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Defining the Spatial Relationship Between Hepatitis C Virus Infection and Interferon-Stimulated Gene Induction in the Human Liver

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Hepatitis C virus (HCV) is a hepatotropic RNA virus that chronically infects approximately 170 million people worldwide.[1] HCV infection activates an interferon (IFN) response attributed to detection of HCV by various pattern recognition receptors in the infected liver. Though chronic HCV infection often results in high expression of hepatic IFN-stimulated gene (ISG) messenger RNAs (mRNAs),[2,3] whose protein products are able to exert anti-HCV effects in cell-culture assays,[4] the virus still persists. Why does this activated immune response fail to clear the virus in these patients? Though HCV infection does disrupt various host innate immune defenses, even within the infected liver,[5,6] it is not clear what role this innate immune evasion plays during chronic HCV infection.

Overall, the understanding of the spatial interplay of host immune activation in relationship to viral replication within the infected liver has been limited. In chronically infected livers, does HCV infection directly induce or prevent ISG expression in the infected hepatocyte or in neighboring cells? How does this induction of ISGs limit the spread of the virus within the liver (or not)? These questions can only be addressed by knowing which cells within the liver are infected with HCV, which cells express ISGs, and their spatial relationships during HCV infection. Detecting HCV infection within the liver has been challenging because of high autofluorescence and corresponding low signal-to-noise ratios, as well as uncertain specificities of various staining approaches.[7] However, in recent years, both immunostaining and *in situ* hybridization (ISH) approaches, as well as laser capture microdissection approaches combined with quantitative polymerase chain reaction, have been able to detect HCV RNA and proteins with increasing sensitivity.[8-10]

In this issue of HEPATOLOGY, Wieland et al. expand on these studies to address where interactions between HCV and the innate immune system occur within the chronically infected human liver.[11] By using ISH with isolate sequence-specific probes in liver biopsies, the researchers were able to detect HCV positive-strand RNA signal specifically within hepatocytes, even in infected patients with very low viral loads ($\sim 10^4$ genomes/mL). The signal specificity was demonstrated by lack of background staining and no cross-reactivity of probe sets used with hepatitis B virus–infected livers or targeting either the positive or negative strands of HCV RNA. Additionally, there was little cross-reactivity even between different sequence-specific probes targeted to identical genotypes. Positive HCV ISH signal was indicated by an HCV "signal dot," and the number of signal dots per infected cell positively correlated with the proportion of infected cells per liver quadrant analyzed and with serum and liver viral loads, suggesting that the HCV signal dots are representative of authentic levels of HCV infection. The proportion of HCV-infected cells ranged from 1.3% to 54%, values consistent with other studies that quantified HCV infection within the liver.[8,9] HCV-infected cells were found in clusters, in accord with others,[8-10] supporting the idea that HCV infection occurs efficiently through cell-cell transfer.[12] The clustered pattern of HCV infection suggests that virus spread could be restricted by local activation of ISG responses or, alternatively, that subsets of hepatocytes within the liver may have differing properties that permit or limit HCV infection or replication.

The ultimate aim of the study by Wieland et al.[11] was to identify the distribution of HCV-infected cells in relationship to the cells expressing ISG transcripts within the infected liver. For this, the researchers used a multiplex fluorescence ISH approach, combining

Abbreviations: HCV, hepatitis C virus; IFN, interferon; ISG, IFN-stimulated gene; ISH, in situ hybridization; JAK, Janus kinase; mRNA, messenger RNA; STAT, signal transducer and activator of transcription.

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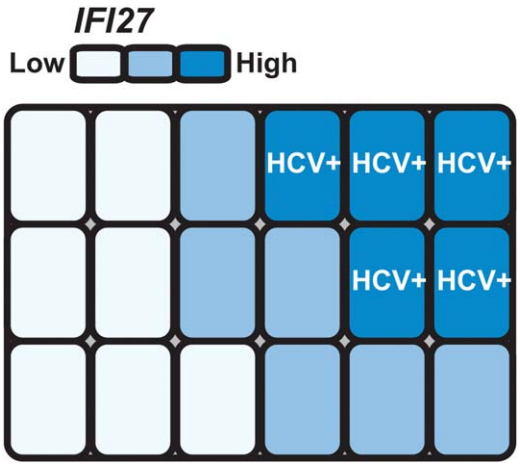

Fig. 1. HCV infection and ISG induction in the chronically infected liver. Differing patterns of HCV-infected cells (denoted by "HCV+") and cells expressing *IFI27* mRNA (darker blue color indicates high expression, medium blue indicates intermediate ISG expression, and light blue indicates low expression) were detected using multiplex fluorescence ISH within a subset of cells in the chronically infected human liver.

ISH for HCV RNA with that for *IFI27*, an ISG whose pretherapy up-regulation in livers of patients with chronic HCV has previously shown by these researchers to be in a four-gene classifier set that strongly predicts probability of sustained virologic response subsequent to HCV therapy.[13] A quantitative analysis of the spatial relationships between HCV RNA and *IFI27*-positive hepatocytes in human liver biopsies found several different expression patterns (see Fig. 1) that suggest that HCV infection induces up-regulation of ISGs primarily in a spatially constrained manner. Uninfected cells with uninfected neighbors generally had low levels of *IFI27* expression, whereas uninfected cells with infected neighbors had moderate levels. Quite interestingly, the highest levels of *IFI27* mRNA were found in cells also positive for HCV genomes. Taken together, this data suggest that Janus kinase/signal transducer and activator of transcription (JAK/STAT)-signaling pathways are, in fact, active and ISG transcription does occur in HCV-infected hepatocytes within livers of chronically infected patients, in contrast to previous reports from the coauthors on this study suggesting that HCV infection blocks JAK/STAT signaling and ISG transcription.[14] The reason for this discrepancy is unclear, although the researchers were able to identify some HCV-positive cells with low to background levels of *IFI27*, as well as cells with high *IFI27* bordering cells that were HCV positive, but did not express *IFI27*, suggesting that there could be differences in how HCV regulates IFN pathway signaling at any give time during acute and chronic infection in the liver. It appears that regulation of IFN signaling and activation by HCV in the chronically infected liver is complex, and looking at regulation of other ISGs in relationship to HCV infection, as well as correlations to treatment outcomes, will be important to understanding how local ISG activation is regulated by HCV and contributes to a functional immune response to HCV.

A number of important questions regarding innate immune regulation by HCV within the liver can be addressed by using methodologies similar to those developed by Wieland et al.[11] For example, which liver cell type produces the IFN that drives ISG mRNA expression? Both Kupffer cells and plasmacytoid dendritic cells may play a role in IFN induction during HCV,[15,16] and staining for markers of these cells, along with ISH for HCV RNA, could begin to address this question. Furthermore, it is still not clear which IFN (IFN-α, IFN-β, or IFN-λ) is the key cytokine that drives hepatic up-regulation of ISGs in nonresponders preceding therapy, and staining methods with higher sensitivities will be required to address this important question. In fact, HCV infection of primary human hepatocytes drives strong IFN-λ induction and only weak induction of type I IFN, suggesting a primary role for IFN- λ in the innate immune response to HCV.[17,18]

Importantly, the question remains as to why ISG up-regulation in the chronically infected level does not result in HCV clearance. Though it has been suggested that, in chronically infected patients, HCV induces a tolerized or refractory state that prevents IFN activity[3,15] or that HCV blocks ISG mRNA translation,[19] the precise mechanisms underlying these hypotheses in the infected patient liver have yet to be determined. Nonetheless, the study by Wieland et al.[11] provides an advance that paves the way for future studies to determine how HCV infection within the architecture of the liver induces local immune activation and how this affects therapy responses to HCV infection.

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