Investigation of Structure-Function Relationship in E2-Antibody Complexes for Immune Prophylaxis of Hepatitis C Virus Infection

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Abstract

Viral proteins often present themselves in various shapes and forms to the host immune system during natural infections. In such a dynamic way, they give the viruses an evolutionary advantage for survival. On the hepatitis C virus (HCV), the E2 protein is one of the most important surface antigens that is highly flexible in its structure for the rather complicated virus entry process. Therefore, a full description of how the structural change of the E2 forms a tie with the E2 function has a great implication for HCV prophylaxis. A series of protein complex structures were determined, respectively, by X-ray crystallography, including a panel of anti-E2 antibodies, mAb#8, mAb#41, mAb#12 and mAb1H8, bound with their corresponding epitopes II (427–446) and III (523-530) on the E2 protein. The structural features were then related to the neutralizing capacities of the antibodies and the functions of the E2. These studies found that Epitope III was in an equilibrium of different conformations and that each conformation could interact selectively with the antibody (mAb1H8) or host receptors, such as CD81 and SR-B1, possibly by the mechanism of shape complementarity. By solving the Epitope II-antibody complex structures, these studies suggested that significant structural changes in the E2 were prerequisite to expose the otherwise deeply buried Epitope II, and in turn, to be accessible to the Epitope-II specific antibodies, including mAb#8, mAb#41 and mAb#12. In addition, by further characterizing intra- and inter-molecular non-covalent bonds at antibody-Epitope II interface, these studies revealed a biophysical linkage between the kinetics of the antibody-antigen complex formation and the antibody's mode of action in the E2 structural context that was critical for antibody-mediated neutralization. These observations have raised the possibility that local and global conformational dynamics of the E2 protein, in conjunction with those virus variants under the immune pressure, may act as a regulatory mechanism to coordinate antibody-mediated immune defenses with host receptor-initiated HCV infections. New approaches can thus be explored by tipping the balance of the E2 conformational states in favor of the recognition of neutralizing antibodies.

Introduction

HCV is a positive-sense, single-stranded, enveloped RNA virus in the Flaviviridae family. Its infection is a major public health problem with approximately 71 million people worldwide who are chronically infected. A significant number of these patients will develop cirrhosis or liver cancer. While the advent of direct-acting antiviral agents has dramatically changed HCV treatment with high cure rate in the majority of patients, immune prophylaxis remains an unmet need. Disease prevention by virus-specific neutralizing antibodies through vaccination is still the most cost-effective and realistic way to control HCV infection.

Among other critical attributes, a successful vaccine counts on its capacity to elicit an antibody that can effectively neutralize the virus. In the case of HCV, the antibody with a broadly neutralizing capacity is rarely detectable, especially at the early stage of HCV infection, and if detectable, the antibody has yet to demonstrate its effectiveness in vivo. As a result, patients become chronically infected even in the presence of appreciable amounts of neutralizing antibody. The underlying mechanism of how HCV manages to escape from antibody neutralization remains a major research challenge.

Materials and Methods, Results and Discussion

The goal of this study is to understand antibody-mediated neutralization by targeting the structural dynamics of the envelope protein E2. The E2 protein has long been regarded as the prime antibody target in efforts to generate a protective immunity. This important undertaking has been corroborated by the studies demonstrating the essential role of E2 in facilitating viral entry into hepatocytes via interacting with various host entry factors, primarily among them, the CD81. Neutralizing antibodies, especially those that can bind to E2, have been linked to viral clearance.

Structurally, E2 is flexible. The flexibility of E2 may affect the optimal presentation of its antigenic sites of interest, which may serve as a potential mechanism for immune evasion. Three major E2 epitopes have been identified for both antibody-mediated neutralization and non-neutralization. The dynamic interactions of these epitopes with their respective antibodies at the virus-host interface provide an ideal experimental model for our investigation.

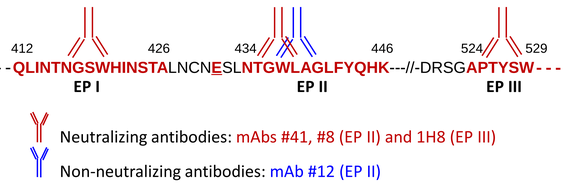

Figure 1. The CD81-binding loop on the E2 protein is a segmental structure mapped to the residues between 523 and 540, of which W529, and, to a certain extent, Y527, are recognized as the most important residues for interacting with CD81. Most neutralizing antibodies target the CD81-binding loop. Additionally, many other individual residues have also been implicated in the CD81 interaction. Among them, several residues scattered in the region encompassing Epitopes I and II, for example, W420 and 436GWLAGLFY443, are of great relevance. To gain molecular insights into the E2-CD81 interface, we developed a panel of monoclonal antibodies recognizing either Epitope II (#41, #8 and #12) or Epitope III (1H8) with the capability of neutralizing (#41, #8 and 1H8) or non-neutralizing (#12).

Table 1. Mutagenesis technology was used for residue mutations to identify the critical amino acid residues from Epitope II and Epitope III for antibody or CD81 binding. The binding experiments were performed using the enzyme-linked immunoassay (ELISA).

Ep II Residue	Neutralizing		Non-neutralizing
	mAb#8	mAb#41	mAb#12
W437	+	+	+
L438	+	+	+
L441	–	–	+
F442	–	–	–

Ep III Residue	1H8	CD81
A524	+	–
P525	+	–
T526	–	+
Y527	+	+
S528	±	–
W529	+	+

+ Critical, – Non-critical, ± Residue-specific

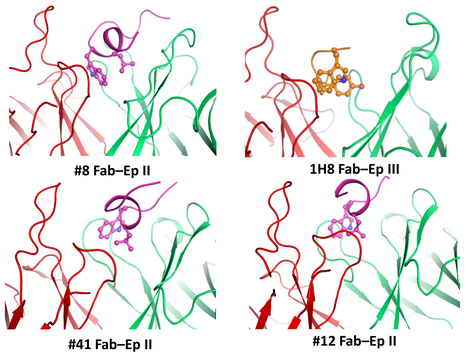

Figure 2. The structures of #8 Fab, #41 Fab and #12 Fab in complex with Epitope II and 1H8 Fab bound to Epitope III were determined by X-ray crystallography. The key residues W437 and L438 of Epitope II and Y527 and W529 of Epitope III were all buried into the antigen binding grooves.

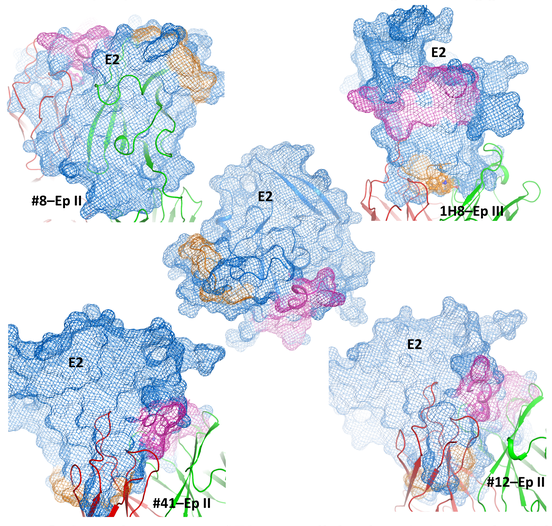

Figure 3. Several structures of E2 extracellular domain in complex with various neutralizing antibodies are available, all showing very similar overall conformation of E2 as shown in the middle (PDB code: 6meh was used as a representative). However, superpositions of Epitope II and Epitope III from our antibody/epitope complexes onto the corresponding region of E2 resulted in significant steric clash between our antibodies and E2, suggesting that our antibodies recognize a very different conformation of E2; and E2 appears to present itself in various conformations.

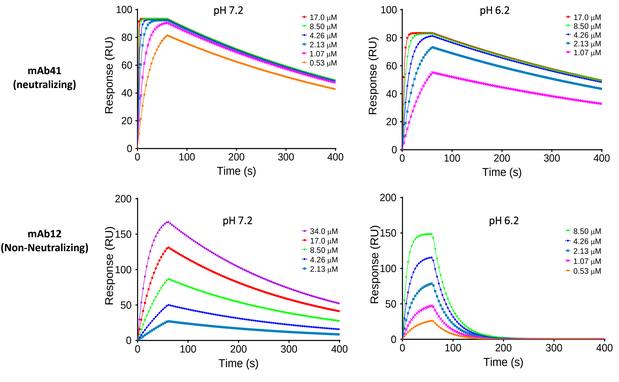

Figure 4. Affinity measurements by surface plasmon resonance. Antibodies were immobilized on the chip at random orientation by amine-coupled immobilization, with unrelated antibody used as a negative control for non-specific binding. Epitope II peptide was 2-fold serially diluted in the range of 34 - 0.5 µM in buffers with different pH and applied on the chip for 60 seconds at the flow rate of 50 µL/min. Dissociation phase was measured for no less than 450 seconds after association ended. the Equilibrium dissociation constant (K_D) as an indicative of the binding affinity between the antibody and its corresponding epitope by measuring the ratios of k_{off}/k_{on} for neutralizing #41 and non-neutralizing #12 under pH of 7.2 and 6.2. The dissociative half-life of the ternary complex is inversely related to the K_{off}, i.e., $T_{1/2} = \ln 2/k_{off}$. #41 had about two orders of magnitude higher binding affinity to Epitope II than #12 at both pH values, which agrees with the neutralizing capabilities of these two antibodies.

Conclusion

Our study suggests that HCV appears to use the structural flexibility of E2 protein to escape our immune surveillance, presenting itself to our immune system in certain shapes to gain an evolutionary advantage for survival. Antibody-mediated neutralization of HCV could thus be viewed as the outcome from: (1) blocking HCV virus access through the structurally defined E2–host receptor interface when Epitope II is hidden; (2) locking Epitope II in a particular conformation that inhibits virus entry into the liver cell when Epitope II is fully exposed; (3) if this transition process is taken advantage by the virus, the virus may present an Epitope II conformation that is capable of avoiding neutralization, thus allowing it to persist in patients. (4) local Epitope III conformational dynamics, in conjunction with sequence variations, may act as a regulatory mechanism to coordinate “1H8-like” antibody-mediated immune defenses with CD81-initiated HCV infections. The vulnerabilities in the HCV structural dynamics may be served as targets for the elimination of the virus by neutralizing antibodies.