Interferon-alpha treatment rapidly clears Hepatitis E virus infection in humanized mice

Martijn D. B. van de Garde1, Suzan D. Pas2, Gertine W. van Oord1, Lucio Gama4, Youkyung Choi5, Robert A. de Man1, Andre Boonstra1 & Thomas Vanwolleghem1,3

Antiviral treatment options for chronic Hepatitis E Virus (HEV) infections are limited and immunological determinants of viral persistence remain largely unexplored. We studied the antiviral potency of pegylated interferon-α (pegIFNα) against HEV infections in humanized mice and modelled intrahepatic interferon stimulated gene (ISG) responses. Human gene expression levels in humanized mouse livers were analyzed by qPCR and Nanostring. Human CXCL10 was measured in mouse serum. HEV genotype 3 (gt3) infections were cleared from liver and feces within 8 pegIFNα doses in all mice and relapsed after a single pegIFNα injection in only half of treated animals. Rapid viral clearance by pegIFNα was confirmed in HEV gt1, but not in Hepatitis B Virus infected animals. No ISG induction was observed in untreated HEV gt3 and gt1 infected humanized livers compared to control chimeric mice, irrespective of the human hepatocyte donor, viral isolate or HEV infection duration. Human specific ISG transcript levels in mouse liver increased significantly after pegIFNα treatment and induced high circulating human CXCL10 in mouse serum. In conclusion, HEV gt1 and gt3 infections do not elicit innate intrahepatic immune responses and remain highly sensitive to pegIFNα in immunocompromised humanized mice.

Hepatitis E Virus (HEV) infections are emerging in western countries1. HEV is a non-enveloped positive-sense single-stranded RNA virus, belonging to the family Hepeviridae within the genus Orthohepevirus2. Transmission mainly occurs through the fecal-oral route via contaminated water in developing countries or through the consumption of undercooked meat in industrialized countries3. Seven different genotypes have been described so far, of which genotype (gt) 1 and 3 are most prevalent in humans2. In healthy individuals, HEV mostly resolves spontaneously without severe symptoms, but pregnant women seem to be at risk of developing fulminant liver failure by HEV gt1 with mortality rates up to 25%4, 5. On the other hand, increasing rates of chronic gt3 infections have been described in immunocompromised patients in Europe, resulting in progressive liver fibrosis and cirrhosis6–8. These data indicate that host pathogen interactions differ between both genotypes.

Antiviral treatment options for chronic HEV infected immunocompromised patients are limited. Ribavirin (RBV) leads to sustained viral responses in roughly 75% of patients, but is hampered by RBV-induced anemia and the need for recombinant erythropoietin injections or transfusions in more than half of patients9, 10. As an alternative, pegylated interferon-alpha (pegIFNα) has been administered to a few patients in doses comparable to Hepatitis C virus (HCV) treatment regimens10, 11. However, factors associated with interferon (IFN)-susceptibility, the optimal pegIFNα dose or treatment duration have not been investigated in vivo.

The anti-HEV effects of IFNα in vitro differ according to the target cell and viral strain used. In vitro HEV models consist of human hepatoma and lung adenocarcinoma cell-lines, in which replication of subgenomic or full length replicons and seldom intact patient-derived viruses are studied12–16. Patient-derived HEV gt3 cultures show slow viral propagation, whereas HEV gt1 can only be cultured in vitro after induction of endoplasmic reticulum stress in the host cell line15–17. While HEV gt1 replication has been shown to be adequately suppressed by...
exogenous IFNαs, HEV gt3 replication has not18–20. In addition, viral inhibition of the interferon stimulated gene (ISG) responses have been described as a determining factor for IFNα susceptibility in vitro21. As the studied host cells are either no target cells in vivo (A549 cells) or are hampered by defects in their innate immune signaling (Huh7 and Huh7.5), the host response towards genuine patient-derived HEV in differentiated human hepatocytes remains to be established18,22. In addition, several clinical observations are not matched by in vitro viral replication data. HEV containing an in vivo RBV acquired mutation (K1383N), showed conflicting results in vitro with decreased viral replication and increased RBV-sensitivity23. Furthermore, the antiviral efficacy of sofosbuvir against HEV showed discrepancies in different in vitro and in vivo studies24–27.

Recently, we and others have shown that human-liver chimeric mice can be used to study HEV infection in differentiated human hepatocytes in vivo15,20,29. Here, we examined baseline ISG expression levels and susceptibility to pegIFNα in HEV gt1 and gt3 infected humanized mice. We demonstrate that HEV gt1 infections lead to higher virus loads in mouse feces, bile and liver compared to HEV gt3 infections, without the induction of intrahepatic innate immune responses. Both HEV genotypes, but not Hepatitis B virus (HBV), are cleared after a few doses of pegIFNα in vivo, an effect accompanied by a clear increase of human ISG transcript levels in liver and of circulating human CXCL10 levels in mouse serum.

Results

Higher viral burden in HEV gt1 compared to gt3 infected human-liver chimeric mice. Humanized UPA+/−/NOG mice were i.v. inoculated with a filtered feces suspension containing either HEV gt3 or HEV gt1 and were observed for 2, 6 or 14 weeks until euthanization. Infected mice were housed individually to prevent inter-mice contamination. During the infection course a higher percentage of HEV gt1 infected mice presented viremia, but the peak viral load in serum was similar to HEV gt3 infected mice (2.6 ± 0.4 and 1.4 ± 0.4 log HEV RNA IU/ml, respectively, Fig. 1a). The peak HEV RNA load in feces was significantly higher in HEV gt1, compared to HEV gt3 infected mice (5.9 ± 0.2 and 4.2 ± 0.5 log HEV RNA IU/gr, respectively, P = 0.029; Fig. 1b). HEV gt1 infected mice also had higher viral loads in bile (6.1 ± 0.2 vs. 5.2 ± 0.4 log HEV RNA IU/ml, respectively, P = 0.038; Fig. 1c) and liver (6.8 ± 0.2 vs 5.8 ± 0.3 log HEV RNA IU/gr, respectively, P = 0.015; Fig. 1d) at euthanasia, despite similar levels of serum human albumin compared to HEV gt3 infected mice, indicative for similar degrees of human chimerism (1.6 ± 0.4 and 1.9 ± 0.5 mg/ml, respectively, Fig. 1e). Despite lower absolute HEV gt1 inocula compared to HEV gt3, animals challenged with undiluted feces suspensions demonstrated similar results reaching higher HEV gt1 RNA levels in bile (P = 0.038), liver (P = 0.006), and feces (P = 0.06) compared to HEV gt3 RNA levels. These results point to a higher in vivo virulence of HEV genotype 1 compared to genotype 3.

No induction of intrahepatic innate immune responses in HEV gt1 or gt3 infected human-liver chimeric mice. Because of the HEV gt1 and gt3 clinical differences4–6,9 and different viral burdens in humanized mice, we examined the human host response in chimeric livers 2, 6, or 14 weeks after infection with either HEV gt1 or gt3. Using qRT-PCR we could not detect a significant increase in transcript levels of alpha or beta IFNs (data not shown), pathogen recognition receptors TLR3 and DDX58 (Fig. 2a), transcription factor STAT1 (Fig. 2b), or ISGs CXCL9, CXCL10, ISG15, RSAD2, OAS1, MX1, and IFT1 (Fig. 2c). Furthermore, longer duration of HEV gt3, but not HEV gt1 infection led to significantly decreased STAT1, RSAD2 and MX1 expression levels in the liver (Fig. 2b+c). None of these human transcripts were detected in non-chimeric mouse livers.

In order to evaluate a broader number of genes, Nanostring analysis of 594 human specific immunology-related genes was performed on chimeric (serum hAlb 2.5 ± 0.8 mg/ml) gt3 HEV-infected livers (6.1 ± 0.25 log HEV RNA IU/gram) at different time points post infection. Human transcript specificity was confirmed by including RNA from 3 non-chimeric livers and led to the removal of 50 cross-reactive genes from further analyses. Based on set criteria (<100 relative RNA counts and below four times the standard deviation in all samples), 255 genes were defined as non-expressed. Principal component analyses did not reveal clustering of samples (Fig. 2d). Of 18 genes related to interferon signaling and response, none showed consistent upregulation compared to...
non-infected chimeric mice (Fig. 2e). Down regulation of STAT1 and MX1 as observed by qRT-PCR, was confirmed in the Nanostring gene expression data (Fig. 2b+c,e). Taken together, these data show that ongoing HEV gt1 or gt3 replication for up to 14 weeks does not elicit an innate immune response in human hepatocytes in vivo.

Figure 2. Minimal intrahepatic interferon-stimulated gene induction in HEV infected human-liver chimeric mice, between weeks 2 to 14 post infection. Whole chimeric-liver RNA was isolated from HEV gt3 and gt1 infected mice and analyzed for the human specific gene expression of sensing molecules TLR3 and DDX58 (a), transcription factor STAT1 (b), and interferon stimulated genes CXCL9, CXCL10, ISG15, RSAD2, OAS1, MX1 and IFIT1 (c) using qRT-PCR. Groups consist of n = 4, 6, 4, 3, 6 and 4 mice from left to right (a–c). Given values on y-axes are fold changes over HEV RNA negative chimeric-livers transplanted with the same hepatocyte donor. X-axes shows weeks post infection, HEV genotype, and hepatocyte donor. Significance was assessed within groups of the same hepatocyte donor using Kruksal-Wallis one-way Anova with Dunnett’s Multiple comparison test. *P < 0.05, Gray bars indicate HEV gt1, black bars HEV gt3 (a–c). In-depth human gene expression analysis was performed on RNA from chimeric mouse livers before infection, and after 2, 6 and 14 weeks of HEV gt3 infection using nCounter® Human Immunology V2 panel. Principal component 1 (x-axis) and 2 (y-axis) comprise 49% of the variance between samples using all non-cross reactive genes (d). Uninfected samples are indicated in green, infected samples are indicated in blue, red (HEV0069), purple (HEV0122) and yellow and by the number of weeks infected HEV2, HEV6, HEV6, HEV14, respectively (d). Heatmap shows fold change over average of 4 uninfected mice for interferon signaling and response genes (e). Gene legend is indicated on the right side and sample legend below the heatmap (e). Dark red indicates ≥ 5 fold change, and dark blue ≤ 5 fold change (e).
HEV but not HBV is sensitive to peglFNα-2a treatment in human-liver chimeric mice. Baseline ISG expression in hepatocytes has been shown to predict the response to IFNα treatment in chronic HCV infected patients. As HEV did not induce an ISG response in vivo, we examined the HEV-sensitivity to peglFNα treatment. As a negative antiviral control, we applied the same treatment to HBV gtA infected mice, which has been shown to only slightly reduce serum HBV DNA levels in a similar humanized mouse model. After 1 to 2 peglFNα injections, HEV gt3 RNA became undetectable in feces of all treated animals (Fig. 3a–c). Complete viral clearance in liver and bile was observed in all mice at euthanasia 24 hours after 4 or 8 peglFNα injections (Fig. 3d). To examine whether a single dose of 30 µg/kg peglFNα would suffice to clear HEV gt3 in vivo, 4 animals received one injection after 6 weeks of ongoing HEV gt3 replication and were observed for an additional 4 weeks. This led to a complete viral clearance in 2 out of 4 mice and relapse in feces in the remainder 2 (Fig. 3c+d). Four weeks after the initial single peglFNα dose, the latter 2 animals received repetitive 10-fold lower peglFNα doses for 2 weeks. Again a steep decline in fecal HEV RNA loads was noted, but HEV RNA reemerged in feces and was detectable in bile and liver at euthanasia one day after the second peglFNα treatment course (Fig. 3c+d). The high in vivo HEV IFNα sensitivity was corroborated in HEV gt1 infected animals. Again rapid suppression of HEV replication was noted in feces (Fig. 3e), liver and bile (data not shown) after a 2 week treatment course with 30 µg/kg peglFNα. In contrast, a similar treatment regimen of HBV gtA infected mice induced a maximum decline of 0.7 ± 0.2 log HBV DNA copies/ml in serum with high intrahepatic viral loads at necropsy (6.9 ± 0.6 log HBV DNA copies/gr liver) (Fig. 3f). Non-treated HEV gt1, HEV gt3 and HBV infected mice never showed spontaneous viral clearance (Fig. 1b–d and Suppl. Fig. 2a–c), nor was loss of human chimerism in peglFNα-treated animals observed, based on persistent detection of human albumin levels in mouse serum (data not shown). These data indicate that HEV, but not HBV is highly sensitive to peglFNα in humanized mice.
IFIT1, cleared HEV. Similar to previous reports, HBV persistence in vivo dose in the two mice that α and 108 ment was associated with an increase in serum human CXCL10 levels of HEV gt1 and gt3 infected mice (59, and IFIT1 MX1 genes in the livers of HEV infected humanized mice (Fig. 4a and Suppl. Fig. 3). In addition, treat-

γ is associated with an intrahepatic IFN- petent woodchucks chronically infected with woodchuck hepatitis virus (a model for chronic HBV infection), but not HBV was found to be sensitive to pegIFN in vivo induced hepatocyte-specific innate responses α.

The antiviral potency of pegIFN α treatment and even after a single pegIFN α injection in 2/4 mice. PegIFN α treatment led to an 20-fold increase treated animals. PegIFN α α gt3, HEV gt1 and HBV infected and pegIFN α treated mouse models. PegIFN α analized mouse model for chronic HEV infection. We demonstrate that (1) HEV is highly sensitive to pegIFN α treatment in vivo; (2) HEV infection in human hepatocytes doesn’t elic a innate immune response; (3) HEV α gt1 presents higher viral loads compared to HEV gt3.

HEV gt1 and gt3, but not HBV, showed to be highly sensitive to pegIFN α treatment in immune deficient human liver chimeric uPA+/−/ NOG mice. Viral clearance in feces, liver and bile was achieved after 4 and 2 weeks treatment and even after a single pegIFN α injection in 2/4 mice. PegIFN α associated viral clearance was companioned by an increase of intrahepatic human ISGs and serum CXCL10 levels. In line with our data (Fig. 3f), the antiviral potency of pegIFN α against other hepatotropic viruses was less pronounced in similar humanized mouse models. PegIFN α reduced HBV viremia by 2.5 log IU/ml and HCV loads with 2.3 log IU/ml after 12 and 4 weeks of treatment respectively, without clearing the infection13-33. Successful IFNα treatment in immunocompetent woodchucks chronically infected with woodchuck hepatitis virus (a model for chronic HBV infection), is associated with an intrahepatic IFN-γ and NK/T cell gene signature, but not an ISG signature36. All together this suggests that pegIFNα has a strong direct anti-viral effect against HEV, whereas HBV and HCV require the immune system to achieve viral clearance or complete suppression.

Ongoing HEV gt1 or gt3 replication did not elic it human-innate immune responses in humanized livers of 30 uPA+/−/NOG mice, irrespective of the infection duration, the human hepatocyte donor or viral isolate used. We specifically addressed the genomic response of human hepatocytes to HEV without that of infiltrating immune cells in our profound immune deficient uPA+/−/NOG mice. In addition, we carefully eliminated cross hybridizing probes by including non-chimeric mice. After prolonged HEV gt3 infection for more than 3 months, significantly lower expression levels of STAT1, RSAD2 and MX1 compared to uninfected controls were observed, suggesting possible viral interference with the host’s cell innate immune signaling. Hepatotropic pathogens have developed different methods to evade innate immune defenses37. In our model, expression of TLR3 and DDX58 was detected in all HEV-infected chimeric livers indicating that these host sensing molecules were not counteracted at the transcription level (Fig. 2a). Several studies in HEK293T, A549 and Huh7 cells have suggested that HEV can directly interfere with phosphorylation of STAT1 and the induction of IFNα30,21, 38. However, most of these

Figure 4. CXCL10 transcripts and protein are induced after pegIFNα treatment in HEV infected mice. RNA was isolated from non-treated and 2 weeks pegIFNα treated HEV gt1, gt3 and HBV infected mouse livers and was analyzed for the expression of human CXCL10 (a). X-axes indicate treatment dosage and virus genotype (a). Given values on y-axes are RNA levels in fold changes over uninfected non-treated mice (a). Human CXCL10 levels were measured using ELISA in mouse serum of uninfected, HEV-infected and HEV-infected pegIFNα treated mice (b). Dotted line indicates lower limit of detection (LLOD) (b). Gray bars indicate HEV gt1, black bars HEV gt3 and striped bar uninfected (a + b).
The clinical experience with pegIFN based therapies for chronic HEV is minimal. Eight cases have been published of which 5 showed a suppression of viremia at the first measured timepoint after initiation of pegIFNα treatment. PegIFNα treatment in HEV infected humanized mice modelled the viral decline seen in these 5 patients. It remains however unclear why some chronic HEV patients show slow viral declines upon IFN-treatment. We observed a viral relapse in feces, liver and bile of 2 humanized mice after a second pegIFNα treatment course (Fig. 3c-d). While animals received a 10-fold lower pegIFNα dose, the relapse might be partially ascribed to elevated intrahepatic ISG levels before retreatment. Increased CXCL10 levels were measured in the liver of 2 mice 4 weeks after a single pegIFNα injection, which corresponds to the timepoint at which retreatment human-liver chimeric mice were inoculated intravenously (i.v.) with 200 µl feces suspension containing HEV gt3 (8.8 log IU/ml or diluted to 6.8 log IU/ml), HEV gt1 (7.9 log IU/ml or diluted to 6.2 log IU/ml) or 200 µl

| Donor ID | Gender | Age  | Race     |
|----------|--------|------|----------|
| HD1      | Male   | 2 years | Caucasian |
| HD2      | Female | 2 years | Caucasian |
| HD3      | Female | 7 months | Caucasian |

Table 1. Hepatocyte donors.

In conclusion, despite higher viral loads for HEV gt1 in human-liver chimeric mice, both HEV gt1 and gt3 do not induce an intrahepatic innate immune response. HEV, but not HBV, is highly sensitive to pegIFNα treatment in humanized mice.

**Material and Methods**

**Ethics, consent and permissions.** The use of patient material was approved by the medical ethical committees of Erasmus Medical Center and Antwerp University Hospital. Informed consent was obtained from all subjects. All animal work was conducted according to relevant Dutch national guidelines. The study protocol was approved by the animal ethics committee of the Erasmus Medical Center (DEC nr. 141-12-11).

**Mouse origin and genotyping.** Urokinase-type plasminogen activator (uPA)/NOD/Shi-scid/IL-2Rγnull (NOG) mice were kindly provided by the Central Institute for Experimental Animals (Kawasaki, Japan)45. Mice were bred at the Central Animal Facility of the Erasmus Medical Center. Zygosity of mice was determined as described previously46. Mice were co-housed with a maximum of 4 mice per individually ventilated cage and were fed normal chow ad libitum.

**Human hepatocyte transplantation.** Six to twelve week old male uPA-homozygous mice were transplanted as described previously46. In short, mice were anesthetized and transplanted via intrasplenic injection with 0.5 × 10⁶ to 2 × 10⁶ viable commercially available cryopreserved human hepatocytes from 1 of 3 donors (Corning, NY, USA; Lonza, Basel, Switzerland; Table 1). Graft take was determined by human albumin in mouse serum using an ELISA with human albumin cross-adsorbed antibody (Bethyl laboratories, Montgomery, TX, USA) as previously described46.

**Viral strains, mouse infection and treatment.** HEV gt3 was derived from feces of one of two chronic HEV patients (HEV0069 and HEV0122) as described previously47. HEV gt1 Sar-55 was derived from feces of a Rhesus macaque that had been originally inoculated with the human Sar-55 strain47. Eight weeks after transplantation human-liver chimeric mice were inoculated intravenously (i.v.) with 200 µl feces suspension containing HEV gt3 (8.8 log IU/ml or diluted to 6.8 log IU/ml), HEV gt1 (7.9 log IU/ml or diluted to 6.2 log IU/ml) or 200 µl
patient serum containing HBV gtA (7.7 log IU/ml). After viral inoculation, mice were housed individually. Mice were treated with a single subcutaneous pegIFNα-2a (30 µg/kg unless stated otherwise, Pegasys, Roche, Basel, Switzerland) injection or every 3–4 days for 2 or 4 weeks. Overview of viral isolates are shown in Table 2. An overview of experimental groups is shown in Table 3 and Suppl. Figure 4.

**HEV RNA and HBV DNA detection.** The presence of HEV RNA in mouse serum, feces, bile and liver was determined by an ISO15189:2012-validated, internally controlled quantitative real-time RT-PCR, described previously7, 15. Cycle threshold (Ct) values above 38 were considered background, which corresponds to a lower limit of detection of 2.16 log10 HEV RNA units/ml in undiluted human serum. HEV RNAs detected in samples with Ct values below 38 are indicated with their calculated values. HBV viral load was measured in mouse serum and liver using a dual target approach, using primers and probes targeting preS-gen, as described before48, 49, and the X gene (HBV XJfwd12 5′-ggtctgtgccaagtgtttgst-3′, HBV XJprobe 5′-FAM-acgcaacccccactggctggg-BHQ1–3′, HBV XJrev12, 5′-tycgcagtatggatcgsc-3′).

**RNA isolation of whole liver, generation of cDNA and real-time qPCR.** Whole liver RNA was isolated using RNeasy mini kit (Qiagen, Hilden, Germany) including DNAse treatment according to manufacturer’s protocol starting with homogenization of liver tissue in RLT buffer. cDNA was generated by using an iScript cDNA synthesis kit (Bio-Rad Laboratories, Hercules, CA, USA) according to the manufacturer’s protocol. Human specific gene expression was measured using Taqman primer/probe quantitative PCR, in TaqMan® Gene Expression Master Mix (Thermo Fisher Scientific, Waltham, MA, USA). Primer/probe combinations were purchased from Thermo Fisher Scientific; CXCL10 (Hs01124251_g1), CXCL9 (Hs00171065_m1), DDX58 (Hs01061436_m1), GAPDH (Hs002656705_g1), IFIT1 (Hs01911452_s1), ISG15 (Hs01921425_s1), IFNA1 (Hs00855471_g1), IFNA4 (Hs01681284_sh), IFNB1 (Hs01077958_s1), MX1 (Hs00895608_m1), OAS1 (Hs00973637_m1), RSAD2 (Hs00369813_m1), STAT1 (Hs01013996_m1), TLR3 (Hs01551078_m1). Expression of target genes was normalized to the expression of GAPDH using the formula 2^−ΔΔCt, ΔΔCt = Ctarget−CgADPH.

**Cytokine measurement.** Human CXCL10 was measured in 1:5 diluted mouse serum samples using the Human CXCL10/IP10 Quantikine ELISA Kit (R&D Systems, Minneapolis, MN, USA) according to manufacturer’s protocol.

**Nanostring analyses.** RNA was isolated from chimeric mouse livers as described above. The nCounter GX human Immunology V2 Kit (NanoString Technologies, Seattle, WA, USA) was used to measure the expression of 594 human genes in the RNA of these samples. Following hybridization, transcripts were quantitated using the nCounter Digital Analyzer. Samples were run at the Johns Hopkins Deep Sequencing & Microarray Core facility. To correct for background levels, the highest negative control value for each sample was subtracted from each count value of that sample, as described previously48, 51. Following background subtraction, any negative

| Treatment | Chimeric liver | Virus | n= | Hepatocyte donor |
|-----------|---------------|-------|----|-----------------|
| none      | None          | 3     | n/a*|
| yes       | None          | 8     | HD1, HD2 |
| yes       | HEV gt1       | 10    | HD1 |
| yes       | HEV gt3 (HEV0069) | 16  | HD1, HD2, HD3 |
| yes       | HEV gt3 (HEV0122) | 4    | HD1 |
| yes       | HBV gtA       | 5     | HD3 |
| none      | None          | 2     | n/a*|
| yes       | None          | 2     | HD2 |
| yes       | HEV gt1       | 3     | HD2 |
| yes       | HEV gt3 (HEV0069) | 11  | HD2 |
| yes       | HBV gtA       | 6     | HD2 |

Table 3. Overview of experimental groups. *n/a, not applicable.
count values were considered as 0. The geometric mean of 5 housekeeping genes provided by the company panel was calculated and used to normalize expression values. RNA from non-chimeric mouse livers was used as control to test cross-reactivity of genes. Fifty cross-reactive genes were removed prior to analyses of the data set. Non-expressed genes were defined as < 100 relative RNA counts and below four times the standard deviation in all samples.

**Statistics.** Differences between groups were calculated using two tailed Mann-Whitney test or Kruskal-Wallis one-way ANOVA with Dunn’s all column comparison post-test (GraphPad Prism version 5.01; GraphPad Software). Differences were considered significant when P < 0.05. Results are presented as the mean ± SEM. Principal component analyses was performed on log 2 transformed data set and heatmap of IFN signaling/ response genes was generated using Multi-experiment viewer (MeV) software version 4.9.

**References**

1. Dalton, H. R. & Seghatchian, J. Hepatitis E virus: Emerging from the shadows in developed countries. *Transfusion and apheresis science: official journal of the World Apheresis Association: official journal of the European Society for Haemapheresis*. doi:10.1016/j.transci.2016.10.016 (2016).
2. Smith, D. B. et al. Consensus proposals for classification of the family Hepeviridae. *The Journal of general virology* 95, 2223–2232, doi:10.1099/vir.0.068429-0 (2014).
3. Aggarwal, R. & Jameel, S. Hepatitis E. *Hepatology* 54, 2218–2226, doi:10.1002/hep.24674 (2011).
4. Khuroo, M. S. & Kamili, S. Aetiology, clinical course and outcome of sporadic acute viral hepatitis in pregnancy. *Journal of viral hepatitis* 10, 61–69 (2003).
5. Kran, L. J., Nelson, K. E. & Labrique, A. B. Host immune status and response to hepatitis E virus infection. *Clinical microbiology reviews* 27, 139–165, doi:10.1128/CMR.00062-13 (2014).
6. Kamar, N. et al. Factors associated with chronic hepatitis in patients with hepatitis E virus infection who have received solid organ transplants. *Gastroenterology* 140, 1481–1489, doi:10.1053/j.gastro.2011.02.050 (2011).
7. Pas, S. D. et al. Hepatitis E virus infection among solid organ transplant recipients, the Netherlands. *Emerging infectious diseases* 18, 869–872, doi:10.3201/eid1805.1111712 (2012).
8. Nijhuis, J. et al. Hepatitis E virus genotype 3 infection in a tertiary referral center in the Netherlands: Clinical relevance and impact on patient morbidity. *Journal of clinical virology: the official publication of the Pan American Society for Clinical Virology* 74, 82–87, doi:10.1016/j.jcvi.2015.11.038 (2016).
9. Kamar, N. et al. Ribavirin for chronic hepatitis E virus infection in transplant recipients. *The New England journal of medicine* 370, 1111–1120, doi:10.1056/NEJMoa1215246 (2014).
10. Peters van Ton, A. M., Govers, T. J. & Drenth, P. J. Antiviral therapy in chronic hepatitis E: a systematic review. *Journal of viral hepatitis* 22, 965–973, doi:10.1111/jvh.12403 (2015).
11. Alric, L., Bonnet, D., Laurent, G., Kamar, N. & Liope, J. Chronic hepatitis E virus infection: successful virologic response to pegylated interferon-alpha therapy. *Annals of internal medicine* 153, 135–136, doi:10.7326/0003-4819-153-2-20100720-00025 (2010).
12. Berto, A. et al. Replication of hepatitis E virus in three-dimensional cell culture. *Journal of virological methods* 187, 327–332, doi:10.1016/j.jviromet.2012.10.017 (2013).
13. Nguyen, H. T., Shukla, P., Torian, U., Faulk, K. & Emerson, S. U. Hepatitis E virus genotype 1 infection of swine kidney cells in vitro is inhibited at multiple levels. *Journal of virology* 88, 868–877, doi:10.1128/JVI.02203-15 (2014).
14. Nguyen, H. T. et al. A naturally occurring human/hepatitis E recombinant virus predominates in serum but not in faeces of a chronic hepatitis E patient and has a growth advantage in cell culture. *The Journal of general virology* 93, 526–530, doi:10.1099/vir.0.037259-0 (2012).
15. van de Garde, M. D. et al. Hepatitis E Virus (HEV) Genotype 3 Infection of Human Liver Chimeric Mice as a Model for Chronic HEV Infection. *Journal of virology* 90, 4394–4401, doi:10.1128/JVI.00114-16 (2016).
16. Tanaka, T., Takahashi, M., Kusano, E. & Okamoto, H. Development and evaluation of an efficient cell-culture system for Hepatitis E virus. *The Journal of general virology* 88, 903–911, doi:10.1099/vir.0.82535-0 (2007).
17. Nair, V. P. et al. Endoplasmic Reticulum Stress Induced Synthesis of a Novel Viral Factor Mediates Efficient Replication of Genotype-1 Hepatitis E Virus. *PLoS pathogens* 12, e1005521, doi:10.1371/journal.ppat.1005521 (2016).
18. Dehmare, P. B., Desai, S. & Loke, K. S. Innate immune responses in human hepatocyte-derived cell lines alter genotype 1 hepatitis E virus replication efficiencies. *Scientific report* 6, 26827, doi:10.1038/srep26827 (2016).
19. Toud, D. et al. Antiviral Activities of Different Interferon Types and Subtypes against Hepatitis E Virus Replication. *Antimicrobial agents and chemotherapy* 60, 2132–2139, doi:10.1128/AAC.02427-15 (2016).
20. Zhou, X. et al. Disparity of basal and therapeutically activated interferon signalling in constraining hepatitis E virus infection. *Journal of virology* 23, 294–304, doi:10.1111/jtvh.12491 (2016).
21. Dong, C. et al. Suppression of interferon-alpha signalling by hepatitis E virus. *Hepatology* 55, 1324–1332, doi:10.1002/hep.25530 (2012).
22. Keskinen, P. et al. Impaired antiviral response in human hepatoma cells. *Virology* 263, 364–375, doi:10.1016/S0042-6822(99)00008-1 (1999).
23. Debing, Y. et al. Hepatitis E virus mutations associated with ribavirin treatment failure result in altered viral fitness and ribavirin sensitivity. *Journal of hepatology* 65, 499–508, doi:10.1016/j.jhep.2016.05.002 (2016).
24. van der Valk, M., Zaatijer, H. L., Kater, A. P. & Schinkel, J. Sofosbuvir shows antiviral activity in a patient with chronic hepatitis E virus infection. *Journal of hepatology*. doi:10.1016/j.jhep.2016.09.014 (2016).
25. Donnelly, M. C. et al. Sofosbuvir and Daclatasvir Anti-Viral Therapy Fails to Clear HEV Viremia and Restore Reactive T Cells in a HEV/HCV Co-Infected Liver Transplant Recipient. *Gastroenterology*. doi:10.1053/j.gastro.2016.05.060 (2016).
26. Wang, W. et al. Distinct Antiviral Potency of Sofosbuvir Against Hepatitis C and E Viruses. *Gastroenterology* 151, 1251–1253, doi:10.1053/j.gastro.2016.09.061 (2016).
27. Dao Thi, V. L. et al. Sofosbuvir Inhibits Hepatitis E Virus Replication In Vitro and Results in an Additive Effect When Combined With Ribavirin. *Gastroenterology* 150, 82–85 e84, doi:10.1053/j.gastro.2015.09.011 (2016).
28. Sayed, I. M. et al. Study of hepatitis E virus infection of genotype 1 and 3 in mice with humanised liver. *Gut*. doi:10.1136/gutjnl-2015-311109 (2016).
29. Allweiss, L. et al. Human liver chimeric mice as a new model of chronic hepatitis E virus infection and preclinical drug evaluation. *Journal of hepatology* 64, 1033–1040, doi:10.1016/j.jhep.2016.01.011 (2016).
30. Chen, L. et al. Hepatic gene expression discriminates responders and nonresponders in treatment of chronic hepatitis C viral infection. *Gastroenterology* 128, 1347–1444 (2005).
31. Mcgilvray, I. et al. Hepatic cell-type specific gene expression better predicts HCV treatment outcome than IL28B genotype. *Gastroenterology* 142, 1122–1131 e1121, doi:10.1053/j.gastro.2012.01.028 (2012).
32. Feld, J. J. et al. Hepatic gene expression during treatment with peginterferon and ribavirin: Identifying molecular pathways for treatment response. *Hepatology* **46**, 1548–1563, doi:10.1002/hep.21853 (2007).
33. Allweiss, L. et al. Immune cell responses are not required to induce substantial hepatitis B virus antigen decline during pegylated interferon-alpha administration. *Journal of hepatology* **60**, 500–507, doi:10.1016/j.jhep.2013.10.021 (2014).
34. Kneteman, N. M. et al. Anti-HCV therapies in chimeric scid-Ab/lupA mice parallel outcomes in human clinical application. *Hepatology* **43**, 1346–1353, doi:10.1002/hep.21209 (2006).
35. Watanabe, T. et al. Hepatitis C virus kinetics by administration of pegylated interferon-alpha in human and chimeric mice carrying human hepatocytes with variants of the IL28B gene. *Gut* **62**, 1340–1346, doi:10.1136/gutjnl-2012-305253 (2013).
36. Fletcher, S. P. et al. Intrahepatic Transcriptional Signature Associated with Response to Interferon-alpha Treatment in the Woodchuck Model of Chronic Hepatitis B. *PLoS pathogens* **11**, e1005103, doi:10.1371/journal.ppat.1005103 (2015).
37. Proctor, U., Maini, M. K. & Knolle, P. A. Living in the liver: hepatic infections. *Nature reviews. Immunology* **12**, 201–213, doi:10.1038/nri3169 (2012).
38. Nan, Y. et al. Hepatitis E virus inhibits type I interferon induction by ORF1 proteins. *Journal of virology* **88**, 11924–11932, doi:10.1128/JVI.01935-14 (2014).
39. Tateno, C. et al. Morphological and microarray analyses of human hepatocytes from xenogeneic host livers. *Laboratory investigation; a journal of technical methods and pathology* **93**, 54–71, doi:10.1038/labinvest.2012.158 (2013).
40. Tournay, K. G., Depraetere, S., Meuleman, P., Leroux-Roels, G. & Pauwels, R. A. Murine IL-2 receptor beta chain blockade improves human leukocyte engraftment in SCID mice. *European journal of immunology* **28**, 3221–3230, doi:10.1002/(SICI)1521-4411(199810)28:10<3221::AID-IMMU3221#623.0.CO;2-S>1.0.CO;2-S (1998).
41. Yu, C. et al. Pathogenesis of hepatitis E virus and hepatitis C virus in chimpanzees: similarities and differences. *Journal of virology* **84**, 11264–11278, doi:10.1128/JVI.01205-10 (2010).
42. Evans, D. T., Serra-Moreno, R., Singh, R. K. & Guatelli, J. C. BST-2/tetherin: a new component of the innate immune response to enveloped viruses. *Trends in microbiology* **18**, 388–396, doi:10.1016/j.tim.2010.06.010 (2010).
43. Sunetha, P. V. et al. Hepatitis E virus (HEV)-specific T-cell responses are associated with control of HEV infection. *Hepatology* **55**, 695–708, doi:10.1002/hep.24738 (2012).
44. Brown, A. et al. Characterization of the Specificity, Functionality, and Durability of Host T-Cell Responses Against the Full-Length Hepatitis E Virus. *Hepatology* **64**, 1934–1950, doi:10.1002/hep.28819 (2016).
45. Suehazu, H. et al. Establishment of a humanized model of liver using NOD/Shi-scid IL2Rγnull mice. *Biochemical and biophysical research communications* **377**, 248–252, doi:10.1016/j.bbrc.2008.09.124 (2008).
46. Vanwolleghem, T. et al. Factors determining successful engraftment of hepatocytes and susceptibility to hepatitis B and C virus infection in uPA-SCID mice. *Journal of hepatology* **53**, 468–476, doi:10.1016/j.jhep.2010.03.024 (2010).
47. Tsarev, S. A. et al. Characterization of a prototype strain of hepatitis E virus. *Proceedings of the National Academy of Sciences of the United States of America* **89**, 559–563 (1992).
48. Pas, S. D., Fries, E., De Man, R. A., Osterhaus, A. D. & Niesters, H. G. Development of a quantitative real-time detection assay for hepatitis B virus DNA and comparison with two commercial assays. *J Clin Microbiol* **38**, 2897–2901 (2000).
49. Pas, S. D. & Niesters, H. G. Detection of HBV DNA using real-time analysis. *Journal of clinical virology: the official publication of the Pan American Society for Clinical Virology* **25**, 93–94 doi:S138665320000161 [pii] (2002).
50. Movita, D. et al. Inflammatory monocytes recruited to the liver within 24 hours after virus-induced inflammation resemble Kupffer cells but are functionally distinct. *Journal of virology* **89**, 4809–4817, doi:10.1128/JVI.03733-14 (2015).
51. van de Garde, M. D. et al. Liver Monocytes and Kupffer Cells Remain Transcriptionally Distinct during Chronic Viral Infection. *PLoS one* **11**, e0166094, doi:10.1371/journal.pone.0166094 (2016).

**Acknowledgements**

We would like to thank Vincent Vaes and Vincent Duiverman for their assistance in performing biotechnical manipulations, and Jolanda Voermans and Claudia Mulders for excellent technical assistance. This study was supported by the Virgo consortium, funded by the Dutch government project number FES0908 and the Netherlands Genomics Initiative (NGI) project number 050-060-452. TV is recipient of an Erasmus MC fellowship 2011 and a 2014 research mandate of the Belgian Foundation against Cancer (2014–087).

**Author Contributions**

Designed research: M.D.B.v.d.G., A.B., T.V. Performed experiments: M.D.B.v.d.G., S.D.P., G.v.O., L.G., T.V. Analysis and interpretation of the data: M.D.B.v.d.G., S.D.P., A.B., T.V. Wrote manuscript: M.D.B.v.d.G., T.V.
Provided essential research tools: S.D.P., L.G., Y.C., R.A.d.M., T.V. Critical revision of the manuscript for important intellectual content: S.D.P., R.A.d.M., A.B., T.V. Approved final version of manuscript: all.

**Additional Information**

**Supplementary information** accompanies this paper at doi:10.1038/s41598-017-07434-y

**Competing Interests:** The authors declare that they have no competing interests.

**Publisher’s note:** Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

© The Author(s) 2017