Editorial

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Learning from a clinical cohort for HCV vaccine development

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About 71 million people worldwide suffer from chronic hepatitis C virus (HCV) infection. Without available treatment the large majority of HCV-infected patients progress to chronic disease. Chronic HCV infection is one of the most important risk factors for liver disease, including liver fibrosis, cirrhosis and hepatocellular carcinoma. Despite the development of efficient direct-acting antivirals with success rates of more than 95%, HCV incidence rates are still increasing, which is in part due to the ongoing opioid epidemic that is currently taking its toll in the US. High costs of treatment and the possibility for reinfection, which is frequent among intravenous drug users, warrant the development of a protective vaccine.[1]

The small but significant rate of spontaneous HCV clearance, in some rare cases even after years of chronic infection, suggests that that the development of a protective vaccine is not an impossible task. Despite large progress, the immune correlates of protection against infection are still only partially understood. Early vaccine studies involving chimpanzees[2] have shown that long-lasting CD4 and CD8 T-cell responses play a significant role in HCV clearance, with loss of robust CD4+ response resulting in disease progression.[3] On the other side, B-cell responses and broadly neutralizing antibodies have been associated with HCV clearance.[4–8] Broadly neutralizing antibodies, meaning antibodies that neutralize more than 1 HCV genotype (GT) can be found in both patients with chronic HCV and in clearers. However, individuals clearing the infection were characterized by more rapid development of broadly neutralizing antibodies compared to those ultimately progressing to chronic HCV infection.[5] While a large number of patient-derived broadly neutralizing monoclonal antibodies effectively protect against or control an already established infection in liver humanized mice (reviewed in[9,10]), the development of a protective vaccine has been a major challenge. A prospective HCV vaccine does not necessarily need to provide complete sterilizing immunity, although this certainly would be the most desirable outcome. It might be sufficient to delay development of chronic infection to give the immune system a head start towards the elicitation of broadly neutralizing antibodies. This would appear to be similar to a spontaneous clearance of HCV infection and could be sufficient to help the host immune response stay ahead of the virus during the acute phase of HCV infection. This is further supported by the finding that spontaneous clearance rates increase from about 25% for a primary infection to 30 to 60% for those with 1 or multiple reinfections.[11]

While several studies link the early development of neutralizing antibody responses with HCV clearance,[4,5] the B-cell repertoire of individuals spontaneously clearing HCV infection and specific epitopes associated with protection have not yet been analyzed in detail. In that regard, individuals clearing multiple singular infections could be of particular interest as they could provide crucial information on correlates of protective immunity. Consequently, a thorough breakdown of B-cell responses associated with viral clearance will be paramount for HCV vaccine development.

Merat and Bru *et al.* from AIMM Therapeutics and the University of Amsterdam aimed to shine a light on the role of the B-cell responses during spontaneous HCV clearance and long-term immunity.[12] To perform a detailed analysis of the B-cell repertoire of patients with chronic HCV compared to spontaneous clearers, they took advantage of the long-term Amsterdam Cohort Study among injection drug users. Of the 13 individuals analyzed from the cohort, 5 were chronically infected, either after primary infection or reinfection, while the other 8 remained HCV negative after primary or after 1 or even multiple reinfections.

By performing multiplex flow-cytometry analyzing antibody binding to HCV envelope glycoproteins E1E2 of different GTs, the authors revealed that antibodies isolated from clearers present a much broader reactivity than those isolated from chronically infected patients, which confirms previous studies.[4,5] Out of 8 clearers, 7 had antibodies that showed reactivity against at least 2 HCV GTs and 5 of these had antibodies that showed cross-reactivity against at least 5 different GTs. Only 3 out of 5 chronically infected individuals showed reactivity against 2 or more GTs, with none of these reacting with more than 4 GTs.

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To obtain a more detailed understanding of the cross-neutralizing B-cell response in individuals clearing the infection, the authors analyzed the epitope specificity of antibodies secreted from B-cell cultures obtained from the 4 clearers with the highest frequency of cross-neutralizing antibodies. Most binding sites of broadly neutralizing antibodies cluster into 4 epitopes on the viral envelope protein designated as antigenic regions or domains (see ref[9] for a description of overlap between the AR and antigenic domain regions). Alanine mutant scanning of these 4 epitopes (epitope I/antigenic domain E, epitope II/antigenic domain D, AR3/antigenic domain B and AR4) revealed that the vast majority of antibodies (73%) targeted the AR3/antigenic domain B region. Additionally, antibodies targeting AR4 were present in 3 out of 4 individuals while no antibodies were found detecting epitope I/antigenic domain E or II/antigenic domain D only. Other studies will be necessary to confirm these observations and determine whether broadly neutralizing antibodies to other regions are involved in spontaneous clearance.

The predominance of AR3- and AR4-directed B-cell responses in HCV clearers was confirmed using antibodies derived from monoclonal B-cell culture. Out of 12 isolated antibodies, including all antibodies directed against AR3, 10 showed broad neutralization of GTs 1a, 1b, 3a and 4a. Furthermore, 8 out of 12 antibodies neutralized at least 1 neutralization-resistant HCVpp with 2 of these, 1 AR3- and 1 AR4-specific antibody neutralizing all analyzed strains. In 1 of the clearers, AR3-specific memory B-cells were still present 5.2 years post clearance of primary infection suggesting long-lasting immunity against HCV infection. However, it should be noted that not all AR3/antigenic domain B antibodies have broad neutralization profiles.[13]

What is the clinical impact of these findings? By directly comparing the B-cell responses of individuals clearing 1 or multiple HCV infection(s) with those of individuals who develop chronic infection, Merat and Bru *et al.* confirm that the early development of broadly neutralizing antibodies can confer protective immunity against HCV and thus reinforces the concept that the development of a protective vaccine is feasible. Furthermore, by identifying the broadly neutralizing epitopes associated with viral clearance they provide highly valuable information for structure guided vaccine design: Antigens containing the antigenic regions AR3/antigenic domain B and AR4 could serve as a starting point for the development of novel vaccine candidates.[14] This is supported by previous findings that antibodies targeting AR3 and AR4 act synergistically[15,16] and that some antibodies directed against AR3/antigenic domain B have been shown to present a high barrier against escape mutations.[13,17] The experience with other HCV B-cell vaccine candidates has shown that it is possible to elicit neutralizing B-cell responses with HCV E1/E2 based approaches. However, many of these responses have shown to be isolate-specific.[18] On the plus side, structure guided vaccine development has shown to be a successful strategy to elicit broadly neutralizing antibodies for other pathogens such as HIV,[19] influenza A virus and respiratory syncytial virus. It remains to be determined whether B-cell focused approaches are able to provide immunity to HCV infection or whether an efficacious vaccine will also have to induce CD4 and CD8 based T-cell responses, as indicated by some of the previous studies.

Ultimately, the findings provided by Merat and Bru *et al.* support the concept that a B-cell vaccine against HCV infection is feasible and most likely will have to induce broadly neutralizing antibodies targeted against AR3/antigenic domain B and preferably also against AR4. The latter offers the advantage of very high conservation among different HCV genotypes[20] and thus might provide a very broad protection profile.

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Conflict of interest

The authors declare no conflicts of interest that pertain to this work.

Please refer to the accompanying ICMJE disclosure forms for further details.

Supplementary data

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Author names in bold designate shared co-first authorship

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