Neutralizing Antibodies Against Hepatitis C Virus and Their Role in Vaccine Immunity

See “An antigenically diverse, representative panel of envelope glycoproteins for HCV vaccine development,” by Salas JH, Urbanowicz RA, Guest JD, et al, on page XXX.

Long-term infection with hepatitis C virus (HCV) is a major cause of chronic liver diseases, terminating in cirrhosis and cancer, and despite the development of curative direct-acting antivirals requiring only 8–12 weeks of treatment, about 400,000 people die due to HCV annually. Furthermore, there are 1–2 million acute infections annually, of which 75% become life-long with the majority not diagnosed. Thus, vaccines would be an important component of measures required for the elimination of HCV. However, the most advanced candidate, a viral vector-based vaccine expressing the HCV nonstructural proteins, failed to prevent chronic infections after HCV exposure in high-risk populations despite induction of HCV-specific T-cell responses. Thus, as for most other viral infections, induction of neutralizing antibodies (NtAb) could be key for obtaining protective immunity after vaccination, and 2 experimental strategies were developed to measure HCV neutralization: retroviral pseudo-particles expressing HCV envelope E1 and E2 proteins (HCVpp) and cell culture-derived infectious HCV (HCVcc). It is important that cell culture systems used in vaccine studies fully recapitulate the global heterogeneity and antigenicity of HCV, as well as the intrinsic features of circulating HCV particles, and thus aim at capturing efficient NtAb with broad activity. In this issue of Gastroenterology, Salas et al defined a small panel of HCVpp that uniquely captures the broadness of NtAb in patients with HCV infection and in well-defined human monoclonal HCV antibodies, thus expanding the possibility of exploring vaccine-induced immunity.

In vitro neutralization assays in patients with acute HCV infection evidenced an important role of NtAb in viral clearance, implicating early timing and potency, and studies in animal models (chimpanzees and human liver chimeric mice) showed that NtAb can prevent HCV infection, at least with the homologous virus. Furthermore, an inactivated HCVcc vaccine candidate induced NtAb in mice that could prevent HCV infection in human liver chimeric mice. There is some evidence that NtAb with broad activity in vitro, which is the capacity to neutralize diverse virus variants, contributes to viral clearance in patients with HCV and could even clear experimental HCV infection in human liver chimeric mice. However, there is also evidence from a study of patients with acutely infected HCV that these responses are narrowly focused against the homologous or closely related isolates, and studies in chimpanzees and human liver chimeric mice indicate that even NtAb with broad cross-genotype in vitro activity primarily protects against the homologous virus. The complexity of defining the role of NtAb in protection from HCV is further increased by differential features of the virus particle linked to changes in the E1–E2 sequence or associated host factors, which can dramatically alter sensitivity to NtAb. Also, neutralization is frequently not linked to viral genome-sequence relatedness, and universally conserved epitopes might be protected from NtAb. Finally, constant high HCV evolution creates NtAb escape mutants. Add to this that the viral particle envelope structure has only been partly uncovered and that broadly protective NtAb responses apparently are represented by specific germline heavy chain variable genes, it remains a challenge to generate vaccine antigens that will induce broadly protective NtAb. Thus, the availability of representative HCVpp and HCVcc assays to better define NtAb responses in natural infections and in vaccine studies is of high importance.

To address this challenge, Salas et al tested a large number of genotype 1 E1–E2 HCVpp functional clones aimed at representing the heterogeneity identified in >2500 deposited HCV E1–E2 sequences, and further included representative isolates of genotypes 2–6. Finally, 65 HCVpp constructs (56 genotype 1 and 9 genotypes 2–6) with high representation of observed E1–E2 sequence polymorphisms and efficient entry into Huh7 hepatoma cells were selected for neutralization studies against a panel of monoclonal HCV antibodies targeting a variety of conformational epitopes and with varying degree of cross-reactivity against existing panels of HCVpp and HCVcc. Subsequently, the HCVpp were divided into 2 tiers below and 2 tiers above the mean neutralization titer of all tests. Furthermore, the antigenic relationship among HCVpp was determined based on clustering of patterns of neutralization titer, thus defining 15 antigenic groups of HCVpp. Based on the defined tiers and mean of neutralization for each monoclonal antibody, the defined HCVpp antigenic groups, HCV genotype and subtype, and the HCVpp entry efficiency, the authors succeeded in defining a panel of only 15 representative HCVpp, which remarkably gave similar neutralization results as obtained with the larger panel of 65 HCVpp. The utility of the 15 HCVpp panel was evaluated against samples from patients infected with genotype 1–6 isolates, as well as additional monoclonal HCV antibodies with unique or overlapping epitopes compared with those tested initially. The observation that samples from genotype 4–6 patients had greater neutralization breadth (greatest in genotype 6 patients) compared with genotype 1–3 patients deserves investigation in a larger number of matched patients. The suggested use of genotype 6 E1–E2 antigens for
future vaccine development is, thus, of interest, in particular considering that recent structures of HCV E2 in association with antibodies were based on a genotype 6 isolate.\textsuperscript{17}

Although the panel of 15 HCVpp represented the larger panel, the associated E1–E2 sequences contained a much lower percentage of the observed polymorphisms among deposited sequences, raising the interesting possibility that these are not involved in determining neutralization sensitivity. It would be interesting to examine the role of specific polymorphisms for virus neutralization using targeted mutational analysis.

Recently, HCVcc variants representing each of 6 neutralization clusters were defined.\textsuperscript{14} Together with the HCVpp panel established by Salas et al,\textsuperscript{3} as well as smaller genotype panels published earlier\textsuperscript{3}, the HCV vaccine field should have the neutralization assays required to fully characterize in vitro the breath of neutralization of antibodies induced by vaccine antigens in animals or in human trials. It should be recognized, however, that in vitro neutralization features might not necessarily reflect in vivo neutralization capacity.\textsuperscript{19} This could be due to inherent features of HCV particles generated in vitro, which might lack essential associated factors such as lipoproteins. The human liver chimeric mouse model can be used to test the protective immunity of infused NtAb. However, the HCV field is still missing robust immunocompetent small-animal models for vaccine immunogenicity and challenge studies.\textsuperscript{30} An alternative could be the use of a controlled human infection model, which, given the high cure rate with current drug regimens, is considered a possible future option to test the protective immunity of vaccine candidates.\textsuperscript{30}

The studies of NtAb against the panels of HCVpp and HCVcc confirmed a lack of correlation between genetic relatedness and detected neutralization titers in samples from patients infected with HCV or in various human monoclonal antibodies, thus highlighting the importance of using HCV isolates that represent the antigenic diversity in test panels.\textsuperscript{14,13} It remains to be determined whether the proposed minimal HCVpp and HCVcc panels fully represent the global heterogeneity and antigenicity of HCV, as divergent isolates, subtypes, and genotypes are discovered worldwide, primarily as part of the efforts to expand treatment of HCV globally. Furthermore, it remains to be shown that antibodies associated with clearance of natural infections and vaccine-induced antibodies that show broad neutralization in tests with minimal HCVpp and HCVcc panels will be associated with protection against HCV infection and chronicity.\textsuperscript{19,20}

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