



Editorial

New insights into hepatitis B and C virus co-infection☆Volker Brass¹, Darius Moradpour^{2,*}¹Department of Medicine II, University of Freiburg, Freiburg, Germany²Division of Gastroenterology and Hepatology, Centre Hospitalier Universitaire Vaudois, University of Lausanne, Rue du Bugnon 44, 1011 Lausanne, Switzerland

See Article, pages 446–457

More than 500 million people worldwide are chronically infected with the hepatitis B (HBV) or hepatitis C virus (HCV). Infections with these viruses are the leading cause of chronic hepatitis, liver cirrhosis and hepatocellular carcinoma (HCC). As a consequence, major research efforts have been devoted to HBV and HCV. However, relatively little is known about interactions between HBV and HCV in patients co-infected with both viruses.

Co-infection with HBV and HCV is common due to shared modes of transmission (reviewed in [1,2]). Available evidence indicates more frequent development of cirrhosis and an increased incidence of HCC in HBV/HCV co-infection as compared to mono-infection by either virus. As a result, studies have reported an excess liver-related mortality in HBV/HCV co-infection as compared to HBV or HCV infection alone [3].

While liver disease activity and fibrosis progression are generally more severe in the presence of double infection, an inverse relationship in the replicative levels of the two viruses has been noted, suggesting direct or indirect viral interference [1,2]. Challenging this notion, longitudinal studies revealed that the two viruses may replicate independently from each other in some patients, with fluctuations in the serum level of one virus that appear unrelated to the viremia of the other [4]. However, suppression of HBV replication was found

in patients with chronic hepatitis B when they developed acute hepatitis C and, conversely, inhibition of HCV replication has been observed in patients with chronic hepatitis C superinfected with HBV [5,6]. Finally, HBV reactivation was observed in co-infected patients after successful treatment of HCV with pegylated interferon- α (PEG-IFN- α) and ribavirin [7,8]. Therefore, while liver disease appears to be enhanced, the two viruses seem to inhibit each other at the replicative level.

From the basic perspective, HBV/HCV co-infection implies the exciting scenario of two pathogens with completely divergent life cycles meeting in the same host. HBV belongs to a family of hepatotropic DNA viruses (*Hepadnaviridae*) (reviewed in [9]). The viral genome consists of an incompletely double-stranded circular DNA of about 3.2-kbp length. The viral RNAs are transcribed in the nucleus from the covalently closed circular DNA template. Replication occurs within nucleocapsids via the reverse transcription of packaged pregenomic RNA.

HCV is classified in the *Hepacivirus* genus within the *Flaviviridae* family. It contains a 9.6-kb positive-strand RNA genome that encodes a polyprotein precursor of about 3000 amino acids (reviewed in [10]). The polyprotein precursor is co- and posttranslationally processed by cellular and viral proteases to yield the mature structural and nonstructural proteins. HCV replication takes place in a cytoplasmic membrane-associated complex (membranous web).

Interactions between HBV and HCV have been difficult to study because of the lack of appropriate model systems. Indeed, robust infection of cultured cells by HBV remains a challenge, with only few cell types, including HepaRG and primary human or tupaia hepatocytes

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at disposition [11–13]. However, HepaRG cells do not appear to be susceptible to efficient HCV infection and replication (George Koutsoudakis and Ralf Bartenschlager, personal communication) and primary human as well as tupaia cells support only low level HCV replication [14,15]. Therefore, the few studies that have addressed interactions between HBV and HCV were based on heterologous overexpression of viral proteins and have yielded conflicting results. For example, some studies demonstrated that HCV core inhibits HBV replication [16,17] while others did not [18]. Similarly, HCV NS5A was found to enhance [19] or inhibit [20] HBV replication. Thus, it remains unclear whether there is a direct interference between HBV and HCV.

Against this background, the study by Eyre et al. published in this issue of the Journal provides important new insights into HBV/HCV co-infection [21]. The authors used Huh-7 human HCC cells to study interactions between HBV and HCV. Huh-7 cells support HBV replication and virion formation as well as the entire HCV life cycle, including viral entry, RNA replication, and infectious particle release [22–25]. As these cells cannot be infected by HBV, the authors used a recombinant adenovirus vector (AdHBV) to intracellularly deliver a replication-competent HBV genome. A recombinant adenovirus expressing the green fluorescent protein (AdGFP) served as control for nonspecific effects. Huh-7 cells were also infected with cell culture-derived HCV (HCVcc) [23–25]. Importantly, HCV replication and virion production were not affected when cells were co-infected simultaneously with AdHBV and HCVcc or when HCVcc-infected cells were subsequently exposed to the recombinant adenovirus. For unknown reasons, HCV RNA replication was even increased when the cells were preinfected with AdHBV. On the other hand, HBV DNA levels in cells and supernatants were either unaffected or slightly increased by HCVcc infection. Thus, in this replicating context, there appeared to be no or only a minor influence of HBV on HCV and *vice versa*.

The key findings of this study are in line with a recent study by Bellecave et al. [26]. In this study, Huh-7 cells were engineered to inducibly replicate and produce infectious HBV using a tetracycline-regulated gene expression system. HCV was introduced into these stable cell lines as well as into control Huh-7 cells inducibly expressing the GFP either in the form of selectable HCV replicons or HCVcc. In this highly reproducible model system, HBV and HCV were found to replicate in the same cell without overt interference. In addition, specific inhibition of one virus did not affect the replication and gene expression of the other. Finally, cells harboring replicating HBV could be infected by HCVcc, arguing against superinfection exclusion, and they also supported efficient production of infectious HCV.

Taken together, the studies by Eyre et al. and Bellecave et al. reveal the absence of direct interference

between HBV and HCV *in vitro*. This remarkable observation indicates that – at least in Huh-7 cells – the two viruses rely on distinct and non-competing sets of host factors for their replication. At the same time, these results suggest that the viral interference observed in co-infected patients is likely due to indirect mechanisms mediated by innate and/or adaptive host immune responses. In this context, both HBV and HCV replication are susceptible to antiviral cytokines, including type I and type II IFNs as well as tumor necrosis factor- α , that are produced by the infected cell or infiltrating T-cells [27,28]. An alternative explanation for the viral interference observed *in vivo* may relate to host factors that become limiting in human hepatocytes in the liver but not in Huh-7 cells *in vitro*. Clearly, the development of *in vitro* and *in vivo* model systems that will allow to study the effect of innate and adaptive responses in HBV/HCV co-infection as well as the establishment of experimental conditions that more closely resemble human hepatocytes or the naturally infected liver represents the next challenge.

In conclusion, the study by Eyre et al. appearing in this issue of the Journal [21] and the study by Bellecave et al. published earlier this year [26] demonstrate that HBV and HCV can replicate in the same cell without evidence for direct interference *in vitro*. Therefore, the viral interference observed in co-infected patients is probably due to indirect mechanisms mediated by innate and/or adaptive host immune responses. These findings provide new insights into the pathogenesis of HBV/HCV co-infection and may contribute to its clinical management in the future.

Acknowledgments

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