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4 **Structure of hepatitis C virus envelope glycoprotein E1 antigenic site 314-324 in complex**
5 **with antibody IGH526**
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Abstract

Hepatitis C virus (HCV) is a positive-strand RNA virus within the *Flaviviridae* family. The viral “spike” of HCV is formed by two envelope glycoproteins, E1 and E2, which together mediate viral entry by engaging host receptors and undergoing conformational changes to facilitate membrane fusion. While E2 can be readily produced in the absence of E1, E1 cannot be expressed without E2 and few reagents, including monoclonal antibodies, are available for study of this essential HCV glycoprotein. A human MAb to E1, IGH526, was previously reported to cross-neutralize different HCV isolates and, therefore, we sought to further characterize the IGH526 neutralizing epitope to obtain information for vaccine design. We found that MAb IGH526 bound to a discontinuous epitope, but with a major component corresponding to E1 residues 314-324. The crystal structure of IGH526 Fab with this E1 glycopeptide at 1.75Å resolution revealed that the antibody binds to one face of an α -helical peptide. Single mutations on the helix substantially lowered IGH526 binding but did not affect neutralization, indicating either that multiple mutations are required or that additional regions are recognized by the antibody in the context of the membrane-associated envelope oligomer. Molecular dynamics simulations indicate the free peptide is flexible in solution, suggesting that it requires stabilization for use as a candidate vaccine immunogen.

Highlights:

- **IGH526 is a cross-neutralizing antibody that recognizes HCV E1 glycoprotein**
- **IGH526 binds a discontinuous epitope with a linear component near the terminal membrane proximal region of E1**
- **The linear component of the epitope is α -helical when bound to IGH526**
- **Molecular dynamics suggest the unliganded E1 linear peptide is flexible in solution**
- **This study defines the first target on HCV E1 for structure-based immunogen design**

Key words: HCV; E1 envelope glycoprotein; IGH526; vaccine design

Introduction

Hepatitis C virus (HCV) infects more than 150 million people worldwide and is a leading cause of liver cirrhosis and hepatocellular carcinoma in developed countries^{1, 2}. It is estimated that 3-4 million people are newly infected each year^{2, 3}. Recent advances in direct-acting anti-viral (DAA) drug development have given hope for curing this devastating human disease⁴⁻⁶. However, as many of the new infections are associated with injecting drug usage (IDU), particularly in young adults in developed countries and in regions along illegal drug trafficking routes^{7, 8}, it is unclear how the new treatments could be effectively disseminated to these marginalized and sometimes mobile populations. Furthermore, recent studies have shown that treated patients are not entirely immune to reinfection^{9, 10}, indicating DAA treatment alone will not be sufficient in controlling the HCV endemic. Considering the socio-economic factors associated with HCV infection, an effective vaccine will be needed to combat this human pathogen.

HCV is a single-stranded, positive-sense RNA virus in the *Flaviviridae* family. The 9.6-kb virus genome encodes a single open reading frame and the translated polypeptide is processed further by host and viral proteases into 10 viral proteins¹¹. A T-cell based vaccine, based on adenovirus and poxvirus vectors expressing the non-structural proteins NS3, NS4A, NS4B, NS5A and NS5B, is currently being evaluated in a phase II clinical trial¹². Another vaccine candidate, based on the structural envelope glycoproteins E1 and E2, has been evaluated in different animal models including chimpanzees¹³ and in a phase I study¹⁴. This candidate subunit vaccine was safe in humans and elicited moderate level of virus neutralizing antibodies in some of the test subjects^{15, 16}. However, the results suggest that, for a subunit vaccine to be efficacious, the antigenicity and immunogenicity of the vaccine antigens must be improved through rational vaccine design.

The E1 and E2 glycoproteins form a heterodimer (E1E2) on the viral surface and mediate viral entry^{11, 17}. E2 is the receptor binding protein, but the function of E1 is currently unknown. Developing an HCV vaccine is challenging due to the high antigenic variability of E1 and E2^{18, 19}. Structural characterization of cross-reactive neutralizing antibody (NAb) epitopes on E1 or E2 can therefore provide templates for vaccine design to circumvent this variability.

E1 is known to be less immunogenic compared to E2²⁰. However, two E1 regions targeted by monoclonal antibodies (MAb) have been identified: residues 192-202, which are recognized by the weakly neutralizing MAb H-111²¹; and residues 313-328, which interact with the cross-reactive NAb IGH526 and IGH505²². MAb IGH526, the subject of this study, is of particular interest because it has been shown to cross-react with and cross-neutralize several HCV genotypes²². Its epitope has been mapped to E1 residues 313-328 using a library of overlapping

peptides of E1 and site-directed mutagenesis. Residues 313-328 are nearly universally conserved²³ and are recognized by 30 of 92 HCV patient sera²⁴, and 15 of 41 vaccinee sera¹⁶, thereby representing a promising antigenic target for vaccine design. Previously, NMR was used to characterize E1 residues 314-342²⁵, with results indicating that residues 319-323 adopt a helical conformation, while residues 314-315 and 324-328 do not have regular secondary structure. Although this NMR study suggested a helical propensity for residues 313-328, the NMR measurements were performed in the presence of 50-80% hexafluoroisopropanol, which can induce helical conformation in peptides^{26, 27}. Also, for vaccine design, it is critical to determine the conformation of epitopes recognized by NABs. Therefore, in the present study, we sought to characterize MAb IGH526, which could be of great utility for studying HCV E1, and for obtaining structural information on this major E1 antigenic site for rational vaccine design.

Results

MAb IGH526 is a neutralizing antibody recognizing a discontinuous epitope that includes a linear component on E1

MAb IGH526 was cloned and expressed in mammalian FreeStyle™ 293F cells as full-length IgG1. First, we examined its biological activity. In virus neutralization assays, IGH526 neutralized the prototypic HCV strain H77 albeit less potently than the well-characterized anti-E2 MAb AR3A or anti-E1E2 MAb AR4A (Fig. 1a)²⁸. Second, we showed that two overlapping peptides spanning E1 region 313-327 specifically blocked its binding to E1E2 (Fig. 1b), confirming the epitope mapping results from a previous study²². However, when E1E2 was reduced and denatured, binding of MAb IGH526 in ELISA was nearly abolished (Fig. 1c). Furthermore, the antibody also did not recognize E1 in immunoblots (data not shown). Together, the observations suggest IGH526 recognizes a linear region at residues 313-327 on E1, but that there might be additional regions on E1 or E2 involved since binding is severely decreased on E1/E2 denaturation. In an attempt to find these regions, the antibody was tested against a panel of overlapping peptides spanning the viral structural proteins Core (residues 1-191), E1 and E2. The results showed that IGH526 bound only to the previously identified E1 peptide (Fig. 1d). For comparison with a positive control, we performed the same experiment for MAb HCV1, a broadly neutralizing antibody that is known to recognize a linear region in E2²⁹, which this assay clearly confirmed. This is in agreement with the observation above that binding of MAb IGH526 to E1E2 could be blocked entirely by peptides encoding the putative linear epitope (Fig. 1). Together, the results indicate that MAb IGH526 recognizes a discontinuous epitope on either E1 or E1E2, with a major component appears to be a linear epitope spanning E1 region 313-327.

Crystal structure of Fab IGH526 bound to an E1 peptide reveals a helical epitope

We used x-ray crystallography to characterize how MAb IGH526 interacts with the linear component of its epitope. MAb IGH536 Fab was generated and its crystal structure was determined at 1.75Å resolution in complex with an 11-residue peptide (amidated at the C-terminus), corresponding to E1 residues 314-324) (Table S1 and Fig. S1). The peptide (TGH_RMAWDMMM) adopts a 2.5-turn α -helix when bound to IGH526 Fab (Fig. 2a). The peptide is wedged against an extended 16-residue CDR H3 loop and inserted into a shallow cleft formed by the CDR H1, H2, L1, and L3 loops. Lack of CDR L2 contact is not uncommon for small molecule or peptide antigens³⁰. Overall, IGH526 Fab buries 530Å² of the peptide surface; the binding interface consists of 126 van der Waals interactions, four hydrogen bonds and one aromatic hydrogen bond (Tables S2 and S3). Four hydrogen bonds between the antibody and the peptide main chain form interactions with the peptide in a side-chain independent manner (Fig. 2b). The antibody-antigen union is further enhanced by specific interactions with His316 and Trp320, two of the bulkier residues on the antigen, with the antibody (Fig. 2b and 2c): (i) an aromatic hydrogen bond³¹ is formed between the backbone amide of Phe^{H98} of CDR H3 and the electronegative phenyl ring of the Trp320 indole (Fig. 2b, bottom left); (ii) water-mediated hydrogen bonds are made to both imidazole nitrogens of His316 by Asp^{H95} of CDR H3 and Thr^{L32} of CDR L1 (Fig. 2c, top); and (iii) a water-mediated hydrogen bond is made between the backbone carbonyl of Asp^{H95} in CDR H3 and the indole N-H of Trp320 (Fig. 2c, bottom). The structure suggests that this E1 helix cannot be extended further in the intact protein as any extension of the helix on the N-terminal or C-terminal side would quickly result in severe clashes with the antibody (Fig. S2).

Mutagenesis of IGH526 epitope residues severely inhibits IGH526 binding to membrane-extracted E1E2 but does not disrupt IGH526 neutralizing activity

Previous epitope mapping using peptides showed that substitution of His316, Ala319, Trp320 Met323, and Met324 to alanine or glycine had the most impact on IGH526 binding²² in general agreement with the structure (Fig. 2d-e), which shows these residues contact multiple antibody residues and are substantially buried within the antibody-antigen interface. Arg317 at the perimeter of the interface has a substantial interaction with the antibody, but mutation to alanine only modestly reduced binding. To further investigate this epitope, we introduced single mutations into E1E2 for binding and neutralization studies. The binding assays with membrane-extracted E1E2 show that alanine/glycine replacement at His316, Trp320 and Met323 had the most impact, followed by Ala319, Met324 and also Arg317 (Fig. 3a). These residues all face the antibody in the peptide-complex structure, while mutations on the non-binding side of the helix (Met318, Asp321 and Met322) had less impact on antibody binding. In a functional study, alanine mutation at Gly315, Met318, Asp321, Met322 or Met323 completely abolished viral infectivity (Fig. 3b), suggesting these residues may be important in protein folding by interacting with other portions of E1E2. Surprisingly, despite severe loss of binding to the antibody in ELISA, the H316A and W320A single mutants were less sensitive to IGH526 neutralization,

(Fig. 3b-d), indicating that the single mutations did not disrupt the epitope on the virus to the same extent as observed in membrane-extracted E1E2 or in linear peptides²². However, a H316A/W320A double mutant could not be neutralized by IGH526 even at high antibody concentrations (Fig. 3c-d).

Molecular dynamics suggests the linear component of the IGH526 epitope is highly flexible

While structural characterization of the E1 ectodomain or the E1E2 complex will be required to fully define the IGH526 epitope, the crystal structure of the peptide complex revealed the high affinity linear component of the epitope, which could serve as a starting point for vaccine design. Therefore, we sought to further characterize the stability and conformational dynamics of this E1 peptide. Molecular dynamics (MD) simulations were conducted for 25 ns on the bound and unbound peptides starting from the crystal structure coordinates (Fig. 4 and Fig. S3). When bound, the peptide maintained a similar conformation as in the crystal structure with a C α root mean square deviation (RMSD) of 0.42 ± 0.1 Å, calculated over the last 15 ns (Fig. 4a and 4c). When unbound, the peptide unfolded at the N-terminus during 3-8 ns, but remained helical at Ala319-Met324 throughout most of the simulation (Fig. 4b and 4c). Accordingly, the C α RMSD, calculated over the last 15 ns, across the whole peptide, relative to the crystal structure, was 3.4 ± 0.1 Å, while the C α RMSD of just Ala319-Met324 was 0.38 ± 0.1 Å (Fig. 4b and Fig. S3). This simulation is consistent with Ala319-Met325 containing three methionines and one alanine, residues that have high helical propensity³². In the synthetic peptide, the C-terminal region is further stabilized by the amidated C-terminus, which forms a hydrogen bond with the backbone carbonyl of Asp321.

Discussion

In this study, we sought to characterize the epitope of the anti-E1 MAb IGH526. Although this antibody is not as potent as some anti-E2 MAbs isolated, it cross-reacts with multiple HCV isolates by recognizing an immunogenic and highly conserved region on E1^{16,22}. Furthermore, it is one of the few anti-E1 MAbs available and, therefore, an invaluable reagent for study of this recalcitrant protein, whose function for the virus remains unknown. E1 is challenging to study because it requires co-expression with E2 and forms heterogeneous soluble aggregates of E1E2 even when expressed without the transmembrane domain³³⁻³⁶. The absence of a folded E1 independent of E2 renders it difficult to determine whether IGH526, isolated from infected patients, interacts with E1 only or with the E1E2 complex. The recently published structure of the E1 N-terminus domain reveals a unique cross-linked organization, suggesting E1E2 could be presented as a multimeric oligomer on the virus³⁷. While it is unclear how closely the membrane extracted E1E2 used for binding studies here resemble the functional oligomer on the virus, as shown above, IGH526 appeared to distinguish between them since it retained neutralizing ability against viruses harboring mutations that abrogated binding to extracted E1E2. These results and

the observation that the antibody failed to bind denatured E1E2 highlight the complexity of the IGH526 epitope; a complete definition would require structural characterization of the functional E1E2 oligomer. Because this is hindered by technical challenges in producing well-behaved, soluble protein, we focused on the linear component of the IGH526 epitope initially identified by peptide scanning.

The crystal structure of the antibody-bound peptide complex revealed a helical conformation of the linear component interacting with IGH526 across one face. This finding corroborates with the previously published NMR study of a longer peptide indicating helical propensity for the same region²⁵. Of note, E1 region is primarily hydrophobic, suggesting it may be proximal to the viral membrane³⁸. Furthermore, the residues in this region are nearly universally conserved across all HCV strains despite high sequence variability, consistent with membrane proximal regions of other viruses like HIV^{22, 39}. In this regard, IGH526 may resemble anti-HIV MAb 4E10, which binds to a highly conserved membrane proximal helix of gp41⁴⁰. MAb 4E10 displays reactivity towards lipid, which raises the same possibility for IGH526, and could be an explanation for why IGH526 recognizes membrane-bound E1E2 differently. Again, further study of the antibody in a more functional context is required to test this possibility.

Finally, by elucidating the binding surface of IGH526 on the α -helical peptide, we have provided a first template of E1 for immunogen design. Furthermore, MD simulations suggested the free peptide is flexible, particularly at its N-terminus, which matches the previous NMR study²⁵. Therefore, it will be critical to stabilize the free peptide as a short helix and enhance the presentation of the binding surface through multimerization of this E1 antigenic site on a nanoparticle. Although this linear region does not appear to represent the complete epitope of IGH526, stabilized immunogens may be useful in boosting the immune response towards this highly conserved antigenic site after an initial prime by soluble E1E2.

Materials and Methods

Antibody production

The V_H and V_L domains of human antibody IGH526²² were synthesized (GenScript) and fused with human C_H1-3 and C_κ domains using the pIgG1 vector⁴¹ for high-level protein expression of a complete IgG by transient transfection of 293F cells. To prepare the Fab fragment for crystallization studies, C_H2-3 domains in the expression vector were deleted and soluble Fab was produced by transient transfection of 293F cells with the recombinant DNA. The Fab was purified by affinity chromatography using a rabbit anti-human Fab antibody column followed by size exclusion chromatography with a Superdex 200 column (GE Healthcare).

Synthesis of the TGHMAWDMMM.amide peptide

Rink Amide AM resin (282 mg, 0.2 mmol) (Novabiochem, 200-400 mesh, 0.71 mmol/g substitution) was weighed directly into a disposable solid-phase peptide synthesis (SPPS) reaction vessel (CSPS Pharmaceuticals) and swelled with *N,N*-Dimethylformamide (DMF) for one hour. Fmoc protecting group removal was accomplished by two treatments of the resin with 20% 4-methylpiperidine in DMF for a duration of two minutes each. After thorough washing of the resin with DMF, the appropriate Fmoc amino acid (1.0 mmol) was dissolved in a solution of 1H-Benzotriazolium 1-[bis(dimethyl-amino)methylene]-5-chloro-hexafluorophosphate (1-),3-oxide (HCTU) in DMF (2.5 mL, 0.4 M) in a scintillation vial. Diisopropylethylamine (261 µL, 1.5 mmol) was added to the vial. After 30s of preactivation, the contents of the vial were added to the drained but wetted resin. Coupling was performed for 45 minutes. At the end of the synthesis, the resin was washed with dichloromethane (DCM) and dried under vacuum for 1 hour. Cleavage from the solid-phase was achieved using 95% trifluoroacetic acid/2.5% H₂O/2.5% triisopropylsilane for 2h. The resin was filtered and the TFA was concentrated with a stream of nitrogen prior to the peptide being precipitated by the addition of cold diethyl ether. Peptide was pelleted by centrifugation and dissolved in 30% acetonitrile in water containing 0.05% TFA prior to being flash frozen and lyophilized. Crude peptide was purified by RP-HPLC. Electrospray mass spectrometry confirmed the identity of the peptide as the expected product (expected: 1365.6 amu, found: 1365.4 amu).

Enzyme-linked immunosorbent assay (ELISA)

(i) To determine whether the antibodies recognized native, reduced or mutant E1E2, the antigens were captured onto ELISA wells (Maxisorp ELISA plates, Corning) that were pre-coated with *Galanthus nivalis* lectin (5 µg/ml, Sigma) (folded protein), or unfolded with 0.1% SDS, 50 mM DTT and incubated at 56°C for 15 min before capture onto ELISA wells (unfolded protein). E1E2 and mutant antigens were prepared from cell lysates of 293T cells transfected with H77 E1E2-expression plasmid⁴². Non-fat milk (4%, BioRad) in PBS and 0.05% Tween 20 was used as a blocker in assays using lectin-captured antigens. The ELISA plates were washed after 1 h incubation and binding of antibodies was detected by peroxidase (HRP)-conjugated goat anti-human IgG Fc or anti-mouse IgG secondary antibody (1:2000) (Pierce) and TMB substrate (Pierce). (ii) To study the ability of peptides in inhibiting MAb IGH526 binding to E1E2, the peptides and mAb were mixed at the specified ratio and incubated for 30 min before adding to ELISA microwells coated with lectin-captured E1E2. After 1 h incubation, the plates were washed and bound antibody was detected with secondary antibody and chromogenic substrate as above. (iii) To study the peptide specificity of antibodies, a series of overlapping peptides consisting of 18-mers, with an overlap of 12 amino acids covering the E1E2 HCV glycoprotein region was used. The peptides were obtained from NIH AIDS Research & Reference Reagent Program, as HCV type 1a H77 (18-mer) Peptides - Complete Set (Cat# 7620, LOT# 4). The

peptides were dissolved in DMSO at 1 mg/ml and diluted in PBS for ELISA. Briefly, microwell plates were coated with 200 ng/well of peptide in duplicate, after blocking the plates were incubated with 10 μ g/ml of antibody for an hour and binding signals were developed and measured as above.

Virus neutralization assay

Neutralization assays were performed in Dulbecco's Modified Eagle Medium (D-MEM) supplemented with 10% fetal calf serum (FCS) (Invitrogen). HCVpp was generated by co-transfection of 293T cells with pNL4-3.LucR^E ^{43, 44} and the corresponding expression plasmids encoding the E1E2 genes at 4:1 ratio by polyethylenimine ⁴⁵. Virus infectivity was detected using the firefly luciferase assay system (Promega) and % neutralization was calculated as residual virus infectivity at the indicated antibody concentrations divided by infectivity without antibody after background subtraction. Background infectivity of the pseudotype virus was determined using cells transfected with pNL4-3.lucR^E only and VSVpp was a control for non-specific activity. In the neutralization assay, the virus was incubated with the diluted antibodies for 1 h at 37° C before adding to Huh-7 cell monolayers and incubated for another 6 h. For virus with low infectivity, the 6h incubation step was replaced with spinoculation at 800 g for 1 h. Expression of the luciferase reporter gene in the infected cells was measured 3 days post infection.

Complex formation and crystallization

The IGH526 Fab was concentrated to 8-12 mg/mL using an Amicon centrifugal ultrafiltration unit (Millipore) with a 10 kDa molecular weight cutoff. The concentrated Fab and E1 peptide were incubated at a 1:10 molar ratio overnight at 4° C in 150 mM NaCl, 20 mM Tris-HCl pH 7.2 to form the complex. Samples were screened for crystallization using the 384 conditions of the JCSG Core Suite (Qiagen) at both 277 and 293 K using the JCSG robotic Crystallization system (Rigaku). Crystallization was performed using the nanodroplet vapor diffusion method ⁴⁶ with standard JCSG crystallization protocols ⁴⁷. Sitting drops composed of 200 nl of protein sample were mixed with 200 nl of crystallization reagent in a sitting drop format and were equilibrated against a 50 μ l reservoir of crystallization reagent. After approximately 7 days at 20° C, crystals formed in 20% (w/v) PEG 6000, 1M lithium chloride and 0.1 M citric acid, pH 4.0 (JCSG Core Suite 1, well H10).

X-ray data collection

IGH526-peptide complex crystals diffracted to 1.75 Å at beamline 23ID-D of the Advanced Photon Source (APS). Data were collected at 100 K to a completeness of 100% with an overall R_{sym} of 9% and R_{pim} of 4% (Table 1). Data were indexed, processed and scaled with HKL-2000

⁴⁸ in orthorhombic space group $P2_12_12_1$ with unit cell parameters $a = 66.3\text{\AA}$, $b = 67.6\text{\AA}$, $c = 120.7\text{\AA}$. One protein-peptide complex is present per asymmetric unit with a Matthews' coefficient (V_m) of $2.71\text{\AA}^3/\text{Da}$ and solvent content of 54.6%.

Structure determination and refinement

The structure was determined by molecular replacement using Phaser ⁴⁹ with the HCV1 Fab structure (PDB ID: 4DGV) as an initial model. Model building was carried out using Coot-0.6.2 ⁵⁰ and refinement was implemented with Phenix ⁵¹. Final R_{cryst} and R_{free} values are 18.4 % and 21.5 %, respectively. Buried molecular surface areas were analyzed with MS ⁵² using a 1.7 $\text{\AA}$ probe radius and standard van der Waals radii, and van der Waals contacts and hydrogen bonds were evaluated with CONTACSYM ^{53, 54} and HBPLUS ⁵⁵. Surface potential and electrostatics were calculated using APBS ⁵⁶ and all structural visualizations were generated with PyMOL (The PyMOL Molecular Graphics System, Version 1.2r3pre, Schrödinger, LLC.). For the Fab, the residues were numbered according to the Kabat scheme ⁵⁷.

Molecular dynamics (MD) simulations

(i) Peptide and peptide-antibody structure preparation for MD. The starting structural coordinates for unbound peptide and peptide-antibody were obtained from the IGH526 complex structure solved in this study. The structures were prepared by assigning bond orders and adding hydrogen atoms using default protocol from the protein preparation wizard interface with Maestro 8.5 in Schrödinger biologics suite (www.schrodinger.com). Force field parameters for standard amino acid residues were obtained from OPLS-AA (Optimized Potentials for Liquid Simulations-All Atom) force field ⁵⁸.

(ii) MacroModel energy optimizations. Optimized structures were subsequently minimized in MacroModel 9.7 in Schrödinger biologics suite using steepest descent method with maximum of 500 iterations with gradient convergence threshold of 0.05 and constant dielectric with dielectric constant of 1. The OPLS-AA force field with extended cutoff values (van der Waals = $8.0\text{\AA}$; electrostatic = $20.0\text{\AA}$; H-bond = $4.0\text{\AA}$) was used for the minimizations.

(iii) System preparation for simulations. MD simulations were performed using OPLS-AA force field in Desmond MD package with Maestro-Desmond interoperability tool, version 2.0 ⁵⁹. The systems were set up for explicit solvent simulations. The solute was placed in an orthorhombic box containing SPC water ⁶⁰ at 1.5 nm from solute boundaries. The physiological salt concentration of 0.15 M was used.

(iv) MD simulations. MD simulations for a period of 25 ns were performed for both structures using the following three steps protocol: minimization; equilibration; and production run.

Minimization. The setup systems were minimized to remove close contacts between solute-solvent molecules using the Limited Memory Broyden-Fletcher-Goldfarb-Shanno (LBFGS) method with maximum iterations of 200 cycles and convergence threshold of $1.0\text{ Kcal mol}^{-1}\text{\AA}^{-1}$.

The step size was 0.005 ps and the switch criterion was 25.0 Kcal mol⁻¹ Å⁻¹. The short-range interactions used cutoff radius of 9.0 Å, whereas the long-range Coulombic interactions were taken into account using smooth Particle Mesh Ewald (PME) with Ewald tolerance of 1e⁻⁰⁹ Å.

Equilibration protocol. During equilibration, 2000 steps of steepest descent minimization of water molecules and ions were performed to allow water molecules to assume a lower energy geometry, while the solute was restrained with a force constant of 50 kcal/mol Å². The resulting systems were then subjected to 2000 steps of minimization with no restraints, reaching a root mean square gradient of 0.1 to assure the relaxation of the structures, followed by 12 ps of heating from 10 to 300 K in a constant volume ensemble with restraints on the solute heavy atoms (50 kcal/mol Å²). Annealing was followed by 12 ps of constant pressure unrestrained simulation at 300 K, where convergences of energies, temperature, pressure and density of the systems was monitored.

Production runs protocol. The output coordinates and velocities from the equilibrated run were subsequently used in production runs over a period of 25 ns at 300 K using an isothermal-isobaric (NPT) ensemble. Hydrogen bonds were constrained with a variant of the M-SHAKE algorithm⁶¹. To maintain 1 atm at 300 K in NPT ensemble, the system was coupled to a Martyna-Tobias-Klein barostat⁶² with relaxation time of 2 ps with isotropic coupling style, and a Nose-Hoover thermostat with relaxation time of 1 ps⁶³. Long-range electrostatic interactions were modeled using a Particle Mesh Ewald method⁶⁴ and a 64 × 64 × 64 Fourier space mesh with fifth-order B spline interpolation. The van der Waals (VDW) interactions and real space contributions to the electrostatics were truncated at 10 Å, and estimated the long-range VDW contributions to the energy and the pressure by assuming a homogeneous distribution of VDW spheres with dispersion coefficient 69.5 kcal/mol/Å. RESPA integrator⁶⁵ with steps of 2 fs was used for bonded and short-range non bonded interactions, and 6 fs for long-range electrostatics. During the course of simulations, quality checks of the system were performed by monitoring the total energy profile and the Cα RMSD of structures in MD trajectories.

Accession numbers:

The atomic coordinates have been deposited in the Protein Data Bank, www.pdb.org (PDB ID code 4N0Y).

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Competing interests statement

The authors declare no conflict of interest.

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FIG. 1. Biological activities of recombinant MAb IGH526. (a) Neutralization of HCV by MAb IGH526. The MAb neutralized HCV pseudotype virus particles (HCVpp) displaying the genotype 1a E1E2, but not the control envelope glycoprotein G from vesicular stomatitis virus (VSVpp). MAbs AR3A and AR4A are control neutralizing MAbs to E2 and E1E2 complex, respectively ⁶⁶. (b) Binding of MAb IGH526 to E1E2 in ELISA can be blocked effectively by peptide H312 (HITGHRMAWDMMMWNWS) or NIH 6971 (YPGHITGHRMAWDMMMWNW). (c) Binding of MAb IGH526 to E1E2 in ELISA. E1E2 antigens expressed in 293T cells in their native (closed symbols) or reduced (open symbols) form were captured onto microwells by lectin. The control MAbs A4 and AR3A recognize a continuous E1 epitope ⁶⁷ and a discontinuous E2 epitope ⁴¹, respectively. (d) Pepscan analysis of MAb IGH526. The antibody only bound a specific E1 peptide in a library of overlapping peptides (18-mer, 11-residue overlap, 250 ng/well) spanning the viral structural proteins: Core, E1 and E2. MAb HCV1 is a control that binds the E2 412-423 region.

FIG. 2. Crystal structure of MAb IGH526 in complex with its E1 peptide epitope. (a) The overall structure of the IGH526 complex is shown in ribbon representation. The peptide epitope (red) is bound between the heavy (dark grey) and light (light grey) chains of the Fab. The CDR loops (H1-H3, L1-L3) are shown in different colors. (b) Hydrogen bonds and an aromatic hydrogen bond (bottom left) between residues on the E1 peptide (red) and antibody (gray) are shown. (c) Water molecules (green spheres) mediate hydrogen bonds between the E1 peptide and antibody. (d) The E1 peptide is represented as a helical wheel with the antibody residues that make van der Waals interactions and hydrogen bonds shown below each E1 contacting residue. The electrostatic character of each epitope residue is color-coded. (e) Burial of residues on the E1 peptide by IGH526 (above) and burial of CDR loops of IGH526 by the E1 peptide are shown.

FIG. 3. Characterization of the IGH526 epitope. (a) Alanine scanning mapping of the IGH526 epitope on E1E2 by ELISA. Left: Expression of the mutants was confirmed by the anti-E2 MAb AR2A (1 µg/mL) ⁴¹. Center and right: Binding of MAb IGH526 to the variants. Note enhanced binding was observed for the W326A mutant, while the majority of the mutants bound at similar (filled symbols) or reduced (grey symbols) levels compared to wildtype E1E2. Binding was abolished for the mutants H316A, W320A and M323A. (b) Effects of alanine substitution on viral infectivity and antibody neutralization. HCV pseudotype virus particles (HCVpp) bearing the specific alanine/glycine substitutions were generated as described previously ⁴¹. The infectivity of the mutant panel was evaluated based on the activity of the HCVpp reporter gene luciferase (relative light unit, RLU) (Left). Mutants that produced HCVpp with detectable signals were titrated against MAb IGH526 in a neutralization assay (Right). (c) Binding and (d) Neutralization of the H316A/W320A single and double mutants. The data are presented in the same manner as in (a) and (b), respectively.

FIG. 4. Conformational flexibility of E1 peptide 315-324. The IGH526 helical peptide from the crystal structure was submitted to molecular dynamics (MD) simulations for 25 ns both with and without the antibody. **(a)** The C α root mean square deviation (RMSD) of the antibody-bound (blue) and unbound (green) peptides relative to the conformation in the crystal structure is plotted over the 25 ns simulation. **(b)** The C α root mean square fluctuation (RMSF) for each residue of the antibody-bound (blue) and unbound (green) peptides is plotted over the 25 ns simulation. RMSF is calculated relative to the crystal structure. The models at various time points were aligned on Ala319-Met324, the region on the peptide that remains helical throughout the simulation. **(c)** The bound peptide conformation from the crystal structure (red) is compared to the free peptide after 25 ns of molecular dynamics either in the bound state (top) or the unliganded state (bottom). Ala319 and Trp320 at the N-terminus of the stable portion of the helix are highlighted.

Figure 1**(a)** **(b)** **(c)** **(d)**

Figure 2