Elucidating the Differences in Pathogenicity Between Hepatitis E Virus Genotypes: The Quest Continues

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Hepatitis E virus (HEV) is an emerging pathogen and the most common cause of acute hepatitis.[1] Five genotypes have been described to be capable of infecting humans (genotype 1, gt2, gt3, gt4, and gt7), and two genotypes (gt5 and gt6) have been described that only affect other animals.[1] HEV typically causes an acute self-limiting illness, and not all infections develop overt clinical signs. After an incubation period of 2 to 6 weeks, nonspecific symptoms, such as fever, nausea, abdominal pain, vomiting, anorexia, jaundice, and malaise, can develop.

Abbreviations: gt, genotype; HEV, hepatitis E virus; IFNα-2a, interferon alpha-2a; RBV, ribavirin.

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Excess mortality can be seen in individuals with an underlying chronic liver disease, in immunosuppressed patients, and in pregnant women. HEV infection during pregnancy can take a fulminant course, resulting in fulminant hepatic failure, membrane rupture, spontaneous abortion, and stillbirth.[2] Numerous studies have shown a high mortality (20%-25%) in pregnant women, especially during the third trimester, and children who survive have increased neonatal morbidity and mortality. Importantly, HEV-induced complications during pregnancy are uniquely seen in developing countries and seem to be restricted to infections of gt1 and gt2.[1,2] Only two cases of gt3 infection in pregnant women have been reported in industrialized countries, one occurring at the end of the second trimester and the second during week 34 of gestation.[3,4] In both cases, no hospitalization was required and mother and child had a positive outcome.

The cause of excess maternal mortality in HEV-infected pregnant women is currently not known and has been the subject of many studies. In this issue, Knegendorf et al.[5] describe a placental-derived cell culture system (JEG-3) capable of supporting HEV replication and assessed potential genotype-dependent differences. While JEG-3 cells supported subgenomic HEV replication of gt3 (strain Kernow-C1 clone p6) to a similar level as HepG2 hepatocellular carcinoma cells, gt1 (strain Sar55) replication occurred but at a much lower rate compared to that in HepG2 cells.

The authors also successfully demonstrated HEV infection in JEG-3 cells after electroporation of full-length HEV RNA of gt3, including virion assembly and release of infectious viral particles that have similar biophysical characteristics as HEV particles typically produced in HepG2 cells.[6] Infection with full-length gt1 HEV was not successful, but it remains unclear whether this is typical for gt1 viruses in JEG-3 cells or rather due to the inherent low replicative capacity of Sar55 in cell culture; most likely it is due to the latter. Interestingly, we recently showed that Sar55 propagates more efficiently and replicates at a much higher level in immune-deficient mice with humanized liver

compared to the gt3 isolate p6,[7] highlighting the difference in gt1 HEV replication *in vivo* and *in vitro*.

Currently, there is no treatment available for HEV infection during pregnancy; the main treatment is merely supportive. Ribavirin (RBV), a broad-spectrum antiviral with proven activity against HEV in cell culture, preclinical animal models, and in patients (but unfortunately contraindicated during pregnancy),[1,2,7,8] also effectively inhibited HEV replication in JEG-3 cells, thereby validating this cell culture model for the discovery of novel direct antiviral agents. The added value of sofosbuvir in this context is questionable.

Another discrepancy between gt1 and gt3 viruses became prominent during interferon alpha-2a (IFNα-2a) treatment. While IFNα-2a was equally effective in inhibiting HEV reporter viruses of gt1 and gt3 in hepatoma cells, it only appeared effective against HEV of gt3 in JEG-3 cultures. The lack of activity of IFNα-2a in gt1-infected JEG-3 cultures is intriguing and warrants further investigation, especially because RBV therapy was effective. RBV has been shown to exert its antiviral activity through multiple ways, such as depletion of cellular guanosine triphosphate pools, upregulation of IFN-stimulated genes, direct inhibition of the viral polymerase, inhibition of viral RNA capping, RNA mutagenesis, and modulation of the adaptive immune system.[9] Which of these contribute to its activity against HEV is not fully understood, but at least it has been shown that the depletion of guanosine triphosphate pools and the induction of mutagenesis are involved.[8,10] In addition, the mode of action of RBV could be different depending on the cell type of interest.

The lack of activity of IFNα-2a against gt1 in JEG-3 cells cannot directly be explained by intrinsic defects of the cellular innate immune response of the placental-derived JEG-3 cells. Signal transducer and activator of transcription 1 phosphorylation occurred with exogenous IFN administration, suggesting at least successful activation of the Janus kinase-signal transducer and activator of transcription signaling pathway. However, the authors did observe specific downregulation of IFN-stimulated gene expression by HEV both in JEG-3 and HepG2, and for both genotypes. It is remarkable that the gt1 virus could significantly block (or decrease) type I IFN responses in placental-derived cells, especially given its low replicative capacity in these cells. This insinuates that this interference must occur through a very efficient process.

Although the study by Knegendorf and colleagues nicely highlights the complex nature of genotype-specific and cell type-specific virus–host interactions, much more research and insight are needed to understand the pathogenesis of gt1 during pregnancy. Another complicating factor is that the outcome of HEV in pregnant women seems to be influenced by geographic region. Both a high incidence and severe course have been observed in HEV-endemic countries, such as northern India, while in other HEV-endemic areas, such as Egypt, a benign course was seen with little or no morbidity.[11] Moreover, no fatalities were observed in the small number of cases reported in industrialized countries.[12] The reason(s) for these geographic differences remain unclear but could be due to a different virulence of the circulating viruses, genetic traits of the resident population, environmental factors, and/or better medical care in the case of more industrialized countries.[2,11]

This study provides an important first step, but the researchers' described cell culture model could be further improved on several fronts. First, it remains important to search for additional gt1 isolates that can more efficiently replicate in placental and hepatic cell lines. Additionally, more permissive JEG-3 subclones could perhaps be identified as well as other unrelated placental-derived cell lines of distinct ethnic origins. Second, it is known that an increase in progesterone, estrogen, and human chorionic gonadotropin levels (negatively) influence the immunologic state during pregnancy and thus, at least indirectly, have a favorable impact on viral replication.[2] Cultured JEG-3 cells are known to only have limited endogenous hormonal production,[13] and while hormonal changes during pregnancy are considered to only affect cellular immunity, this model now allows the study of the potential direct impact on viral replication of exogenously added hormones and other relevant cytokines.

In conclusion, this JEG-3-based cell culture model provides an excellent tool to identify the factors that determine HEV pathogenicity during pregnancy. Mapping the virus–host interactions that impede innate immunity and revealing the crucial pathways that support HEV replication in a genotype-specific and tissue type-specific manner will enable the search for specific antiviral medication and therapeutic approaches that can be used in pregnant women.

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