REVIEW 10.1111/1469-0691.12493

Why is it so difficult to develop a hepatitis C virus preventive vaccine?

C. Zingaretti, R. De Francesco and S. Abrignani
Istituto Nazionale Genetica Molecolare (INGM), Milano, Italy

Abstract

With an estimated 3% of the world's population chronically infected, hepatitis C virus (HCV) represents a major health problem for which an efficient vaccination strategy would be highly desirable. Indeed, chronic hepatitis C is recognized as one of the major causes of cirrhosis, hepatocarcinoma and liver failure worldwide and it is the most common indication for liver transplantation, accounting for 40–50% of liver transplants. Much progress has been made in the prevention of HCV transmission and in therapeutic intervention. However, even if a new wave of directly acting antivirals promise to overcome the problems of low efficacy and adverse effects observed for the current standard of care, which include interferon-α and ribavirin, an effective vaccine would be the only means to definitively eradicate infection and to diminish the burden of HCV-related diseases at affordable costs. Although there is strong evidence that the goal of a prophylactic vaccine could be achieved, there are huge development issues that have impeded reaching this goal and that still have to be addressed. In this article we address the question of whether an HCV vaccine is needed, whether it will eventually be feasible, and why it is so difficult to produce.

Keywords: Chronic infection, envelope glycoproteins, hepatitis C, hepatitis C virus, vaccine
Article published online: 19 December 2013
Clin Microbiol Infect 2014; **20** (Suppl. 5): 103–109

Corresponding author: S. Abrignani, Via Francesco Sforza 35, Milano 20122, Italy
E-mail: abrignani@ingm.org

Introduction

No vaccine for the prevention of hepatitis C virus (HCV) infection is yet available. The current standards of care, based on the combination of interferon-α (IFN-α), ribavirin (RBV) and—for genotype 1 infections—NS3 protease inhibitors, still have suboptimal rates of success, are burdened by considerable side effects, and are unlikely to be widely used because of very high costs [1]. Given that HCV is responsible for a substantial proportion of chronic liver diseases worldwide, including cirrhosis and hepatocellular carcinoma, and that more than 170 million people are infected [2], a prophylactic vaccine represents an unmet medical need that would be of great public health benefit. Indeed, vaccination has proved to be the most successful and cost-effective strategy to prevent infections and associated diseases.

Among the difficulties that have hampered the development of a vaccine against HCV there are its extreme genetic variability, the lack of small animal models for testing vaccines, and the fact that a cell culture system supporting the production of infectious HCV and allowing studies on virus neutralization *in vitro* became available only recently [3–5].

Despite these issues, much progress has been made toward the identification of promising prophylactic vaccine candidates, and some of these candidate vaccines have entered clinical trials. However, several issues concerning development and efficacy assessment of HCV vaccines remain to be addressed. Should these be overcome, the control of HCV spread would appear a realistic goal to achieve.

The Virus

Hepatitis C virus is a small, enveloped, single-stranded RNA virus classified as a member of the Hepacivirus genus within the Flaviviridae family [6]. The 9.6-kb positive-sense RNA genome contains a single open reading frame encoding a polyprotein of about 3000 amino acids, which, upon translation, is cleaved into ten different structural (core and envelopes gpE1 and gpE2) and non-structural (p7, NS2, NS3, NS4A, NS4B, NS5A and NS5B)

proteins [7]. An impressively high replication rate (10^{12} virions/day) [8] and the lack of proof-reading activity by the viral RNA-dependent RNA polymerase account for the high genetic variability of the virus [9]. Seven major genotypes (with 30–35% variability in nucleotide sequence) and many subtypes (15–20% variability) have been distinguished phylogenetically. Moreover, HCV circulates within an infected individual as distinct 'quasi-species' [10].

Acute HCV infection is usually subclinical and 50–85% of infected individuals develop chronic infection, a condition leading to cirrhosis and, in some cases, to liver failure or hepatocellular carcinoma over a period of 10–30 years [11]. Chronic HCV infection is one of the main causes of liver cirrhosis and cancer [12,13] and >350 000 people die from hepatitis C-related liver diseases every year. Moreover, chronic HCV infection has been associated with several extrahepatic diseases [14,15] including mixed cryoglobulinaemia [16] and non-Hodgkin's B-cell lymphoma [17].

Transmission of HCV occurs via direct parenteral exposure to contaminated blood. HCV epidemiology has changed over the last two decades: transfusion-associated transmission has disappeared with the advent of serological tests based on the detection of antibodies or HCV RNA. At present, a history of intravenous drug injection represents the major risk factor for newly acquired HCV infection (65%) [18], followed by sexual (15%) [19] and occupational (4%) risks. Vertical transmission occurs particularly in mothers with high viral load or who are co-infected with human immunodeficiency virus (HIV) (<5%) [20–22].

Do We Need a Preventive HCV Vaccine?

Despite a substantial decline in HCV transmission due to improved prevention strategies and the introduction of new powerful targeted therapies, hepatitis C remains a huge health problem and there are several reasons for continuing to pursue the objective of a vaccine. On the one hand, mortality and morbidity due to HCV are not going to decrease. HCV has surpassed HIV as a cause of mortality in the USA [23] and the burden of HCV-related liver disease is projected to increase in the next decade [24]. Indeed, the pool of asymptomatic chronic HCV carriers who represent an infectious reservoir will remain substantial for many years and *c.*50 000 new infections still occur annually in the USA and Europe (CDC data). On the other hand, therapeutic approaches to HCV raise many concerns: current treatments, based on the IFN-α/RBV backbone, are expensive, poorly tolerated and lead to sustained virological response in about half of patients. New directly acting antivirals that target specific HCV proteins are emerging for the treatment of hepatitis C and results of late-stage clinical trials with IFN-free combinations of directly acting antivirals suggest that highly effective therapy to cure HCV infection will be available in the near future [1]. However, the very high costs make new antiviral therapy unlikely to be sustainable for public health systems. Moreover, the treatment rate is very low for HCV-infected patients. Indeed, <30% of patients with chronic hepatitis C are aware of the infection and only about 10% of patients are currently treated [25–27]. Therefore, even if new antivirals could cure 90% of patients there would still be a considerable percentage of patients that would be excluded.

An efficient HCV vaccine would represent the most cost-effective and realistic means to significantly reduce the worldwide mortality and morbidity associated with HCV infection. An HCV vaccine would be initially indicated for persons at risk of coming into contact with contaminated blood, whereas a universal vaccination strategy could be recommended later.

Is a Preventive HCV Vaccine Doable?

The observation that effective immune responses may occur during the natural course of HCV infection supports the feasibility of an effective vaccine. Indeed, in contrast to HIV infection, a sizable proportion (20–35%) of infected persons can clear the virus spontaneously. Generally, this is observed in the first 6–9 months of infection [28,29], and it has been shown that individuals who have cleared infection are less likely to develop chronic infection when re-exposed [30,31]. Collectively these data provide evidence that natural immunity to HCV exists and that cross-protective immunity within and between common HCV genotypes can be achieved.

Adaptive immunity is unequivocally mediated by one or more of the following: neutralizing antibodies, $CD4^+$ T-cell-mediated inflammation and $CD8^+$ T-cell-mediated cytotoxicity. In the case of HCV, there is strong evidence that the rapid induction of high-titre cross-neutralizing antibodies targeting HCV envelope proteins correlates with viral clearance and protects from re-infection [31–34]. On the other hand, the importance of broad HCV-specific $CD4^+$ T helper and $CD8^+$ cytotoxic responses in the resolution of infection has been suggested by many studies [28,29,35–40]. Therefore, an optimal HCV vaccine probably needs to elicit broad cross-reactive cellular immune responses together with cross-neutralizing antibodies.

As it is the development of chronic infection that causes clinically evident disease, whereas acute infection is mostly silent and without clinical consequences, an HCV vaccine that

prevented chronic infection, allowing a self-limited acute infection, would be acceptable.

Several vaccine strategies, listed in Table 1 and reviewed elsewhere [41–43], have been explored to identify suitable candidates, including subunit vaccines and genetic vaccines (plasmid DNA and viral vector vaccines).

Promising results have been obtained with adjuvanted recombinant gpE1/gpE2 heterodimer vaccine (Tables 2 and 3). When used to immunize naive chimpanzees that were then challenged with a heterologous HCV strain, only one out of nine developed chronic infection [44], whereas most control animals became chronically infected following viral challenge. Vaccination induced inflammatory $CD4^+$ T-cell responses as well as high titres of anti-gpE1/gpE2 antibodies capable of blocking the binding to CD81, the main HCV receptor on hepatocytes. Moreover, although immunization with polypeptide subunits does not result in direct stimulation of $CD8^+$ T cells, a high number of intrahepatic, HCV-specific $CD8^+$ cytotoxic T lymphocytes secreting IFN-γ was observed in vaccinated chimps 10 weeks after the challenge, and the percentage of these cells was inversely correlated with viral loads [45]. However, not all animals with high anti-E1E2 titres and/or high $CD8^+$ T-cell activity were able to eradicate the infection even though acute viraemia was suppressed. As the result of encouraging preclinical results, the recombinant gpE1/gpE2 heterodimer, formulated in an oil–water adjuvant MF59, was among the earliest preventive vaccine candidates to enter clinical trials. A phase I study has revealed that this vaccine is safe and well tolerated and that it elicits anti-gpE1/gpE2 antibodies able to cross-neutralize major HCV genotypes and $CD4^+$ T-lymphocyte responses to HCV envelope proteins (Table 3) [46,47].

It should be pointed out that the relevance of a humoral response in resolving HCV infection is still controversial. Anti-envelope antibodies are detected in nearly all chronically infected patients but do not always have neutralizing properties. However, the advancements in design and selection of immunogens may greatly improve the efficacy of a vaccine based on the elicitation of antibody response. For instance, it is now possible to interrogate in great detail the B-cell repertoire of HCV-infected patients, in particular those patients that have cleared acute HCV infection, and to identifiy several HCV cross-neutralizing antibodies that in turn could lead to the identification of E1E2 broadly neutralizing epitopes. This approach may guide the structural design of novel E1E2 antigens with improved efficacy. The same approach has been extensively adopted in the HIV field with impressive results, and a few HCV broadly neutralizing antibodies have already been described and reviewed [48].

T-cell-based vaccines have also been proposed as vaccine candidates [43,49]. Adenoviral vectors are particularly attractive for this approach because they are the most potent at T-cell priming in primates and humans [50]. The main problem of adenoviral vectors is pre-existing immunity to the vector, which can lead to its clearance before a response is elicited to the inserted immunogen. However, this issue has been overcome by using chimpanzee adenovirus as the gene vehicle [51]. Good results in terms of elicitation of broad $CD4^+$ and $CD8^+$ T-cell response in preclinical studies have been obtained for one of these vaccines, consisting of a chimpanzee adenoviral vector delivering non-structural genes NS3–NS5B, which contain many well-defined $CD4^+$ and $CD8^+$ epitopes (Table 3). After heterologous viral challenge, all animals became HCV-infected but those that were vaccinated presented a 100 times lower average peak of viraemia than controls. Moreover, three out of four chimpanzees presented viraemia of short duration [52]. This T-cell-based vaccine has been shown to be safe, well tolerated

TABLE 1. Clinical-stage vaccine candidates for hepatitis C virus (HCV)

Investigator	Vaccine type	Immunogen	Stage	Application
Chiron/Novartis [46,62,63]	Recombinant protein	Recombinant gpE1/gpE2 in oil/water adjuvants	Phase I [46,63]	Prophylactic vaccine
Okairos [64]	Adenoviral vector vaccine	Adenovirus vectors expressing HCV NS 3, 4 and 5	Phase 2	Prophylactic/Immunotherapeutic vaccine
Innogenetics/ GenImmune [65]	Recombinant protein	Alum-adjuvanted gpE1 glycoprotein	Phase 2 [66] Programme stopped as no effects on viraemia or fibrosis progression	Immunotherapeutic vaccine
Intercell AG (IC41) [67,68]	Peptide	HCV peptide cocktail with polyarginine	Phase 2 in combination with pegylated interferon [69,70]	Immunotherapeutic vaccine
Globe Immune (GI-5005)	Recombinant yeast cells	Heat-killed yeast expressing Core-NS3 fusion protein [71]	Phase 2 in combination with pegylated interferon [72,73]	Immunotherapeutic vaccine
Tripep (ChronVac-C)	Plasmid DNA	DNA-based vaccination with NS3/4A-expressing plasmid	Phase 1b	Immunotherapeutic vaccine
CIGB-230 [74]	Plasmid DNA and recombinant protein	Mixture of plasmid-expressing HCV structural antigens with a recombinant HCV core protein	Phase 1b [75]	Immunotherapeutic vaccine
Transgene (TG4040)	Adenoviral vector vaccine	Modified vaccinia Ankara virus expressing NS proteins (NS3-NS5B)	Phase I	Immunotherapeutic vaccine

TABLE 2. Outcome of chimpanzee vaccination/challenge studies performed with adjuvanted recombinant gpE1/gpE2 formulations

	gpE1/gpE2	Unimmunized control	Statistical significance[b] (p)
Homologous challenge			
Acute infections	7/12[a]	10/10	
Chronic infections (%)	2/12 (17)	7/10 (70)	0.03
Heterologous challenge			
Acute infections	9/9	14/14	
Chronic infections (%)	1/9 (11)	8/14 (57)	0.04
Total			
Acute infections	16/21	24/24	
Chronic infections (%)	3/21 (14)	15/24 (63)	0.002

Animals were immunized with 30–80 μg gpE1/gpE2 in various oil/water adjuvants on months 0, 1 and 6 approximately, followed by intravenous challenge 2–4 weeks later with hepatitis C virus 1 (HCV-1) or HCV-H. Both strains belong to the 1a subtype. Circulating levels of viraemia were measured using RT-PCR assays for HCV genomic RNA for at least 1 year post-challenge.
Adapted from reference [44].
[a]In five vaccinees challenged with homologous HCV-1, no viraemia could be detected at any time after challenge in either plasma peripheral blood mononuclear cells or liver biopsy samples and so were considered to have been sterilized.
[b]p-values (Fishers exact test) refer to chronic carrier rates between controls and vaccinees.

and highly immunogenic in phase I studies and has now moved to a phase II trial [53].

Overall, these results suggest that the goal of an effective vaccine, protecting from different HCV strains and preventing chronic infection, should be achievable and could be obtained by strategies that combine the elicitation of broadly cross-neutralizing antibodies with the induction of strong HCV-specific T-cell responses.

Why Is It So Difficult to Make One?

In contrast to another epidemic hepatotropic virus, hepatitis B virus (HBV), for which a vaccine has been successfully developed, a vaccine against HCV represents a significant challenge. Many viral and non-viral factors can be taken into account to explain why it is so difficult to develop an HCV vaccine (Table 4).

Hepatitis C virus displays extremely high genetic diversity (30% of nucleotide divergence between genotypes) compared with HBV (8% divergence between different genotypes) [54]. HCV has evolved several effective immune escape strategies, leading to a very high rate of chronic hepatitis following acute infection (50–80%). Conversely, only 5–10% of immunocompetent adults develop chronic hepatitis after acute infection with HBV [55]. The mechanisms of HCV evasion from the host immune system affect both innate and adaptive immune responses. Evasion from the innate immune system includes impairment of dendritic and natural killer cell responses and

TABLE 3. Summary of main results obtained for adjuvanted gpE1/gpE2 heterodimer vaccine and for NS3-5B adenoviral vaccine

Vaccine	Vaccine type	Study type	Description	Status	Main results	Elicited immune response
Chiron corp gpE1/gpE2 Heterodimer	Subunit vaccine	Preclinical	Prophylactic efficacy in Chimpanzee model	Completed	Protection from chronic hepatitis after challenge with homologous and heterologous strain	High titres of anti-E1E2 nAb, which correlate inversely with viral titres; hepatitis C virus (HCV) specific $CD4^+$ T-cell response
		Phase I	Blinded randomized dose-ranging study in 60 healthy volunteers	Completed	Satisfactory safety and tolerability	Anti-E1E2 antibodies able to cross-neutralize major HCV genotypes with titres between 100 and 2000 (protected chimps range); very strong $CD4^+$ T-cell responses to E1E2
		Phase II	Proof-of-concept field trial in high-risk subjects	Stopped at planning stage		
Okairos NS3-5B	Adenoviral vector vaccine	Pre-clinical	Prophylactic efficacy in Chimpanzee model	Completed	Amelioration of acute hepatitis and acute viraemia in vaccinees after challenge with heterologous strain. No significant difference in carrier rates between vaccinees and controls	Broad $CD4^+$ and $CD8^+$ T lymphocytes
		Phase I	Blinded randomized study in 40 healthy volunteers	Completed	Safe and highly immunogenic	Broad $CD4^+$ and $CD8^+$ T lymphocytes secreting multiple cytokines (interleukin-2, interferon-γ, tumour necrosis factor-α) targeting multiple epitopes and recognizing heterologous strains. Presence of polyfunctional and proliferative long-term memory population after 1 year
		Phase I/II	Randomized, double-blind, placebo control in intravenous drug user population; (Phase I: 68 volunteers; Phase II: 276 additional volunteers)	Ongoing (results expected in 2015/2016)		

TABLE 4. Summary of the main obstacles to the development of an effective hepatitis C virus (HCV) vaccine as compared to the successfully developed hepatitis B virus (HBV) vaccine

	HCV	HBV
Viral factors	High rate of development of chronic infection following asymptomatic acute infection in most immunocompetent hosts; High genetic variability between different genotypes (30–35%); High variability and heavy N-glycosylation of external proteins; Several mechanisms of evasion from host innate and adaptive immune system evolved	Asymptomatic or clinical acute infection with rare chronic infection development in most immunocompetent hosts; Low genetic variability between different genotypes (8%); The envelope protein (hepatitis B surface antigen) is very conserved and have minor post translational modifications
Efficacy assessment factors	No clear correlate of protection identified; No simple cohort available for efficacy trials; No immunocompetent small animal model available; Prevention of chronic infection is technically difficult to assess	Neutralizing antibodies represent correlate of protection; Efficacy trials relatively easy and doable in high incidence populations at time of vaccine development
Technical issues	The glycoproteins E1/E2 are very complex to scale up, because they have to be purified as intracellular material from the endoplasmic reticulum of mammalian cells	Recombinant hepatitis B surface antigen is easily produced in yeast cells
Other factors	The myth has been spread that HCV will disappear in a few years and that in any case new antiviral agents will finish the job; as a result there private or public investments in the HCV vaccine field are scarce	
	Vaccine still under development	Highly efficacious vaccine available (estimated >1 000 000 deaths prevented worldwide)

reduction of type-I IFN induction [56–59]. Among the mechanisms used to escape from the adaptive immune response, of crucial importance are the continuous generation of new viral variants occurring during HCV infection, allowing the virus to escape the action of HCV-specific $CD8^+$ T cells and neutralizing antibodies, and the exhaustion of $CD8^+$ T cells observed during chronic infections, which leads to a reduced ability both to secrete antiviral cytokines (including IFN-γ) and to proliferate in response to antigen stimulation [56]. Another evasion mechanism is the ability of HCV to be transferred to naive cells through direct cell–cell contact in a CD81-independent way, so avoiding the effect of neutralizing antibodies [60]. Finally, single point mutations, glycosylation site modifications and conformational changes occur in HCV glycoproteins during HCV evolution and contribute to the virus' adaptation to humoral pressure. Conversely, the HBV envelope protein (i.e. the hepatitis B surface antigen, or HBsAg), is highly conserved and undergoes minor post-translational modification.

Apart from these intrinsic obstacles, the exact correlate of immune protection against HCV infection has not been identified, possibly because of the heterogeneity contained within small study populations of patients together with the limitations in terms of animal models of HCV infection.

The planning and execution of clinical trials for efficacy assessment pose many questions as well. Because of the relatively low incidence of new infections in the developed world, it is not trivial to identify the appropriate at-risk population to enrol. Post-transfusion infection risk has been eliminated by donor screening, and other high-risk groups suitable for efficacy testing can have inherent difficulties such as lack of compliance (in intravenous drug users), low incidence of infection (healthcare workers), lack of supporting infrastructure (in many developing countries where incidence of infection is high) and ethical issues (in prisoner populations where prevalence and incidence of infection are both high). However, some of these cohorts have been used successfully for testing HBV vaccine, and so these obstacles should not be insuperable.

As discussed above, in the case of HCV, a vaccine that allowed only a 'transient infection', while preventing the development of chronic HCV infection, would be as beneficial as one that provided sterilizing immunity. However, the design of an efficacy trial intended to measure prevention of chronic infection is not straightforward, in particular in the absence of a strong correlate of immunity. In fact, studies on patients with acute hepatitis C have shown that HCV infections treated with pegylated IFN-α during the acute phase are eradicated in virtually all patients [61]. As the chronic state of the infection is defined starting from 6 months after onset, it becomes very difficult to conclude an efficacy trial. When an infected vaccinee is detected, IFN treatment will have to be started without delay and it will be impossible to establish whether the infection will become chronic. For this reason, for example, a phase II, proof-of-concept trial with adjuvanted E1/E2 heterodimer was stopped at planning stage.

Finally, developmental issues exist for gpE1/gpE2 vaccine. Indeed, the heterodimer has to be purified as intracellular material from endoplasmic reticulum of mammalian cells, making it very difficult to scale up its production.

Conclusions

Only a few years ago, the prospects for effective vaccination against HCV were considered remote. The situation today is more optimistic for several reasons. First, we now know that the spontaneous eradication of virus occurs in a consistent fraction of acute infections and is associated with specific immune responses to the virus. Second, clear evidence

indicates that, despite the high percentage of chronic HCV infections, the immune system is capable of a response that leads to HCV clearance and protection against re-infection.

In light of this, several efforts have been made to identify and develop vaccine candidates recapitulating these immune responses. Some of these candidates have given promising results in the chimpanzee model of infection and have subsequently moved to clinical trials. However, many issues still remain to be resolved before a vaccine against HCV can be achieved. A clear correlate of protection has not yet been identified. Moreover, not only is it difficult to identify the appropriate at-risk population to enrol in an efficacy trial for a preventive HCV vaccine, but it may also be very difficult to conclude an efficacy trial designed to measure prevention of chronic infection. If these obstacles are removed, the prospect of a vaccine appears a realistic objective for the near future. Considering that HCV is the first cause of primary liver cancer, this would mean that an important cause of global morbidity and mortality would be controlled.

Transparency Declaration

The authors declare no conflicts of interest.

References

1. Aghemo A, De Francesco R. New horizons in hepatitis C antiviral therapy with direct-acting antivirals. *Hepatology* 2013; 58: 428–438.
2. Shepard CW, Finelli L, Alter MJ. Global epidemiology of hepatitis C virus infection. *Lancet Infect Dis* 2005; 5: 558–567.
3. Lindenbach BD, Evans MJ, Syder AJ *et al.* Complete replication of hepatitis C virus in cell culture. *Science* 2005; 309: 623–626.
4. Pietschmann T, Kaul A, Koutsoudakis G *et al.* Construction and characterization of infectious intragenotypic and intergenotypic hepatitis C virus chimeras. *Proc Natl Acad Sci USA* 2006; 103: 7408–7413.
5. Wakita T, Pietschmann T, Kato T *et al.* Production of infectious hepatitis C virus in tissue culture from a cloned viral genome. *Nat Med* 2005; 11: 791–796.
6. Lindenbach BD, Murray CL, Thiel H-J, Rice CM. Flaviviridae: The Viruses and Their Replication. In: Knipe DM, Howley P, eds. *Fields virology*, 6th edn. New York: Lippincott Williams & Wilkins, 2013; 712–746.
7. Bartenschlager R, Penin F, Lohmann V, Andre P. Assembly of infectious hepatitis C virus particles. *Trends Microbiol* 2011; 19: 95–103.
8. Neumann AU, Lam NP, Dahari H *et al.* Hepatitis C viral dynamics *in vivo* and the antiviral efficacy of interferon-α therapy. *Science* 1998; 282: 103–107.
9. Bowen DG, Walker CM. Mutational escape from $CD8^+$ T cell immunity: HCV evolution, from chimpanzees to man. *J Exp Med* 2005; 201: 1709–1714.
10. Simmonds P. The origin of hepatitis C virus. *Curr Top Microbiol Immunol* 2013; 369: 1–15.
11. Alter HJ, Seeff LB. Recovery, persistence, and sequelae in hepatitis C virus infection: a perspective on long-term outcome. *Semin Liver Dis* 2000; 20: 17–35.
12. Levrero M. Viral hepatitis and liver cancer: the case of hepatitis C. *Oncogene* 2006; 25: 3834–3847.
13. Jacobson IM, Davis GL, El-Serag H, Negro F, Trepo C. Prevalence and challenges of liver diseases in patients with chronic hepatitis C virus infection. *Clin Gastroenterol Hepatol* 2010; 8: 924–933, e117.
14. Hoofnagle JH. Course and outcome of hepatitis C. *Hepatology* 2002; 36: S21–S29.
15. Sene D, Limal N, Cacoub P. Hepatitis C virus-associated extrahepatic manifestations: a review. *Metab Brain Dis* 2004; 19: 357–381.
16. Charles ED, Dustin LB. Hepatitis C virus-induced cryoglobulinemia. *Kidney Int* 2009; 76: 818–824.
17. Marcucci F, Mele A. Hepatitis viruses and non-Hodgkin lymphoma: epidemiology, mechanisms of tumorigenesis, and therapeutic opportunities. *Blood* 2011; 117: 1792–1798.
18. Alter MJ. Prevention of spread of hepatitis C. *Hepatology* 2002; 36: S93–S98.
19. Piazza M, Sagliocca L, Tosone G *et al.* Sexual transmission of the hepatitis C virus and efficacy of prophylaxis with intramuscular immune serum globulin. A randomized controlled trial. *Arch Intern Med* 1997; 157: 1537–1544.
20. Hershow RC, O'Driscoll PT, Handelsman E *et al.* Hepatitis C virus coinfection and HIV load, $CD4^+$ cell percentage, and clinical progression to AIDS or death among HIV-infected women: Women and Infants Transmission Study. *Clin Infect Dis* 2005; 40: 859–867.
21. Zanetti AR, Tanzi E, Romano L *et al.* Multicenter trial on mother-to-infant transmission of GBV-C virus. The Lombardy Study Group on Vertical/Perinatal Hepatitis Viruses Transmission. *J Med Virol* 1998; 54: 107–112.
22. Weiner AJ, Thaler MM, Crawford K *et al.* A unique, predominant hepatitis C virus variant found in an infant born to a mother with multiple variants. *J Virol* 1993; 67: 4365–4368.
23. Ly KN, Xing J, Klevens RM, Jiles RB, Ward JW, Holmberg SD. The increasing burden of mortality from viral hepatitis in the United States between 1999 and 2007. *Ann Intern Med* 2012; 156: 271–278.
24. Davis GL, Alter MJ, El-Serag H, Poynard T, Jennings LW. Aging of hepatitis C virus (HCV)-infected persons in the United States: a multiple cohort model of HCV prevalence and disease progression. *Gastroenterology* 2010; 138: 513–521, 521 e511–516.
25. Russmann S, Dowlatshahi EA, Printzen G, Habicht S, Reichen J, Zimmermann H. Prevalence and associated factors of viral hepatitis and transferrin elevations in 5036 patients admitted to the emergency room of a Swiss university hospital: cross-sectional study. *BMC Gastroenterol* 2007; 7: 5.
26. Irving WL. Acute hepatitis C virus infection: a neglected disease? *Gut* 2006; 55: 1075–1077.
27. Morrill JA, Shrestha M, Grant RW. Barriers to the treatment of hepatitis C. Patient, provider, and system factors. *J Gen Intern Med* 2005; 20: 754–758.
28. Thimme R, Oldach D, Chang KM, Steiger C, Ray SC, Chisari FV. Determinants of viral clearance and persistence during acute hepatitis C virus infection. *J Exp Med* 2001; 194: 1395–1406.
29. Lechner F, Wong DK, Dunbar PR *et al.* Analysis of successful immune responses in persons infected with hepatitis C virus. *J Exp Med* 2000; 191: 1499–1512.
30. Mehta SH, Cox A, Hoover DR *et al.* Protection against persistence of hepatitis C. *Lancet* 2002; 359: 1478–1483.
31. Osburn WO, Fisher BE, Dowd KA *et al.* Spontaneous control of primary hepatitis C virus infection and immunity against persistent reinfection. *Gastroenterology* 2010; 138: 315–324.
32. Fafi-Kremer S, Fauvelle C, Felmlee DJ *et al.* Neutralizing antibodies and pathogenesis of hepatitis C virus infection. *Viruses* 2012; 4: 2016–2030.
33. Pestka JM, Zeisel MB, Blaser E *et al.* Rapid induction of virus-neutralizing antibodies and viral clearance in a single-source outbreak of hepatitis C. *Proc Natl Acad Sci USA* 2007; 104: 6025–6030.

34. Raghuraman S, Park H, Osburn WO, Winkelstein E, Edlin BR, Rehermann B. Spontaneous clearance of chronic hepatitis C virus infection is associated with appearance of neutralizing antibodies and reversal of T-cell exhaustion. *J Infect Dis* 2012; 205: 763–771.
35. Dubuisson J, Penin F, Moradpour D. Interaction of hepatitis C virus proteins with host cell membranes and lipids. *Trends Cell Biol* 2002; 12: 517–523.
36. Gruner NH, Gerlach TJ, Jung MC *et al.* Association of hepatitis C virus-specific $CD8^+$ T cells with viral clearance in acute hepatitis C. *J Infect Dis* 2000; 181: 1528–1536.
37. Missale G, Bertoni R, Lamonaca V *et al.* Different clinical behaviors of acute hepatitis C virus infection are associated with different vigor of the anti-viral cell-mediated immune response. *J Clin Invest* 1996; 98: 706–714.
38. Rosen HR, Miner C, Sasaki AW *et al.* Frequencies of HCV-specific effector $CD4^+$ T cells by flow cytometry: correlation with clinical disease stages. *Hepatology* 2002; 35: 190–198.
39. Tsai SL, Liaw YF, Chen MH, Huang CY, Kuo GC. Detection of type 2-like T-helper cells in hepatitis C virus infection: implications for hepatitis C virus chronicity. *Hepatology* 1997; 25: 449–458.
40. Frese M, Schwarzle V, Barth K *et al.* Interferon-γ inhibits replication of subgenomic and genomic hepatitis C virus RNAs. *Hepatology* 2002; 35: 694–703.
41. Abrignani S, Reali E, Hoghton M, De Francesco R. Hepatitis C vaccines. In: Plotkin SA, Orenstein WA, Offit PA, eds., *Vaccines*, 6th edn. Philadelphia: ELSEVIER Saunders, 2012; 1074–1084.
42. Fauvelle C, Lepiller Q, Felmlee DJ *et al.* Hepatitis C virus vaccines—progress and perspectives. *Microb Pathog* 2013; 58: 66–72.
43. Swadling L, Klenerman P, Barnes E. Ever closer to a prophylactic vaccine for HCV. *Expert Opin Biol Ther* 2013; 13: 1109–1124.
44. Houghton M, Abrignani S. Prospects for a vaccine against the hepatitis C virus. *Nature* 2005; 436: 961–966.
45. Erickson AL, Houghton M, Choo QL *et al.* Hepatitis C virus-specific CTL responses in the liver of chimpanzees with acute and chronic hepatitis C. *J Immunol* 1993; 151: 4189–4199.
46. Frey SE, Houghton M, Coates S *et al.* Safety and immunogenicity of HCV E1E2 vaccine adjuvanted with MF59 administered to healthy adults. *Vaccine* 2010; 28: 6367–6373.
47. Stamataki Z, Coates S, Abrignani S, Houghton M, McKeating JA. Immunization of human volunteers with hepatitis C virus envelope glycoproteins elicits antibodies that cross-neutralize heterologous virus strains. *J Infect Dis* 2011; 204: 811–813.
48. Kong L, Giang E, Robbins JB *et al.* Structural basis of hepatitis C virus neutralization by broadly neutralizing antibody HCV1. *Proc Natl Acad Sci USA* 2012; 109: 9499–9504.
49. Lauer GM. Immune responses to hepatitis C virus (HCV) infection and the prospects for an effective HCV vaccine or immunotherapies. *J Infect Dis* 2013; 207 (suppl 1): S7–S12.
50. Casimiro DR, Chen L, Fu TM *et al.* Comparative immunogenicity in rhesus monkeys of DNA plasmid, recombinant vaccinia virus, and replication-defective adenovirus vectors expressing a human immunodeficiency virus type 1 gag gene. *J Virol* 2003; 77: 6305–6313.
51. Colloca S, Barnes E, Folgori A *et al.* Vaccine vectors derived from a large collection of simian adenoviruses induce potent cellular immunity across multiple species. *Sci Transl Med* 2012; 4: 115ra112.
52. Folgori A, Capone S, Ruggeri L *et al.* A T-cell HCV vaccine eliciting effective immunity against heterologous virus challenge in chimpanzees. *Nat Med* 2006; 12: 190–197.
53. Barnes E, Folgori A, Capone S *et al.* Novel adenovirus-based vaccines induce broad and sustained T cell responses to HCV in man. *Sci Transl Med* 2012; 4: 115ra111.
54. Kidd-Ljunggren K, Miyakawa Y, Kidd AH. Genetic variability in hepatitis B viruses. *J Gen Virol* 2002; 83: 1267–1280.
55. Hyams KC. Risks of chronicity following acute hepatitis B virus infection: a review. *Clin Infect Dis* 1995; 20: 992–1000.
56. Thimme R, Binder M, Bartenschlager R. Failure of innate and adaptive immune responses in controlling hepatitis C virus infection. *FEMS Microbiol Rev* 2012; 36: 663–683.
57. Lambotin M, Raghuraman S, Stoll-Keller F, Baumert TF, Barth H. A look behind closed doors: interaction of persistent viruses with dendritic cells. *Nat Rev Microbiol* 2010; 8: 350–360.
58. Cheent K, Khakoo SI. Natural killer cells and hepatitis C: action and reaction. *Gut* 2011; 60: 268–278.
59. Rehermann B. Hepatitis C virus versus innate and adaptive immune responses: a tale of coevolution and coexistence. *J Clin Invest* 2009; 119: 1745–1754.
60. Timpe JM, Stamataki Z, Jennings A *et al.* Hepatitis C virus cell–cell transmission in hepatoma cells in the presence of neutralizing antibodies. *Hepatology* 2008; 47: 17–24.
61. Wiegand J, Buggisch P, Boecher W *et al.* Early monotherapy with pegylated interferon α-2b for acute hepatitis C infection: the HEP-NET acute-HCV-II study. *Hepatology* 2006; 43: 250–256.
62. Choo QL, Kuo G, Ralston R *et al.* Vaccination of chimpanzees against infection by the hepatitis C virus. *Proc Natl Acad Sci USA* 1994; 91: 1294–1298.
63. Ray R, Meyer K, Banerjee A *et al.* Characterization of antibodies induced by vaccination with hepatitis C virus envelope glycoproteins. *J Infect Dis* 2010; 202: 862–866.
64. Strickland GT, El-Kamary SS, Klenerman P, Nicosia A. Hepatitis C vaccine: supply and demand. *Lancet Infect Dis* 2008; 8: 379–386.
65. Leroux-Roels G, Depla E, Hulstaert F *et al.* A candidate vaccine based on the hepatitis C E1 protein: tolerability and immunogenicity in healthy volunteers. *Vaccine* 2004; 22: 3080–3086.
66. Nevens F, Roskams T, Van Vlierberghe H *et al.* A pilot study of therapeutic vaccination with envelope protein E1 in 35 patients with chronic hepatitis C. *Hepatology* 2003; 38: 1289–1296.
67. Firbas C, Boehm T, Buerger V *et al.* Immunogenicity and safety of different injection routes and schedules of IC41, a hepatitis C virus (HCV) peptide vaccine. *Vaccine* 2010; 28: 2397–2407.
68. Firbas C, Jilma B, Tauber E *et al.* Immunogenicity and safety of a novel therapeutic hepatitis C virus (HCV) peptide vaccine: a randomized, placebo controlled trial for dose optimization in 128 healthy subjects. *Vaccine* 2006; 24: 4343–4353.
69. Klade CS, Wedemeyer H, Berg T *et al.* Therapeutic vaccination of chronic hepatitis C nonresponder patients with the peptide vaccine IC41. *Gastroenterology* 2008; 134: 1385–1395.
70. Wedemeyer H, Schuller E, Schlaphoff V *et al.* Therapeutic vaccine IC41 as late add-on to standard treatment in patients with chronic hepatitis C. *Vaccine* 2009; 27: 5142–5151.
71. Habersetzer F, Baumert TF, Stoll-Keller F. GI-5005, a yeast vector vaccine expressing an NS3-core fusion protein for chronic HCV infection. *Curr Opin Mol Ther* 2009; 11: 456–462.
72. Jacobson IM, McHutchinson JG, Boyer TD *et al.* GI-5005 therapeutic vaccine plus PEG-IFN/RIBAVIRIN in genotype 1 chronic HCV patients. *J Hepatol* 2010; 52: A2006.
73. McHutchinson JG, Jacobson IM, Boyer TD *et al.* GI-5005 therapeutic vaccine plus pegIFN/ribavirin improves end of treatment response at 48 weeks versus pegIFN/ribavirin in naive genotype 1 chronic HCV patients. *Hepatology* 2009; 50 (suppl 1): 229A.
74. Bacardi D, Amador-Canizares Y, Cosme K *et al.* Toxicology and biodistribution study of CIGB-230, a DNA vaccine against hepatitis C virus. *Hum Exp Toxicol* 2009; 28: 479–491.
75. Alvarez-Lajonchere L, Shoukry NH, Gra B *et al.* Immunogenicity of CIGB-230, a therapeutic DNA vaccine preparation, in HCV-chronically infected individuals in a Phase I clinical trial. *J Viral Hepat* 2009; 16: 156–167.