

Hepatitis C Virus Plays Hide and Seek With Neutralizing Antibodies

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Hepatitis C virus (HCV) evades immune responses to establish a chronic infection. These viral evasion mechanisms pose a formidable challenge to vaccine development. In this issue of HEPATOLOGY Prentoe et al. report important observations that guide the development of vaccination strategies for induction of potent cross-neutralizing antibodies.⁽¹⁾

While the importance of cellular immunity for control of HCV has been appreciated for a long time, more recent evidence also supports a vital role of neutralizing antibodies. For instance, among women accidentally infected with HCV, acutely resolving patients displayed significantly stronger neutralizing antibody responses early after infection compared to those developing a chronic course.⁽²⁾ Moreover, in mouse models neutralizing antibodies can prevent or even clear an HCV infection.^(3,4) These results encourage vaccination approaches aiming for induction of neutralizing antibody responses. However, the

viral epitopes that should be targeted are only beginning to emerge, and strategies to elicit such antibodies are largely elusive.

It is well established that the amino-terminal domain of the viral envelope protein 2 (E2) is immunogenic and induces antibodies that neutralize HCV. However, this domain is flexible and tolerates mutations. Thus, it evolves rapidly—in fact, it is the most variable portion of the HCV genome and, therefore, called hypervariable region 1 (HVR1)—and serves as a “decoy” for antibodies. Not only this but HVR1 also occludes viral protein domains that are not so flexible and are thus highly conserved.^(5,6) This occlusion impedes access of antibodies to these epitopes and limits neutralization. The epitopes affected by HVR1 occlusion include, for instance, the conformational binding site to the key HCV entry factor cluster of differentiation 81 (CD81).

HCV attaches to the cell surface primarily by binding to glycosaminoglycans and scavenger receptor class B type 1. These initial contacts may elicit conformational changes that expose the binding site to CD81, thus facilitating interactions with CD81 and downstream entry factors (Fig. 1). Thus, highly “vulnerable,” conserved epitopes would only be fully uncovered when HCV has reached its target cell’s surface and is poised to enter. This entry mechanism would limit access of antibodies targeting conserved protein domains and therefore contribute to immune evasion and maintenance of a chronic infection.

Prentoe and coauthors now add new information to our understanding of the interplay between antibodies and HVR1.⁽¹⁾ They used a panel of HCV strains representing all major HCV genotypes and a set of the most powerful available cross-neutralizing monoclonal antibodies targeting a spectrum of distinct subdomains of the E1-E2 protein heterodimer (antigenic domains A-D and antigenic region 1 [AR1]-AR5; Fig. 1) and compared virus antibody binding and neutralization between the parental viruses and the respective variants lacking HVR1. Using this approach, they first convincingly show that the respective viruses lacking HVR1 are much more susceptible to neutralization by

Abbreviations: AR, antigenic region; CD81, cluster of differentiation 81; E1/E2, envelope proteins 1 and 2; HCV, hepatitis C virus; HVR1, hypervariable region 1.

Received July 27, 2016; accepted August 8, 2016.

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View this article online at wileyonlinelibrary.com.

DOI 10.1002/hep.28760

Potential conflict of interest: Nothing to report.

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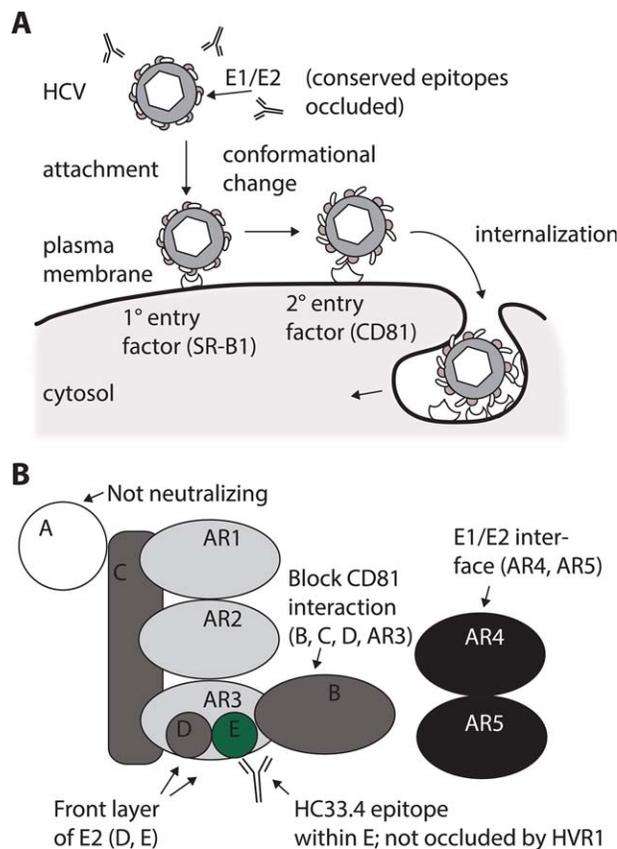


FIG. 1. (A) Simplified HCV cell entry model highlighting a putative receptor-dependent conformational switch that discloses otherwise (partially) occluded conserved epitopes required for subsequent virus receptor interactions like CD81. (B) Schematic drawing of antigenic regions (not to scale) of the HCV E1-E2 heterodimer subdomains A-D and AR1-AR5 examined by Prentoe et al. and which are occluded by HVR1. The HR33.4 antibody epitope maps to subdomain E (or a portion of it) and is exceptional as it seems to be not well protected by HVR1. This site may be uniquely suited for antibody-based therapeutic or prophylactic approaches and as a target for vaccine development. Abbreviation: SR-B1, scavenger receptor class B type 1.

a given antibody compared to the parental virus with HVR1. These results indicate that the HVR1 of these diverse viruses globally protects the epitopes targeted by these antibodies. This confirms previous observations^(5,6) and extends them because it indicates that HVR1-dependent epitope occlusion encompasses all of the above-mentioned subdomains comprising conserved cross-neutralizing epitopes (Fig. 1). Notably, the HC33.4 antibody (anti-domain E⁽⁷⁾) seems to be the sole exception to this as the three virus strains tested were similarly susceptible to neutralization irrespective of the presence of HVR1.⁽¹⁾

Second, employing an immunoprecipitation assay to quantify antibody binding, the authors show that deletion of HVR1 globally increases virus immunoprecipitation. Therefore, deletion of HVR1 unmasks previously inaccessible (or poorly accessible) epitopes—again, across multiple different cross-neutralizing epitopes

(at least AR2-AR5 that were evaluated here). Third, by correlating antibody binding with neutralization, they observe only a poor correlation for the parental viruses. However, for viruses with deleted HVR1 the efficiency of immunoprecipitation (i.e., antibody binding) strongly correlated with virus neutralization. This argues that once HVR1 is deleted, there is a very simple relationship between antibody binding and virus neutralization in the sense that the higher the binding efficiency, the greater the neutralization. For wild-type viruses there is no such direct correlation, and the situation is more complex. This implies that determinants of HVR1 modulate the efficacy of antibody neutralization at least in part independently of the efficiency of antibody binding to its cognate epitope. Notably, this HVR1-dependent modulation of neutralization is strain-dependent as Prentoe and colleagues show that, for instance, the antibody HC84.26 neutralizes several

HVR1-deleted variants with essentially indistinguishable efficiency. In contrast, the cognate parental viruses are neutralized by the same antibody with greater than 40-fold differences in efficiency. This illustrates that strain-specific properties of HVR1 have a pronounced impact on neutralization efficacy, although the actual binding epitope of the antibody is conserved between the strains. Remarkably, this phenomenon occurs for several cross-neutralizing antibodies examined here, indicating that strain-specific HVR1 determinants also modulate access to these epitopes. Future work will have to address which HVR1 features are responsible for this and how they achieve this. The crystal structures of the E2 core domain provided first important insights into the structure of key HCV cross-neutralizing epitopes.^(8,9) However, these structures do not encompass HVR1. Additional structural information also encompassing HVR1 or even the full E1-E2 heterodimer will help an understanding of the hide and seek between HCV and neutralizing antibodies.

What are the implications of these findings for vaccine development? First, and encouragingly, there are a number of highly conserved cross-neutralizing epitopes that are targeted by different monoclonal antibodies (as inferred by the powerful cross-neutralization of HVR1-deleted viruses). Second, and discouragingly, most of these epitopes are occluded by HVR1 in case of wild-type viruses so that neutralization is modest. Ultimately, combinations of such antibodies may be powerful enough to overcome the HVR1 occlusion, and vaccine approaches inducing a spectrum of these could establish cross-protective humoral immunity. Alternatively, if it was possible to induce antibodies that bind HCV and induce conformational changes that disrupt HVR1-dependent epitope occlusion, these could much improve the potency of the above-mentioned cross-protective antibodies. On the other hand, antibodies that bind HVR1 can also impede neutralization—possibly by obstructing access to conserved epitopes.⁽¹⁰⁾ Finally, the viral epitope represented by the HC33.4 antibody is exceptional: first, it is cross-protective and, second, it is not occluded by HVR1—possibly because it bridges a conserved portion of E2 with the C-terminal segment of HVR1.⁽¹⁰⁾ This makes it a very interesting, potentially unique target for vaccine development.

Considering these implications, the study by Prentoe et al. is groundbreaking and will kindle and guide new approaches in HCV vaccine development.

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