Transmission of hepatitis E virus infection to human-liver chimeric FRG mice using patient plasma

Ibrahim M. Sayeda, b, Lander Foqueta, Lieven Verhoeyea, Florence Abravanelc, d, e, Ali Farhoudia, Geert Leroux-Roelsa, Jacques Izopetc, d, e, Philip Meulemana, *

a Dept. Clinical Chemistry, Microbiology and Immunology, Faculty of Medicine and Health Sciences, Ghent University, Ghent, Belgium
b Microbiology and Immunology Department, Faculty of Medicine, Assiut University, Assiut, Egypt
c INSERM U1043, IFR-BMT, CHU Purpan, Toulouse, France
d Université Paul-Sabatier, Toulouse, France
e Laboratory of Virology, CHU Purpan, Toulouse, France

A B S T R A C T

Hepatitis E virus (HEV) is considered as an important pathogen in developing countries but there is growing evidence of its increasing significance and prevalence in the Western world. Although most acute HEV infections resolve spontaneously, chronicity has been observed in immunocompromised patients. The study of HEV has been hampered by the absence of practical animal models. Because the in vivo study of HEV was essentially limited to primates and pigs we recently established the human-liver chimeric uPA-SCID mouse model as a useful tool to study HEV infection. Because the humanized FRG mouse model, another type of mouse with humanized liver, is more easily accessible to the scientific community, we investigated its susceptibility to HEV infection. FRG mice were transplanted with human hepatocytes and challenged with different HEV genotypes using different routes of exposure. Our data clearly shows that the humanized FRG mouse is an alternative animal model for the study HEV infection. As observed in the uPA-SCID model, controlled oral inoculation did not lead to active infection. However, intrasplenic injection of genotype 3-infected patient plasma did result into persistent infection. Although the efficiency of transmission was low, this observation corroborates previously published case reports of blood transfusion-associated HEV transmission.

The hepatitis E virus (HEV) has become an important pathogen in developed countries (Dalton and Seghatchian, 2016; Hartl et al., 2016; Sayed et al., 2015a, 2015b). It belongs to the Hepeviridae family and isolates that can infect humans are classified into 4 genotypes (gt) of the Orthohepevirus A species (Smith et al., 2014). HEV of gt1 and 2 only infect humans and are responsible for acute HEV infections in Asia, Africa and Mexico, predominantly via fecal-oral transmission. HEV of gt3 and 4 comprise zoonotic strains that can infect both humans and pigs, but also include strains isolated from rabbits, deer and mongoose. Genotype 3 viruses are responsible for most autochthonous infections in Europe and are mainly transmitted through consumption of raw or undercooked contaminated food especially from pigs, wild boar and deer (Doceul et al., 2016; Sayed et al., 2015a). Transmission of gt3 HEV has also been linked to blood transfusion (Andonov et al., 2014; Hewitt et al., 2014).

Gt1 HEV infection usually results in a self-limiting acute icteric hepatitis. However, in certain patient groups it is associated with progressive liver disease leading to fulminant hepatitis and death. Overall mortality rates are 1–4% in adults, higher in infants under 2 years and up to 10–20% in pregnant women (Aggarwal, 2013). Most gt3 infections cause either no or only mild non-specific flu-like symptoms such as myalgia, arthralgia, weakness, abdominal pain and vomiting; and resolve spontaneously. Persistent infection with gt3 HEV may occur in immunocompromised persons, such as transplant recipients, and can rapidly lead to liver cirrhosis (Behrendt et al., 2014). Chronic HEV can be treated with a 3–6 month ribavirin therapy, but mutant viruses with reduced
sensitivity to ribavirin have recently been described (Debing et al., 2014; Todt et al., 2016).

Although certain HEV isolates, mainly of gt3 and gt4 have been adapted to grow in vitro (Shukla et al., 2011; Tanaka et al., 2007, 2009), HEV of gt1 is responsible for most infections worldwide but only poorly grows in cell culture (Nguyen et al., 2014). In addition, the lack of a suitable small animal model has also significantly hindered the progress of the HEV field. However, our group and others recently reported that immune deficient human-liver chimeric Alb-uPA mice are an appropriate model to study HEV infection, virus-host interactions and drug efficacy (Allweiss et al., 2016; Sayed et al., 2016; van de Garde et al., 2016). Humanized uPA-SCID mice could be infected with fecal preparations containing HEV of gt1 and gt3. HEV RNA and viral protein could be detected in fecal preparations, plasma and liver of infected mice. Treatment of infected mice with ribavirin for 2 weeks lead to a substantial reduction in both viral RNA titer and HEV ORF3 protein in mouse plasma, fecal and liver samples, thereby validating the human-liver chimeric uPA-SCID mouse as a suitable model for the evaluation of novel anti-HEV therapies. However, the uPA-SCID model is technically very challenging to work with and it is not commonly available to the research community. Therefore, alternative models that are simpler to handle and more easily accessible are of high interest. FAH−/−–Rag-2−/−–IL-2Rγ−/− (FRG) mice are immunodeficient mice that suffer from a genetic metabolic liver disease. Knockout of the fumarylacetoacetate hydrolase (FAH) gene, an essential enzyme of the tyrosine catabolic pathway, causes accumulation of hepatotoxic tyrosine metabolites. Oral administration of 2-[(2-nitro-4-trifluoromethylbenzoyl)1,3-cyclohexadiene (NTBC) via the drinking water prevents the accumulation of these toxic metabolites, thereby maintaining the mice in a healthy state (Grompe et al., 1995). Nevertheless, the induction of liver disease is essential to achieve high-level liver engraftment and repopulation of transplanted hepatocytes. Knockout of the Rag-2 and IL-2γ genes results in depletion of T, B and NK cells. This immunodeficiency allows for the transplantation of human hepatocytes without the risk of graft rejection. Compared to uPA-SCID mice, FRG mice, and in general, immune deficient FAH−/− mice, are less subjected to pre-weaning mortality, are less fragile in general and the window for transplantation is not limited to the first weeks after birth but can be freely chosen by simple withdrawal of NTBC (Azuma et al., 2007; Bissig et al., 2007). In addition, researchers can easily obtain non-transplanted breeder pairs to start their own immunodeficient FAH−/− colony and immunodeficient FAH−/− mice with humanized liver can be purchased. Similar to humanized uPA-SCID mice, immune deficient human liver chimeric FAH−/− mice are susceptible to HBV, HCV and Plasmodium falciparum infection (Bissig et al., 2010; de Jong et al., 2014; Foquet et al., 2014, 2015a, 2015b; Meuleman et al., 2005; Mikolajczak et al., 2015; Ortega-Prieto and Donner, 2016; Siu and Ploss, 2015; Vaughan et al., 2012; Vercauteren et al., 2014, 2015). Here we evaluated whether humanized FRG mice are also be susceptible to HEV infection.

FRG mice were transplanted with primary human hepatocytes as described in detail in (Foquet et al., 2017). Humanized FRG mice were inoculated via intrasplenic or oral route with HEV preparations of gt3F (stool and plasma obtained from a French chronic HEV patient) and gt1 (strain Sar-55; kindly provided by Dr. Suzanne Emerson and Dr. Robert H. Purcell, NIH). Detection and quantification of HEV RNA was done on mouse stool (10% w/v fecal suspension), plasma and liver samples using an in-house RT-qPCR that targets ORF3 as described previously (Sayed et al., 2016). In addition, a nested PCR that targets the HEV ORF2 region was performed to confirm borderline positive samples and to allow sequencing of the viral genome. Non-transplanted FRG mice served as negative control. All details on mouse production, infection and molecular analysis are available in an online supplement.

When humanized FRG mice (n = 3) were inoculated intrasplenically with a filtered patient stool suspension containing HEV gt3F, chronic infection was established in all mice. HEV RNA was detected in fecal samples within one to two weeks post inoculation, and the virus titer increased with time indicating active replication of the virus (Fig. 1a). Viremia was delayed in two mice and weakly intermittently positive in the third mouse. The viral titer of 10% stool suspensions was generally 10–100-fold higher as compared to that in plasma. The viral particles excreted into the mouse stool were infectious since transfer of a 10% (w/v) filtered stool suspension onto an HuH7.5 cell culture resulted in active infection of the hepatoma cells (Fig. 2). In addition, high intrahepatic HEV RNA levels were detected at the time of sacrifice, which correlated with the degree of liver humanization (up to 5.6 × 105 IU/30 mg of liver tissue, Table 1). Similar findings were also reported in uPA-based human liver chimeric mice inoculated with the same inoculum or inoculated with other fecal preparations derived from genotype 3-infected patients (Allweiss et al., 2016; Sayed et al., 2016; van de Garde et al., 2016). Human albumin levels were monitored on a regular basis and did not indicate direct hepatotoxicity of HEV (data not shown). This confirms previous observations made in HEV-infected humanized uPA mice (Allweiss et al., 2016; Sayed et al., 2016). HEV RNA was never detected in stool nor plasma of non-transplanted FRG mice that were injected with the same viral inoculum, indicating that the human hepatocyte is the main site of HEV replication and that mouse hepatocytes do not support HEV infection and/or replication.

To confirm that HEV infection in FRG mice was not limited to genotype 3 viruses, one humanized mouse was inoculated intrasplenically with a preparation containing 2.5 × 103 IU HEV of genotype 1 (Sar-55 strain; originally obtained from an infected chimpanzee but later on passaged in humanized uPA-SCID mice (Purcell et al., 2013; Sayed et al., 2016)). HEV RNA could already be detected in fecal samples at week 1 post-infection and reached levels between 103 and 104 IU/ml from week 2 onwards (Fig. 1b). In general, the HEV RNA content of the stool suspensions of this mouse was relatively low and no virus could be detected in plasma. However, at the time of inoculation the human albumin level in the plasma of the mouse was below 2 mg/ml, indicating a rather low degree of humanization of the liver (estimated at around 7% according to (Bissig et al., 2010)).

Two humanized FRG mice, with estimated liver chimerism of 10 and 27% respectively (Bissig et al., 2010), were inoculated by intrasplenic injection with HEV gt3 infected patient plasma (obtained from the same chronic HEV infected patient mentioned above). While the feces and plasma of the first mouse remained negative (limit of detection 200 IU/ml) throughout the 16-week observation period, viral RNA was intermittently detectable in the feces of the second mouse after 6–8 weeks (Fig. 3a). From week 9 onwards the fecal viral shedding reached a level of 9 × 103 IU/ml (10% w/v suspension) and persisted until the end of the experiment. The plasma of the second mouse tested negative for HEV RNA at all moments examined. Since the level of viral RNA in the fecal suspension of the infected mouse was rather low, we wanted to confirm the results with a nested RT-PCR targeting a region in the HEV genome that differs from that of the real-time PCR. HEV ORF2 was strongly amplified in all fecal samples collected at week 8, 12 and 16, while a sample collected at week 4 post-inoculation was negative (Fig. 3b). Previous experiments by our group and others failed to prove the presence of infectious viral particles in the plasma of infected patients (Allweiss et al., 2016; Sayed et al., 2016; van de Garde et al., 2016). However, in the current study we succeeded in establishing an infection in one of two mice injected intrasplenically with HEV RNA containing plasma. The appearance
of HEV RNA in fecal material of the mouse was delayed, but additional confirmation of active HEV infection was provided by successful amplification of HEV RNA in three separate liver fragments isolated at sacrifice (average viral load $2.1 \times 10^3$ IU HEV RNA/30 mg liver tissue), thereby confirming the risk of HEV transmission by transfusion of contaminated blood (Andonov et al., 2014; Hewitt
et al., 2014).

To investigate whether oral inoculation, the presumed natural route of transmission, would also result in infection of humanized FRG mice we examined two different strategies. The first strategy consisted of a multiple forced-feeding protocol with an HEV-containing solution of proven infectivity, while the second strategy was prolonged co-housing of a highly humanized naïve mouse with two HEV infected ones.

Two highly humanized FRG mice (human albumin level of 7.3 and 7.0 mg/ml respectively, which corresponds to about 40% liver humanization according to (Bissig et al., 2010)) were challenged with a Sar-55-containing mouse stool suspension via a repeated oral gavage strategy. Specifically, on three consecutive days the mice received a daily oral dose containing $2.5 \times 10^5$ IU HEV RNA. Despite this repetitive administration, HEV RNA could not be detected in stool or plasma of both mice during the 13-week observation period. Human albumin levels were monitored during the entire experiment and indicated that the human liver graft was stable. Interestingly, one of the mice was subsequently injected intrasplenically with the gt3 infected patient stool sample used in the above described experiments and became readily infected. HEV RNA was detected in both plasma and feces of the re-challenged mouse. In order to confirm that the active infection was caused by the second intrasplenic injection (gt3 virus) and not by the first oral administration (gt1 virus), the viral RNA isolated from the two inocula and 3 different samples collected at week 4 (plasma), week 5 (stool) and week 7 (stool) after the intrasplenic inoculation was amplified using primers targeting the ORF2 region of the viral genome. Sequence analysis of all amplicons revealed that the infection was indeed caused by the second gt3 challenge.

In our second approach, a highly humanized sentinel mouse was cohoused for 16 weeks with two HEV infected mice (HEV gt3, range fecal viral titer $10^5$–$10^6$ IU/ml). All samples collected from the sentinel mouse (stool, plasma and liver) tested negative using our sensitive RT-qPCR. To rule out that the absence of viral transmission was attributed to a loss of liver chimerism, human albumin levels and the degree of liver chimerism were tested. The results of these analyses clearly indicated that the human liver graft was stable (data not shown).

The failure to introduce a productive HEV infection in humanized FRG mice through forced oral inoculation is in agreement with published data by Allweiss et al. and our group using the uPA-based model (Allweiss et al., 2016; Sayed et al., 2016). However, Allweiss et al. also described transmission via co-housing but one cannot rule out that this occurred through small skin injuries, caused by e.g. a fight between animals or bleeding, which came in contact with highly infectious virus present in the feces originating from the infected animals. Since controlled oral gavage has so far never lead to active HEV infection of humanized mice we speculate that oral transmission requires certain essential (human) receptors that are lacking or are different in the mouse intestine. On the other hand, similar experiments performed in pigs and cynomolgus monkeys indicate that oral transmission is at least 10,000-fold less efficient than intravenous inoculation (Kasorndorkbua et al., 2002, 2004; Tsarev et al., 1994). Further studies should therefore be conducted to finally conclude on the possibility of oral HEV transmission in humanized mice.

In conclusion, we here report that humanized FRG mice are susceptible to HEV infection of different genotypes and that the data obtained is similar to previous reports utilizing uPA-based humanized mice. Humanized FRG mice are easily accessible to the research community and can be used for the in vivo study of the hepatitis E virus infection and the evaluation of novel antiviral compounds. In addition, we show for the first time that human liver chimeric mice can be infected with plasma obtained from infected individual, indicating that HEV contaminated blood products may indeed pose a risk for blood transfusion recipients, especially those that are under immunosuppressive regimens.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.antiviral.2017.02.011.

References