Research Article

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Hepatitis Delta co-infection in humanized mice leads to pronounced induction of innate immune responses in comparison to HBV mono-infection

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Background & Aims: The limited availability of hepatitis Delta virus (HDV) infection models has hindered studies of interactions between HDV and infected hepatocytes. The aim was to investigate the antiviral state of HDV infected human hepatocytes in the setting of co-infection with hepatitis B virus (HBV) compared to HBV mono-infection using human liver chimeric mice.

Methods: Viral loads, human interferon stimulated genes (hISGs) and cytokines were determined in humanized uPA/SCID/beige (USB) mice by qRT-PCR, ELISA and immunofluorescence.

Results: Upon HBV/HDV inoculation, all mice developed viremia, which was accompanied by a significant induction of hISGs (i.e. hISG15, hSTATs, hHLA-E) compared to uninfected mice, while HBV mono-infection led to weaker hISG elevations. In the setting of chronic infection enhancement of innate defense mechanisms was significantly more prominent in HBV/HDV infected mice. Also the induction of human-specific cytokines (hIP10, hTGF-ß, hIFN-ß and hIFN-λ) was detected in HBV/HDV co-infected animals, while levels remained lower or below detection in uninfected and HBV mono-infected mice. Moreover, despite the average increase of hSTAT levels determined in HBV/HDV infected livers, we observed a weaker hSTAT accumulation in nuclei of hepatocytes displaying very high HDAg levels, suggesting that HDAg may in part limit hSTAT signaling.

Conclusions: Establishment of HDV infection provoked a clear enhancement of the antiviral state of the human hepatocytes in chimeric mice. Elevated pre-treatment ISG and interferon levels may directly contribute to inflammation and liver damage, providing a rationale for the more severe course of HDV-associated liver disease. Such antiviral state induction might also contribute to the lower levels of HBV activity frequently found in co-infected hepatocytes.

Introduction

Approximately 20 million people worldwide are chronically infected with the hepatitis Delta virus (HDV), which is considered the most severe form of viral hepatitis and is associated in up to 70% of patients with an accelerated course of fibrosis progression and liver cirrhosis [1]. The pathogenic agent is a small single-stranded RNA virus with a genome of 1679 nucleotides in length, which hijacks the host RNA polymerase II to propagate the viral genome in an RNA-directed RNA replication via a rolling cycle amplification process [2]. Within the infected hepatocytes the viral replication leads to accumulation of three different HDV RNAs: genomic RNA, antigenomic RNA and the mRNA, which translates into the only known viral protein, the hepatitis delta antigen (HDAg). There are two isoforms of the HDAg: a 24KDa small isoform which is needed for replication and the 27KDa long variant which is generated by an RNA editing event and is essential for viral assembly [3,4]. HDV is a defective virus that needs the envelope proteins of the HBV to infect the human hepatocytes [5], as well as to envelope the newly synthesized viral RNA, generate infectious viral particles and hence to complete the viral life-cycle [6]. Therefore, infectious HDV virions can only be assembled and released if a hepatocyte is co-infected with HBV or after being rescued by HBV infection [7].

Viral components, such as genomic DNA, single and double stranded RNA and viral proteins, can be recognized by host cells through pattern recognition receptors (PRRs) which in turn respond by activating intracellular signaling cascades associated with antiviral immunity [8]. Different types of PRRs, e.g. toll-like-receptors (TLRs) and retinoic acid-inducible gene

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Abbreviations: HDV, Hepatitis Delta Virus; HBV, Hepatitis B Virus; ISGs, interferon stimulated genes; UPA, Urokinase Plasminogen activator; SCID, Severe combined immunodeficiency.

(Rig-I)-like receptors (RLRs), can detect viruses and are involved in the induction of interferon stimulated genes (ISGs), production of type I interferons (IFN) and other cytokines [9]. Type I interferons (IFNα and IFNß) lead via the JAK STAT pathway to the induction of ISGs, which are known to be the hallmark of antiviral immunity and play a fundamental role in counteracting viral infections [10,11]. On the other hand, elevated pre-treatment ISG levels have been associated with weaker responses to IFNα treatment in hepatitis C virus (HCV) infected patients [12] and in some cases were even shown to support viral replication [13].

The limited availability of HDV infection models has hindered studies of interactions between HDV and infected human hepatocytes. Therefore, the aim of our study was to investigate the antiviral state of the human hepatocytes in the setting of HBV/HDV co-infection compared to HBV mono-infection using human liver chimeric mice [14].

Materials and methods

Generation of humanized mice and viral infection

Humanized liver chimeric mice were generated as previously described. Briefly, one million viable thawed cryopreserved human hepatocytes were injected intra-splenically in young homozygous Alb-uPA/SCID/beige (shortly USB) mice as previously reported [14]. Humanized mice that were generated by transplanting human hepatocytes obtained from a single human donor were used for the

Table 1. ISG and cytokine induction.

Gene	Fold difference: HBV *vs.* uninf (median/median)	*p* value	Fold difference: HBV/HDV *vs.* uninf (median/median)	*p* value	Fold difference: HBV/HDV *vs.* HBV (median/median)	*p* value
hMxA	1.0	n.s.	3.0	<0.0001***	3.5	0.0014**
hOAS1	2.2	0.0031**	3.3	<0.0001***	1.8	0.0039**
hUSP18	1.5	0.037*	3.0	<0.0001***	2.5	<0.0001***
hISG15	1.0	n.s.	4.3	<0.0001***	3.5	0.0006***
hISG20	1.4	n.s.	4.5	0.0017**	2.9	<0.0001***
hHLA-E	1.4	n.s.	3.2	<0.0001***	2.0	<0.0001***
hTAP1	2.0	0.0045**	5.1	<0.0001***	2.6	<0.0001***
hADAR	1.3	n.s.	2.2	<0.0001***	1.6	0.0006***
hIP10	6.9	0.0091**	40.2	<0.0001***	5.3	<0.0001***
hNTCP	1.2	n.s.	0.8	0.0383*	0.7	0.0179*
hSTAT1	2.1	0.0054**	3.5	0.0002***	1.8	0.0226*
hSTAT2	1.7	0.0277*	2.0	<0.0001***	1.5	0.0179*
hSTAT3	1.4	n.s.	2.9	<0.0001***	2.0	0.0076**
hPIAS3	1.4	0.0236*	1.6	0.0078**	1.0	n.s.
hPIAS4	1.4	0.0205*	1.9	0.0006***	1.3	0.0111*
hRIG-I	1.6	0.0426*	2.9	<0.0001***	1.9	0.0022**
hTLR2	1.7	n.s.	2.8	0.0021**	1.6	0.0238*
hTLR3	1.5	0.0239*	1.3	0.0193*	1.1	n.s.
hTLR4	3.7	0.0370*	1.3	n.s.	0.5	n.s.
hTLR7	n.d.	n.s.	n.d.	n.s.	n.d.	n.s.
hTLR8	n.d.	n.s.	n.d.	n.s.	n.d.	n.s.
hTLR9	0.4	n.s.	1.2	n.s.	1.4	n.s.
hAPOBEC3A	n.d.	n.s.	n.d.	n.s.	n.d.	n.s.
hAPOBEC3B	3.5	0.0006***	6.4	<0.0001***	1.4	0.0350*
hAPOBEC3C	n.d.	n.s.	n.d.	n.s.	n.d.	n.s.
hAPOBEC3F	1.9	0.0021**	3.2	0.004**	1.5	n.s.
hAPOBEC3G	2.3	0.0012**	4.5	0.0006***	2.2	0.0111*
hCasp8	n.d.	n.s.	n.d.	n.s.	n.d.	n.s.
hHSPA6	1.9	0.0175*	2.3	<0.0001***	1.2	n.s.
hIFN-α	n.d.	n.s.	n.d.	n.s.	n.d.	n.s.
hIFN-β	n.d.	n.s.	d	0.0199*	d	0.0066**
hTGF-β	8.2	0.0045**	22.7	<0.0001***	1.8	n.s.
hIL28A+B	n.d.	n.s.	d	0.0334*	d	0.0036**
hIL29	n.d.	n.s.	d	0.002**	d	0.0004***

The RNA expression of classic ISGs, signaling genes, and cytokines was comparatively analyzed in stably HBV mono-infected, HBV/HDV co-infected and uninfected mice using a larger cohort of mice reconstituted with human hepatocytes derived from four different human donors. The last column indicates the relative expression differences determined in HBV/HDV co-infected mice compared to HBV mono-infected mice. Values are median fold inductions (median infected/median uninfected) and *p* values are calculated with the non-parametric Mann-Whitney U test. n.s., not significant; n.d., not detectable; d, detectable (i.e., when RNA expression in uninfected mice is below the lower limit of detection and fold inductions cannot be calculated).