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Inhibiting cell-to-cell transmission to reach HDV cure: the importance of IFN α

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While hepatitis B virus (HBV) persists in cells through the establishment of a stable episomal DNA [1], hepatitis delta virus (HDV) is thought to be maintained in infected organisms likely by viral spreading. This hypothesis arose from our knowledge of the HDV life cycle that includes the production of an edited genome and a viral protein (L-HDAg) necessary for HDV morphogenesis but inhibiting its replication [2]. This is now enforced by Zhang and colleagues reporting a short half-life of HDV RNAs [3] that would not allow HDV maintenance into non-dividing cells. Although the existence of long term HDV persistence in cells was suspected in mice [4, 5], HDV spreading was thought to occur exclusively thanks to the hijacking of HBV envelope for its secretion and re-infection of naïve cells [2].

Several therapeutic options aiming to cure HDV infection have entered the clinic (reviewed in [6] and **Figure 1**). Bulevirtide (previously called Myrcludex-B®) is a peptide that block HDV entry into cells by competing with the virus for its binding to the NTCP receptor (**Figure 1**). Lonafarnib, a farnesyl transferase inhibitor (FTI) was initially designed to decrease the secretion of HDV particles since L-HDAg needs to be farnesylated to interact with the HBV envelop proteins (**Figure 1**). A recent study reported that the remaining released particles (escaping the canonical function of FTI) are mostly non-infectious notably because they contain an edited version of the HDV genome [7] (**Figure 1**). Nucleic acids polymers (NAPs) were also reported to potentially act on viral egress by blocking HBsAg release [8] (**Figure 1**). However, a spreading of HDV occurring exclusively through its secretion with HBV envelope would not explain how HDV can be maintained in patients experiencing liver transplantation and receiving anti-HBsAg therapies [9] or in HDV mono-infected mice [4]. Moreover, results from clinical

trials with bulevirtide, lonafarnib or NAPs and the first real-life studies for bulevirtide (that received an conditional authorization for the use in patients in the European Union since 2020) suggested that antiviral effects of either of the drugs are rather limited when used in monotherapy and become very potent for HDV patients as soon as combined to interferon alpha (IFN- α) [10].

A new publication from Stephan Urban's laboratory at the University Hospital Heidelberg, published in this issue of the journal sheds light on the functional role of IFN- α for HDV spread and propagation which may have important implications for the clinical development of novel combination therapies for HDV cure. In an elegant study, Zhang et al. unraveled that HDV propagates from mother to daughter cells upon mitosis and that exogenous IFN- α treatment (but neither bulevirtide nor lonafarnib) specifically blocks this cell-to-cell spreading [3] (**Figure 1**). Moreover, by comparing two HDV infection models, they observed a marked inhibition of HDV spreading following cell division in differentiated HepaRG (dHepaRG) cells compared to HuH7-NTCP cells, that do not exhibit MDA5-mediated innate immune response upon HDV infection [11]. Of note, the knock-down of MDA5 expression in dHepaRG cells induced virus spreading, confirming that not only exogenous IFN treatment, but also HDV-induced cellular type I IFN expression restrict cell-to-cell transmission of HDV [3]. Of note, to exclude spreading induced by the release of viral particles, the entire study was performed from HDV mono-infected cells *in vitro* [3] and the importance of this cell-to-cell transmission (over transmission *via* secreted particles) and its inhibition by IFN- α treatment in HBV/HDV co-infected patients has not been addressed yet. Indeed, the presence of HBsAg might favor HDV assembly over cell-to-cell spreading and as human hepatocytes are competent for IFN production upon HDV infection [11], HDV cell-to-cell propagation could be as limited in patients as observed in HepaRG cells.

The study from Zhang et al. uncovers a new mechanism of how IFN affects the HDV life cycle. Besides activation of the innate immune cells, IFN- α has been described to directly affect numerous steps of the HDV life cycle including entry [12], replication and secretion [13]. Here, Zhang et al. demonstrated that IFN- α also abrogates cell-to-cell propagation [3] (**Figure 1**). In theory, IFN- α combines more antiviral effects than bulevirtide, lonafarnib and NAPs and should thereby be very potent at inhibiting HDV. However, IFN- α -based monotherapies were shown to suppress HDV viremia only in a limited number of patients with relapses after arrest of treatment being very often reported [14]. The molecular bases of the low responsiveness of HBV/HDV co-infected patients to IFN- α therapies are unknown. Type I IFNs initiate a signaling cascade *via* IFN- α/β receptors (IFNAR) through the JAK-STAT

pathway, which transcribes hundreds of IFN-stimulated genes (ISGs) and it has been suggested that both HBV and HDV could inhibit the JAK-STAT signaling pathway and hence the response to exogenous IFN [15]. Moreover, whereas HBV is considered as a weak inducer of innate responses [16, 17], HDV strongly activates the IFN pathway *via* MDA5 as mentioned above [11, 13]. The low effectiveness of IFN-based therapies in patients may therefore be due to the basal up-regulation of ISGs expression, as previously shown in the case of HCV-infected patients [18]. Nevertheless, the efficacy of the current trials using innovative molecules are strongly enhanced when combined with IFN- α , suggesting the existence of synergistic effects of these molecules in patients. Zhang et al. propose that the efficacy of the combined treatment may be due to the paralleled inhibition of both cell-to-cell- (by IFN- α) and viral particles-mediated spreading (by either Isonafarnib or bulevirtide).

Assuming a crucial role of IFN- α in combination therapy, adverse effects induced by this broad antiviral molecule still remain a clinical limitation. Even though the use of IFN-lambda might be an alternative with lower side effects (reviewed in [6]), the understanding of the comprehensive role of type I IFN in the HDV life cycle, and notably the identification of ISGs with antiviral effector functions would help to consider more targeted antiviral options. Surprisingly, only proviral ISGs have been described as involved in the HDV life cycle so far, such as ADAR-1 and MOV-10 (reviewed in [2]). ISGs with an antiviral effect against HDV remains to be identified in order to provide insights into resistance to IFN- α treatment as well as to allow the development of new antiviral strategies with less side effects than IFN- α based therapies. To this end, lessons from chronic HBV infection shade light into putative strategies that may be considered. In chronic HBV-infected patients, correlations between the response to IFN treatment and single nucleotide polymorphisms (SNP) within the coding sequence of ISGs have been reported, including IFIT1 [19]. Genome Wide Association Studies (GWAS), analyzing the association between gene polymorphism and treatment responses in chronically HDV infected patients might therefore be another step toward the identification of effector genes specifically targeting HDV, and notably preventing cell-to-cell spreading.

In conclusion, the study from Zhang et al provides new knowledge on the interplay between HDV and the innate immune response, advancing previous studies from the same group [11, 20]. Finally, by providing a previously undiscovered mechanism of action of type I IFNs against HDV, the study by Zhang et al. will likely contribute also to the clinical development of novel combination therapies tackling this global health threat.

Figure Legend:

Figure 1: Current antiviral strategies against HDV infection.

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