

## Interferon responses and spontaneous HCV clearance: Is it all a matter of fat?

Jordan J. Feld

Toronto Western Hospital Liver Centre, University Health Network, University of Toronto, Toronto, Canada

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Shortly after the discovery of the hepatitis C virus (HCV), it was recognized that the virus interacts intimately with the lipid machinery of the cell. Lipids are involved in almost all stages of the HCV lifecycle beginning with viral entry and including RNA replication, virion assembly and secretion of mature infectious viral particles [1]. The HCV core protein associates directly with cellular lipid droplets leading to recruitment of the non-structural (NS) proteins NS5A and NS3 to form the replicase complex, which serves as a scaffold for the RNA-dependent-RNA polymerase (NS5B) to begin replication of the viral genome [2]. Viral assembly then takes place and seems to parallel the processing of endogenous very low density lipoproteins (VLDL) with transfer of apolipoprotein B (apoB) and then apoE to form infectious low-density viral particles termed lipoviral particles (LVP) [3]. Impaired lipidation of viral particles results in production of non-infectious high-density virions, whereas increased abundance of LVP is associated with increased HCV infectivity [4]. The presence of increased apoE on the surface of LVP is associated with increased infectivity, possibly by facilitating interaction with lipoprotein receptors on hepatocytes [5]. ApoE may be important for more than infectivity. ApoE expression influences immune responses targeting lipid antigens and therefore its presence on HCV viral particles may be important for virus–host interactions [6].

HCV infection triggers innate immune pathways leading to the production of interferons and the establishment of an intracellular antiviral state [7]. Successful viral clearance however requires the subsequent emergence of a broad and potent HCV-specific adaptive immune response [8]. If infection is not cleared in the months following infection, the adaptive immune response wanes and the infection progresses to a chronic state with stable viral titers but progressive liver disease, after which viral clearance does not occur without antiviral therapy [8]. Responses to interferon-based treatment are largely dictated by the baseline expression of intra-hepatic interferon-stimulated genes (ISGs) [9]. Patients with low baseline ISG expression respond well to interferon with high rates of viral clearance while those with

preactivated ISGs show no further gene induction with therapy, leading to interferon non-response and treatment failure [10,11]. What controls the degree of ISG preactivation remains poorly understood.

The identification of single-nucleotide polymorphisms (SNPs) near the *IL28B* gene that are strongly associated with spontaneous [12] and treatment-induced viral clearance [13–15] raised the possibility that levels of interferon lambda (IL28B) were driving baseline ISG expression. However, despite the long and growing list of associations between the *IL28B* genotype and many aspects of HCV infection, the mechanisms underlying these relationships remain remarkably elusive. Hepatic ISG expression is associated with the *IL28B* genotype, however most reports have not found differing levels of IL28B itself across the different genotypes [16,17]. Furthermore, there are some seemingly paradoxical associations. One might have expected that a robust interferon response at baseline would lead to higher rates of spontaneous viral clearance. However, patients with the CC genotype have lower baseline ISG expression [16–18], perhaps suggesting reduced interferon production, yet have higher rates of spontaneous HCV clearance. Despite their favourable response to therapy, CC patients also tend to have higher baseline HCV RNA titres, a factor consistently shown to be associated with interferon non-response [13]. Finally, patients with the CC genotype have also been shown to have higher cholesterol but less hepatic steatosis [19].

In this issue of the *Journal*, Sheridan and colleagues explore possible links between interferon responses and HCV–lipid interactions [20]. They hypothesize that HCV may take advantage of the effects of interferon on lipid homeostasis. In a cohort of 72 patients with genotype 1 HCV infection, they evaluated the correlations between levels of the infectious low-density HCV fraction (LVP), lipid parameters (apoE, apoB and cholesterol) and the response to interferon-based therapy. They also examined the role of the *IL28B* genotype and measured serum levels of interferon-gamma-inducible protein 10 (IP10) as a surrogate for ISG expression. They first show that, as might be expected, apoE levels correlate well with the amount of lipid-rich LVP HCV but not with the higher density, lipid-poor HCV fractions. Lower apoE levels were found in patients with a good response to therapy and in those with the treatment-favourable *IL28B* genotype (CC). Finally, they found a modest correlation with levels of apoE and IP10, but this was not replicated in a small validation cohort.

Keywords: Lipoviral particles (LVP); Apolipoprotein E (apo E); Interferon-responses; IL28B; Spontaneous clearance.

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E-mail address: jordan.feld@uhn.on.ca



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## Editorial

Based on these findings, the authors wonder if higher levels of IP10, which they interpret to indicate greater interferon and ISG activation, lead to more apoE, which in turn favours the more infectious LVP form of the virus resulting in greater HCV infectivity and reduced treatment response.

An evaluation of the HCV fractions by *IL28B* genotype yielded interesting results. The CC genotype was associated with higher total HCV RNA levels, as has been shown before [13], but interestingly the correlation was entirely driven by an association with the less infectious non-LVP form of the virus. This may at least partially explain the paradoxical association between the treatment-responsive CC genotype and higher viral loads. The higher viral loads seen in CC patients are composed of the less infectious non-LVP virus and therefore do not negatively affect the response to treatment. Presumably the association of higher viral levels with poor treatment response relates to levels of the more infectious LVP fraction and would likely disappear after controlling for the *IL28B* genotype. The cohort in this study was too small for such an analysis.

This study raises some interesting possibilities. The authors suppose that endogenous interferon stimulates higher levels of apoE, which ultimately promotes infectious LVP HCV and thus reduces responses to interferon-based therapy. Although this is an interesting hypothesis, it is premature to draw causal links. The association between apoE levels and IP10 was marginal at best and IP10 is only a modest surrogate for ISG activation [21]. Notably, although there was a trend, IP10 levels did not correlate with treatment outcome in this cohort and did not differ by *IL28B* genotype. ApoE and LVP titre also did not differ by treatment response. However, the differences in levels of apoE, apoB and LDL by *IL28B* genotype were significant. Rather than an explanation for treatment non-response, the authors might have identified a factor relevant to spontaneous viral clearance. The association between the CC genotype and lower apoE but higher apoB and LDL levels was quite robust. If the CC genotype is associated with lower apoE levels and therefore less infectious virus, this may explain why patients with this genotype are more likely to clear infection. The infectiousness of the virus is likely much more important at the time of acute exposure when viral spread is rapidly progressing than during interferon treatment. It may well be true that the LVP virus is less interferon-responsive, in which case the hypothesis about treatment may also be correct, but this has yet to be shown. The data supporting LVP-related infectivity from this group [22] and others [23] are strong and may be very relevant to spontaneous clearance. In fact, an explanation for spontaneous clearance with the CC genotype that does not require more interferon production would potentially explain why these patients go on to have lower levels of ISG preactivation, a paradox that has until now been unexplained. A theory allowing for lower interferon production in CC patients, would also potentially explain the higher viral titres seen with the CC genotype, whether LVP or non-LVP HCV.

To clarify whether the findings truly relate to interferon will require some additional work. It would be helpful to know if apoE is induced by exogenous (and ideally endogenous) interferon directly and if so what mechanisms are involved. To support the concept that higher apoE leads to interferon non-response, it would be helpful to determine if there is a difference in interferon sensitivity between the LVP and non-LVP viral fractions. It would also be useful to delve further into the issue of spontaneous clearance and the association with the CC genotype. Do all individuals with the CC genotype have lower apoE but higher

apoB and LDL levels or is this a phenomenon that requires the presence of HCV, or possibly, as the authors suppose, endogenous interferon? If the *IL28B* genotype affects apoE levels independent of interferon, the question of the underlying mechanism arises once again. Do patients who clear acute HCV have lower levels of apoE and LVP? If so, would modulation of lipid balance at the time of acute infection be a potential therapeutic strategy?

As with many provocative studies, the work by Sheridan and colleagues may have raised more questions than it answers but it is certainly a valuable contribution. These data offer some potential explanations to some puzzling aspects of the complicated but slowly emerging picture of HCV, lipids and interferon responses. The possibility that interferon non-response is related to effects on lipids is certainly intriguing and merits further study and exploration of the potential relevance for spontaneous HCV clearance, may be even more important.

### Conflict of interest

Consulting for Abbott Laboratories, Gilead Sciences, Hoffmann-LaRoche Pharmaceuticals, Merck Pharmaceuticals, Tibotec Pharmaceuticals, Vertex Pharmaceuticals. Speaking Abbott Laboratoires, Merck Pharmaceuticals.

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