaneous resolution of acute hepatitis C is unknown. CD4⁺ T cells targeting a broad array of class II HCV epitopes are detected during acute infection[44], but are dysfunctional and ultimately fail in individuals with a persistent outcome[12,13]. HCV-specific CD4⁺ T cells do not select for escape mutations in class II epitopes and are difficult to detect during chronic infection for reasons that are not yet understood[12,13]. CD8⁺ T cells are detectable in liver during the chronic phase of infection but the response is ineffective because of mutations in class I HCV epitopes or exhaustion[12,13], defined as loss of effector functions and failure to differentiate[45]. CD8⁺ T cells targeting class I epitopes that do not escape are the most profoundly exhausted and express high levels of co-inhibitory receptors like PD-1 that are thought to contribute to dysfunction[12,13]. The

pervasive nature of defective CD8⁺ T cell immunity is highlighted by studies to restore the response in chronically infected humans and chimpanzees. As an example, recombinant virus vectors encoding HCV non-structural proteins that generated strong, multi-functional CD8⁺ T cell activity in uninfected humans [42,43] and chimpanzees[40] failed to prime effective CD8⁺ T cells in persistently infected individuals, even when replication was suppressed by concurrent treatment with DAA[46]* or IFN and ribavirin[47]* [48]*. CD8⁺ T cells that did expand recognized class I epitopes that had developed escape mutations earlier in chronic infection or were unique to the vaccine sequence. Antibody-mediated blockade of inhibitory PD-1 signaling in CD8⁺T cells also failed to control HCV infection in most chronically infected humans[49] and chimpanzees[50].

HCV viremia, and by extension antigen production, drop rapidly with initiation of DAA therapy. These are important factors contributing to immune exhaustion[45] and so at least conceptually DAA therapy should facilitate immune recovery. Innate immune responses and inflammatory markers recover at least partially in DAA-treated individuals. Interferon-stimulated gene (ISG) expression in liver and blood is reduced by DAA therapy[51,52] and recovery of function by circulating NK cells[51] has been described. However, recent profiling of serum cytokines and chemokines indicated that some inflammatory mediators do not normalize after DAA cure[53] and ongoing dysfunction of mucosal associated invariant T (MAIT) cells has been described[54].

Recent studies extended this analysis to T cell immunity. Improved proliferation of HCV-specific CD8⁺ T cells after antigen-driven expansion from peripheral blood has been described in patients successfully treated with DAA[55]**. Recovery of proliferation was most noticeable in expanded CD8⁺ T cells targeting intact class I epitopes, as might be expected if they are most profoundly impaired by chronic antigen stimulation. Expression of the co-inhibitory PD-1 receptor declined after successful treatment, and expression of CD127, the IL-7 α receptor that is marker of memory CD8⁺ T cells, increased[55]. A second study also noted a small but statistically significant decrease in PD-1 expression on circulating HCV-specific CD8⁺ T cells from 7 subjects who were cured with a combination of three DAA agents[56]*. Another study reported no improvement in IFN- γ production by HCV-specific T cells in patients treated with DAA alone, DAA with ribavirin, and DAA with ribavirin and pegIFN[57]**. Why studies that measured T cell recovery by IFN- γ production [57] versus proliferation and phenotype [55]) reached different conclusions isn't clear. The studies differed in experimental design. As noted above, T cell proliferation[55] was measured after antigen-driven expansion from blood, while IFN- γ [57] was measured immediately after *ex vivo* antigen stimulation of circulating T cells. Differences in study populations and antiviral regimens may also be a factor. Fully functional T cell responses mediate multiple effector functions, including production of IFN- γ and TNF- α , cytotoxic activity and the capacity to proliferate[45]. It will be necessary to assess multiple effector functions in direct *ex vivo* assays to fully compare the quality of HCV-specific CD8⁺ T cells after DAA-mediated cure versus spontaneous resolution of infection.

Reinfection studies should provide the most definitive measure of whether protective immunity is generated after DAA cure. To date, protection has been assessed in one chimpanzee re-challenged with HCV after DAA cure of chronic hepatitis C[58]**. CD8⁺ T

cells targeting intact and escaped HCV epitopes were visualized in liver during chronic infection and for two years after termination of virus replication by DAA therapy. Re-challenge of the cured animal with the same virus used to establish the first infection resulted in a second persistent infection. HCV replication was only transiently controlled by a memory HCV-specific T cell response. Detailed analysis of the response revealed that the expanded CD8⁺ T cells targeted class I epitopes that escaped in the first infection. They rapidly selected for escape mutations after reinfection, and this coincided with the rebound in viremia and HCV persistence. CD8⁺ T cells targeting epitopes that remained intact during the first infection failed to expand after reinfection, presumably because exhaustion was not reversed after DAA cure[58]. Importantly, there was no apparent broadening of the CD8⁺ T cell response to new epitopes not targeted in the first chronic infection[58]. The study, although very preliminary, does make a case for vaccination to broaden immune responses after DAA cure. Detailed studies of human subjects after DAA cure are needed to assess the effectiveness of recall immune responses and whether they prevent persistence. Reinfection studies in humans would provide an important test of the breadth and function of antibody and T cell responses, especially against heterologous strains of HCV that may present new epitopes that broaden the immune repertoire beyond that established by earlier chronic infection (Figure 1). Importantly, recovery of CD4⁺ T cell immunity was not assessed in this reinfection study[58] or in the earlier *ex vivo* studies of T cell function[55] [57] in individuals successfully treated with DAA. It is difficult to predict whether helper activity can recover spontaneously after DAA cure (Figure 1).

Summary

HCV vaccine development is unique from many perspectives. Most notably, the goal of vaccination is not to prevent HCV infection by eliciting sterilizing immunity, but instead to prevent HCV persistence. It might also provide the first instance of preventive vaccination in individuals cured of a chronic infection with antiviral therapy. As proposed in Figure 2, the hurdles to successful vaccination may be different in HCV naïve and cured individuals. Vaccination to skew infection outcome towards resolution and away from persistence in HCV naïve humans may require a better understanding of how antibodies interfere with virus replication infection, and mechanisms of premature CD4⁺ T helper cell failure, a reliable predictor of progression to chronic hepatitis C. Vaccination to prevent persistence after reinfection may have additional requirements. Very little is known about the breadth or fate of neutralizing antibodies after chronic HCV infection is cured with DAA, or if an established humoral response can be boosted and refocused by vaccination to provide better protection against reinfection. Similarly, it may be necessary to broaden the repertoire of targeted class I and class II epitopes beyond those recognized by T cells during chronic infection.

Restoring and broadening ineffective antibody and/or T cell responses are typically the goals of therapeutic vaccination. Comparing antibody and T cell responses in vaccinated humans who are HCV naïve or DAA cured should be a priority for HCV vaccine development. The two vaccines designed to prevent HCV persistence after primary infection could be an important starting point. Vaccination with the recombinant envelope glycoproteins E1 and E2[32] [33] would provide a direct test of whether the antibody repertoire established by

chronic infection can be broadened and sustained, and whether the breadth and function of HCV-specific CD4+ T cell responses can be restored to levels observed in vaccinated HCV naïve individuals. Vaccination with the recombinant virus vectors that express non-structural HCV proteins[42,43] would provide a robust test of whether CD4+ and CD8+ T cells can differentiate into multifunctional memory populations after years of exhaustion caused by inflammation and high antigen load. Finally, the unique convergence of preventive and therapeutic vaccination in cured chronic HCV infection could provide an ideal setting to test new experimental vaccines that substantially broaden immune responses. As an example, a recombinant cytomegalovirus vector expressing SIV/HIV genes can prime CD4+ and CD8+ T cells against epitopes not recognized during natural infection with these viruses[59]*. Assessing immunogenicity of preventive vaccines in small numbers of individuals after cure of chronic infection could provide large advances in our general understanding of how immune responses are dysregulated and conditions for their restoration in other chronic viral infections and cancer.

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Abbreviations

HCV	hepatitis C virus
DAA	direct acting antiviral

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Highlights

- Protection from HCV persistence, not sterilizing immunity, is the goal of HCV vaccine development.
- An HCV vaccine eliciting T cells but not antibodies is in clinical testing to prevent chronic infection.
- Whether HCV-specific immunity recovers after antiviral cure of chronic infection remains uncertain.
- A vaccine may be needed to restore dysfunctional immunity and prevent reinfection in cured individuals.

Vaccine to prevent:	Target population:	HCV immune status:	Vaccine objective:
primary infection	Wide- anyone at risk for HCV infection.	Naïve- no T cell impairment.	Accelerate T cell immunity compared to natural infection.
reinfection	Narrow- anyone at risk of reinfection after DAA cure.	Primed- but potential for residual T cell impairment .	Broaden T cell repertoire to new epitopes.

Figure 1. Comparison of vaccines to prevent primary infection (top) and reinfection (bottom) with HCV after antiviral cure

A vaccine to prevent primary HCV infection would have wide distribution to individuals at risk for primary infection, most commonly people who inject drugs. For T cell priming, the objective is to accelerate and sustain responses so that they do not fail before eradication of the virus. A vaccine to prevent reinfection would have more targeted distribution to individuals cured of chronic hepatitis C with DAA. If T cell responses primed during chronic infection do not afford protection, vaccination to broaden the response to new epitopes may be an additional requirement to prevent persistence.

Figure 2. Predicted patterns of T cell recovery after DAA cure

Based on observations in humans and chimpanzees with chronic hepatitis C, CD8+ T cells that are profoundly exhausted by chronic stimulation with intact class I epitopes may be least likely to recover after DAA cure. CD8+ T cells targeting epitopes that develop escape mutations early in the course of infection are predicted to recover more readily. Recovery of CD4+ T cell immunity is almost certainly essential to protection from reinfection. Whether they will recover or can be restored by vaccination is more difficult to predict given the poor understanding of mechanisms leading to their loss during HCV infection.