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Meta-Analysis of Hepatitis C Virus Vaccine Efficacy in Chimpanzees Indicates an Importance for Structural Proteins

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Abstract

Background & Aims—Studies in patients and chimpanzees that spontaneously cleared hepatitis C virus (HCV) infections demonstrated that natural immunity to the virus is induced during primary infections and that this immunity can be cross protective. These discoveries led to optimism about prophylactic HCV vaccines and several studies were performed in chimpanzees, although most included fewer than 6 animals. To draw meaningful conclusions about the efficacy of HCV vaccines in chimpanzees, we performed statistical analyses of data from previously published studies from different groups.

Methods—We performed a meta-analysis that compared parameters among naïve (n=63), vaccinated (n=53), and rechallenged (n=36) animals, including peak RNA titer post-challenge, timepoints of peak RNA titer, duration of viremia, and proportion of persistent infections.

Results—Each vaccination study induced immune responses that were effective in rapidly controlling HCV replication. Levels of induced T-cell responses did not indicate vaccine success. There was no reduction in the rate of HCV persistence in vaccinated animals, compared with naïve animals, when non-structural proteins were included in the vaccine. Vaccines that contained only structural proteins had clearance rates that were significantly higher than vaccines that contained non-structural components (P=0.015).

Conclusions—The inclusion of non-structural proteins in HCV vaccines might be detrimental to protective immune responses and/or structural proteins might activate T-cell responses that mediate viral clearance.

Keywords

correlates of protection; viral kinetics; Elispot responses

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Hepatitis C virus (HCV) is an enveloped virus with a single stranded, plus-sense RNA genome (~9.6 kb) consisting of ~341-base 5' non-translated region (NTR), a single open reading frame encoding all virus-specific proteins (~3011 amino acids), and a 3' NTR. The polyprotein is cleaved, co- and post-translationally by host and viral proteases to produce structural proteins (core and envelope glycoproteins (E1,E2)) and nonstructural (NS) components: p7; NS2-3 (protease); NS3 (serine protease and RNA helicase); NS4A; NS4B; NS5A and NS5B (RNA-dependent RNA polymerase, RdRp)^{1,2}.

Transmission of HCV is typically by the parenteral route, persistent infections occur in 70-80% of acutely infected individuals, the majority of which will develop chronic hepatitis and will be at risk for cirrhosis, end-stage liver disease and/or hepatocellular carcinoma³. HCV is associated with 40-60% of chronic liver disease in the U.S. Of these patients, one third goes on to develop progressive fibrosis and cirrhosis⁴ making hepatitis C the major disease leading to liver transplantation⁵.

HCV sequences are continually evolving during an infection due to the error-prone NS5B RdRp, which generates an estimated 10^{-5} - 10^{-4} errors/nucleotide/replication cycle^{6,7}, and the high production and clearance rate of the virus, estimated at $\sim 10^{12}$ virions/day⁸. Consequentially, HCV exists as several closely related but distinct viruses within a host; referred to as a quasispecies population. Seven major genotypes (GT) (designated 1-7) have been defined for HCV, differing from each other by ~30-35% over the complete genome^{9,10}. The greatest genetic variability is observed in the E1 and E2 glycoproteins and the NS5A region⁹. This genetic diversity poses problems for vaccine development from the perspective of target antigens and the potential for escape from vaccine-induced immune responses. Immune escape has been shown directly and indirectly for natural infections in both T-cell¹¹⁻¹³ and B-cell¹⁴⁻¹⁶ epitopes.

There is no licensed vaccine for HCV and prophylactic vaccine development has been hampered by the fact that the only animal model for pathogenesis or immune control of viral infection is the chimpanzee. This model has been highly important for understanding mechanisms of viral clearance and especially the role of T-cells in control of viral replication¹⁷⁻²¹. Based on clinical and chimpanzee studies demonstrating the role of T-cells in natural clearance, T-cell-based vaccines have received a great deal of focus, particularly given that these vaccines can target more conserved regions of HCV.

The data generated from chimpanzee vaccine studies is the most comprehensive available to assess the success or shortcomings of HCV vaccine approaches. However, the majority of studies have used small numbers of animals (1 to 6 per study) (Table 1). We have employed statistical methodology to quantitatively examine the published data and compare the course of HCV infection in naïve; vaccinated and rechallenged animals. The results from these analyses have been used to assess how well vaccines against HCV are functioning at controlling viral replication, which areas still require further investigation, and to establish biomarkers and parameters that could be used to assess the success of vaccines in the clinic.

Materials and Methods

Data Search and Inclusion Criteria

Searches of the Scopus database (www.scopus.com) using the terms “hepatitis C virus” and “chimpanzee” or “hepatitis C virus” and “chimpanzee” and “vaccine” resulted in 341 and 93 publications, respectively. These were reviewed and, where available, data was extracted on i) peak HCV RNA titer (the highest titer following infection) (ii) time to peak titer (representing the day post-infection at which viral replication is controlled) (iii) duration of viremia (the time from infection until viremia was consistently undetectable) and (iv)

outcome of infection (persistence or clearance) in 152 chimpanzees consisting of 63 naïve; 53 vaccinated and 36 rechallenged animals. The virus titers in the different studies were not all determined using the same quantitative assays. However, studies have used commercial tests (e.g. Amplicor; bDNA assay) or real-time PCR assays that have been shown to be consistent with WHO standards^{22;23} such that the titers determined can be considered comparable. Data from studies using mutated viruses, such as the deleted HVR1 region²⁴, and cell culture derived HCV^{25;26} were not included as the viral kinetics were not considered representative of natural infections. Data from studies reporting viral kinetics in naïve^{17;21;27-47} and rechallenged^{17;18;48-51} chimpanzees together with unpublished data (A. Prince, M. Major personal communications) were used for comparisons with vaccinated animals. No differentiation was made between homologous and heterologous challenges. Heterologous challenges were considered as GTs different from the primary challenge or immunizing antigens. As shown in Table 1 only one study³¹ used a different subtype (GT1a) from that represented by the immunizing antigens (GT1b). Some studies used different isolates of the same GT but these were not considered heterologous. The majority of vaccine studies (7/12) (Table 1) used delivery systems designed to induce T-cell responses to NS proteins. Of these 7 studies, 3 targeted NS proteins alone and 4 additionally targeted structural proteins (core and/or E1E2). Structural region proteins alone were targeted by 5/12 studies.

Statistical Approach to Data Analyses

We used non-parametric tests throughout as it was unclear that the data were normally distributed. To compare categorical variables, the two-tailed Fisher exact test was applied. To perform pairwise comparisons the exact Wilcoxon Signed-Rank Test was used. Multiple comparisons were adjusted using the Bonferroni correction; a two-sided P-value of <0.05 was considered significant except where this was corrected for multiple comparisons to a two-sided P-value of ≤ 0.017 . The specific tests used and related P-values (SPSS; V.17, Chicago, IL) are noted in the Figures and Tables.

Results

Primary and Secondary HCV Infections

We initially analyzed matched data on viral kinetics during primary and secondary infections of recovered chimpanzees. We performed pairwise comparisons of peak RNA titers, time to peak titer and duration of viremia in 11 animals. Figure 1 shows that for each of these parameters the differences between primary and secondary infections are significant ($P < 0.004$). The median peak RNA titer (\log_{10} RNA copies/mL) (Figure 1A) was significantly different between primary and secondary infections ($p = 0.0038$); median difference of $2.0 \log_{10}$ (Interquartile Range (IQR) = 1.2). The median peak RNA titer for primary infections was calculated as 5.9 (IQR = 0.5), and for secondary infections as 4.0 (IQR = 1.7). The median difference in time post infection that this peak titer was measured (Figure 1B) was also significant ($p = 0.0037$); occurring earlier during secondary infections (13 days; IQR = 12) compared to primary infections (56 days; IQR = 26); median difference of 42 days (IQR = 14). Similarly, the duration of viremia (Figure 1C) was shortened significantly ($p = 0.0038$) during secondary infections from 84 days (IQR = 75) to 21 days (IQR = 33); median difference 63 days, (IQR = 38). Although persistent infections can occur in chimpanzees upon rechallenge^{27;49;51} this was not observed in any of the animals for which paired data was available.

Evaluation of Vaccine Efficacy

The type of immune control induced by natural infection with HCV could be taken as the minimum standard expected of any prophylactic vaccine. In order to assess how successful

HCV vaccine trials have been in the chimpanzee model we compared peak viral RNA titer; time to peak titer and duration between naïve, rechallenged and vaccinated chimpanzees. Data from all studies listed in Table 1 were included in the statistical analyses. The studies performed by Houghton and co-workers^{52;53} and Esumi et al⁵⁴ did not include data for RNA titers therefore these studies were excluded from our statistical comparisons of viral kinetics but were included in analyses of clearance rates.

HCV RNA Kinetics Independent of Outcome—Data obtained comparing peak titer and time to peak titer for the 3 groups of animals is shown in Figure 2. All animals for which kinetics data were available were included in this comparison regardless of infection outcome (persistence or clearance). As expected from the pairwise comparisons performed for Figure 1 there is a significant decrease ($P<0.001$) for these two parameters in rechallenged animals compared to naïve animals. The median values for each parameter were similar to those seen for paired data. Specifically, 5.8 (IQR=1.1) and 4.6 (IQR=1.1) for peak RNA titer (\log_{10} RNA copies/mL) and 49 days (IQR=42) and 14 days (IQR=14) for peak date in naïve and rechallenged animals, respectively. More encouraging is the fact that we found significant decreases ($P<0.001$) for each parameter in vaccinated animals compared to naïve animals, median values for peak titer and peak date in vaccinated animals were 4.6 \log_{10} RNA copies/mL (IQR=1.4) and 21 days (IQR=21), respectively. There was no significant difference in peak RNA titers ($P=0.312$; Figure 2A) or control of viral replication ($P=0.160$; Figure 2B) between rechallenged and vaccinated animals.

HCV RNA Kinetics Based on Outcome—We performed the same statistical analyses using data only from naïve ($n=24$); rechallenged ($n=28$) and vaccinated ($n=21$) animals that cleared HCV with additional analysis to assess whether vaccination reduces the duration of viremia. Comparisons of peak titer and time to peak titer show significant decreases for rechallenged ($P<0.0001$) and vaccinated animals ($P\leq 0.005$) compared to naïve animals (Figure 3), similar to that seen when all animals are included (Figure 2). The median values for peak titer in naïve, rechallenged and vaccinated animals were calculated as 5.9 (IQR=1.3); 4.1 (IQR=1.1) and 4.6 (IQR=2.0) \log_{10} RNA copies/mL, respectively. The median values for peak date in naïve, rechallenged and vaccinated animals were calculated as 46 (IQR=25); 14 (IQR=14) and 21 (IQR=33) days, respectively. However, there is a trend towards higher (Figure 3A; $P=0.112$) and later (Figure 3B; $P=0.135$) peak RNA titers in vaccinated animals that clear compared to rechallenged animals that clear. This data suggests higher levels of viral replication occur in vaccinees prior to clearance. In addition, the duration of viremia in vaccinated animals that clear (median 77 days; IQR=75) is not significantly ($P=0.141$) shorter than naïve animals (102 days; IQR=51), and is significantly longer ($P=0.0001$) than that in rechallenged animals (28; IQR=39) (Figure 3C). This further indicates that even when virus is cleared in vaccinees this clearance is not as efficient as in the rechallenged group.

Vaccination and Outcome of Infection—Despite the encouraging data showing control of viral replication in vaccinated animals (Figures 2 and 3) the crucial question is whether the rate of clearance decreases as a result of vaccination. Comparing all vaccinated animals independent of vaccine antigen there is a significant ($P<0.0001$) decrease in the proportion of animals that develop persistent infections in vaccinees (28.3%) compared to naïve animals (61.9%) (Table 2). The data also shows the rate of clearance in vaccinees is similar to rechallenged animals (16.7%) ($P=0.214$). However, if the proportion of persistent infections in vaccinated animals is analyzed with respect to the regions targeted, structural region alone compared to NS region alone or in addition to structural proteins, a different picture emerges. We find that a significantly ($P=0.015$) higher proportion of persistent infections (45.8%) are found when antigens to NS proteins are included in the vaccine

(Table 3). The proportion of chronic infections in animals that receive vaccines targeting only the structural region (13.8%) is similar to that seen in rechallenged animals (16.7%, Table 2) while the proportion of chronic infections in animals that received vaccine containing NS components (Table 3;45.8%) is not significantly different ($P=0.227$) from naïve animals (Table 2;61.9%).

T-cell Responses at Challenge do not Predict Outcome

The analyses above suggest that T-cell responses induced by vaccination are in some way inferior to those induced by natural infection; possibly at the level of T-cell function or phenotype. This inferiority of the vaccine-induced T-cell response does not appear to be a question of magnitude. Independent of infection outcome Elispot responses (expressed as spot forming units/ 10^6 peripheral blood mononuclear cells) were not significantly different ($P=0.38$) between rechallenged and vaccinated animals at challenge (Figure 4A). The \log_{10} median values at challenge were calculated as 2.5 (IQR=0.48) (rechallenged) and 2.7 (IQR=0.9) (vaccinated). At 2-4 weeks post challenge the Elispot values are also not statistically different ($P=0.44$) (Figure 4B); 3.1 (IQR=1.3) (rechallenged) and 2.9 (IQR=0.7) (vaccinated). Despite a lack of statistical difference the numbers suggest there is a better recall of the T-cell responses in the rechallenged group than in the vaccinated group. The statistical comparisons for immune responses are less powerful than the comparisons of RNA titers due to few results available for rechallenged animals ($n=5$) and due to the variation of antigens and immune response study techniques between laboratories. Nonetheless, these results indicate that vaccination induces comparable levels of HCV-specific T-cells, relative to natural infection, which are recalled upon challenge.

If Elispot responses between vaccinated animals are compared based upon the outcome of infection we found that these were not significantly different ($P=0.21$) at challenge between vaccinated animals that cleared ($n=16$) and those that developed persistent infections ($n=10$) (Figure 4C). The median \log_{10} responses were 2.95 (IQR=0.8) and 2.7 (IQR=0.8) for cleared and chronic animals, respectively. However, there is a trend ($P=0.08$) for the recall response in vaccinated animals that cleared HCV (3.1; IQR=0.9) to be higher than that in vaccinated animals that developed persistent infections (2.8; IQR=0.8) (Figure 4D). These data further suggest that the magnitude of the vaccine-induced T-cell response cannot be used to predict success of a vaccine and that within vaccinees the quality of the response possibly accounts for more efficient proliferation of cells upon exposure to virus that then leads to clearance.

Discussion

This meta-analysis provides important data for future development, expectations and assessment of HCV prophylactic vaccines. We show statistically significant differences in viral kinetics between primary and secondary infections in recovered chimpanzees. This statistical analysis is consistent with previous observations demonstrating that although infection can occur upon re-exposure following clearance, viral kinetics are very different with rapid clearance in the majority of cases^{18;48;55;56}. Similar significant decreases in maximum HCV RNA titer and duration of infection for secondary infections in intravenous drug users were recently reported⁵⁷. Interestingly, in both chimpanzees (Table 2) and humans⁵⁷ the HCV clearance rate was 83% of reinfecting subjects, this compares to ~25% in patients⁵⁷ and ~38% in chimpanzees (Table 2) with primary infections. These data together with studies on immune correlates of clearance support the argument that memory immune responses are induced during primary infections, that these responses are recalled following secondary infections and that the responses are primarily T-cell based (reviewed in ref 58).

All of the chimpanzee vaccine approaches listed in Table 1 induced HCV-specific immune responses and HCV kinetics were significantly impacted in vaccinees compared to naïve animals. The peak viral titers were significantly decreased ($P < 0.001$) and viral replication was controlled earlier ($P < 0.0001$) regardless of the final outcome of infection. This shows that vaccination against HCV induces memory immune responses that are recalled upon exposure to virus and are effective at controlling viral replication. However, the duration of viremia in vaccinated animals was not significantly improved over naïve animals; suggesting vaccination was not able to consistently produce immune responses qualitatively similar to those induced during spontaneous clearance. Thus, not only does previous infection with HCV induce immune responses that protect from secondary infections but this natural immunity is superior to vaccine-induced immunity.

Our meta-analysis indicates that the rate of viral persistence is not increased following vaccination. However, the T-cell vaccine approach that includes NS proteins does not significantly ($P = 0.227$) decrease the rate of persistence (45.8%) compared to naïve animals (61.9%) and results in a significantly higher rate compared to vaccines that target the structural region alone ($P = 0.015$). Vaccines with the greatest success at leading to resolved infections included all or part of the HCV envelope region inducing either neutralizing antibody^{33;52-54}, E1E2 T-cell responses³⁴ or both²⁸, suggesting that neutralizing antibodies can play a role in protection but also that this region may contain T-cell epitopes that are important for clearance. However, the induction of neutralizing antibodies or T-cell responses to the envelope region does not necessarily guarantee success of a vaccine and absence of these antigens from a vaccine was not always associated with persistence.

There are a number of mechanisms that may account for our observed difference in outcome based upon the vaccine antigen. The choice or use of adjuvants may have influenced the quality of the immune response and memory cells induced. Most of the vaccines targeting structural proteins used adjuvants (Table 1) but a number of vaccines targeting the NS proteins also included adjuvants e.g. CpGs or recombinant viruses expressing co-stimulatory molecules. An analysis of animals vaccinated with adjuvants compared to those vaccinated without adjuvants showed no significant difference ($p = 0.51$) in the rate of persistence. The type of recombinant virus used for the T-cell based vaccines also did not impact the outcome of infection after challenge. The rate of persistence in animals immunized with Vaccinia virus vectors compared to those immunized with Adenovirus vectors was not significantly different ($p = 0.64$). It is more difficult to analyze the use of viral vectors on the outcome of infection as the vaccines designed to induce T-cells mainly used recombinant viruses (Table 1). This approach is unlikely to change unless significant drawbacks can be associated with viral vectors as these are one of the most efficient means of inducing T-cell responses. It is also possible that antibody plays a role in successful clearance through antibody-dependent cell-mediated cytotoxicity (ADCC). This has been shown to be important in a number of viral infections^{59;60} and also to be induced by a recent HIV vaccine that showed a reduced risk of HIV infection in vaccinees^{61;62}.

It is not possible to predict from this meta-analysis which vaccines should be developed for future clinical trials, but vaccines with a T-cell component targeting NS proteins should not be dismissed. It should be remembered that all the vaccines trials induced immune responses that modified viral replication and in order to protect against heterologous viruses responses to the more conserved NS proteins will almost certainly be required. However, more extensive studies need to be performed at the level of T-cell function in order to obtain a better understanding of the types of T-cells induced and provide reliable biomarkers to predict vaccine success.

Vaccine failure may also be caused by immune escape from the induced response. The persistence of HCV in some vaccine studies has been associated with immune escape from CD4⁺⁶³ or CD8⁺³¹ T-cells and with higher viral mutation rates⁶⁴ although immune escape from neutralizing antibody has not yet been demonstrated in vaccine studies. These data suggest that in cases where the immune response cannot rapidly clear the virus there remains an environment for immune pressure.

Ideally, the goal of prophylactic vaccines is to prevent infection upon exposure. However, from our data analysis sterilizing immunity seems to be unrealistic for hepatitis C. Most efficacy trials use reduction of clinical disease as the endpoint and not elimination of all evidence of infection. With the highly sensitive assays such as PCR, it is likely that low level replication of the infectious agent may be detected in successfully vaccinated individuals. HCV differs from many viruses in that it causes persistent infection which leads to chronic liver disease. A vaccine that leads to low-level HCV titers long-term would probably not be considered successful. Although low-level HCV titers increases the chances of virus elimination under the current standard of care (SOC)⁶⁵ the risk to benefit ratio in vaccinees would be considered too high and a clinical trial demonstrating a significant beneficial effect in vaccinees when combined with treatment would also be difficult. However, eliminating persistent infections while reducing acute phase viral titers would eventually prevent the major disease burden and most transmissions^{66;67} such that any prophylactic hepatitis C vaccine could target prevention of chronic infections as the primary endpoint.

Clinical development of HCV vaccine candidates is challenging. It is now known that treatment of acute HCV infections has a high probability of success. At present, the SOC recommends monitoring patients for ~12 weeks after diagnosis of acute HCV infection prior to initiating treatment with pegylated interferon- α , usually in combination with ribavirin, if the individual has not cleared the virus⁶⁸. In clinical trials there is active monitoring; if treatment is initiated at the time of diagnosis it would be difficult to determine if the vaccine can prevent chronic infections. Our meta-analysis suggests that this difficult problem is not unsolvable. In clinical vaccine trials, the subjects would be monitored periodically after the last dose of vaccine, typically at 6 month intervals. Given our data analysis, the median duration of viremia in vaccinees that clear HCV is 77 days (IQR=75); for rechallenged animals it is 28 days (IQR=39) and naive animals it is 102 days (IQR=51). These data indicate that if vaccine-induced immune responses are as efficient at clearing HCV as the immunity induced by natural infection there is a high probability that vaccinees will have cleared HCV within 3 months of exposure and significant differences would be seen between vaccine and placebo groups. If vaccines are developed that do not decrease the duration of viremia but decrease the rate of virus persistence differences could still be seen between vaccine and placebo groups if sufficient numbers were included in the studies.

This meta-analysis provides values for viral kinetic parameters that can be used to judge the success of future vaccine studies. However, it also demonstrates that there are scientific questions that still need to be resolved in HCV vaccine development, specifically correlates of protection. However, substantial progress has been made: vaccines can induce immune responses that impact viral replication; clinical studies are feasible and we have the means to judge efficacy. The development of effective HCV vaccines is a very real possibility.

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Abbreviations

CID50	chimpanzee infectious doses 50
GT	Genotype
IFN	Interferon
IQR	Interquartile Range
NS	Non-structural
NTR	Non-translated region
PBMCs	Peripheral blood mononuclear cells
SFU	Spot forming units

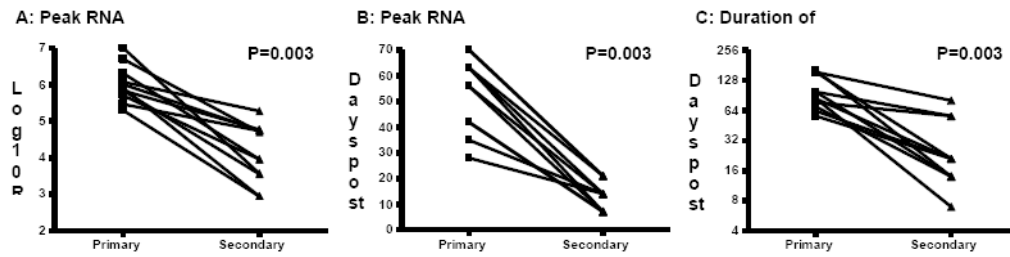


Figure 1. Comparison of HCV kinetics during primary and secondary infections in chimpanzees. A) Maximum RNA titer detected in serum samples. B) Time post-challenge (days) that maximum RNA titer was measured. C) Duration of viremia (days; Log₂ scale). $P < 0.05$ indicates a difference between primary and secondary infections (exact Wilcoxon Signed-Rank Test). Data obtained from^{48-50;69}.

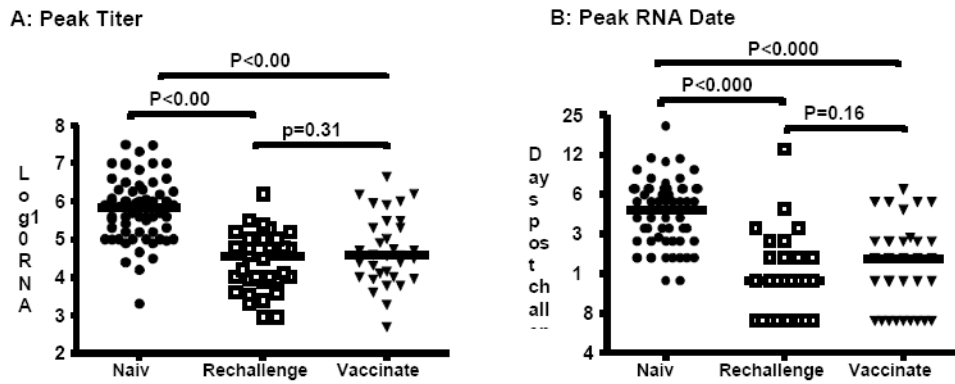


Figure 2.

Comparison of HCV kinetics in naïve; rechallenged and vaccinated chimpanzees, regardless of infection outcome. A) Maximum RNA titer detected in serum samples. B) Time post-challenge (days) that maximum RNA titer was measured. Closed circles: naïve; open squares: rechallenged; closed triangles: vaccinated. Horizontal bars represent median values. $P < 0.017$ indicates a difference between groups (Mann-Whitney U Test).

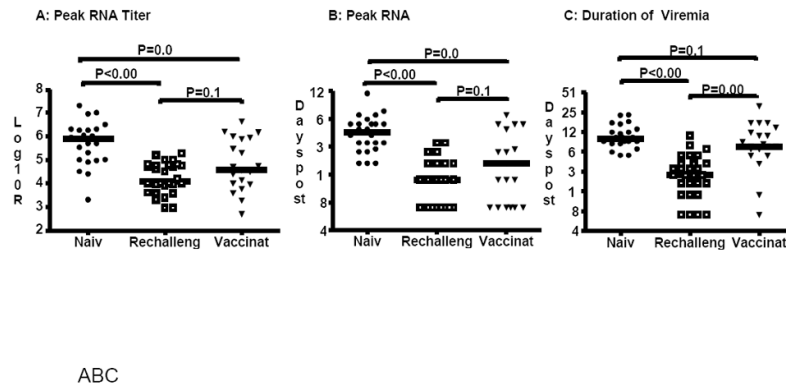


Figure 3. Comparison of HCV kinetics in naïve; rechallenged and vaccinated chimpanzees that cleared HCV. A) Maximum RNA titer detected in serum samples. B) Time post-challenge (days) that maximum RNA titer was measured. C) Duration of viremia (days; Log₂ scale). Closed circles: naïve; open squares: rechallenged; closed triangles: vaccinated. Horizontal bars represent median values. P<0.017 indicates a difference between groups (Mann-Whitney U Test).

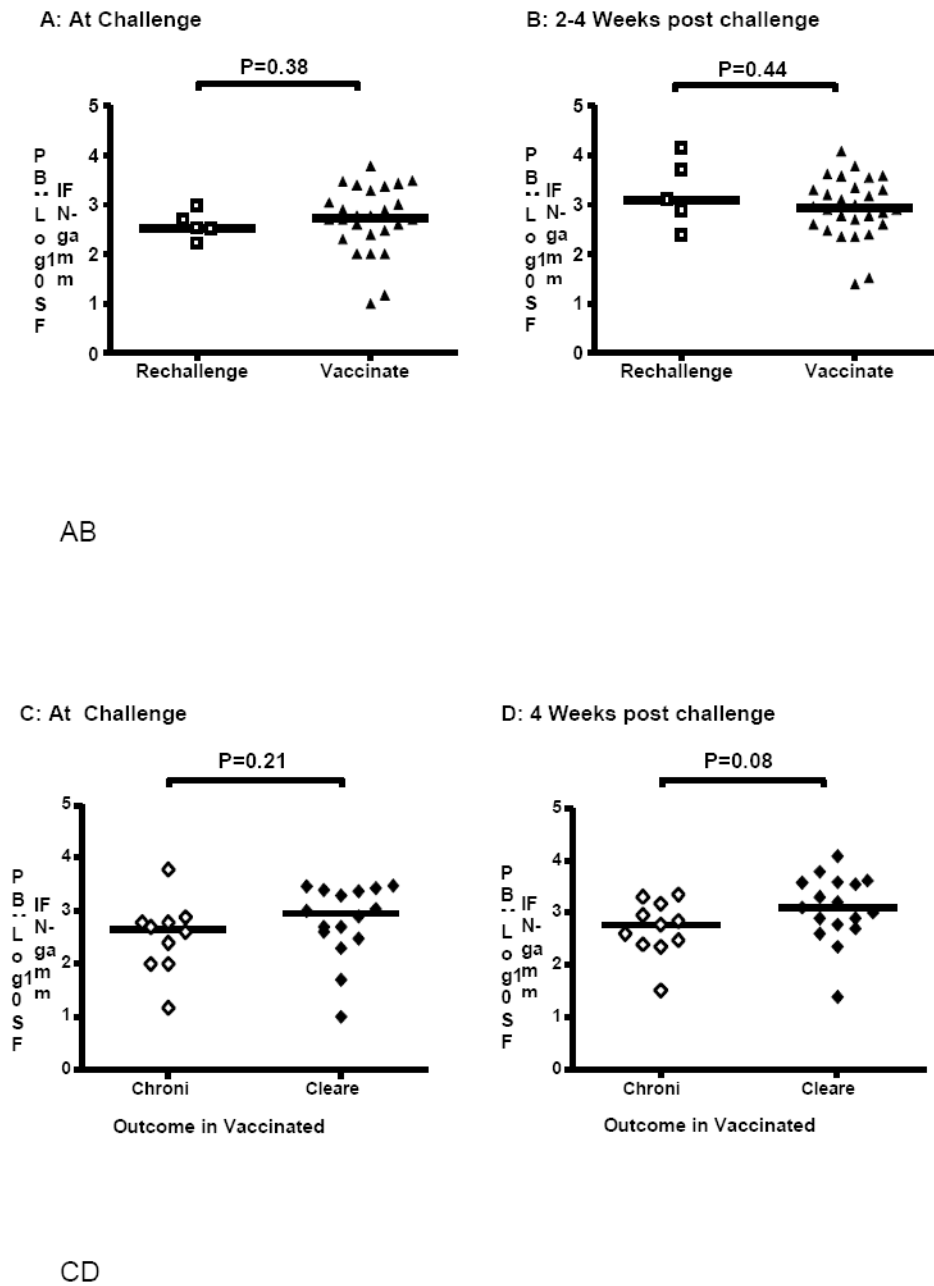


Figure 4. Comparison of Elispot responses. A) Rechallenged and vaccinated at challenge. B) Rechallenged and vaccinated 2-4 weeks post challenge. C) Vaccinated animals at challenge. D) Vaccinated animals 4 weeks post challenge. Data is shown as IFN- γ spot forming units (SFU) per 10^6 peripheral blood mononuclear cells (PBMCs). (A) and (B): Open squares: rechallenged; closed triangles: vaccinated. (C) and (D): Open diamonds: persistent infections; closed diamonds: cleared infections. Horizontal lines represent median values. $P < 0.05$ indicates a difference between groups (Mann-Whitney U Test).

Table 1

Prophylactic HCV vaccine studies in chimpanzees

Vaccine (Genotype sequence used) (Number of animals vaccinated)	Immunogenicity	Challenge Inoculum* Dose (CID50 [§])	Outcome	Ref.
DNA prime and protein boost (using C, gpE1, gpE2 and NS3) (GTs 1a (core, NS3) and 1b (core, E1E2, NS3)) (N=2) Adjuvant: Alum	Induced specific T-cell responses and antibody to E1 and E2.	GT 1b Homologous 25CID50	Modifies infection, protects from chronic infection 1 resolved infection 1 developed persistent infection	32
DNA prime and Recombinant Adenovirus expressing core, E1,E2 and NS3 to NS5B (GT 1b) (N=6) Adjuvant: Human IL-12-expressing plasmid	Induced specific T-cell responses and anti-E2 antibody (neutralizing)	GT 1b Homologous 100CID50	Modifies infection, protects from chronic infection 1 protected from infection 1 resolved infection 4 developed persistent infection	27
DNA prime and Recombinant VV expressing NS3,NS5A,NS5B (GT 1a) (N=1) Adjuvant: CpGs and VV expressing co-stimulatory molecules B7.1; ICAM-1; LFA-3..	Induced specific T-cell responses	GT 1a Homologous 100CID50	Modifies infection 1 developed persistent infection	63
DNA prime and Recombinant Adenovirus expressing NS3 to NS5B (GT 1b) (N=5) Adjuvant: None	Induced specific T-cell responses	GT 1a Heterologous 100CID50	Modifies infection, protects from chronic infection 4 resolved infection 1 developed persistent infection	31
DNA prime and rMVA boost (using C, gpE1, gpE2 and NS3) (GT 1b) (N=4) Adjuvant: None	Induced specific T-cell responses and antibody to E1 and E2.	GT 1b Homologous 25CID50	Modifies infection, protects from chronic infection 1 resolved infection 3 developed persistent infection	70
Recombinant VV core, E1, E2, p7, NS2 and NS3 (GT 1b) (N=4) Adjuvant: None	Induced specific T-cell responses. Weak anti-E1E2 response.	GT 1b Homologous 2.5 and 24CID50 [†]	Modifies infection, protects from chronic infection 4 resolved infection	28
DNA prime and Recombinant Adenovirus expressing NS3,NS5A,NS5B (GT 1a)	Induced specific T-cell responses	GT 1a Homologous	Modifies infection or protects from chronic infection 1 resolved infection	64

Vaccine (Genotype sequence used) (Number of animals vaccinated)	Immunogenicity	Challenge Inoculum* Dose (CID50 [§])	Outcome	Ref.
(N=2) Adjuvant: Human IL-12-expressing plasmid		100CID50	1 developed persistent infection	
Recombinant gpE1/gpE2 (GT 1a)	Induced antibodies to E1E2	GT 1a Homologous	Protects against infection or chronic infection 5 protected from infection	53 52
(N=21) Adjuvant: MF59 or MF75 oil-water emulsion		10 to 100CID50	14 resolved infection 2 developed persistent infection	
Recombinant gpE1/gpE2 + HVR1 peptides (GT 2)	Induced antibodies to E1E2 and HVR1	GT 2 Homologous	Protects from chronic infection 1 resolved infection	54
(N=1) Adjuvant: Complete and Incomplete Freund's		10 CID50		
DNA vaccine expressing E2 protein (GT 1a)	Induced antibody and T cell responses to E2.	GT 1a Homologous	Modifies infection, protects from chronic infection 2 resolved infection	33
(N=2) Adjuvant: None		100CID50		
Recombinant gpE1/gpE2 (GT 1a)	Induced antibodies and cellular responses to E1E2	GT 1a Homologous	Delayed/modified infection 1 developed persistent infection	71
(N=1) Adjuvant: RIBIs		100CID50		
Recombinant VLPs containing C, E1 and E2 (GT 1b)	Induced specific T-cell responses. No detectable anti-E1E2 Ab response.	GT 1b Homologous	Modifies infection, protects from chronic infection 4 resolved infection	34
(N=4) Adjuvant: AS01B (N=2)		100CID50		

Homologous and heterologous refer to the same or different GT, respectively.

[§] CID50=50% chimpanzee infectious doses;

[†] Animals erroneously received 2.5CID50 which did not lead to infection in control animals. Animals were then challenged with 24CID50.

Table 2

Outcome and rates of clearance in naïve; rechallenged and vaccinated animals.

Group	Total	Cleared Infections	Chronic Infections	% Chronic Infection [95% CI]	
Naïve	63	24	39	61.9 [48.8 – 73.6]	} P<0.0001
Rechallenged	36	30	6	16.7 [7.0 – 33.5]	
Vaccinated	53	38	15	28.3 [17.2 – 42.6]	} P=0.214

} P<0.0001

Fisher's Exact Test; P<0.017 indicates a difference between groups; CI, confidence intervals.

Table 3

Outcome and rates of clearance in vaccinated animals based upon the genome regions included in the vaccine.

Group	Total	Cleared Infections	Chronic Infections	% Chronic Infection [95%CI]	
NS proteins in the vaccine	24	13	11	45.8 [26.2 – 66.8]	} P=0.015
Structural proteins alone (Core and/or E1E2)	29	25	4	13.8 [4.5 – 32.6]	

Fisher's Exact Test; P<0.05 indicates a difference between groups; CI, confidence intervals.