Research Article
Viral Hepatitis

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Recombinant H77C gpE1/gpE2 heterodimer elicits superior HCV cross-neutralisation than H77C gpE2 alone

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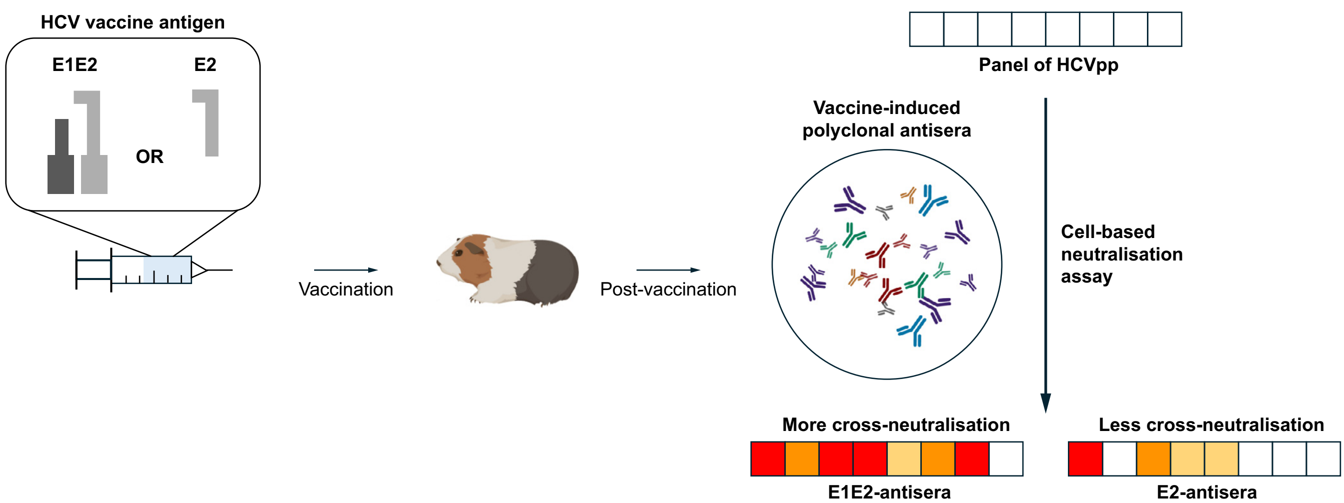
Graphical abstract



Highlights

- Glycoprotein-based HCV vaccines are immunogenic.
- We compared the antigenicities of E1E2 and E2.
- HCV E1E2-based vaccine induced a broader cross-neutralising profile.

Impact and implications

An HCV vaccine is urgently required to prevent the high global incidence of HCV infection and disease. Since HCV is a highly heterogeneous virus, it is desirable for a vaccine to elicit antibodies that neutralise the infectivity of most global strains. To this end, we have compared the immunoreactivity and antigenicity of recombinant H77C E1E2 heterodimer with that of H77C E2 alone and show that the former exhibits more cross-neutralising epitopes and demonstrates a broader cross-neutralisation profile *in vitro*. In addition, our data suggests a way to further broaden cross-neutralisation using a combination of E1E2 antigens derived from a few different HCV clades. Our work is relevant for the development of an effective global HCV vaccine.

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Recombinant H77C gpE1/gpE2 heterodimer elicits superior HCV cross-neutralisation than H77C gpE2 alone

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Background & Aims: An optimal HCV vaccine requires the induction of antibodies that neutralise the infectivity of many heterogenous viral isolates. In this study, we have focused on determining the optimal recombinant envelope glycoprotein component to elicit cross-neutralising antibodies against global HCV genotypes. We compared the immunoreactivity and antigenicity of the HCV genotype 1a strain H77C-derived envelope glycoprotein heterodimer gpE1/gpE2 with that of recombinant gpE2 alone.

Methods: Characterisation of the envelope glycoproteins was accomplished by determining their ability to bind to a panel of broadly cross-neutralising monoclonal antibodies. Immunogenicity was determined by testing the ability of vaccine antisera to neutralise the infectivity *in vitro* of a panel of pseudotyped HCV particles in which gpE1/gpE2 derived from representative isolates of the major global HCV genotypes were displayed.

Results: gpE1/gpE2 binds to more diverse broadly cross-neutralising antibodies than gpE2 alone and elicits a broader profile of cross-neutralising antibodies in animals, especially against more heterologous, non-1a genotypes. While not all heterologous HCV strains can be potently inhibited *in vitro* by gpE1/gpE2 antisera derived from a single HCV strain, the breadth of heterologous cross-neutralisation is shown to be substantial.

Conclusions: Our work supports the inclusion of gpE1/gpE2 in an HCV vaccine in order to maximise the cross-neutralisation of heterogenous HCV isolates. Our data also offers future directions in formulating a cocktail of gpE1/gpE2 antigens from a small selection of HCV genotypes to further enhance cross-neutralisation of global HCV strains and hopefully advance the development of a globally effective HCV vaccine.

Introduction

Despite the availability of curative HCV antivirals, the incidence of HCV infection is rising in many countries due to the increased frequency of injecting drug use and the inhibitory cost of HCV antivirals. In comparison, an HCV vaccine delivered to people-who-inject-drugs (PWIDs) would be highly cost-saving to healthcare budgets around the world and at present, remains the main hope in achieving the World Health Organization's goal of eliminating HCV infection as a major infectious disease.[1] There is now persuasive cumulative evidence for the protective role of HCV neutralising antibodies[2–9] and HCV-specific T helper and cytotoxic lymphocyte responses.[10–13] In the only reliable, fully immunocompetent HCV chimpanzee model, the recombinant E1/E2 heterodimeric envelope glycoprotein antigen remains the only prophylactic vaccine candidate, demonstrating statistically significant efficacy against the development of persistent viremia and associated disease.[3] The vaccine was comprised of the two recombinant envelope glycoproteins E1 and E2 derived from a genotype 1a strain HCV1, that fold into a native heterodimer inside the endoplasmic reticulum of transfected mammalian cells.[2,14] Further, an HCV vaccine candidate that elicits just cellular immune responses against HCV, without the production of any virus-neutralising antibodies, failed to demonstrate any efficacy against the persistent carrier state,[15] leading the field to now focus on candidates that induce cross-neutralising antibodies that are a well-established correlate of protection for all viral vaccines approved for human use so far.

HCV is extremely heterogeneous with eight major genotypes identified phylogenetically around the world with each genotype comprising numerous subtypes.[16] HCV genotypes 1 through 6 predominate.[17,18] Of the two envelope glycoproteins encoded by

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the HCV genome, E2 is known to be the main target for virus-neutralising antibodies. As a result, many groups are developing vaccines using E2 alone.[19,20] Our work focuses on the use of the E1E2 heterodimer because of its efficacy in reducing the rate of chronicity in the chimpanzee model, and importantly, it was more immunogenic than E2 alone in chimpanzees and in phase I clinical trials.[3] Large-scale production of the E1E2 heterodimer has been a challenge because its intracellular location in transfected mammalian cells leads to cellular toxicity, low yield, and difficulties with large-scale purification from the complex cellular milieu. However, we have now solved these issues by expressing E1E2 using specially designed conditions in large bioreactors. We also improved purification by utilizing a Fc fusion precursor that allows mature E1E2 to be purified on a large-scale using Protein A-based affinity columns followed by a protease cleavage to remove the Fc.[21] The work reported herein aims to address the important question of the breadth of cross-neutralisation afforded by E1E2 and E2 alone and to understand the underlying mechanisms. We now report data showing that E1E2 contains more diverse cross-neutralising epitopes than E2 alone, leading to a superiority of E1E2 in its ability to cross-neutralise the infectivity of many heterogenous, global HCV genotypes and subtypes. Furthermore, our work offers a roadmap to producing a cocktail of E1E2 antigens derived from different genotypes to further enhance cross-neutralisation and, hopefully, advance the development of a potent global vaccine.

Materials and methods

Cell culture and antibodies

HEK 293T cells were grown in DMEM (Gibco, Grand Island, NY, USA) supplemented with 10% heat-inactivated FBS (Sigma-Aldrich, St Louis, MO, USA), 100 units/ml penicillin and 100 μg/ml streptomycin (Invitrogen, Carlsbad, CA, USA). Human hepatoma Huh7.5 cells were propagated in DMEM (Gibco, Grand Island, NY, USA) containing 10% heat-inactivated FBS (Omega Scientific, Tarzana, CA,USA), 0.1 mM non-essential amino acids (Invitrogen, Carlsbad, CA, USA), and penicillin (100 units/ml) and streptomycin (100 μg/ml) (PenStrep; Invitrogen, Carlsbad, CA, USA) in an incubator supplemented with 5% CO_2 at 37 °C. CHO cells stably expressing recombinant gpE1/gpE2 (amino acids 192 to 746) or gpE2 construct (amino acids 384 to 661) derived from the genotype 1a H77C infectious clone (GenBank accession number AF009606)[22] were propagated in Iscove's modified Dulbecco's medium (Thermo Fisher Scientific, Waltham, MA, USA) containing 10% heat-inactivated FBS (Thermo Fisher Scientific, Waltham, MA, USA), 0.1 mM sodium hypoxanthine – 0.016 mM thymidine (HT supplement; Thermo Fisher Scientific, Waltham, MA, USA), 0.002 mM methotrexate, 100 U/ml penicillin, and 100 μg/ml streptomycin (PenStrep; Invitrogen, Carlsbad, CA, USA). Anti-HCV monoclonal antibodies (mAbs) (H-111, HC33.4, HC84.26, AR3A, HC-1AM, AR4A and AR5A) were previously described from various co-authors of this study.[23–27]

Expression and purification of recombinant glycoproteins E1E2 and E2

E1E2 or E2 were purified from cell pellets expressed as Fc-tagged proteins. For Fc-tagged E1E2, a tPA leader was inserted upstream of E1 (amino acids 192-383), followed by a duplication of amino acids 384 to 385 (ET), a human IgG1 Fc tag (227 amino acid residues), a human rhinovirus protease 3C (HRV3C) sequence (LEVLFQGP) and the coding sequence for E2 (amino acids 384-746).

For Fc-tagged E2, we inserted a tPA leader sequence upstream of E2 (amino acids 384-661). Additionally, at the carboxyl-terminus of E2, we inserted a HRV3C sequence, followed by a human IgG1 Fc tag.

Purification of Fc-tagged glycoproteins was as previously described.[21] Briefly, the cell extract was applied to Protein G Sepharose 4 Fast Flow (GE Healthcare, Piscataway, NJ) and then washed. Subsequently, the resin was digested with His6-GST-HRV3C protease (Thermo Fisher Scientific) overnight at 4 °C. The digested material was applied to glutathione sepharose 4B (GE Healthcare) to remove the protease and the flowthrough was then passed through a hydroxyapatite column and subsequently concentrated.[21] The final antigens (E1E2 and E2) reached at least 90% purity (Fig. S1).

ELISA

Microtiter plates (Thermo Scientific CAT # 439454) were coated with recombinant E1E2 or E2 antigens (50 ng/well) in PBS 1X overnight at 4 °C. Plates were washed with PBS containing 0.2% Tween 20 (PBST, 1X) and blocked for 1 h in 5% bovine serum albumin (Sigma-Aldrich, St. Louis, MO) in PBST. mAb was added for 1 h and detected by an alkaline phosphatase-conjugated anti-human secondary antibody (1:10,000; Jackson Immuno Research, West Grove, PA) and KPL peroxidase substrate (SeraCare Life Sciences, Milford, MA). The absorbance at 450 nm was read using an Enspire plate reader (Perkin-Elmer, Waltham, MA, USA)

Immunisation of animals and collection of serum samples

Hartley guinea pigs (Medimabs, Montreal, QC, Canada), 5 to 7 weeks old, were cared for in accordance with Canadian Council on Animal Care guidelines. Experimental methods were reviewed and approved by the University of Alberta Health Sciences Animal Welfare Committee (AUP00000392). Purified E1E2 or E2 antigen (4 μg) were mixed at a 1:1 equi-volume ratio with 75 μg alum and 7.5 μg monophosphoryl lipid A (Vaccigrade; InvivoGen, San Diego, CA, USA). The final antigenic preparation (100 μl) of E1E2 or E2 was administered via intramuscular injection to each guinea pig on days 0, 30 and 90. Pre-vaccination blood samples were collected at day 0, and post-vaccination blood samples (terminal bleeds) were obtained 14 days after the final immunisation. After clotting, whole blood samples were centrifuged at 5,000 × *g* for 15 min, and sera were collected, and heat inactivated at 56 °C for 30 min. Serum samples were stored in aliquots at -80 °C until use.

Production of HCVpp

Plasmids encoding a panel of various HCV glycoprotein E1E2 were described previously.[28–30] HCV pseudoparticles (HCVpp) were generated by co-transfection of HEK 293T cells with two plasmids encoding HCV glycoprotein gpE1/gpE2, pNL4.3.Luc.R-E containing the luciferase reporter and env-defective HIV proviral genome (National Institute of Health AIDS Reagent Program), and pAdvantage (Promega, Madison, WI) using Lipofectamine 2000 (Thermo Fisher Scientific)

Table 1. HCV bNabs used in this study.

Monoclonal antibody	Epitope	Specificity	Capacity to block E1E2 binding to CD81	Neutralising activity	Ref.
AR4A	Conformational discontinuous E1 (201-206), E2 (459-487), E2 (543-597), E2 (652-698)	E1E2	No	1a,1b,2a,3a,4a,5a,6a (HCVcc); 1a,1b,2a,2b,3a,4,5,6 (HCVpp)	24,45
AR5A	Conformational discontinuous E1 (201-206), E2 (459-486), E2 (513-597), (639-692)	E1E2	No	1a,2a,4a,5a,6a (HCVcc); 1a,1b,4,5,6 (HCVpp)	24,45
AR3A	Conformational discontinuous E2 (394-424), E2 (437-447), E2 (523-540)	E2	Yes	1a,1b,2a,2b,3a,4,5,6 (HCVpp); 1a,1b,2a,3a,4a,5a,6a (HCVcc)	27
HC33.4	Linear E2 (413,418,420,421)	E2	Yes	1a,2a,3a,4a,5a,6a (HCVcc)	46
HC84.26	Conformational discontinuous E2 (418–446); E2 (611–616)	E2	Yes	1a,2a,3a,4,5,6 (HCVcc)	23
HC-1AM	Conformational E2 (523-540)	E2	Yes	1a,1b,2a,2b,3a (HCVcc)	47
H-111	Linear E1 (192-205)	E1	N/A	N/A	26

bNAbs, broadly cross-neutralising monoclonal antibodies; HCVcc, HCV cell culture; HCVpp, HCV pseudoparticles; N/A, not available.

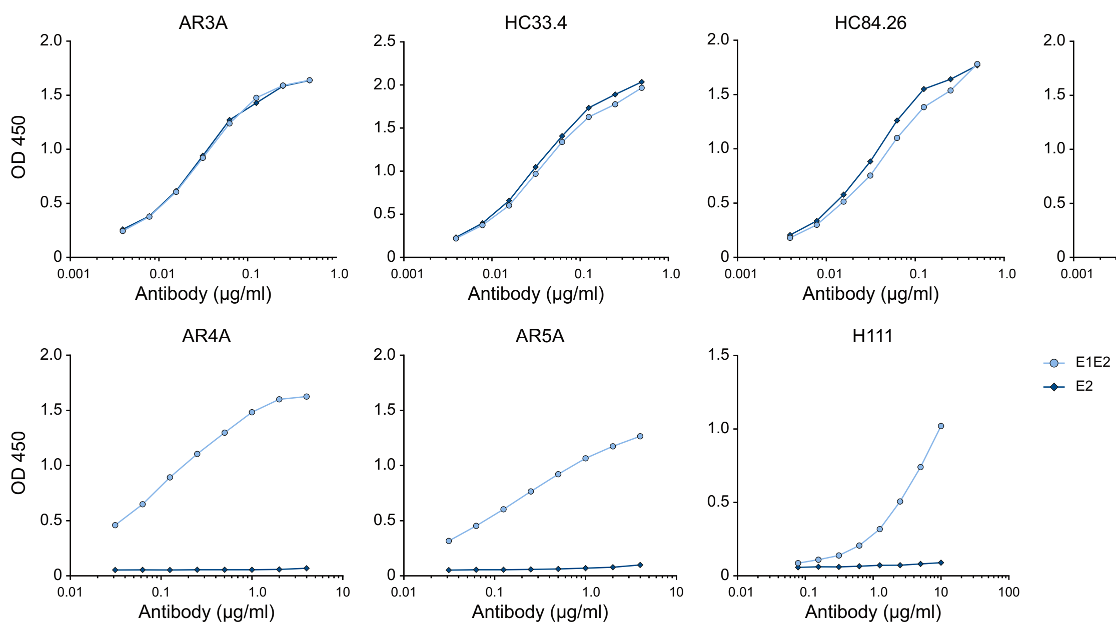

Fig. 1. Binding of HCV cross-neutralising human MAbs to purified E1E2 and E2 antigens. Microtiter plates were coated either with purified recombinant E1E2 (circle, light blue) or E2 (diamond, dark blue) and probed with the 2-fold decreasing concentrations of HCV neutralising human mAbs (starting at 0.5 μg/ml to 10 μg/ml). Bound antibodies were detected by an alkaline phosphatase-conjugated anti-human secondary antibody. The mean optical densities measured at 450 nm for each mAb tested in two independent experiments are plotted *vs.* mAb concentration (μg/ml). E2-specific antibodies are AR3A, HC33.4, HC84.6 and HC-1AM. E1E2-specific antibodies are AR4A, AR5A. E1-specific antibody is H-111. mAbs, monoclonal antibodies.

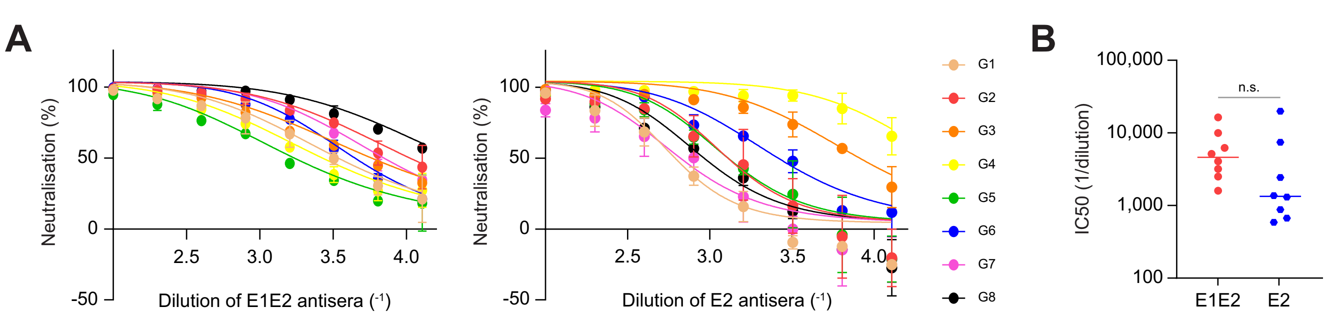

Fig. 2. Homologous neutralisation activity against H77C HCVpp by E1E2 and E2-immunised guinea pig antisera. (A) Antisera from guinea pigs (G1-G8) either immunised with E1E2 (left) or E2 (right) were serially diluted and their abilities to block entry of HCV pseudoparticles pseudotyped with H77C E1E2 were determined as described. (B) IC_{50} of these antisera were determined using GraphPad Prism software (version 9). The Mean of IC_{50} between antisera from E1E2-and E2-immunised guinea pigs were compared. Unpaired Student's *t* test showed *p* values >0.05 (non-significant (n.s.)).

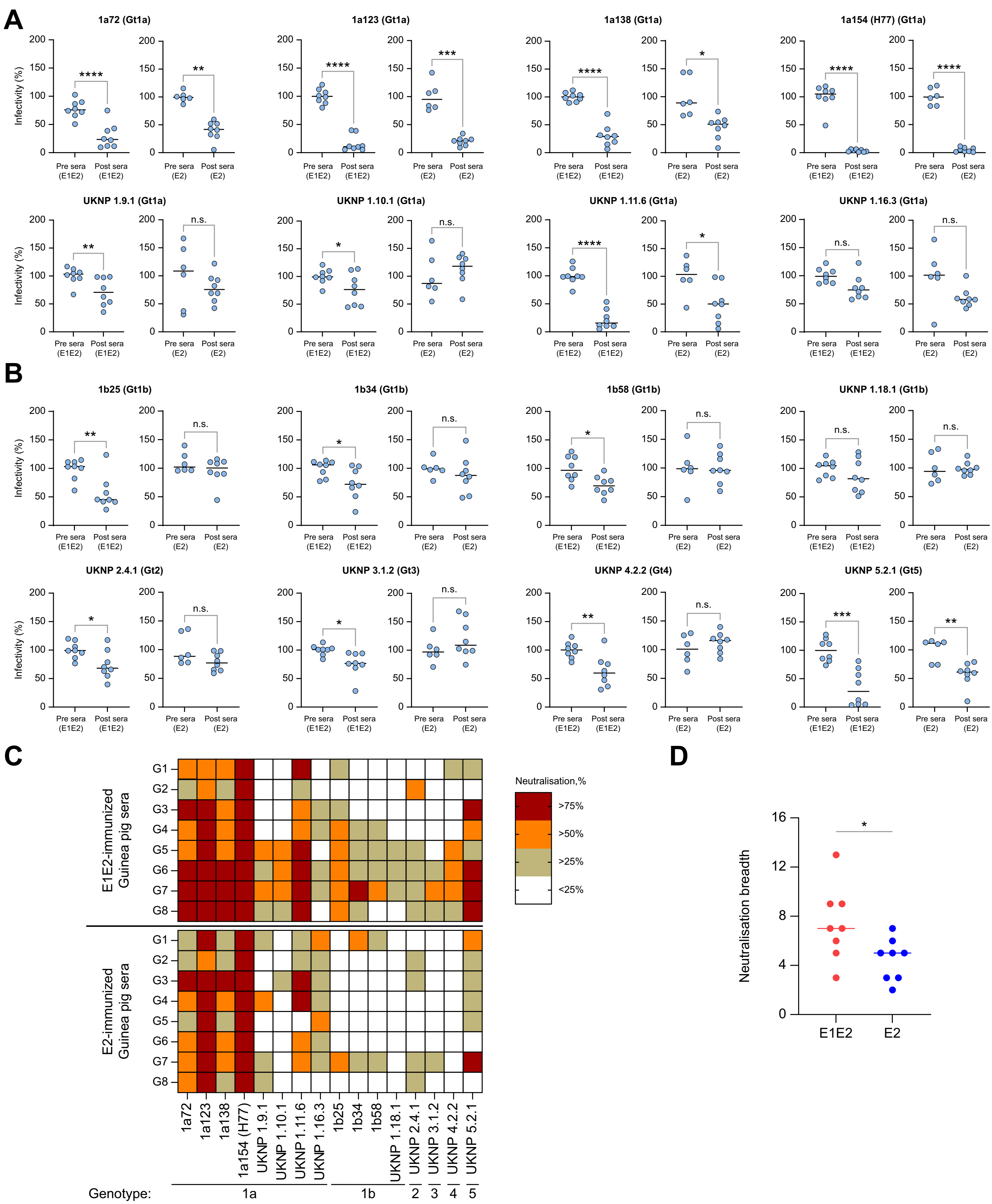

Fig. 3. Comparison of the neutralisation activity between antisera from E1E2-and E2-immunized guinea pigs. The infectivity of sera from guinea pigs pre- or post-vaccination with E1E2 and E2 was tested against a panel of eight genotype 1a HCVpp (A) and eight non genotype 1a HCVpp (B) in Huh7.5 cells. Pre- and post-vaccinated sera were diluted at a 1:100 ratio. The amount of virus entry was measured by quantifying the HCVpp-encoded luciferase activity 48 h post incubation and the proportion of infectivity was normalized with HCVpp incubated without serum. Results were shown from at least two independent experiments in triplicate. Mean proportion of infectivity is denoted by a solid line. Student's *t* tests were performed to compare between pre- and post-vaccination. *****p* <0.0001; ****p* <0.001; ***p* <0.01

according to the manufacturer's protocol. Twenty-four hours post-transfection, the medium was replaced by DMEM containing 3% FBS. At 48 h and 72 h post-transfection, the cell culture supernatants containing HCVpp were harvested by passing through 0.45 μm filters and stored at -80 °C for future use.

Neutralisation assays

For neutralisation assays, 1×10^4 human hepatoma (Huh7.5) cells were plated onto poly-lysine-coated 96-well plates 1 day prior to infection. HCVpp was pre-mixed with heat-inactivated sera from immunised guinea pigs at 1:100 dilutions for 1 h at 37 °C, followed by addition to Huh7.5 cells. At 5 h post-infection, the immune sera-virus inoculum was replaced with fresh culture medium and was incubated for an additional 72 h at 37 °C. Pre-immune sera (Pre) were used as controls. For E1E2 antisera, each dot represents pre-immunisation serum from each animal; for E2 antisera, each dot in pre-immunisation sera indicates a technical replicate. Luciferase activity was measured according to the manufacturer's protocol (Promega Inc, Madison, WI). Briefly, cells were lysed using Cell Lysis Buffer, followed by the addition of substrate buffer for 5 min. The luminescence (relative light units [RLUs]) was measured using the EnSpire 2300 multilabel reader (Perkin-Elmer). The percent neutralisation was calculated[29]: % Neutralisation = [1-(RLU $_{\text{post-vaccinated/}}$ RLU $_{\text{Pre-vaccinated sera}}$)] x 100%.

Statistical analysis

Statistical analysis (paired Student's *t* test) was performed using GraphPad Prism 9 software. *p* values less than (<) 0.05 were considered statistically significant.

Results

Intracellular H77 E1E2 (aa 192-746) and intracellular E2 (aa 384-661) were each expressed and purified from CHO cell pellets to greater than 90% purity. Each glycoprotein was then used to coat enzyme immunoassay wells and the relative immunoreactivity determined using seven broadly cross-neutralising monoclonal antibodies (bNAbs), which broadly neutralise the *in vitro* infectivity of many heterogeneous HCV genotypes (Table 1). Fig. 1 shows that E1E2 strongly bound all MAbs tested whereas E2 only bound four strongly. Of the three bNAbs showing specificity for the E1E2 heterodimer, two (AR4A and AR5A) have been described as recognizing conformational epitopes formed by the E1 and E2 interaction, while H-111 recognizes E1 and not E2 (Table 1). As expected, H-111 binds only to the E1E2 heterodimer (Fig. 1). The remaining four bNAbs that bind equally well to E1E2 and E2 (AR3A, HC-33.4, H84.26, HC-1AM) have been reported previously to bind to different epitopes within E2 (Table 1).

Next, we assayed the ability of the guinea pig antisera to neutralise the *in vitro* infectivity of HCVpp derived from the same 1a strain as the vaccine antigens (H77C sequence). Fig. 2A shows that guinea pigs immunised with E1E2 elicited high titres of neutralising antibodies against homologous H77C HCVpp although there was not a statistically significant difference compared with E2 alone antisera (Fig. 2B).

In terms of cross-neutralising antibodies against heterologous HCVpp, we observed that the cross-neutralising profile of E1E2 and E2 antisera against HCVpp derived from eight different genotype 1a strains (the same basic genotype from which the vaccines were derived) were similar, although E1E2 tended to elicit stronger and broader cross-neutralising antibodies (Fig. 3A). Interrogation of a representative panel of non-genotype 1a HCVpp revealed a greater differentiation of these two antigens. E1E2 elicited significant neutralising antibodies against 3/4 different genotype 1b HCVpp, whereas E2 did not significantly neutralise any 1b HCVpp (Fig. 3B). Of four more genetically distinct HCVpp derived from HCV genotypes 2, 3, 4, and 5, E1E2 antisera significantly inhibited the infectivity of all four, whereas E2 only significantly neutralised one derived from genotype 5, albeit less potently than E1E2 antisera (Fig. 3B). Fig. 3C shows a relative heat map of these data, further illustrating this differentiation of E1E2 and E2 antisera. To compare the breadth of cross-neutralisation, we have tallied the number of isolates that each antiserum can neutralise by over 50%, as described by Osburn *et. al.*.[7] E1E2 antisera exhibited a broader neutralisation breadth than E2 antisera (Fig. 3D). Finally, we interrogated the cross-neutralising activity of E1E2 antisera against a wider selection (addition of 14 more) heterogeneous HCVpp (Fig. 4A). The relative heat map (combined with data in Fig. 3C, total of 30) is shown (Fig. 4B). It revealed that 7/9 different genotype 1a HCVpp were partially but significantly neutralised by a 1:100 dilution of guinea pig antisera. Three of five genotype 1b HCVpp were also significantly and partially neutralised, whereas 1/5, 3/5, 3/4, and 2/2 HCVpp were significantly neutralised from genotypes 2, 3, 4 and 5, respectively, using 1:100 dilutions of E1E2 antisera. This demonstrates the ability of the E1E2 antigen derived from a single HCV strain to prime bNAbs against the very heterogeneous human Hepacivirus genus.

Discussion

Our previous findings from vaccinating small animals, chimpanzees and humans have shown that neutralising antibodies elicited by E1E2 derived from a single HCV 1a strain (HCV1) are *not* restricted to the homologous strain used.[31–35] The current study expands upon these findings by showing that E1E2 of another genotype 1a strain (H77) binds numerous discrete bNAbs (Fig. 1) and that E1E2 antisera from immunised guinea pigs exhibits significant cross-neutralising antibodies against a wide variety of HCVpp derived from a broad spectrum of

and *p <0.05. n.s. indicates no significance. (C) The normalized neutralisation activity of post-vaccination antisera was represented in a heat map. Color codes: >75% neutralisation (red), >50% neutralisation (orange), >25% neutralisation (light green), and <25% neutralisation (white). Patterns of neutralisation from individual guinea pigs immunised with either E1E2 (upper panel) or E2 (lower panel) against a panel of 16 HCVpp are shown. Another representation of this figure is shown in Fig. S2 by arranging the HCVpp as tiers of neutralisation resistance as described.[29] (D) The breadth of the cross-neutralisation conferred by E1E2-or E2-induced antisera was compared. The breadth of cross-neutralisation is determined by the number of isolates (out of the 16 HCVpp tested) that were neutralised by >50%. The mean of this number between guinea pigs immunised with E1E2 or E2 was compared by Student's *t* tests. *Indicates statistical significance, *p* <0.05. HCVpp, HCV pseudoparticles.

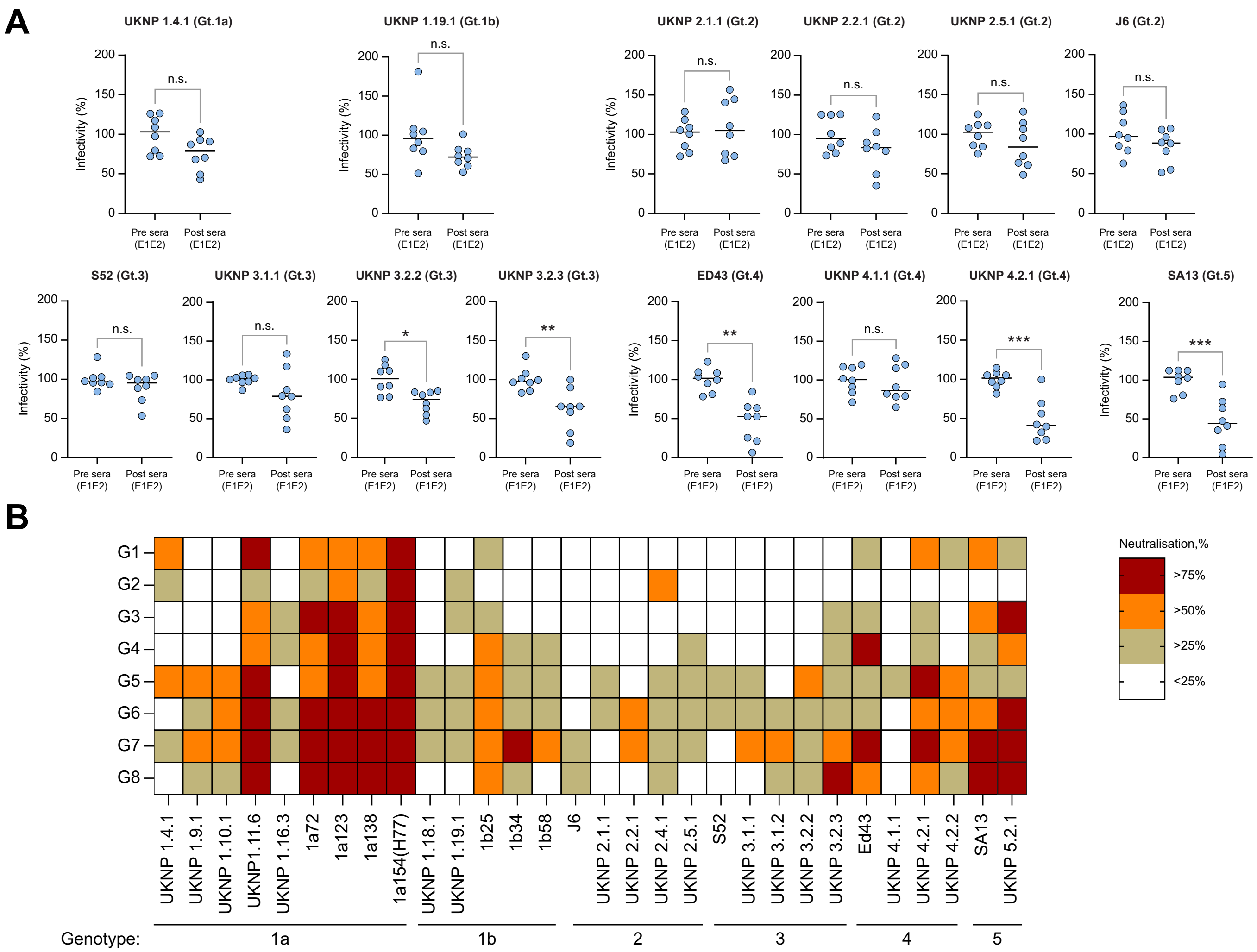

Fig. 4. The cross-neutralisation profile of antisera from E1E2-immunised guinea pigs. The neutralisation activity of sera from guinea pigs pre- or post-vaccination with E1E2 was tested against an expanded panel of heterologous HCVpp in Huh 7.5 cells. Pre- and post-vaccinated sera were diluted at 1:100. The amount of virus entry was measured by quantifying the HCVpp-encoded luciferase activity 48 h post incubation and the proportion of infectivity was normalized with HCVpp incubated without serum. The data was calculated from three independent experiments, each performed with triplicate wells. Means of infectivity % are indicated. Student's *t* tests were performed to compare between pre- and post-vaccination. *****p* <0.0001; ****p* <0.001; ***p* <0.01 and **p* <0.05. n.s. indicates no significance. The normalized neutralisation activities of post-vaccination antisera against 30 HCVpp are represented in a heat map. The same scale was used as indicated in Fig. 3. HCVpp, HCV pseudoparticles.

predominant global genotypes (Fig. 4). In addition, the current study shows that H77C E1E2 outperforms H77C E2 alone in terms of binding to various highly cross-neutralising bNAbs and in the breadth and titre of elicited cross-neutralising antibodies against highly heterogenous HCV genotypes. Importantly, in both vaccinated chimpanzees and human volunteers, HCV-1 E1E2 has been shown to be substantially more immunogenic than HCV-1 E2 alone ([3] & MH, unpublished).

Neutralising antibodies correlate with protection against heterologous HCV genotypes in chimeric mice harbouring human hepatocytes,[4] in chimpanzees,[2,6] and in various patient cohorts[5,7–9] and adjuvanted E1E2 has been demonstrated to be capable of substantially reducing the chronic carrier state in vaccinated chimpanzees subsequently challenged with homologous and heterologous 1a strains.[2,3,14] No other vaccine candidate has demonstrated such efficacy in the reliable chimpanzee model[36] or in any other animal model. Given that severe clinical manifestations of HCV infection are predominantly associated with viral persistence over many years, our current work provides further encouragement that an effective, broadly neutralising, global vaccine can be produced which, if necessary, can be further enhanced relatively easily by producing an optimal cocktail of E1E2 antigens derived from just a few different genotypes. Herein, we report only partial *in vitro* neutralisation of most HCVpp at a 1:100 dilution of antisera and so it remains to be seen if this activity will translate to broad protection in humans. Protection against HCV infection, as in the case of the approved HBV and HPV vaccines, will likely rely more heavily on the generation of vaccine-mediated HCV-specific B and T cell memory responses rather than maintaining high circulating levels of antibodies and activated T cells required for optimal protection against fast-acting respiratory viruses like SARS-CoV-2, influenza, and respiratory syncytial virus.[37–41] The observed priming of bNAbs by E1E2 implies the simultaneous generation of broad memory B and T cells that could be cross-protective, but efficacy trials in human volunteers will be required to definitively address these questions, possibly through rapid human challenge trials.[42]

As has been common practice in developing effective vaccines against heterogeneous viruses and bacteria in the past, combinations of vaccine antigens derived from different HCV genotypes/strains can be readily produced to enhance the potency of a global HCV vaccine and our data reported in this study offer some pointers on what combinations may be appropriate to this end. Vaccines against HCV and HIV that only elicit cellular immune responses in the absence of neutralising antibodies have proven to be unsuccessful in human efficacy trials.[15,36,43,44] Our data indicates that the use of E1E2 antigens in either recombinant form or encoded using RNA or vectored platforms remains a promising approach for preventing global HCV disease and transmission. Finally, it should be noted that the work reported here compared H77C E1E2 with H77C E2 derived from the common global HCV genotype 1a. Given the large heterogeneity of HCV and its complex cell entry mechanisms, it remains to be seen if the superiority of E1E2 applies to all other HCV genotypes.

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Abbreviations

bNAbs, broadly cross-neutralising monoclonal antibodies; HCVpp, HCV pseudoparticle; mAbs, monoclonal antibodies; RLU, relative light unit; HBV, hepatitis B virus; HPV, human papillomavirus

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Conflict of interest

Authors (JK, HTL, ML, DH, AL, KC, MW, JJ, JKK, DLT, MH, JL) own stock in Aurora Vaccines Inc., which is developing a HCV vaccine for clinical and commercial use.

Please refer to the accompanying ICMJE disclosure forms for further details.

Authors' contributions

JK, DLT, MH, JL conceived and designed the experiments. JK, HTL, ML, DH, AL, KC, MW, JJ, EAT, RAU, JL performed the experiments. JK, HTL, JKK, MH, JL analyzed the data. EAT, RAU, JKB, JRB, JB, ML, SF contributed reagents/materials/analysis tools. JK, MH, JL wrote the paper.

Data availability statement

The numerical source data for all applicable graphs is provided in the excel file named "Rawdata-Kunduetal2024".

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Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jhep.2024.06.029.

References

Author names in bold designate shared co-first authorship

[1] WHO. Global hepatitis report 2017.. Published online April 28. 2017. p. 1–83.

[2] 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(4):1294–1298.

[3] Houghton M. Prospects for prophylactic and therapeutic vaccines against the hepatitis C viruses. Immunol Rev 2011;239(1):99–108. https://doi.org/10.1111/j.1600-065x.2010.00977.x.

[4] Meuleman P, Bukh J, Verhoye L, et al. In vivo evaluation of the cross-genotype neutralizing activity of polyclonal antibodies against hepatitis C virus. Hepatology 2011;53(3):755–762. https://doi.org/10.1002/hep.24171.

[5] Pestka JM, Zeisel MB, Bläser 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(14):6025–6030. https://doi.org/10.1073/pnas.0607026104.

[6] **Morin TJ**, **Broering TJ**, Leav BA, et al. Human monoclonal antibody HCV1 effectively prevents and treats HCV infection in chimpanzees. Plos Pathog 2012;8(8):e1002895. https://doi.org/10.1371/journal.ppat.1002895.

[7] Osburn WO, Snider AE, Wells BL, et al. Clearance of hepatitis C infection is associated with the early appearance of broad neutralizing antibody responses. Hepatology 2014;59(6):2140–2151. https://doi.org/10.1002/hep.27013.

[8] Osburn WO, Fisher BE, Dowd KA, et al. Spontaneous control of primary hepatitis C virus infection and immunity against persistent reinfection. Gastroenterology 2010;138(1):315–324. https://doi.org/10.1053/j.gastro.2009.09.017.

[9] Lavillette D, Morice Y, Germanidis G, et al. Human serum facilitates hepatitis C virus infection, and neutralizing responses inversely correlate with viral replication kinetics at the acute phase of hepatitis C virus infection. J Virol 2005;79(10):6023–6034. https://doi.org/10.1128/jvi.79.10.6023-6034.2005.

[10] Diepolder HM, Scholz S, Pape GR. Influence of HLA alleles on outcome of hepatitis C virus infection. Lancet 1999;354(9196):2094–2095. https://doi.org/10.1016/s0140-6736(99)00327-x.

[11] Cooper S, Erickson AL, Adams EJ, et al. Analysis of a successful immune response against hepatitis C virus. Immunity 1999;10(4):439–449. http://pubmed.gov/10229187.

[12] Grakoui A, Shoukry NH, Woollard DJ, et al. HCV persistence and immune evasion in the absence of memory T cell help. Science 2003;302(5645):659–662. https://doi.org/10.1126/science.1088774.

[13] Shoukry NH, Grakoui A, Houghton M, et al. Memory CD8+ T cells are required for protection from persistent hepatitis C virus infection. J Exp Med 2003;197(12):1645–1655. https://doi.org/10.1084/jem.20030239.

[14] Houghton M, Abrignani S. Prospects for a vaccine against the hepatitis C virus. Nature 2005;436(7053):961–966. https://doi.org/10.1038/nature04081.

[15] Page K, Melia MT, Veenhuis RT, et al. Randomized trial of a vaccine regimen to prevent chronic HCV infection. New Engl J Med 2021;384(6):541–549. https://doi.org/10.1056/nejmoa2023345.

[16] Borgia SM, Hedskog C, Parhy B, et al. Identification of a novel hepatitis C virus genotype from Punjab, India: expanding classification of hepatitis C virus into 8 genotypes. J Infect Dis 2018;218(11):1722–1729. https://doi.org/10.1093/infdis/jiy401.

[17] Petruzziello A, Marigliano S, Loquercio G, Cozzolino A, Cacciapuoti C. Global epidemiology of hepatitis C virus infection: an up-date of the

distribution and circulation of hepatitis C virus genotypes. World J Gastroenterol 2016;22(34):7824–7840. https://doi.org/10.3748/wjg.v22.i34.7824.
[18] Bukh J. The history of hepatitis C virus (HCV): basic research reveals unique features in phylogeny, evolution and the viral life cycle with new perspectives for epidemic control. J Hepatol 2016;65(1):S2–S21. https://doi.org/10.1016/j.jhep.2016.07.035.
[19] Donnison T, McGregor J, Chinnakannan S, et al. A pan-genotype hepatitis C virus viral vector vaccine generates T cells and neutralizing antibodies in mice. Hepatology 2022;76(4):1190–1202. https://doi.org/10.1002/hep.32470.
[20] Li D, Wang X, Schaewen M von, et al. Immunization with a subunit hepatitis C virus vaccine elicits pan-genotypic neutralizing antibodies and intrahepatic T-cell responses in non-human primates. J Infect Dis 2017. https://doi.org/10.1093/infdis/jix180. Published online April 8.
[21] Logan M, Law LMJ, Wong JAJX, et al. Native folding of a recombinant gpE1/gpE2 heterodimer vaccine antigen from a precursor protein fused with Fc IgG. J Virol 2016. https://doi.org/10.1128/jvi.01552-16. Published online October 19 JVI.01552-16.
[22] Yanagi M, Purcell RH, Emerson SU, et al. Transcripts from a single full-length cDNA clone of hepatitis C virus are infectious when directly transfected into the liver of a chimpanzee. Proc Natl Acad Sci 1997;94(16):8738–8743. https://doi.org/10.1073/pnas.94.16.8738.
[23] Keck ZY, Xia J, Wang Y, et al. Human monoclonal antibodies to a novel cluster of conformational epitopes on HCV E2 with resistance to neutralization escape in a genotype 2a isolate. In: MS Diamond, editor. PLoS pathog. vol. 8; 2012, e1002653. https://doi.org/10.1371/journal.ppat.1002653. 4.
[24] Giang E, Dorner M, Prentoe JC, et al. Human broadly neutralizing antibodies to the envelope glycoprotein complex of hepatitis C virus. Proc Natl Acad Sci 2012;109(16):6205–6210. https://doi.org/10.1073/pnas.1114927109.
[25] Keck Z, Wang W, Wang Y, et al. Cooperativity in virus neutralization by human monoclonal antibodies to two adjacent regions located at the amino terminus of hepatitis C virus E2 glycoprotein. J Virol 2013;87(1):37–51. https://doi.org/10.1128/jvi.01941-12.
[26] Keck ZY, Sung VMH, Perkins S, et al. Human monoclonal antibody to hepatitis C virus E1 glycoprotein that blocks virus attachment and viral infectivity. J Virol 2004;78(13):7257–7263. https://doi.org/10.1128/jvi.78.13.7257-7263.2004.
[27] Law M, Maruyama T, Lewis J, et al. Broadly neutralizing antibodies protect against hepatitis C virus quasispecies challenge. Nat Med 2008;14(1):25–27. https://doi.org/10.1038/nm1698.
[28] Urbanowicz RA, McClure CP, Brown RJP, et al. A diverse panel of hepatitis C virus glycoproteins for use in vaccine research reveals extremes of monoclonal antibody neutralization resistance. J Virol 2016;90(7):3288–3301. https://doi.org/10.1128/jvi.02700-15.
[29] Salas JH, Urbanowicz RA, Guest JD, et al. An antigenically diverse, representative panel of envelope glycoproteins for hepatitis C virus vaccine development. Gastroenterology 2022;162(2):562–574. https://doi.org/10.1053/j.gastro.2021.10.005.
[30] Meunier JC, Engle RE, Faulk K, et al. Evidence for cross-genotype neutralization of hepatitis C virus pseudo-particles and enhancement of infectivity by apolipoprotein C1. Proc Natl Acad Sci 2005;102(12):4560–4565. https://doi.org/10.1073/pnas.0501275102.
[31] Stamataki Z, Coates S, Evans MJ, et al. Hepatitis C virus envelope glycoprotein immunization of rodents elicits cross-reactive neutralizing antibodies. Vaccine 2007;25(45):7773–7784. https://doi.org/10.1016/j.vaccine.2007.08.053.
[32] 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(5):811–813. https://doi.org/10.1093/infdis/jir399.
[33] Law JLM, Chen C, Wong J, et al. A hepatitis C virus (HCV) vaccine comprising envelope glycoproteins gpE1/gpE2 derived from a single isolate elicits broad cross-genotype neutralizing antibodies in humans. In: Bourgeois C, editor. PLoS ONE. vol. 8; 2013, e59776. https://doi.org/10.1371/journal.pone.0059776. 3.
[34] Meunier JC, Gottwein JM, Houghton M, et al. Vaccine-induced cross-genotype reactive neutralizing antibodies against hepatitis C virus. J Infect Dis 2011;204(8):1186–1190. https://doi.org/10.1093/infdis/jir511.
[35] 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(6):862–866. https://doi.org/10.1086/655902.
[36] 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(2):190–197. https://doi.org/10.1038/nm1353.
[37] Goldblatt D, Alter G, Crotty S, et al.. Correlates of protection against SARS-CoV-2 infection and COVID-19 disease. Immunol Rev 2022;310(1):6–26. https://doi.org/10.1111/imr.13091.
[38] Plotkin SA. Correlates of protection induced by vaccination. Clin Vaccine Immunol : CVI 2010;17(7):1055–1065. https://doi.org/10.1128/cvi.00131-10.
[39] Plotkin SA. Recent updates on correlates of vaccine-induced protection. Front Immunol 2023;13:1081107. https://doi.org/10.3389/fimmu.2022.1081107.
[40] Hoes J, Pasmans H, Schurink-van't Klooster TM, et al. Review of long-term immunogenicity following HPV vaccination: gaps in current knowledge. Hum Vaccin Immunother 2022;18(1):1908059. https://doi.org/10.1080/21645515.2021.1908059.
[41] Turner TB, Huh WK. HPV vaccines: translating immunogenicity into efficacy. Hum Vaccin Immunother 2016;12(6):1403–1405. https://doi.org/10.1080/21645515.2015.1103936.
[42] Liang TJ, Feld JJ, Cox AL, et al.. Controlled human infection model — fast track to HCV vaccine? N Engl J Med 2021;385(13):1235–1240. https://doi.org/10.1056/nejmsb2109093.
[43] Buchbinder SP, Mehrotra DV, Duerr A, et al. Efficacy assessment of a cell-mediated immunity HIV-1 vaccine (the Step Study): a double-blind, randomised, placebo-controlled, test-of-concept trial. Lancet 2008;372(9653):1881–1893. https://doi.org/10.1016/s0140-6736(08)61591-3.
[44] Hammer SM, Sobieszczyk ME, Janes H, et al. Efficacy trial of a DNA/rAd5 HIV-1 preventive vaccine. N Engl J Med 2013;369(22):2083–2092. https://doi.org/10.1056/nejmoa1310566.
[45] Kinchen VJ, Bailey JR. Defining breadth of hepatitis C virus neutralization. Front Immunol 2018;9:1703. https://doi.org/10.3389/fimmu.2018.01703.
[46] Keck ZY, Girard-Blanc C, Wang W, et al. Antibody response to hypervariable region 1 interferes with broadly neutralizing antibodies to hepatitis C virusOu JHJ, editor. J Virol 2016;90(6):3112–3122. https://doi.org/10.1128/jvi.02458-15.
[47] Carlsen THR, Pedersen J, Prentoe JC, et al. Breadth of neutralization and synergy of clinically relevant human monoclonal antibodies against HCV genotypes 1a, 1b, 2a, 2b, 2c, and 3a. Hepatology 2014;60(5):1551–1562. https://doi.org/10.1002/hep.27298.

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