Genetic and structural insights into broad neutralization of hepatitis C virus by human VH1-69 antibodies

Tzarum, Netanel; Giang, Erick; Kong, Leopold; He, Linling; Prentoe, Jannick; Augstad, Elias; Hua, Yuanzi; Castillo, Shaun; Lauer, Georg M.; Bukh, Jens; Zhu, Jiang; Wilson, Ian A.; Law, Mansun

Published in: Science Advances

DOI: 10.1126/sciadv.aav1882

Publication date: 2019

Document version: Publisher's PDF, also known as Version of record

Document license: CC BY-NC

An effective vaccine to the antigenically diverse hepatitis C virus (HCV) must target conserved immune epitopes. Here, we investigate cross-neutralization of HCV genotypes by broadly neutralizing antibodies (bNAbs) encoded by the relatively abundant human gene family $V_{H}1-69$. We have deciphered the molecular requirements for cross-neutralization by this unique class of human antibodies from crystal structures of HCV E2 in complex with bNAbs. An unusually high binding affinity is found for germ line–reverted versions of $V_{H}1-69$ precursor antibodies, and neutralization breadth is acquired during affinity maturation. Deep sequencing analysis of an HCV-immune B cell repertoire further demonstrates the importance of the $V_{H}1-69$ gene family in the generation of HCV bNAbs. This study therefore provides critical insights into immune recognition of HCV with important implications for rational vaccine design.

**RESULTS**

Here, we investigate how $V_{H}1-69$ bNAbs achieve cross-genotype recognition of the E2 neutralizing face and the genetic requirements for the immune system to generate such bNAbs. To overcome the inherent flexibility of E2 (28), we designed an E2 core domain construct (29) of the prototypic genotype 1a isolate, H77, and determined its crystal structure with bNAb AR3C (Fig. 1B and fig. S1A) (29). In parallel, Khan et al. (30) reported the crystal structure of a different E2 core construct derived from a genotype 2a isolate, J6, with nonneutralizing mAb 2A12. Structural analysis of H77 E2 in complex with AR3C Fab and alanine scanning mutagenesis (16, 29, 31) mapped AR3 to the E2 front layer and part of the CD81 binding loop (amino acids 426 to 443 and 529 to 531; Fig. 1A and fig. S1A). Most residues in AR3 are highly conserved among HCV genotypes (fig. S1C and table S1). Negative-stain electron microscopy revealed that in soluble E2, AR3 is exposed on the surface and not masked by the glycan shield or by VRs 1 to 3 (VR1–3; Fig. 1B) (29). To examine the accessibility of the AR3 neutralizing epitopes in soluble E2, we investigated binding of four AR3-directed mAbs
Fig. 1. Crystal structures of HK6a E2c3 and AR3 Fab complexes. (A) Schematic representations of the E2 core domain (amino acids 412 to 645) colored by structural components, with VRs in gray, front layer in cyan, β sandwich in red, CD81 loop in blue, and back layer in green. The E2 HVR1, stalk, and transmembrane region (amino acids 384 to 410 and 646 to 746) are not shown. The E2 N-linked glycans are indicated (29). (B) Modeling of the HVR1, VR2, and the Man 6 GlcNac 2 N-linked glycans in the complex structure of H77 E2c, indicating the accessibility of AR3. The Man 6 GlcNac 2 N-linked glycans were modeled on the basis of the N430 glycan in the H77 E2c structure [Protein Data Bank (PDB) entry 4MWF]. The AR3 is marked by a dashed line. The glycans that surround the AR3 are colored in dark gray. (C) KD values of various AR3 immunoglobulin G (IgG) binding to E2ΔTM, E2c, and E2c3 from genotypes 1a and 6a. (D) Neutralization breadth of AR3A–D, U1, and AR2A mAbs tested by HCVcc assays. Dendrograms shown for each mAb are colored on the basis of their half maximal inhibitory concentration (IC50) values. (E) Superposition of the crystal structures of HK6a E2c3-AR3A (blue), -AR3B (green), or -AR3D (pink) complexes with H77 E2c-AR3C (orange) illustrating the differences in the angle of approach of the AR3A–D mAbs to E2c. The structures of the HK6a E2c3-AR3A, -AR3B, or -AR3D complexes were superimposed with H77 E2c-AR3C on the basis of E2, and for each complex, the angle between the Cα atoms of HC V111, HC F54, and light chain (LC) I106 was calculated. The angle of approach was determined as the relative change in the angle between AR3A, AR3B, and AR3D mAbs to AR3C. For clarity, only the Fab VRs and the HK6a E2c3 structure are shown. (F) AR3 epitopes. The E2c structures are shown in surface representation, and the interacting residues in the epitopes were colored and labeled. (G) Schematic overview of the interactions between E2 and the AR3 HCs. The CDR sequences are aligned, and E2 interacting residues are highlighted in blue (hydrogen bonds) and green (hydrophobic interactions).
It has been shown that V_{H}1-69-derived bNAbs against influenza virus and HIV-1 interact with conserved hydrophobic residues in their antigens via hydrophobic residues at the tip of their CDRH2 loops (20–25). Sequence alignment of the V_{H}1-69-encoded AR3 mAb HC indicates a relatively low identity (62 to 73%) for the V_{H} region (amino acids 1 to 110; fig. S5A) and even less for the CDR loops (35 to 57%), especially CDRH3 (Fig. 1G). Nevertheless, the CDRH2 tip of AR3A–D mAbs still retains a hydrophobic motif of I/V52-P52a-X53-F54 (X = hydrophobic residue; Fig. 2A), which makes hydrophobic contacts with a highly conserved hydrophobic pocket between the front layer and CD81 binding loop, despite some variations in CDRH2 interactions. The AR3 mAbs have longer than average CDRH3 loops (18 to 22 residues) that interact with a flexible region of the front layer (amino acids 430 to 437; Fig. 1G). Their CDRH3 adopts a β hairpin stabilized by intra-CDR hydrogen bonds and further enhanced by a disulfide between the CDRH3 C strands in AR3A and AR3C (fig. S5B). The β hairpin conformations vary depending on CDRH3 length, but the two glycines at its apex contact L427 and W529 (for AR3A–C) of E2 (fig. S5B). Buried surface area (BSA) analysis confirms the indispensable role of the HC (Fig. 2B and fig. S5C). The HC BSA is similar for AR3A, AR3C, and AR3D (648 to 683 Å²), but slightly greater for AR3B (797 Å²). Structural analysis suggests that the optimal CDRH3 length for the AR3-like bNAbs is 18 to 22 residues, because a shorter loop will likely generate steric clashes with E2, especially with glycans surrounding AR3 (fig. S5D); a longer loop would result in E2 being further away (Fig. 2C), causing a reduction in binding and neutralization breadth, as observed for AR3D mAb (Fig. 1, C and D).

Therefore, we further characterized Abs from an HCV-immune Ab library (16, 17). Within 22 E1E2 HC binding groups, 7 were encoded by the V_{H}1-69 germline (GL) gene with CDRH3 loops ranging from 12 to 22 residues (fig. S5E). Abs from groups B (including AR1A and E) have shorter CDRH3s (15 or 12 residues, respectively) and little neutralization activity (16). Notably, CDRH2 of AR1A no longer contains the I/V52-P52a-X53-F54 hydrophobic motif (Fig. 2A). Fabs from group U also target AR3 (17), with a similar CDRH2 hydrophobic motif but a 17-residue CDRH3 (Fig. 2C), causing a reduction in binding and neutralization breadth (16). A similar reduction was observed when CDRH3 of AR3A was shortened by 1 residue to 17 residues (fig. S5D); a longer loop would result in E2 being further away (Fig. 2C), causing a reduction in binding and neutralization breadth, as observed for AR3D mAb (Fig. 1, C and D).

In the HIV field, it is widely acknowledged that maturation of bNAbs from their GL Ab precursors is a long evolutionary process (>2 to 5 years) involving selection for mutations contributing to B cell survival and improved Ab functions. Therefore, an in-depth analysis of how GL precursors recognize their target antigen can provide critical insights into bNAb induction (32). The IMGT V-QUEST program (33) was used to predict GL genes for HC (V_{H}1, D_{H}, and J_{H}) and LC (V_{L} and J_{L}) of AR3A–D mAbs (fig. S6A). The inferred GL precursors (AR3A–DGL) and variants with only the V_{H}1 gene reverted (AR3A–D_{1−69}) were synthesized. In comparison with anti-HIV bNAbs, AR3 mAbs exhibit relatively low levels of somatic hypermutation (SHM), with identity to the V_{H}1 GL of 84 to 91% and 74 to 84% at the nucleotide and amino acid levels, respectively (Fig. 3A and fig. S6B), similar to that of V_{H}1-69 anti-influenza bNAbs (23). Although CDRH2 contains a number of mutations
(amino acid identity of 64 to 76% with GL), the CDRH2 hydrophobic motif that presumably initiates the interaction with the E2 hydrophobic pocket is conserved from the GL (Fig. 3A). Binding of AR3GL and AR31–69 IgGs by enzyme-linked immunosorbent assay (ELISA) reveals that AR3A1–69, AR3B1–69, and AR3CGL interact with both H77 E2c and HK6a E2c (fig. S6C). Further quantification using biolayer interferometry for these GL precursors indicated only a 2- to 8-fold decrease in $K_D$ for H77 and 22- to 120-fold for HK6a (Fig. 3B and fig. S6D). AR3A–D GL and AR3A–D 1–69 (34) exhibited some neutralization above the 50% level for AR3A1–69, AR3ACL, and AR3B1–69, and more weakly for AR3C1–69 and AR3CGL, mainly against genotypes 5a and 6a (Fig. 3C), suggesting that neutralization breadth was acquired during affinity maturation. Thus, HCV binding and neutralization can be readily detected in $V_H$ 1-69 precursors.

Our GL reversion analysis and other studies (17, 18, 26) highlight the important role of the $V_H$ 1-69 gene family in E2 recognition and in broad neutralizing responses against HCV. Since the AR3 mAbs were isolated from an HCV-immune phage display Ab library, we used next-generation sequencing (NGS) to characterize enrichment of AR3-like mAbs during panning. The original and three other libraries from panning against native and epitope-masked E1E2 were subjected to NGS, followed by antibodyomics analysis (table S4 and see also Materials and methods). Quantitative library profiles show distinct patterns indicative of rapid enrichment after three panning steps (Pan1 to Pan3) using E1E2 (Fig. 4A). $V_H$ 1 is predominantly
selected in the converged Fab library, with V_H1-69 accounting for 87% of the total V_H population. A notable shift was observed in SHM, with the peak value increasing from 7–9% to 15–16% in Pan3 (Fig. 4A), and CDRH3 length also increased from 10 to 14 residues to enrichment of 15 to 17 residues (Fig. 4A). Two-dimensional (2D) identity/divergence plots visualize enrichment of AR1–3 mAbs from this Fab library (Fig. 4B and fig. S7A) by showing sequences with CDRH3 identity of 85% or higher (orange dots with number and library percentage labeled). Using AR3C as an example, the 2D plot shows a slow but steady increase of AR3C-like HCs from 0.01% in the prepanning library to 0.5% in the third panning cycle. Similar trends were observed for other AR3 mAbs with long CDRH3s (fig. S7A).

To assess the prevalence of V_H1-69 AR3 mAbs in comparison to those targeting other E1E2 antigenic sites, we also sequenced the Fab libraries panned with an E1E2 antigen that was masked with AR1 and AR3 Abs (17). HCV-specific mAbs of other GL origins were selected, including the V_H1-18, V_H3-30, and V_H7-51 families, some of which are cross-NAbs that recognize AR4 and AR5 on the quaternary E1E2 complex. Other mAb groups include mAbs that bind to epitopes that overlap with AR4 and AR5 (mAbs from groups R1 and S1) and non–cross-NAbs that bind to epitopes overlapping with AR1–3 [groups F, I, K, M, and T; for more details, see (17)]. Quantitative library profiles displayed little change in VH gene usage, SHM, and CDRH3 length during this epitope-masked panning (fig. S7B), confirming that mAbs AR4A and AR5A, although present in the prepanning library, were only slightly enriched during selection (fig. S7C). Thus, NGS analysis of an HCV-immune Fab library following antigen challenge revealed the prevalence of V_H1-69–derived Abs targeting AR1 and AR3 of E2, whereas Abs of other GL origins recognize different E1E2 antigenic sites (Fig. 4C).

To investigate whether this V_H1-69 preference is indeed common to HCV-specific bNAbs, we sequenced Ab repertoires of five patients with chronic HCV and compared their repertoires with those from five healthy individuals as controls. Overall, both repertoires displayed similar V_H1 gene distributions, with three subjects in each group showing relatively high

---

**Fig. 3. The inferred GL of AR3 mAbs.** (A) Alignment of the HC CDRs of AR3, AR3GL (inferred GL), and AR31–69 (reversion of only the VH gene to the VH1-69 sequence). The interacting residues in the mature mAbs are colored in blue (hydrogen bonds) and green (hydrophobic interactions). (B) Biolayer interferometry analysis of AR31–69 and AR3GL IgGs binding to H77 and HK6a E2c. (C) Single-dose (50 μg/ml) neutralization assay of AR31–69 and AR3GL IgGs against the eight HCVcc JFH1-based Core-NS2 recombinants of genotypes 1 to 6. Error bars represent SEM.
frequencies of $V_{H1-69}$ (fig. S8A). The HCV–infected donor repertoires exhibit a slightly broader range of CDRH3 lengths than the uninfected controls (normal distribution peaking around 11 to 14 residues [Fig. 4D]), yet with no significant difference for the average length of the HCDR3 loop between the two groups (fig. S10). Further analysis of the three largest $V_{H}$ gene families suggests that the distribution of some gene families could be affected by HCV infection (Fig. 4E). In contrast, both HCV-infected and healthy donors exhibit normal CDRH3 length distributions for the $V_{H3-23}$ gene family. Some differences are also observed for other $GL$ gene families (fig. S8B). Comparing the SHM between the two groups indicates similar distribution of both the HC and LC (fig. S8C). Comparison of the average SHM for the $V_{H1-69}$ gene family and for the overall $V_{H}$ repertoire indicates no significant difference between the two groups (fig. S10), although the sample size here is too small for conclusive statistical analysis.

**DISCUSSION**

The Centers for Disease Control and Prevention recently reported an almost threefold increase in the number of new HCV infections...
in the United States between 2010 and 2015 (35), a result of the increase in injection drug users caused by the opioid crisis in America (36), underscoring the urgent need for an effective HCV vaccine. The extreme genetic diversity of HCV demands elicitation of cross-genotype immune responses from vaccination. Therefore, structural understanding of bNAb recognition, together with the knowledge on how bNAbs develop from their GL precursors, is essential for the rational design of HCV vaccine immunogens (32, 37). As a main target for bNAbs, AR3 is highly conserved and overlaps with the majority of the E2 neutralizing face (29, 38). It was previously shown that, on native virions, HCV neutralization epitopes are shielded by HVR1 (9, 15) and glycans (39). To overcome the HCV escape by shielding of neutralization epitopes, it is crucial to design an antigen that can elicit a high level of bNAbs to conserved epitopes. Our results indicate that AR3 bNAb recognition of soluble E2 is largely independent of VRs (HVR1, VR2, and VR3) or glycans, highlighting that the AR3 and the neutralizing face are promising targets for inclusion in an HCV vaccine, although a better understanding of the entire E1E2 complex would likely serve to optimize this further.

The E2 structures with bNAbs AR3A–D suggest that flexibility of the front layer facilitates binding of bNAbs encoded by the V_{H1-69} GL gene family. Other critical features for breadth of AR3-like bNAbs include a hydrophobic CDRH2 tip and CDRH3 of around 18 to 22 residues. The absence of one or more of these features can result in reduced neutralization breadth (as suggested for mAb U1; Fig. 1D). In contrast to HIV-1 (32), some AR3 inferred GL mAbs bind E2 with high affinity, and low to medium SHM is sufficient to neutralize virus, although further somatic mutation can improve breadth. Our results are consistent with a recent study (27), in which AR3-like bNAbs of V_{H1-69} origin and with similar SHM were isolated from patients with spontaneous viral clearance, suggesting that HCV bNAbs can achieve broad neutralization with rapid lineage development. A possible reason is that the hydrophobic CDRH2 motif that interacts with critical E2 residues for CD81 binding is GL encoded. The tip of the CDRH2 encoded by the V_{H1-69} gene is the most hydrophobic among the human HC genes (25) and therefore has superiority for targeting the hydrophobic E2 neutralizing face and hydrophobic pockets in the flu hemagglutinin stem region (22, 23). Since most of the interactions of CDRH3 with E2 are main chain—main chain hydrogen bonds, they can tolerate variation in the sequence of the CDRH3 and, therefore, low levels of SHM can be sufficient to optimize the interaction with E2.

While HCV-directed bNAbs can stem from different GL genes, bNAbs encoded by V_{H1-69} have been reported in multiple studies (16, 18, 26, 27), suggesting an important role of this Ab family in the immune response to HCV. Our analysis revealed that V_{H1-69} Abs target AR1 and AR3 on E2 when HCV E1E2 was the panning antigen, but significant enrichment of AR1 non-NAbs suggests that AR1 may be more accessible in this format. However, other GL gene families are also involved in host Ab response to HCV. For rational vaccine design, a preferable strategy would appear to be developing HCV antigens that present the conserved AR3 neutralizing epitopes to engage the V_{H1-69} gene family, which is one of the frequent Ab families in the human Ab repertoire. It was recently reported that naïve B cells with high-avidity GL-encoded B cell receptors produce mainly IgM memory B cells following immunization in mice (40). If this is also true in humans, then one may expect that a GL-targeting strategy and E2-based vaccine antigens will trigger the generation of mostly antiviral V_{H1-69} IgM memory B cells. Such a strategy should be taken into consideration in HCV vaccine studies. Furthermore, HCV appears to be an excellent model for studying host humoral immunity to viral infection.

**MATERIALS AND METHODS**

**Expression and purification of the soluble E2 from genotypes 1 to 6**

The DNA constructs of the prototypic isolate H77 (41) for E2c (truncation of N and C termini and VR2 and removal of N448 and N576 glycosylation sites) and E2c3 (additional removal of VR3) were engineered on the basis of the soluble E2 ectodomain (E2ΔTM, amino acids 384 to 717) as previously described (28, 29). On the basis of sequence similarity to H77, E2c constructs of HCV1 (42), CON1 (43), UKN1b12.6 (44), J6 (45), UKN3A1.28c (44), S52 (46), ED43 (47), SA13 (48), and HK6a (34) isolates and the E2c3 constructs of H77, ED43, and HK6a isolates were engineered and synthesized by GeneArt (Invitrogen, USA; fig. S3A). Notably, the E1E2 envelope glycoprotein complex of the HK6a isolate was previously shown to be fully functional in vitro using the HCVpp and HCVcc systems and in vivo in chimpanzee and human liver chimeric mouse models (34, 49, 50). The E2 constructs were expressed and purified as previously described (29).

**Expression and purification of the AR3 IgGs and Fabs**

IgGs AR1A, AR3A, AR3B, AR3C, AR3D, and U1 were expressed and purified as previously described (16). The AR3A–D_{GL} and AR3A–D_{1-69} variable domain genes were synthesized by GeneArt (Invitrogen, USA), cloned into the pIgG vector, and expressed as previously described (16). Fabs AR3C and AR3D were cloned into the phagemid vector pComb3H and transformed into the BL21 (DE3) bacterial strain (Novagen) for expression in the presence of IPTG (isopropyl-β-D-thiogalactopyranoside) overnight at 16°C. The bacterial cells were treated with lysozyme, sonicated, and the Fabs were purified on a protein G affinity column. Fabs AR3A and AR3B were generated from the corresponding IgGs (16) by papain digestion (Sigma-Aldrich). All Fabs were further purified by size exclusion chromatography using a Superdex 200 column (Pharmacia) in 50 mM NaCl, 20 mM tris-HCl (pH 7.2) buffer.

**Crystallization and structural determination of E2c3 HK6a-Fab complexes**

From the 10 HCV isolates, only 4 isolates [H77 (1a), J6 (2a), ED43 (4a), and HK6a (6a)] gave high yield and were therefore used for the structural studies. The E2-Fab AR3 complexes were formed by overnight incubation of purified E2 and each AR3 Fab in a molar ratio of 1:1.25 (E2:Fab) at room temperature, followed by size exclusion chromatography (Superdex 200) to remove unbound Fab using 20 mM tris and 50 mM NaCl (pH 7.2) buffer. Crystallization experiments were performed using the vapor diffusion sitting drop method at 20°C. Crystallization experiments of the E2c from the H77, J6, ED43, and HK6a isolates in complex with AR3A–D yielded no crystals. However, further engineering the E2c proteins (E2c3; fig. S2A) resulted in crystals of the HK6a isolate in complexes with AR3A–D (table S2) that diffracted to 2.80, 2.60, and 3.30 Å for the AR3A, AR3B, and AR3D complexes, respectively, and to low resolution (~10 Å) for AR3C complexes. Crystals of the E2c3 HK6a-Fab AR3A complex were obtained using a reservoir solution of 20% (w/v) PEG 3500, 0.2 M sodium citrate (pH 8.2); crystals of E2c3
HK6a-Fab AR3B complex from a reservoir solution of 20% (w/v) PEG 3500, 0.2 M Li chloride (pH 6.7); and crystals of E2c3 HK6a-Fab AR3D complex from a reservoir solution of 20% (w/v) PEG 6000, 0.1 M Hepes (pH 7.0). Prior to data collection, all crystals were cryoprotected with 10% ethylene glycol and flash cooled in liquid nitrogen. Diffraction datasets were collected at the Advanced Photon Source (APS) and the Stanford Synchrotron Radiation Lightsource (SSRL) (table S2). Data were integrated and scaled using HKL2000 (51). The E2c3 HK6a-Fab AR3A structure was solved by molecular replacement method using Phaser (52), with the E2c H77-Fab AR3C (PDBe entry 4MWF) as a search model. The E2c3 HK6a-Fab AR3B and E2c3 HK6a-Fab AR3D structures were solved by molecular replacement method using the refined E2c3 HK6a-Fab AR3A structure as a search model. Structure refinement was carried out in Phenix (53), and model building was performed with COOT (54). Final refinement statistics are summarized in table S2.

Enzyme-linked immunosorbent assay

Purified E2 isolates were coated onto microwells at 5 µg/ml overnight at 4°C. After blocking with 4% (w/v) nonfat dry milk in dilution buffer (PBS + 0.02% Tween 20) for 30 min, titrated AR3 Abs (purified Ab for fig. S3B and transfected supernatant of Abs from 293T cells for fig. S6C) in dilution buffer with 1% nonfat dry milk were added to the microwells and incubated for 1 hour at room temperature. Titrated Abs were detected with secondary Ab hors eradish peroxidase–conjugated goat anti-human IgG Fc (Jackson ImmunoResearch) and developed with 3,3′,5,5′-tetramethylbenzidine substrate (Thermo Fisher Pierce). The reaction was stopped with 2 N sulfuric acid, and developed plates were read at absorbance of 450 nm.

\[ K_D \] determination

\[ K_D \] values were determined by biolayer interferometry using an Octet RED instrument (ForteBio Inc.). IgGs at ~10 µg/ml in a kinetics buffer [1x PBS (pH 7.4), 0.01% BSA, and 0.002% Tween 20] were immobilized onto protein G–coated biosensors and incubated with varying concentrations of E2 protein. The \( k_{on} \) and \( k_{off} \) values of each E2 protein for each IgG were measured in real time to determine \( K_D \) values. All binding data were collected at 30°C.

Neutralization assay

In vitro neutralization was carried on Huh7.5 cells using either a single high dose (50 µg/ml) or a dilution series of the relevant Ab as previously described (55). Control Ab b6 at the highest dose did not inhibit the viruses. For the dilution series experiments, IC\(_{50}\) values were calculated in GraphPad Prism 6 using a three-parameter curve fitting.

Panning of the phage display library

The construction of the phage display Ab library was previously described (16, 17). In summary, RNA of mononuclear cells from the bone marrow of a patient with chronic HCV was extracted, and complementary DNA (cDNA) was synthesized. LC and HC fragments were amplified using gene-specific primers and cloned into phagemid vector with randomized HC and LC pairing. For antigen panning, the Ab Fab fragments were expressed as a fusion protein to the phage coat protein III for surface display. The HCV-immune phage display Ab library was panned for three consecutive rounds with increasing washing stringency in two different panning experiments (16, 17). In the first experiment, recombinant E1E2 proteins captured by Galanthus nivalis lectin were used as the panning antigens. In the second experiment, E1E2 antigens captured by mAb AR3A and blocked with mAb AR1B were used (17). In each panning step, antigen was directly coated onto a microtiter plate, followed by blocking with 4% (w/v) milk in PBS. The phage library was added to the wells and incubated for 1 to 2 hours at 37°C, and unbound phages were washed away with PBS/0.5% (v/v) Tween 20. Bound phages were eluted and used to infect freshly grown Escherichia coli (XL1-Blue, Stratagene) for titration on LB agar plates with carbenicillin.

Clinical samples

Normal blood donor samples were procured from the Normal Blood Donor Service (NBDS) of The Scripps Research Institute (TSRI). All healthy donors tested negative for hepatitis B virus, HCV, and HIV (56). Blood samples from patients with HCV with long-term chronic HCV viremia (genotype 1 or genotype 3) were collected at Massachusetts General Hospital. Informed consent was given by all subjects under a protocol approved by the Partners Human Research Committee [Institutional Review Board (IRB) no. 1999P004983]. Peripheral blood mononuclear cells (PBMCs) were extracted by Ficoll-Paque (GE Healthcare Life Sciences) density gradient centrifugation. Plasma samples were evaluated for E1E2-specific Ab by ELISA and for virus neutralizing activity using HCVpp (17). Five HCV+ donor samples with high cross-neutralizing activity and five random normal blood donor samples were selected for sequencing analysis below.

NGS and bioinformatics analysis

NGS was performed on the Ion Torrent Personal Genome Machine (PGM) and S5 systems. The HC regions of the Fab phage libraries were amplified from the plasmid stock using PCR with vector-specific primers (tables S4 and S5 and fig. S9). Notably, the forward primer contained a PGM/S5 full-length P1 adaptor, whereas the reverse primer contained a PGM/S5 A adaptor and an Ion Xpress barcode (Life Technologies) to differentiate Fab phage libraries. A total of 25 PCR cycles were performed, and the PCR products with an expected length of 500 to 600 bp were gel purified (Qiagen). The resulting HC libraries were quantitated using the Qubit 2.0 Fluorometer with the Qubit dsDNA HS Assay Kit. The dilution factor required for PGM template preparation was determined such that the final concentration was 50 pM. For the prepping Fab library (Pan0), template preparation was performed with the Ion PGM Template IA 500 Kit, followed by NGS on the Ion PGM system using the Ion PGM Hi-Q Sequencing Kit and an Ion 316 v2 chip. For the postpanning libraries (Pan1 to Pan3) obtained from two E1E2 panning procedures, template preparation and (Ion 530) chip loading were performed on Ion Chef using the Ion 530 Ext Kit, followed by NGS on the Ion S5 System. For five normal donors and five HCV-infected donors studied here, a previously reported 5′-RACE (rapid amplification of cDNA ends) PCR protocol was used to capture their B cell repertoires in an unbiased manner (table S4) (57). Briefly, total RNA was extracted from 5 to 10 million PBMCs into 30 µl of water with the RNeasy Mini Kit (Qiagen). 5′-RACE was performed with the SMARTer RACE cDNA Amplification Kit (Clontech). The Ig PCRs were set up with the Platinum Taq High-Fidelity DNA Polymerase (Life Technologies) in a total volume of 50 µl, with 5 µl of cDNA as template, 1 µl of 5′-RACE primer, and 1 µl...
of 10 μM reverse primer. The 5’-RACE primer contained a PGM/S5 P1 adaptor, whereas the reverse primer contained a PGM/S5 A adaptor and an Ion Xpress barcode (Life Technologies) to differentiate donor libraries. A similar PCR protocol (25 cycles) was used, and the PCR products with an expected length of 600 bp were gel purified (Qiagen). The Ab HC and LC (x and λ) libraries were quantitated using the Qubit dsDNA HS Assay Kit and mixed at a ratio of 1:1:1 for each donor. The five donor libraries were further mixed at an equal ratio for template preparation and (Ion 530) chip loading on Ion Chef using the Ion 530 Ext Kit. NGS was performed on the Ion S5 System.

**REFERENCES AND NOTES**

- Supplementary material for this article is available at http://advances.sciencemag.org/cgi/content/full/5/1/eaav1882/DC1
- Fig. S1. AR3 epitopes.
- Fig. S2. Binding of AR3Δ-A and AR1Δ IgGs to E2.
- Fig. S3. Binding and neutralization of AR3 mAbs to HCV isolates from genotypes 1 to 6.
- Fig. S4. Crystal structures of HK6a E2c3 with AR3 Fab complex structures.
- Fig. S5. Features that enable broad neutralization by AR3.
- Fig. S6. The inferred GL genes of the AR3 mAbs.
- Fig. S7. Identity/divergence analysis of the phage display library from an HCV-infected donor.
- Fig. S8. B cell repertoire of healthy and HCV-infected donors.

**SUPPLEMENTARY MATERIALS**

**Table S1.** NGS of HCV-immune Ab repertoire of an HCV-infected patient.

**Table S2.** Data collection and refinement statistics for HK6a E2c3 AR3 Fab complex structures.

**Table S3.** Hydrogen bond and hydrophobic interactions between E2 and the HC of AR3 Fabs.

**Table S4.** Genotypic differences in the different panning steps.

**Fig. S4.** Crystal structures of HK6a E2c3 with AR3 Fabs.

**Fig. S6.** The inferred GL genes of the AR3 mAbs.

**Fig. S8.** B cell repertoire of healthy and HCV-infected donors.

**Fig. S10.** Summary of the statistic analysis of the NGS of HCV chronically infected and healthy donors.

**Table S1.** Amino acid sequence variation of the E2 protein of HCV genotypes 1 to 7.

**Table S2.** Data collection and refinement statistics for HK6a E2c3 AR3 Fab complex structures.

**Table S3.** Hydrogen bond and hydrophobic interactions between E2 and the HC of AR3 Fabs.

**Table S4.** NGS of HCV-immune Ab repertoire of an HCV-infected patient.

**Table S5.** NGS of HCV-immune Ab repertoires.

**REFERENCES AND NOTES**


Acknowledgments: We thank R. Stanfield, X. Dai, and M. Eiliger for crystallographic and computational support; H. Tien in the Wilson lab for automated crystal screening; K. Cogburn, A. Honda, and K. Jackson for HIV donor sample analysis; and J. Ball for the UKN HIV constructs. Funding: This work was funded in part by NIH grants RO1AI079301 (to M.L.), RO1AI23365 and RO1AI106005 (to M.L. and I.A.W.), U19AI23861 (to M.L. and J.Z.), U01AI131314 (to G.M.L.), and R01AI123861 (to M.L. and J.Z.); the HIV Vaccine Research and Design (HIVRAD) program RO1AI24337 to J.Z.; the Danish Council for Independent Research DFF–4004-00598 (to J.Z.); the Novo Nordisk Foundation NFF140010253 (to J.Z.); and the Candys Foundation (to J.P., E.A., and J.B.). NBDS at TSRI was supported by the Clinical and Translational Science Center (CTSA) grant UL1 TR001114. X-ray datasets were collected at the APS beamline 23ID-B (GM/CA CAT) and SSRF beamline 12-2. The use of the APS was supported by the U.S. Department of Energy (DOE), Basic Energy Sciences, Office of Science, under contract DE-AC02-06CH11357. The use of the SSRF Structural Molecular Biology Program was supported by the DOE Office of Biological and Environmental Research and by the NIH NIGMS (including P41GM103399) and the National Center for Research Resources (P41RR001209). Authors’ contributions: N.T., J.Z., I.A.W., and M.L. designed the project. N.T., E.G., and Y.H. expressed and purified the E2 proteins and mAbs. N.T. and L.K. determined and analyzed the x-ray structures. N.T., S.C., and E.G. determined Ks and assessed binding. J.Z. and L.H.
performed the NGS. J.P., E.A., and J.B. performed the HCVcc neutralization assays. G.M.L. provided the clinical samples and discussion, and N.T., J.B., J.Z., I.A.W., and M.L. wrote the manuscript with support from all the other authors. Competing interests: The authors declare that they have no competing interests. Data and materials availability: All data and code to understand and assess the conclusions of this research are available in the main text, Supplementary Materials, and the PDB (accession codes 6BKB, 6BKC, and 6BKD). Additional data related to this paper may be requested from the authors. This is manuscript 29614 from The Scripps Research Institute.

Genetic and structural insights into broad neutralization of hepatitis C virus by human V\textsubscript{H}1-69 antibodies
Netanel Tzarum, Erick Giang, Leopold Kong, Linling He, Jannick Prentoe, Elias Augestad, Yuanzi Hua, Shaun Castillo, Georg M. Lauer, Jens Bukh, Jiang Zhu, Ian A. Wilson and Mansun Law

DOI: 10.1126/sciadv.aav1882

SUPPLEMENTARY MATERIALS
http://advances.sciencemag.org/content/suppl/2018/12/21/5.1.eaav1882.DC1

REFERENCES
This article cites 65 articles, 17 of which you can access for free
http://advances.sciencemag.org/content/5/1/eaav1882#BIBL

PERMISSIONS
http://www.sciencemag.org/help/reprints-and-permissions