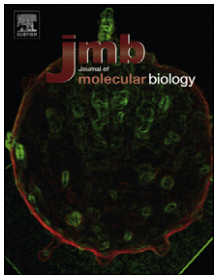

Structure of Hepatitis C Virus Envelope Glycoprotein E1 Antigenic Site 314–324 in Complex with Antibody IGH526

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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 (mAbs), 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 1.75 Å 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 that the free peptide is flexible in solution, suggesting that it requires stabilization for use as a candidate vaccine immunogen.

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 development of direct-acting anti-viral drugs have given hope for curing this devastating human disease [4–6]. However, as many of the new infections are associated with injecting drug usage, 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 that direct-acting anti-viral treatment alone will not be sufficient in controlling the HCV epidemic. Considering the socioeconomic 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 proteins [11]. A T-cell-based vaccine, based on adenovirus and

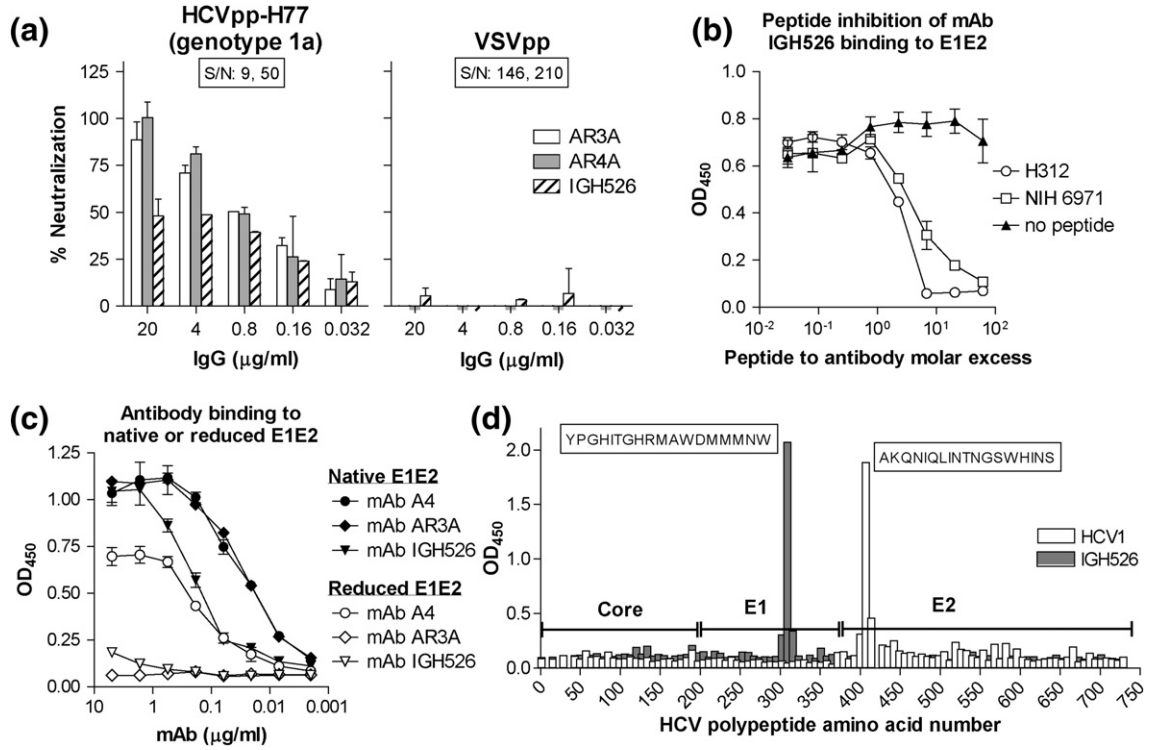

Fig. 1. Biological activities of recombinant mAb IGH526. (a) Neutralization of HCV by mAb IGH526. The mAb neutralized HCV pseudotype virus particles (HCVpp) displaying the genotype 1a E1E2, but not the control envelope glycoprotein G from vesicular stomatitis virus (VSVpp). mAbs AR3A and AR4A are control neutralizing mAbs to E2 and E1E2 complex, respectively [28]. (b) Binding of mAb IGH526 to E1E2 in ELISA can be blocked effectively by peptide H312 (HITGHRMAWDMMMNWS) or NIH 6971 (YPGHITGHRMAWDMMMNW). (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 [66] 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 per well) spanning the viral structural proteins: Core, E1 and E2. mAb HCV1 is a control that binds the E2 412–423 region.

poxvirus vectors expressing the non-structural proteins NS3, NS4A, NS4B, NS5A and NS5B, is currently being evaluated in a phase II clinical trial [12]. Another vaccine candidate, based on the structural envelope glycoproteins E1 and E2, has been evaluated in different animal models including chimpanzees [13] and in a phase I study [14]. This candidate subunit vaccine was safe in humans and elicited a 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 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 E2 [20]. However, two E1 regions targeted by monoclonal antibodies (mAbs) have been identified: residues 192–202, which are recognized by the weakly neutralizing mAb H-111 [21], and residues 313–328, which interact with the cross-reactive mabs IGH526 and IGH505 [22]. The mAb IGH526, which is the subject of this study, is of particular interest because it has been shown to cross-react with and cross-neutralize several HCV genotypes [22]. 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 conserved [23], and are recognized by 30 of 92 HCV patient sera [24] as well as by 15 of 41 vaccine sera [16] in previous studies, thereby representing a promising antigenic target for vaccine design. Previously, NMR was used to