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Challenges and Promise of a Hepatitis C Virus Vaccine

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An estimated 1.5–2 million new hepatitis C virus (HCV) infections occur globally each year. Critical to the World Health Organization's (WHO) HCV elimination strategy is an 80% reduction in incidence of HCV infections by 2030. However, even among high-income countries, few are on target to achieve the WHO's incident infection-reduction goal. A preventative vaccine could have a major impact in achieving incidence-reduction targets globally. However, barriers to HCV vaccine development are significant and include at-risk populations that are often marginalized: viral diversity, limited options for testing HCV vaccines, and an incomplete understanding of protective immune responses. In part because of those factors, testing of only one vaccine strategy has been completed in at-risk individuals as of 2019. Despite challenges, immunity against HCV protects against chronic infection in some repeated HCV exposures and an effective HCV vaccine could prevent transmission regardless of risk factors. Ultimately, prophylactic vaccines will likely be necessary to achieve global HCV elimination.

The introduction of oral, interferon-sparing, direct-acting antivirals (DAAs), which cure hepatitis C virus (HCV) with high efficacy and low toxicity, has fueled optimism for control. However, several limitations make treatment insufficient to eliminate HCV globally. The majority of HCV-infected people remain undiagnosed because HCV infection is rarely symptomatic before the onset of advanced liver disease, and routine HCV screening is not common globally (Gravitz 2011). In addition, the cost of and practical aspects to delivering therapy globally result in a cascade of care that leaves a small subset of those diagnosed cured. HCV treatment rates have been declining globally since the peak in 2015 as the HCV-infected people easiest to access have been treated, leaving those more difficult to access without treatment (J McHutchinson and D Brainard, Gilead Sciences, pers. comm.). A very small subset of those treated develop DAA resistance, and at least some resistant HCV variants are transmissible (Franco et al. 2014). However, antiviral resistance could become more common with expansion of treatment to less compliant populations. HCV cures do not completely reverse the adverse consequences of infection in all patients, and liver disease progression and cancer occur in some patients with cirrhosis despite cure. Last, reinfection following cure in high-risk groups makes HCV elimination even more challenging. Thus, prevention of infection offers significant

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advantages over treatment in eliminating harm from HCV.

Global control will require annual rates of cure that are consistently and significantly higher than new HCV infection rates. However, nearly 60% of surveyed countries had more infections than cures in 2016, and few countries are on target to achieve elimination of HCV as a public health problem by 2030, the goal set by the World Health Organization (WHO) (Hill et al. 2017; WHO 2017). In a 2018 survey of 45 high-income countries, only 11 are on target to achieve the WHO's incidence of infection-reduction goal (Razavi et al. 2019). More high-income countries met screening and treatment targets than incidence-reduction goals. Given that the gap is most significant in infection prevention, an effective vaccine would represent an important weapon in the fight for global HCV control. However, barriers to development remain, including lack of economic incentive for vaccines, limitations to HCV culture systems, viral genetic diversity, limited animal models and at-risk populations for testing vaccines, and an incomplete understanding of protective immune responses. However, both the promise and the challenges of a prophylactic HCV vaccination are vast (Table 1).

ECONOMIC INCENTIVES TO SUPPORT HCV VACCINE ADVANCEMENT

Sound market analysis dictates that development of any product is supported by having a market for that product. Although vaccines represent important tools for disease prevention and have significant social value, they frequently generate lower revenues than drugs and other health care services, and provide a less attractive opportunity for the pharmaceutical industry (Institute of Medicine Committee on the Evaluation of Vaccine Purchase Financing in the United States 2003). In addition to general disadvantages to investment in vaccines, the demographics of the target market of those at risk for HCV infection globally may provide further economic disincentives. In the developed world, the primary risk factor for acquiring HCV is injection drug use. People who inject drugs (PWID) are often not optimally engaged in health care at any stage of drug use. Reaching them soon after initiation of drug use is even more challenging but necessary because young PWID have the highest incidence of HCV infection during the first 2 years of drug use (Villano et al. 1997). An effective vaccine can only reduce the risk of HCV transmission if routinely pro-

Table 1. Challenges and promise of an HCV vaccine

Promise	Challenges
Vaccination prevents HCV regardless of risk factors	Return on financial investment will be limited if vaccination remains restricted to high-risk groups in developed countries
Universal vaccination before initiation of high-risk behavior provides a broad time window in which to offer protection, unlike treatment as prevention	Targeting PWID before they are infected leaves a narrow time window for intervention, arguing for universal vaccination
Protective immunity exists in chimpanzees and humans, making protective vaccination possible	Correlates of protective immunity are unknown
Prevention of infection reduces disease burden and transmission more effectively than treatment	Culturing HCV is difficult, making live attenuated or killed virus vaccines impractical Testing vaccines for efficacy is difficult because of the lack of a predictably high-risk population HCV is a highly variable virus, making selection of an antigen that elicits cross-protective responses difficult

(HCV) hepatitis C virus, (PWID) people who inject drugs.

vided to people before the onset of high-risk behaviors. In developing countries, contaminated injections, blood transfusions, and other exposures represent important risk factors for infection, and universal vaccination will likely be necessary in those nations to prevent HCV and reduce the burden of disease. While this will require prioritizing HCV prevention, modeling forecasting that viral hepatitis will kill more people globally by 2040 than human immunodeficiency virus (HIV) infection, tuberculosis, and malaria combined supports a focus on HCV (Foreman et al. 2018). Importantly, vaccination offers the opportunity to prevent infection regardless of risk factors, and expansion of HCV vaccination from the at-risk population to a universal vaccine once a vaccine has been proven safe and effective will make global control more likely, as well as make vaccine development a more attractive economic prospect.

FEASIBILITY OF TRADITIONAL APPROACHES FOR HCV VACCINE DESIGN

Generation of live attenuated and inactivated whole virus vaccines has been effective for protection against other pathogens, but neither strategy is feasible for HCV. Limitations of HCV culture systems make production of a live-attenuated or inactivated whole HCV vaccine technically very challenging (Thomas and Liang 2016). Current culture strains of HCV have adaptive mutations that enhance replication efficiency in vitro with unknown effects in humans. Live attenuated vaccines against other viruses have been generated by either deletion or inactivation of virulence factors and passage of virus in nonhuman primate cell lines to diminish infectivity. However, the virulence factors for HCV have not been defined, and HCV does not replicate at high levels in nonhuman primate cell lines. Thus, practical production aspects and the potential risk of causing disease limit the utility of live attenuated and inactivated whole HCV vaccines.

HCV GENETIC DIVERSITY

A major challenge for HCV vaccine development is the extraordinary genetic diversity of the virus, with eight known genotypes and more than 100 subtypes (Borgia et al. 2018). HCV strains from different genotypes differ on average ~30% of their amino acids, whereas different subtypes within each genotype differ at an average of about 15% of their amino acids (Smith et al. 2014, 2017; Bukh 2016). In addition to diversity between genotypes and subtypes, immune selection and the error-prone polymerase of the virus generate a diverse quasispecies of related but genetically distinct viral variants within each infected individual, presenting many opportunities for selection of transmissible viral variants with resistance to T-cell and antibody responses (Martell et al. 1992; Erickson et al. 1993; Farci et al. 1997; Forns et al. 1999; Timm et al. 2004; Cox et al. 2005; Liu et al. 2010). Given this viral diversity, vaccine induction of very broadly reactive immune responses or the generation of immune responses that target genetically conserved regions of the viral genome may be required for protection against HCV. One study showed enhanced potential for computer-generated sequences to elicit cross-reactive human T-cell responses versus previously defined T-cell epitopes or circulating HCV variants, supporting the use of a synthetically generated sequence to elicit robust $CD8^+$ T-cell responses (Burke et al. 2012). An HCV vaccine that encodes conserved genetic HCV regions generated T cells that target multiple HCV genotypes (von Delft et al. 2018). However, computational strategies to aid in HCV antigen design have not advanced past small animal testing.

CHALLENGES IN TESTING HCV VACCINES

Chimpanzees played a significant role in understanding the immune response to HCV, but are no longer used in HCV research. Currently, available in vitro systems and immunocompetent small animal models permit more limited assessment of whether vaccine-induced adaptive immune responses will provide protective immunity against HCV (Thomas and Liang 2016). High DAA efficacy introduces the possibility of challenge studies in which vaccinated

humans could be intentionally infected with HCV. Purposeful infection of healthy volunteers with HCV seems at odds with acceptable ethical standards, but controlled human infection studies have been an important contemporary research avenue for the study of other infectious disease vaccines and treatments (Bambery et al. 2016; WHO 2016) However, it is not clear how those infections would be achieved given that primary HCV isolates have limited ability to replicate in tissue culture and do not represent the diversity of the viral quasispecies circulating in natural infection. Direct infusion of HCV-infected human plasma would require careful screening for other pathogens and might also fail to completely recapitulate natural exposure. Thus, vaccine efficacy is difficult to assess without a population at predictably high risk for HCV infection. Although HCV transmission occurs through iatrogenic exposure, predicting who will be at risk and vaccinating them before exposure is not feasible. Rates of HCV transmission in men having sex with men (MSM) are variable and can be high, but the highest risk MSM are HIV infected or at high risk of HIV acquisition, and testing vaccines in an HIV-infected population may underestimate immunogenicty (van de Laar et al. 2009a). In contrast, the incidence of HCV infection in HIV-uninfected PWID is predictably high at 5%–25% per year, identifying PWID as a vaccine test population and showing the continued need for prevention of HCV infection in them (Cox and Thomas 2013). However, successful identification, enrollment, and prospective monitoring of PWID before onset of acute HCV infection has been achieved in few cohorts (Cox et al. 2009; Edlin et al. 2009). These cohorts will likely remain of critical importance to vaccine testing and identification of correlates of protective immunity. Such correlates would facilitate screening of vaccine candidates in volunteers not at risk for HCV with the vaccines that elicit protective immune responses advancing into more difficult-to-conduct clinical trials in at-risk subjects. While the precise correlates of protective immunity against HCV are unknown, there is substantial evidence supporting protective immunity.

EVIDENCE FOR PROTECTIVE IMMUNITY AGAINST HCV

Spontaneous clearance of HCV infection occurs in about 25% of acutely infected individuals (Micallef et al. 2006). Chimpanzees and humans who spontaneously control HCV infection can be infected again, establishing that spontaneous clearance of primary HCV infection does not fully protect from reinfection (Farci et al. 1992; Prince 1994; Bassett et al. 2001; Major et al. 2002; Mehta et al. 2002; Nascimbeni et al. 2003; Shoukry et al. 2003; Prince et al. 2005; Micallef et al. 2007; Page et al. 2009; van de Laar et al. 2009b; Osburn et al. 2010). However, clearance of multiple infections with homologous and heterologous virus has been observed in chimpanzees and humans (Bassett et al. 2001; Prince et al. 2005; Micallef et al. 2007; Page et al. 2009; Osburn et al. 2010; Grebely et al. 2012). Furthermore, clearance occurs far more often in reinfection than in primary infection. Reinfected PWID control secondary HCV infections about 80% of the time, essentially the reverse of persistence rates observed in primary infection (Osburn et al. 2010). Adaptive immune responses would be expected to result in more rapid control of reinfection than occurs in the initial infection, and HCV reinfection is characterized by a reduced peak and duration of viremia compared to initial infection of the same person (Osburn et al. 2010; Sacks-Davis et al. 2015). Reinfection was associated with broadened cellular immune responses compared to primary infection and detectable broadly neutralizing antibodies ([bNAbs], Osburn et al. 2010). Clearance of both homologous and heterologous viral rechallenge was also associated with decreased duration and magnitude of viremia in chimpanzees compared with primary infection (Lanford et al. 2004). These studies suggest that adaptive immunity is induced in spontaneous control of HCV and, although not sterilizing, protects against chronic disease. While additional research aimed at understanding effective immune control of HCV remains important, HCV-specific $CD4^+$ helper T cells, $CD8^+$ cytotoxic T cells, and antibodies are all known to play a role in protection against persistent HCV infection.

CELLULAR IMMUNITY TO HCV INFECTION

Data from both human and chimpanzee studies suggests that HCV-specific $CD4^+$ and $CD8^+$ T cells are crucial in control of primary and secondary HCV infections. Indirect evidence of T-cell control comes from genetic studies, showing associations between HCV clearance and specific class I and class II human leukocyte antigens that present peptides to $CD8^+$ and $CD4^+$ T cells, respectively (Kuniholm et al. 2010; Duggal et al. 2013). The presence of a vigorous and multispecific proliferative $CD4^+$ T-cell response to HCV is a strong immunologic correlate of spontaneous control of acute HCV infection (Diepolder et al. 1996; Chang et al. 2001; Rehermann 2009; Schulze zur Wiesch et al. 2012). Depletion of $CD4^+$ T cells before reinfection of two immune chimpanzees resulted in persistence of HCV despite functional intrahepatic memory $CD8^+$ T-cell responses. Depletion of CD8 T cells in two other immune chimpanzees resulted in prolonged viremia that was controlled only when $CD8^+$ T cells were again detectable in the liver (Grakoui et al. 2003; Shoukry et al. 2003). Thus, both $CD4^+$ and $CD8^+$ memory T cells are important in HCV control in chimpanzees. Similarly, protection against viral persistence in recurrent HCV infection in PWID was associated with broadening of the T-cell response and the expansion of effector memory T cells at the peak of the T-cell response (Osburn et al. 2010; Abdel-Hakeem et al. 2014). Dominant epitope regions of HCV strains isolated from patients with persistent reinfection had sequence variations that were not recognized by preexisting memory T cells. Together, these studies show that memory $CD4^+$ and $CD8^+$ T cells play a crucial role in protective immunity upon reexposure to HCV, and generation of effective memory T-cell responses through vaccination is likely necessary for a successful vaccine.

TRIALS OF VACCINES DESIGNED TO ELICIT CELLULAR IMMUNITY

A variety of strategies have been used to elicit HCV-specific T-cell responses through vaccination (Table 2). One approach is targeting relatively conserved viral proteins within the nonstructural (NS) region of the genome to induce a broad T-cell response, with and without envelope glycoproteins. NS proteins NS3, NS4, and NS5 are more conserved across HCV genotypes than the envelope glycoproteins and are dominant targets of $CD8^+$ T cells (Ward et al. 2002). A variety of strategies have been used to introduce NS proteins in an immunogenic way, including combinations of DNA-based immunization, recombinant virus vectors, adjuvanted HCV proteins, virus-like particles (VLPs), hepatitis B virus surface antigen–HCV recombinants, and pooled synthetic class I peptide epitopes or peptides incorporated in lysosomes (Elmowalid et al. 2007; Youn et al. 2008; Cox and Thomas 2013; Christiansen et al. 2018). Most candidate vaccines have been tested in rodents with a small subset of candidate vaccines tested in macaques and shown to elicit robust T-cell immunity (Polakos et al. 2001; Jeong et al. 2004; Rollier et al. 2005; Fattori et al. 2006; Garrone et al. 2011; Colloca et al. 2012; Lang Kuhs et al. 2012).

Because of cost and limited availability of chimpanzees, fewer vaccine candidates have been assessed for efficacy by protection of chimpanzees from HCV challenge. These vaccines include VLPs comprised of the HCV E1, E2, and core proteins, recombinant NS proteins formulated with the ISCOMATRIX adjuvant, and genetic vaccines that encoded NS proteins (Rollier et al. 2004; Folgori et al. 2006; Elmowalid et al. 2007; Youn et al. 2008; Zubkova et al. 2014). Infection outcome in vaccinated chimpanzees was highly variable, in part, because there were fewer than six vaccinated animals per study (Rollier et al. 2004; Elmowalid et al. 2007; Youn et al. 2008; Dahari et al. 2010; Zubkova et al. 2014). Genetic vaccines encoding the envelope glycoprotein induced antibodies, as well as T-cell responses, but none provided sterilizing immunity (Rollier et al. 2004; Youn et al. 2008). However, these vaccines reduced primary viremia after challenge with HCV by as much as several orders of magnitude (Rollier et al. 2004; Folgori et al. 2006; Elmowalid et al. 2007; Youn et al. 2008; Dahari et al. 2010; Zubkova et al. 2014).

Table 2. Vaccine strategies used for HCV vaccine development

Vaccine recipients for assessing immunogenicity				Immune response–induced	
		Humans			
Rodents or macaques	Chimpanzees	Not at risk for HCV	At risk for HCV	Neutralizing antibodies	T cells
DNA-based	DNA-based			Not tested	Yes
VLPs with various proteins	VLPs with E1, E2, core	VLPs with E1, E2, core		No	Yes
Adjuvanted HCV proteins or truncated E1E2	Adjuvanted HCV proteins	Adjuvanted HCV core, adjuvanted E1E2		Yes, for some	Yes
Recombinant viral vectors	Recombinant viral vectors	Recombinant viral vectors	Recombinant viral vectors	Yes, for some	Yes
Whole virus				Yes	Not tested
Class I HCV peptide epitopes				No	Yes
Peptides incorporated in lysosomes				No	Yes

(HCV) hepatitis C virus, (VLP) virus-like particle.

A meta-analysis of all the data from chimpanzee vaccine trials showed that suppression of acute-phase virus replication was associated with recall of vaccine-primed T cells, but the levels of induced T-cell responses did not correlate with vaccine success (Dahari et al. 2010). The analysis also found that vaccines containing only structural proteins induced significantly higher clearance rates than vaccines containing NS components. However, the vectors and antigens used, the timing of vaccination prime and boost, and the timing and identity of challenge HCV viruses used were highly varied, limiting the ability to compare these studies or conclude that inclusion of NS proteins reduces vaccine efficacy.

The majority of chimpanzee vaccine studies have shown reduced HCV persistence rates in vaccinees versus controls (Dahari et al. 2010). However, some studies of vaccines designed to induce T-cell responses showed increased persistence rates. Vaccination of chimpanzees with recombinant NS3, NS4, and NS5 proteins formulated with the ISCOMATRIX adjuvant resulted in persistent HCV infection on rechallenge in five of five vaccinated animals, despite the appearance of HCV multispecific $CD4^+$ and $CD8^+$ T-cell responses in the liver before experimental challenge and infection (Houghton 2011). A second vaccine study in which naive chimpanzees were immunized with DNA plasmids expressing the core-E1E2 and NS3 for priming and with recombinant modified vaccinia ankara (MVA)-expressing core-E1E2 and NS3 gene sequences as a boost induced HCV-specific antibody and T-cell responses (Rollier et al. 2007). Despite these immune responses, vaccinated animals showed a higher rate of persistence than control animals. That HCV vaccine failures have occurred in the presence of T-cell responses indicates that trials should proceed with caution and include detailed immune response analysis aimed at defining responses associated with control. In addition to the limitations of prior studies as a result of the small number of chimpanzees tested, the diversity of the vaccines tested, and the different methods of immune response analysis, it is also unclear whether the results from nonhuman primates can be translated to humans. More detailed phenotypic and functional analyses performed on existing and future trial specimens will be needed to gain insight into factors that determine whether a vaccine will reduce the rate of persistent infection in humans.

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Human Trials of HCV Vaccines Designed to Elicit T-Cell Responses

Two vaccines designed to prevent infection solely by eliciting T-cell immunity have advanced to immunogenicity trials in human volunteers not at risk for HCV infection. A prototype vaccine with the HCV core protein and ISCOMATRIX adjuvant was assessed for its ability to induce T-cell responses in healthy individuals not at risk for HCV infection (Drane et al. 2009). Although the vaccine was generally well tolerated, $CD8^+$ T-cell responses were only detected in two of the eight participants receiving the highest dose, and the vaccine was thus not advanced into an at-risk population. A second vaccine composed of a replication-defective chimpanzee adenovirus (ChAd) vector encoding NS3, NS4, and NS5 proteins elicited robust T-cell responses in healthy volunteers, and is the foundation of the only HCV vaccine trial completed in at-risk human subjects (Swadling et al. 2014).

Replication-defective adenovirus vectors have long been studied as a means to introduce antigens from other pathogens. Human adenovirus serotype 5 (Ad5) elicits robust and sustained cellular immunity in humans. However, most humans have neutralizing antibodies (NAbs) to Ad5 because of natural exposure, which can reduce immune responses to the antigens carried in Ad5-based vaccines. Replication-defective adenoviral vectors based on serotype 6 (subgroup C) and serotype 24 (subgroup D) have low seroprevalence in humans, reducing immunologic cross-reactivity (Colloca et al. 2012). A segment of DNA coding for NS3-5 of the HCV genotype 1b was delivered by Ad6 followed by Ad24 and, finally, by electroporated plasmid DNA in chimpanzees (Folgori et al. 2006). Following HCV rechallenge with HCV genotype 1a virus, all five vaccinated chimpanzees showed significantly lower alanine amino transferase (ALT) levels and blunted peak of viremia with the average peak more than 100 times lower than in the control group, kinetics similar to those observed in PWID with successful control of repeated HCV exposure (Osburn et al. 2010). Four of the five vaccinated chimpanzees cleared the virus with a significantly shorter duration of viremia versus the control group, whereas one vaccinated chimpanzee maintained low levels of HCV RNA for the duration of the study. A follow-up study revealed that after challenge, vaccinated chimpanzees showed early expansion of $CD8^+$ T cells with higher expression of the memory precursor molecule CD127, lower levels of the inhibitory molecule PD-1, and enhanced effector functions when compared with primary T cells from the mock-vaccinated controls that developed persistent infections (Park et al. 2012). Analogous to this, early expansion of $CD127^+$ HCV-specific T cells with high functionality was shown in chimpanzees that spontaneously control acute HCV infection (Shin et al. 2013).

Replication-defective vectors generated from a subset of novel ChAd serotypes were then screened to identify those not neutralized by human sera and able to grow in human cell lines (Colloca et al. 2012). Of these, ChAd3 expressing the NS region from HCV was found to induce long-lasting T- and B-cell memory responses in mice and macaques. Ad6 and ChAd3 were used as vectors to express NS proteins from HCV genotype 1b and tested as vaccines in a safety and immunogenicity phase 1 clinical trial in healthy volunteers not at risk for HCV infection (Barnes et al. 2012). ChAd3-NS was well tolerated and highly immunogenic, with intracellular cytokine staining showing that ChAd3-NSmut primed a large number of polyfunctional $CD8^+$ T cells. Antigen-specific polyfunctional $CD4^+$ T cells were detected at a lower frequency. Memory $CD8^+$ T cells that expressed CD127, but not PD-1, were sustained in circulation. Although more robust recognition of HCV genotype 1b peptides was observed, T cells recognized genotype 1a and 3a peptide pools, suggesting potential cross-genotypic protection. Central and effector memory T-cell pools retained polyfunctionality and proliferative capacity for at least a year after boosting. Boosting was subsequently found to be more robust with modified MVA than with heterologous adenovirus (Swadling et al. 2014) so the vaccine was advanced to HCV at-risk subjects with ChAd3 prime and MVA boost, both expressing NS3-5.

The ChAd3-NS prime and MVA-NS boost strategy is now being analyzed for efficacy following completion of a staged phase I/II study (see ClinicalTrials.gov, NCT01436357). The primary end point of this study is to prevent HCV persistence in HCV-naive populations of PWID at high risk for infection with immunogenicity and incidence also being assessed. This trial shows the feasibility of conducting HCV vaccine trials in PWID with full results to be released in 2019.

HUMORAL IMMUNITY TO HCV INFECTION

Many licensed prophylactic vaccines against other viral infections induce neutralizing or binding antibody titers that correlate with protection. However, the role on antibodies in HCV infection is defined less clearly. The HCV envelope genes (E1E2) are extremely diverse, and rapid evolution of envelope proteins permits escape from the humoral immune response in persistent infection (Dowd et al. 2009). Several recent studies have shown that antibody resistance can arise from mutations either within or distant from antibody-binding epitopes, providing multiple viral mechanisms of immune escape (Carlsen et al. 2014; Bailey et al. 2015; El-Diwany et al. 2017). However, both humans and animal models of HCV infection show that NAbs can also protect. Clearance of HCV infection is associated with the early development of serum antibodies capable of blocking infection by multiple heterologous HCV strains (Logvinoff et al. 2004; Pestka et al. 2007; Osburn et al. 2014). The appearance later in infection of NAbs has also been associated temporally with spontaneous HCV clearance (Raghuraman et al. 2012). Clearance of reinfection was associated with rapid induction of bNAbs, as well as broadening of T-cell responses (Osburn et al. 2010). Although envelope sequence evolution permits humoral escape, acquisition of resistance to bNAbs by some autologous strains has also been accompanied by progressive loss of envelope protein function and temporally associated with HCV clearance (Kinchen et al. 2018). Thus, bNAbs can mediate clearance of human HCV infection by driving escaped viruses to an unfit state, as well as by neutralizing infecting strains. Combinations of human NAbs targeting multiple epitopes can display complementary neutralizing breadth and, in some cases, neutralizing synergy (Mankowski et al. 2018). Notably, bNAbs displaying low levels of somatic hypermutation similar to those stimulated by vaccination against other viruses have been isolated from the B cells of individuals who cleared HCV infection without treatment, suggesting that vaccine induction of bNAbs against HCV may be feasible (Scherer et al. 2014; Wang et al. 2015; Merat et al. 2016; Bailey et al. 2017).

Antibodies can also provide protection against HCV in animal challenge models. Infusion of immunoglobulin isolated from a chronically infected human before challenge with homologous virus prevented infection of most human liver chimeric mice and infusion before chimpanzee challenge prevented infection with homologous but not heterologous HCV strains (Vanwolleghem et al. 2008; Meuleman et al. 2011; Bukh et al. 2015). Infusion of bNAbs before challenge with heterologous virus could partially or fully prevent infection in humanized mice (Law et al. 2008; Giang et al. 2012; Keck et al. 2016) and chimpanzees (Morin et al. 2012), and combinations of bNAbs also abrogated established HCV infection in human liver chimeric mice (de Jong et al. 2014). Together, these studies show that strain-specific NAbs will not be sufficient, and induction of bNAbs is likely necessary to prevent infection by diverse, heterologous HCV strains.

TRIALS OF VACCINES DESIGNED TO INDUCE HUMORAL IMMUNITY

Strategies developed to induce antibodies against HCV have included protein-based, DNA-based, VLP-based, pox virus–based, and whole virus–based vaccines (Table 2). A vaccine composed of recombinant full-length E1E2 protein from a single genotype 1a HCV strain with oil-in-water adjuvant has been tested in rodents, nonhuman primates, and humans. This vaccine induced heterologous neutralizing activity in guinea pigs (Stamataki et al. 2007), and was protective against homologous HCV challenge in

chimpanzees (Choo et al. 1994). Although it did not prevent infection, it reduced rates of persistence in chimpanzees after challenge with a neutralization-sensitive heterologous virus (Houghton 2011). However, postvaccination bNAb titers in a phase 1a human trial were detectable in only three of 16 vaccinees despite induction of robust $CD4^+$ T-cell proliferation in response to recombinant E1E2, suggesting that further optimization of the vaccine antigen or adjuvant may be needed to maximize bNAb induction (Frey et al. 2010; Law et al. 2013).

Vaccine Other than Recombinant Full-Length E1E2 Protein

Other vaccines designed to induce bNAbs, including those expressing envelope proteins in vivo using DNA vaccination, using DNA priming followed by recombinant MVA boost, or expressing envelope proteins on VLPs have all induced relatively disappointing humoral responses in chimpanzees (for review, see Liang 2013; Bukh 2016). In one study, two chimpanzees vaccinated with DNA designed to express cell-surface E2 protein developed low levels of E2-specific antibodies (Forns et al. 2000). A DNA prime, MVA boost vaccine expressing core, E1, E2, and NS3 proteins failed to induce detectable NAb titers in chimpanzees (Rollier et al. 2007). In another study, chimpanzees vaccinated with VLPs composed of HCV core, E1, and E2 proteins, which induced strong envelope-specific antibody responses in baboons (Jeong et al. 2004), developed no or barely detectable antibody responses despite having robust T-cell responses against core and E1E2 (Elmowalid et al. 2007). Among varied explanations for the weak NAb responses observed in chimpanzees, HCV-infected chimpanzees generally mount less vigorous antienvelope antibody responses than humans upon infection, highlighting a limitation of chimpanzees as a model (Bassett et al. 1998a,b; Lanford et al. 2001).

As the efficiency of production of chimeric replication–competent cell culture viruses (HCVcc) has improved, use of whole inactivated virus has been explored as a vaccine strategy. In one study, vaccination of mice with inactivated J6/JFH-1 HCVcc stimulated NAb against homologous and two heterologous HCV strains, and infusion of purified immunoglobulin from vaccinated mice prevented homologous viral infection of human liver chimeric mice (Akazawa et al. 2013). Other groups have developed multivalent VLPs or purified recombinant E1E2 protein vaccines expressing envelope genes from multiple genotypes as a means to generate bNAbs. Both induced cross-reactive antibodies in mice, and the multivalent E1E2 protein vaccine induced NAbs against one homologous and one heterologous HCV strain (Christiansen et al. 2018; Krapchev et al. 2018). Truncated protein antigens and rationally designed peptide antigens are also at early stages of development. Studies showing that hypervariable region 1 (HVR1) may block conserved bNAb epitopes (Prentoe et al. 2011) prompted testing of an E2 vaccine antigen with three variable regions deleted (Vietheer et al. 2017). This vaccine induced moderately high titers of bNAbs (Vietheer et al. 2017), but another study showed that full-length E1E2 and a variant with truncated HVR1 induced equivalent titers of NAbs against heterologous HCV strains in guinea pigs, suggesting that truncation of HVR1 may not be necessary (Law et al. 2018). A molecular scaffold presenting an epitope targeted by a potent bNAb induced modest antibody responses in vaccinated mice (Pierce et al. 2017). Overall, more research is needed to identify ideal vaccine antigens and platforms for immunization to induce potent bNAbs. Additionally, it is unclear that anti-HCV antibody responses induced in rodents or nonhuman primates are predictive of vaccine responses in humans, highlighting the need for identification of correlates of protective immunity and future human trials.

POPULATIONS TO BENEFIT FROM VACCINATION

Those at higher than average risk of HCV infection include PWID, MSM, health care workers with frequent exposure to blood and bodily fluids, infants born to HCV-infected mothers, and those living in countries with high HCV incidence. In addition, restoration of immunity fol-

lowing treatment is insufficient to prevent HCV reinfection in those at ongoing risk of infection. Reinfection rates vary, but are high when those at greatest risk of HCV exposure and transmission are treated, in part, as a means to interrupt transmission (Martin et al. 2013; Pineda et al. 2015; Midgard et al. 2016; Martinello et al. 2017). A recent study in PWID treated while still actively injecting showed 6-month and 18-month reinfection rates of 12.6 and 17.1 per 100 person-years, respectively (Schulkind et al. 2019). Thus, those previously treated and at ongoing risk of infection should be added to the list of those who would be expected to benefit from a preventative HCV vaccine. The impact of a prophylactic HCV vaccine has been modeled, including models that vary delivery strategy and levels of vaccine efficacy (Hahn et al. 2009; Scott et al. 2015; Stone et al. 2016). Using these models, high vaccination rates of high-risk seronegative PWID using a vaccine with as little as 30% efficacy had significant impact. The most effective vaccines might prevent infection, but vaccines that prevent persistence of infection could decrease HCV morbidity and mortality without reducing incidence. The feasibility of testing vaccines that could reduce rates of chronic HCV infection or HCV RNA levels rather than incidence has been studied, and vaccines reducing viremia during the early acute phase of infection also have potential to reduce HCV transmission by lowering the residual infectious virus titers in injecting equipment (White et al. 2014; Major et al. 2018). Thus, setting goals for decreased persistence rates or population HCV RNA levels following vaccination may be reasonable given the potential to impact disease sequelae and transmission.

CONCLUDING REMARKS

There remain some significant deficits in our tool chest in the quest for an HCV vaccine. Elucidating the mechanisms through which antigen-specific immune cell populations mediate long-term protection will continue to be an important goal. Going forward, successful control of HCV infection will most likely require a combination of mass global screening to identify those with infection, treatment of those infected, and prevention and harm-reduction strategies for those who are uninfected and at risk. Although there remain challenges, a prophylactic HCV vaccine is likely to be required as part of a successful strategy for global control.

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