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ORIGINAL RESEARCH

Analysis of Hepatitis C Virus Particle Heterogeneity in Immunodeficient Human Liver Chimeric *fah-/-* Mice

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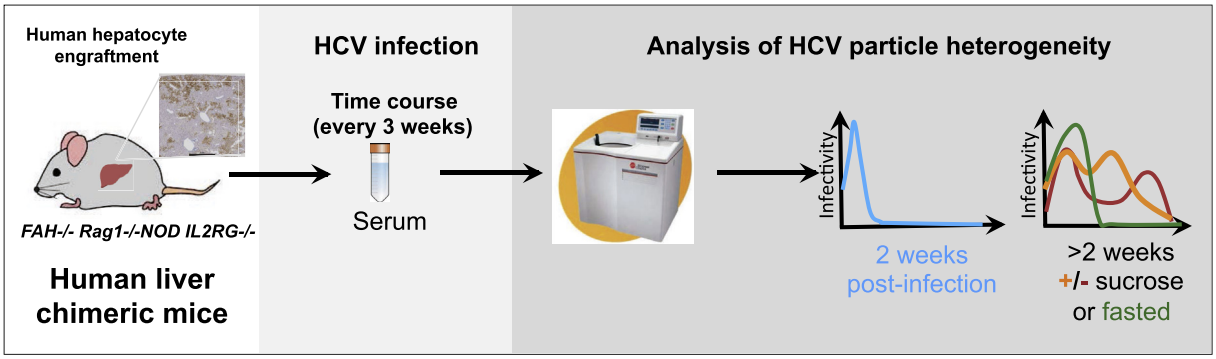

SUMMARY

Our study describes how the biophysical properties of the hepatis C virus evolve over the course of infection using the human liver chimeric FAH-/- mouse model and suggests that metabolic changes impact hepatis C virus biophysical properties.

BACKGROUND & AIMS: Hepatitis C virus (HCV) is a leading cause of chronic liver diseases and the most common indication for liver transplantation in the United States. HCV particles in the blood of infected patients are characterized by heterogeneous buoyant densities, likely owing to HCV association with lipoproteins. However, clinical isolates are not infectious in vitro and the relative infectivity of the particles with respect to their buoyant density therefore cannot be determined, pointing to the need for better in vivo model systems.

METHODS: To analyze the evolution of the buoyant density of in vivo–derived infectious HCV particles over time, we infected immunodeficient human liver chimeric fumaryl acetoacetate hydrolase-/- mice with J6/JFH1 and performed ultracentrifugation of infectious mouse sera on isopicnic iodixanol gradients. We also evaluated the impact of a high sucrose diet, which has been shown to increase very-low-density lipoprotein secretion by the liver in rodents, on lipoprotein and HCV particle characteristics.

RESULTS: Similar to the severe combined immunodeficiency disease/Albumin-urokinase plasminogen activator human liver chimeric mouse model, density fractionation of infectious mouse serum showed higher infectivity in the low-density fractions early after infection. However, over the course of the infection, viral particle heterogeneity increased and the overall in vitro infectivity diminished without loss of the human liver graft over time. In mice provided with a sucrose-rich diet we observed a minor shift in HCV infectivity toward lower density that correlated with a redistribution of triglycerides and cholesterol among lipoproteins.

CONCLUSIONS: Our work indicates that the heterogeneity in buoyant density of infectious HCV particles evolves over the course of infection and can be influenced by diet. *(Cell Mol Gastroenterol Hepatol 2017;4:405–417; http://dx.doi.org/10.1016/j.jcmgh.2017.07.002)*

Keywords: HCV; Lipoprotein; Mouse Model; Human Liver Chimeric Mice.

See editorial on page 443.

Between 130 and 170 million people are chronically infected with hepatitis C virus (HCV) worldwide, putting them at risk for chronic liver disease including steatosis, cirrhosis, and hepatocellular carcinoma.[1] Even with the recent approval of highly effective treatments, HCV remains the primary indication for liver transplantation in the United States and continues to pose a major public health burden. HCV perturbs lipid metabolism, causing liver pathology and potentially contributing to other etiologies including higher risk of cardiovascular diseases,[2,3] the leading cause of death globally. It remains to be determined if the new HCV treatments can revert this phenotype.

HCV is a positive-strand RNA virus that belongs to the *Flaviviridae* family. HCV entry, replication, and assembly are linked closely to host lipid and lipoprotein metabolism.[4–6] It now is understood that the low density and high infectivity of these particles are the result of HCV association with the very-low-density lipoprotein (VLDL) secretion pathway leading to the formation of a lipoviroparticles (LVPs).[7]

Lipoproteins are macromolecular complexes that allow the transport of lipids in the bloodstream. Lipoproteins are composed of a monolayer of phospholipids, in which apolipoproteins are associated. The core of the particle contains cholesterol esters and triglycerides. Lipoprotein subclasses are differentiated by size and buoyant density depending on their triglyceride and cholesterol content. High-density lipoproteins (HDLs) are small dense lipoproteins that are responsible for the transport of cholesterol from peripheral tissues back to the liver in a process called *reverse cholesterol transport*. VLDL are secreted by hepatocytes to mainly transport triglycerides from the liver to extrahepatic tissues. They are transformed in the circulation to intermediate-density lipoprotein and low-density lipoprotein (LDL) after triglyceride hydrolysis by lipoprotein lipase.[8,9]

Despite numerous reports, the precise details of how HCV and host lipoproteins interact, as well as the exact structure of the resulting LVP, remain unknown.[6] Early electron microscopic analyses of patient-derived particles recovered from low-density fractions showed virions with a diameter of 60–70 nm, with immunoreactive viral envelope glycoproteins E1 and E2[10]; viral particles of similar density ($<$1.1 g/mL) from infected chimpanzees are highly infectious.[11] Recent work from Catanese et al[12] reported that cell culture–derived HCV (HCVcc) particles also are heterogeneous, ranging from 40 to 100 nm. Further biochemical characterization of patient-derived HCC and HCVcc particles confirmed the presence of several apolipoproteins (apos) such as apoB, apoC1, as well as apoE.[13] Besides enhancing infectivity, assembly of lipoprotein-associated viral particles also may provide a means of viral escape from potentially neutralizing antibodies by masking viral epitopes in a lipoprotein coat.[14] Several studies have indicated that apoE is required for HCV infectivity and is part of the LVP.[15–18] Interestingly, a recent study showed that apolipoprotein amphipathic α-helices were sufficient to promote HCV particle assembly in cell culture.[19] Other important factors for VLDL lipoprotein assembly, such as the microsomal transfer protein,[16] apoB,[16] and DGAT1[20] have been proposed to play a role in HCV assembly. Even though these in vitro studies have shed some light on the role of lipoproteins in HCV assembly, Huh-7–derived human hepatoma cell lines used to produce HCV particles secrete only partially lipidated immature VLDL,[21] limiting their utility in characterizing the density heterogeneity, composition, and evolution of HCV virions.

There are no fully immunocompetent small-animal models for efficient HCV infection, but several immunodeficient murine xenograft models exist to study HCV infection in vivo. In 1 such model, the severe combined immunodeficiency disease (SCID)/Albumin-urokinase plasminogen activator mouse, in vivo–derived HCV RNA was enriched in the lower density fractions and of higher specific infectivity than in vitro–derived HCV. However, titers were too low to be determined for each fraction.[22] Moreover, the degree of humanization of the lipoprotein profiles in SCID/Alb-uPA liver chimeric mice is reported to correlate with infection success.[23]

In the present study, to analyze the biophysical properties of HCV particles over time, we used an alternative human liver chimeric mouse model that is based on the absence of the tyrosine catabolic enzyme fumaryl acetoacetate hydrolase (*FAH*)[24] on the immunodeficient nod rag γ (NRG) mouse background[25] (designated FNRG mice). FNRG mice infected with J6/JFH1 constitute a suitable model to analyze the buoyant density distribution of infectious HCV particles in a gradient and monitor their evolution over time, as well as establish the susceptibility of the infectious particles in vivo to natural challenges such as feeding and fasting. After infecting these mice with J6/JFH1 and performing an initial analysis of the density of infectious HCV particles, we subjected these mice to a 10% sucrose diet to increase the production of fully lipidated VLDL, and analyzed the HCV virus distribution on a gradient after feeding or fasting. Overall, data in the FNRG mouse model yielded HCV particles of low density and higher infectivity early after infection, supporting previous results in the SCID/Alb-uPA model. However, over the course of the infection, the viral particle density became more heterogeneous with a decreased population of particles of low density, and the overall in vitro infectivity diminished. We further show that a high sucrose diet and a fed vs fasted state affect the buoyant density distribution of HCV particles.

Abbreviations used in this paper: **Alb-uPA, Albumin-urokinase plasminogen activator; apo, apolipoprotein; CETP, cholesterol ester transfer protein; FAH, fumaryl acetoacetate hydrolase; FNRG, absence of fumaryl acetoacetate hydrolase on a immunodeficient NOD Rag gamma IL2 deficient mouse background; FPLC, fast-performance liquid chromatography; HCV, hepatitis C virus; HCVcc, cell culture–derived hepatitis C virus; HDL, high-density lipoprotein; LVP, lipoviroparticle; NRG, nod rag γ; NTBC, nitisinone; PBS, phosphate-buffered saline; SCID, severe combined immunodeficiency disease; VLDL, very low density lipoprotein.**

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