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The genetics of hepatitis C virus underlie its ability to escape humoral immunity

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Hepatitis C virus (HCV) is a leading cause of chronic liver disease, and efforts to develop therapeutic vaccine strategies have been limited by immune escape due to HCV variants that are resistant to current vaccines or HCV variants that rapidly acquire new resistance-conferring mutations. Recently, the crystal structure of the viral envelope protein E2 region was resolved as well as how E2 docks to the host CD81 protein; therefore, antibodies that block this interaction should prevent viral entry into host cells. In this issue of the *JCI*, Bailey and colleagues show that immune escape of HCV can occur by naturally occurring polymorphisms in E2 that are distinct from those at mapped sites of antibody binding. These data reveal alternative mechanisms of resistance that need to be considered in both natural viral escape as well as in rationale vaccine design against HCV.

Hepatitis C virus: treatment options and cost

Hepatitis C virus (HCV) is an enveloped positive strand RNA virus that infects up to 150 million individuals worldwide. HCV-infected individuals are at increased risk of developing cirrhosis and hepatocellular carcinoma. The economic burden of HCV in the US alone is estimated to be \$6.5 billion annually with increasing annual costs projected through at least 2024 (1). Treatment strategies have centered on antiviral drugs, such as ribavirin and type I interferons (2); however, HCV exists as a quasispecies that includes a variety of genotypes that are differentially susceptible to these regimens. Currently, 6 genotypes are recognized, with varying numbers of subspecies within each of these 6 clades. Recently, the drug sofosbuvir, a nonstructural protein 5B (NS5B) RNA polymerase inhibitor, was approved for use in combination with ribavirin to treat HCV genotypes 2 and 3. Sofosbuvir is also approved for use in combination

with ribavirin and pegylated interferon for use against HCV genotypes 2 and 4. These regimens have been shown to achieve up to 90% response rates in patients, particularly in those infected with HCV genotype 1 or 2 (2). The newest FDA-approved oral regimen, which consists of ledipasvir (a NS5A inhibitor) and sofosbuvir, achieved up to 99% cure rate in noncirrhotic patients infected with HCV genotype 1 after 12 weeks of treatment (3).

The search for an effective vaccine

Although much progress has been made in treating HCV, the development of a preventative vaccine remains a major goal in the field. The basis for HCV vaccination is that patients will develop neutralizing antibodies that target HCV viral envelope proteins, thereby inhibiting viral entry into host cells. Multiple monoclonal antibodies with demonstrated ability to neutralize viral attachment or entry into host

cells have been produced (4); however, HCV has a propensity for immune escape, which is associated with the acquisition of mutations in the targeted region of HCV envelope proteins, such as E2 (5). In this issue, Bailey et al. (6) provide new insight into strategies used by HCV for immune escape that will need to be considered for future rational vaccine design.

Specifically, Bailey and colleagues (6) studied the issue of immune escape across a number of HCV envelope-directed monoclonal antibodies and tested these using a panel of human embryonic kidney cell lines (7) that express different HCV pseudoparticles (HCVpp) encoding naturally occurring E1 and E2 variants from HCV genotype 1. The HCVpp-expressing kidney cell library consisted of nineteen clones, and eighteen different monoclonal antibodies were screened for their neutralization ability. Two of the antibodies tested, HC84.26 and AR4A, had the greatest potency and were able to neutralize nearly 90% of the clones. The sensitivity of the different HCVpp-expressing clones to the monoclonal antibody panel varied; however, some clones were extremely resistant to highly effective monoclonal antibodies, including HC84.26.

Bailey et al. (6) then evaluated the envelope proteins in the HCVpp-expressing clones that were resistant or sensitive to neutralization by monoclonal antibodies with known HCV envelope-binding sites. In addition to mutations within the antibody-binding regions of E2, the authors also observed clusters of resistance that mapped to parts of the viral envelope that were distinct from known epitopes and that these clustered in relation to the neutralization resistance/susceptibility profile of the different HCVpp-expressing clones. Together, these data suggest that genetic alterations in envelope proteins that are distinct from known antibody-binding epitopes may confer resistance to antibody-mediated neutralization. Resistance

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mapped to a region in E2 that encompasses amino acids 416–560, which span the front layer, the CD81-binding motif, and the central β -sheet region of the envelope. Specifically, polymorphisms in amino acids 431, 442, and 560 resulted in limited resistance to the antibody panel; however, mutations in the central β -sheet (amino acids 526–569) conferred broad resistance to the antibodies tested. Analysis of the envelope proteins expressed by susceptible clones and the envelope proteins expressed by resistant HCVpp-expressing clones revealed 7 shared variant sites. Introduction of resistance-associated mutations into susceptible HCV envelopes conferred neutralization resistance, and introduction of susceptibility-associated mutations in resistant envelopes resulted in sensitivity to antibody neutralization. Bailey and colleagues (6) identified three critical mutations (I538V, Q546L, and T563V) that mediate resistance to both monoclonal antibodies and polyclonal antibodies. Importantly, these mutations were not in areas of the envelope that mapped to monoclonal antibody-binding sites. Although some of these mutations may confer some negative effect on viral fitness, the identification of these mutations may also explain HCV immune escape. The exact mechanisms of resistance conferred by these mutations are currently not known but may involve alterations in protein folding or glycosylation, which could mask epitopes or create steric hindrance and thereby reduce antibody binding. In addition, receptor-independent transfer of replication-competent HCV RNA by exosomes may also undermine the efficacy of antibody-mediated protection (8). Future studies will need to focus on how these different mutations confer resistance and the overall contribution of exosome-mediated viral entry in immune escape.

Conclusions and future directions

The level of polymorphisms within the HCV envelope that confer antibody resistance makes strategies to achieve thera-

peutic vaccination or to develop broadly effective therapeutic monoclonal antibodies more difficult. Based on the propensity of immune escape, it is unlikely that a vaccine directed against just one region of the HCV envelope will prove effective. This type of multivalent approach to vaccine generation would be similar to the approach used for pneumococcal immunization, which provides protection against multiple pneumococcal serotypes but does not protect against all strains. Potential multivalent HCV vaccines would consist of the most common viral E1E2 variants based on viral genomics. A multivalent approach to HCV immunization will be costly, based on both production costs as well as chemistry, manufacturing, and controls costs, which would be required to ensure the safety of each vaccine component. The work by Bailey et al. (6) clearly demonstrates that monoclonal antibody approaches may not be feasible to provide broad HCV protection, as even bispecific antibodies would likely encounter immune escape. The study by Bailey and colleagues does provide a platform through which new antibodies can be screened. This type of cell-based platform to test the efficacy of vaccine candidates in their ability to neutralize a variety of HCVpp will clearly benefit future clinical development. We are currently in an era in which treatment for HCV-infected individuals has reached new heights in therapeutic efficacy. Further work on identifying envelope variants will hopefully help realize the potential of an HCV vaccine.

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