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Poster for you

Deciphering the cellular response to IFN treatment in HDV-infected cells

Claudie Eber[1,], Roxanne Fouillé[2,*], Emma Gerges[1], Zoé Lebrun[1], David Durantel[2], Thomas F. Baumert[1], Julie Lucifora[2,$], Eloi R. Verrier[1,$]*

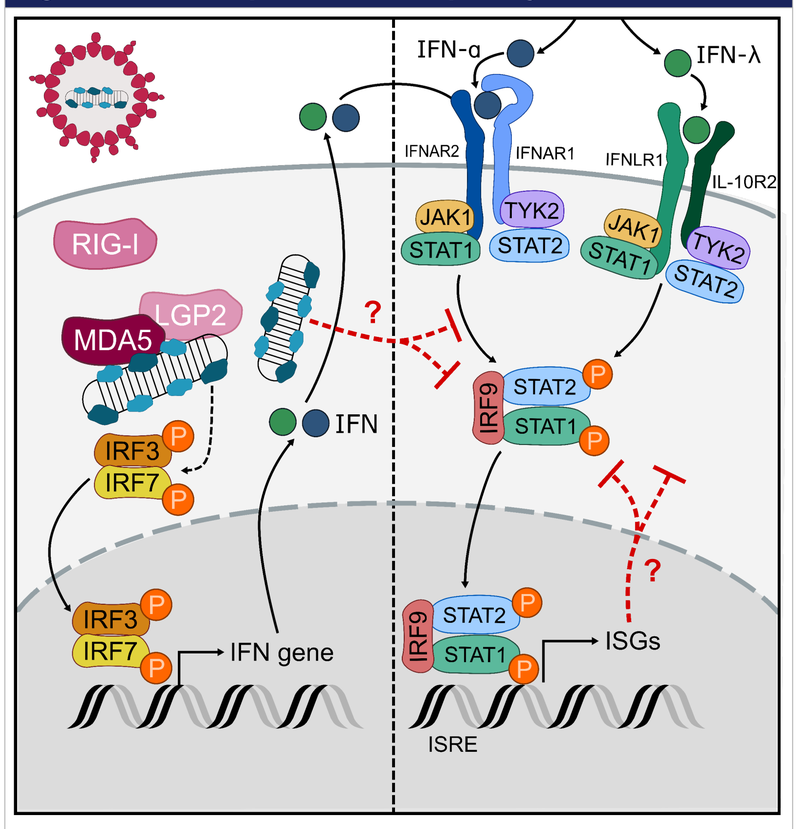
1 University of Strasbourg, Inserm, Institute for Translational Medicine and Liver Disease (ITM), UMR_S1110, Strasbourg; 2 CIRI, Centre International de Recherche en Infectiologie, Univ Lyon, Inserm, U1111, Université Claude Bernard Lyon 1, CNRS, UMR5308, ENS de Lyon, F-69007, Lyon, France; 3 Institut hospitalo-universitaire (IHU). Service d'hépato-gastroentérologie, Hôpitaux Universitaires de Strasbourg, Strasbourg, France; 4 Institut Universitaire de France (IUF), Paris, France.

**These authors contributed equally $These authors contributed equally*

Introduction

- Hepatitis delta virus (HDV) induces an endogenous interferon (IFN) response through the recognition of its ribonucleoprotein by MDA5 and LGP2.
- HDV detection by the cellular sensors leads to the production of IFN-α and IFN-λ which activate the JAK/STAT signaling pathway and induce the expression of interferon-stimulated genes (ISGs), some of them exhibiting antiviral propreties.
- ISG expression is also triggered by the exogenous treatment with IFN-α or IFN-λ.
- **Intrestingly, HDV replication is moderatly affected by both endogenous and exogenous IFN-α *in vivo* and *in vitro.***
- **Furthermore, it has been shown that the ability of IFN-α to trigger ISGs expression is impaired in HDV infected cells[1,2].**
- **The aim of our study is to understand the molecular mechanisms of this inhibition.**

Figure 1: Is The IFN response inhibited by HDV proteins or by an negative feedback loop of the JAK/STAT pathway?



Methods

- We analyzed the transcriptomic impact of IFN-α and IFN-λ on both HDV- and mock- infected dHepaRG cells by RNAseq.
- To evaluate the role of HDV antigens in the interaction with IFN signaling, we overexpressed S-HDAg and L-HDAg by AAV-meditated transduction in dHepaRG cells.
- To assess the impact of the innate immune response, we took advantage of dHepaRG cells KO for RIG-I or MDA5, exhibiting a differential response to HDV infection (Table 1). Cells were infected with HDV for six days followed by treatment with either IFN-α (500 IU/ml) or IFN-λ-1 (IL-29, 100 ng/ml).

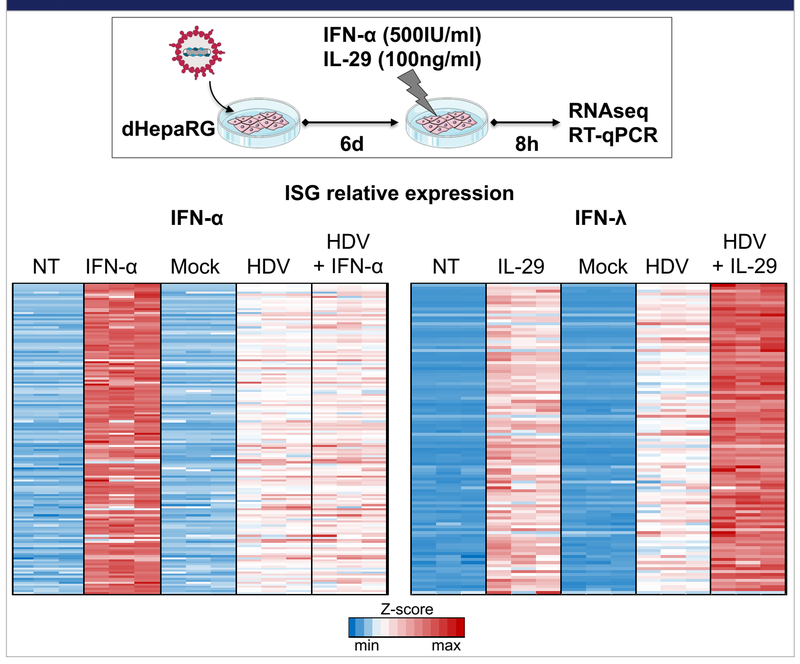
Table 1 **Propreties of the cells used**

	IFN production after HDV infection		IFN response after cell treatment	
	YES	NO	YES	NO
dHepaRG	X		X	
dHepaRG RIG-I_{KO}	X		X	
dHepaRG $MDA5_{KO}$		X	X	

Results

HDV INFECTION SPECIFICALLY DISRUPTS THE IFN-α RESPONSE

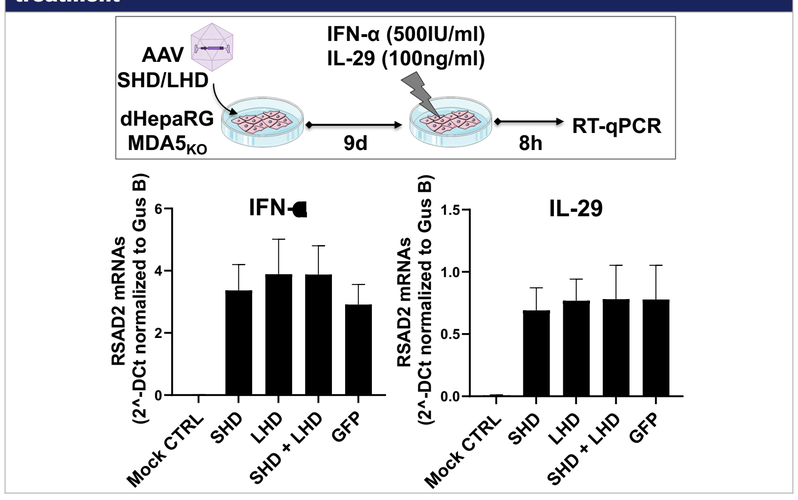
Figure 2: The IFN-α but not IFN-λ is inhibited by HDV infection in dHepaRG



- The cellular response to IFN-α, but not IFN-λ, is impaired in HDV-infected dHepaRG cells.

EXPRESSION OF HDV PROTEINS DOES NOT INTERFERE WITH THE IFN RESPONSE

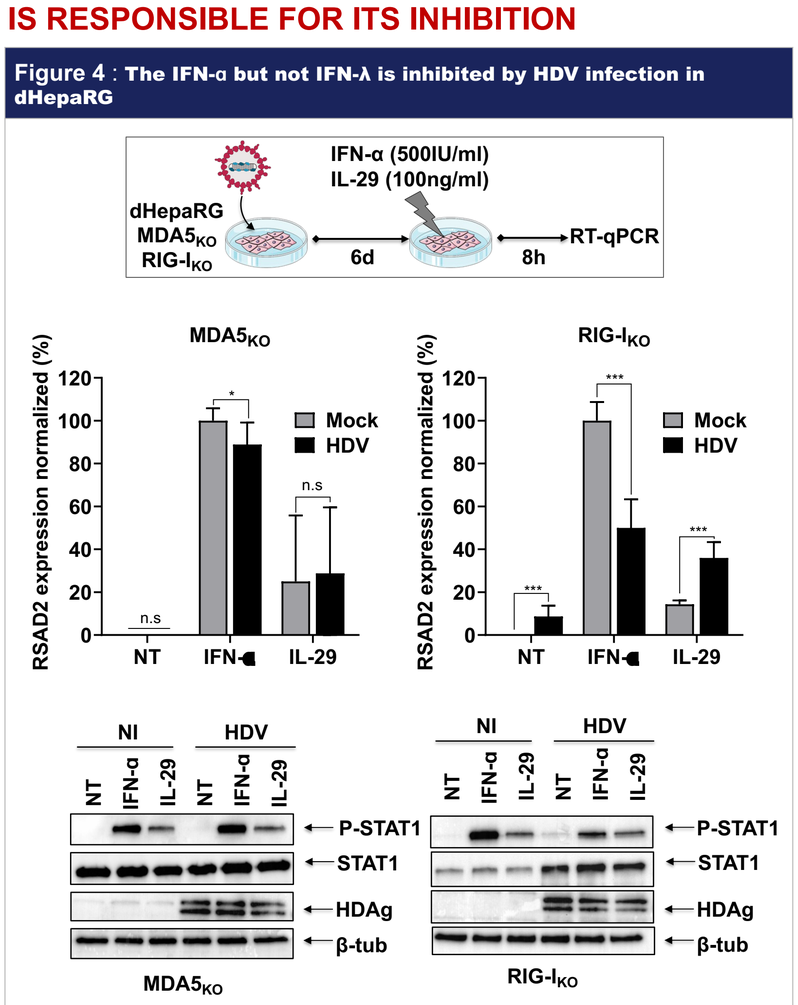
Figure 3: HDV antigens have no direct impact on the response to IFN-α treatment



- HDV antigens expression has no impact on IFN-α-induced ISG expression

HDV-INDUCED PREACTIVATION OF IFN RESPONSE IS RESPONSIBLE FOR ITS INHIBITION

Figure 4 : The IFN-α but not IFN-λ is inhibited by HDV infection in dHepaRG



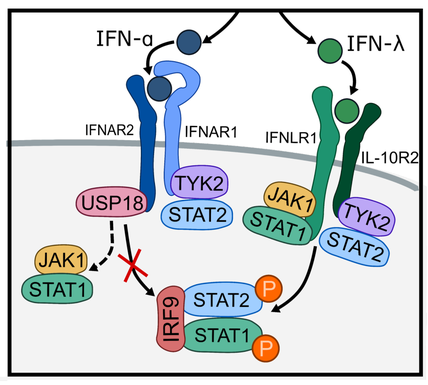
- The IFN-α response is no longer inhibited in absence of HDV sensing in dHepaRG-$MDA5_{KO}$ cells.
- This suggests that the preactivation of the innate immune response induced by HDV infection specifically inhibits the response to IFN-α.

Hypothesis

USP18 IS A KEY INHIBITOR OF THE IFN-α RESPONSE

- USP18 (ubiquitin specific peptidase) is an ISG known to be involved in ISGylation mediated by ISG15.
- This protein was identified as a major inhibitor of the IFN-α mediated response through its association with the IFNAR2 subunit, leading to the displacement of JAK1[3].
- **According to RNA-seq data, USP18 is upregulated in HDV infected cells.**
- **This protein may be involved in the inihibtion of the IFN-α response by HDV.**

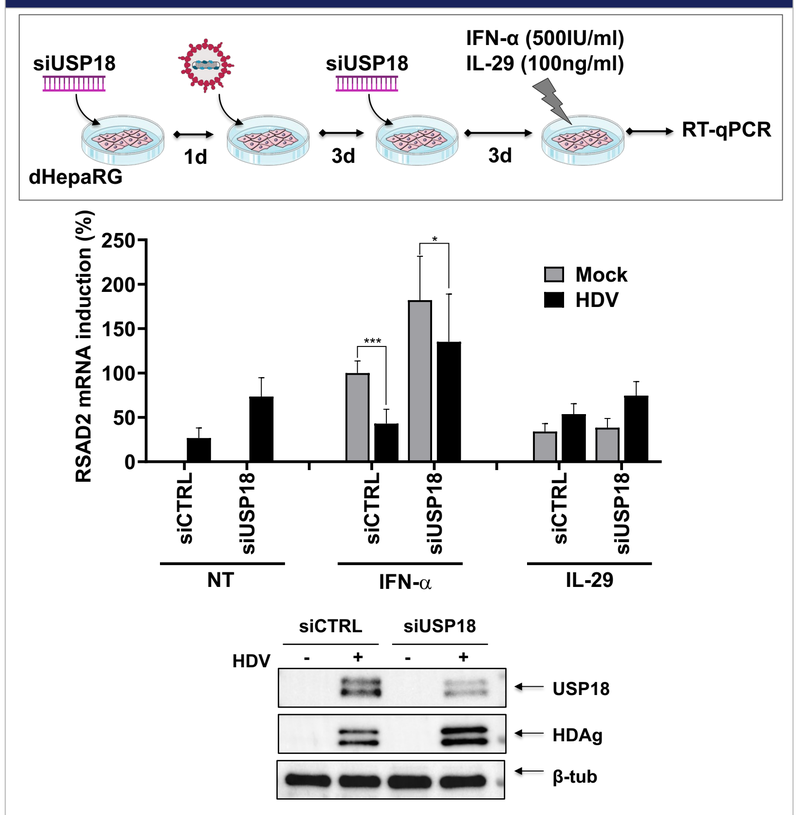
Figure 5: USP18 specifically downregulates IFN-α response



Results

USP18 MODULATE THE TYPE I BUT NOT TYPE III IFN RESPONSE IN HDV-INFECTED CELLS

Figure 6 : USP18 contributes to the negative feedback loop



- Partial restauration of ISG induction upon IFN treatment in USP18 knockdown cells

Conclusion/Perspectives

- The HDV-induced IFN response following HDV infection inhibits the response to IFN-α, but not IFN-λ treatment.
- This innate immune response induces a negative feedback loop which in turn inhibits the activation of STAT proteins.
- The ISG USP18 contributes to this negative feedback loop.
- The partial restauration upon USP18 knockdown raises the question of an alternative mechanism of action that could be involded.

References

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