Research Article

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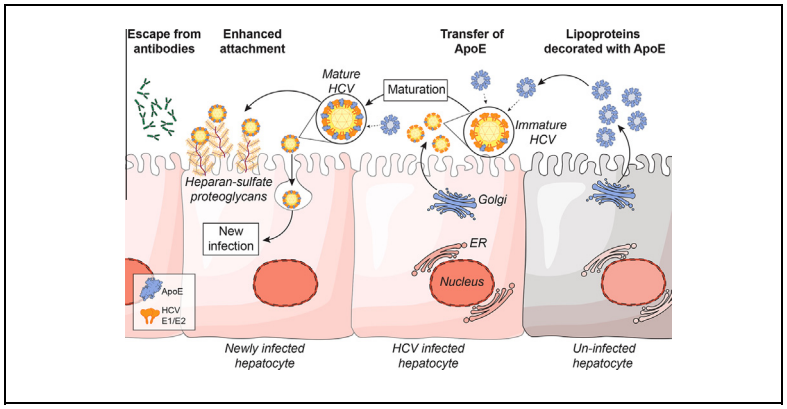
# Maturation of secreted HCV particles by incorporation of secreted ApoE protects from antibodies by enhancing infectivity

## Graphical Abstract




## Highlights

- ApoE secreted from non-infected liver cells incorporates into secreted HCV.
- Physiological quantities of secreted ApoE enhance HCV infectivity.
- Infectivity enhancement is independent of the hypervariable region 1 and SR-B1.
- Secreted ApoE enhance particle interaction with cellular HSPGs.
- Physiological levels of secreted ApoE increase HCV antibody escape.

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## Lay summary

This study shows that HCV particle infectivity is remodeled by secreted ApoE after particle release from cells. Fluctuation of the availability of ApoE likely influences HCV infectivity, antibody escape and transmission.

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# Maturation of secreted HCV particles by incorporation of secreted ApoE protects from antibodies by enhancing infectivity

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**Background & Aims**: Hepatitis C virus (HCV) evades humoral immunity and establishes chronic infections. Virus particles circulate in complex with lipoproteins facilitating antibody escape. Apolipoprotein E (ApoE) is essential for intracellular HCV assembly and for HCV cell entry. We aimed to explore if ApoE released from non-infected cells interacts with and modulates secreted HCV particles.

**Methods**: ApoE secreted from non-infected cells was incubated with HCV from primary human hepatocytes or Huh-7.5 cells. Co-immunoprecipitation, viral infectivity and neutralization experiments were conducted.

**Results**: Physiological levels of secreted ApoE (10–60 μg/ml) enhanced the infectivity of HCV up to 8-fold across all genotypes, which indirectly decreased virus neutralization by antibodies targeting E1 or E2 up to 10-fold. Infection enhancement was observed for particles produced in primary human hepatocytes and Huh-7.5 cells. Selective depletion of ApoE ablated infection enhancement. Addition of HA-tagged ApoE to HCV particles permitted co-precipitation of HCV virions. Serum ApoE levels ranged between 10–60 μg/ml, which is ca 100-fold higher than in Huh-7.5 conditioned cell culture fluids. Serum-derived HCV particles carried much higher amounts of ApoE than cell culture-derived HCV particles. Serum ApoE levels correlated with efficiency of co-precipitation of HCV upon exogenous addition of HA-ApoE. ApoE-dependent infection enhancement was independent of the hypervariable region 1 and SR-B1, but was dependent on heparan sulfate proteoglycans (HSPGs).

**Conclusions**: Physiological quantities of secreted ApoE stimulate HCV infection and increase antibody escape, by incorporating into virus particles and enhancing particle interactions with cellular HSPGs. Thus, secreted particles undergo ApoE-dependent maturation to enhance infectivity and to facilitate evasion from neutralizing antibodies.

**Lay summary:** This study shows that HCV particle infectivity is remodeled by secreted ApoE after particle release from cells. Fluctuation of the availability of ApoE likely influences HCV infectivity, antibody escape and transmission.



## Introduction

Hepatitis C virus (HCV) is a hepatotropic RNA virus of the family *Flaviviridae* that is transmitted parenterally.[1] HCV encodes a single polyprotein that is cleaved by cellular and viral enzymes into the structural proteins core and envelope protein 1 (E1) and E2, the p7 ion channel protein, and the non-structural proteins NS2, NS3, NS4A, NS4B, NS5A and NS5B. The capsid protein encases the viral RNA genome and the E1-E2 proteins are embedded into the viral lipid membrane, where they mediate receptor interactions and viral membrane fusion during cell entry. E1 and E2 are also key targets for neutralizing antibodies and thus key structures for vaccine development. Most people exposed to the virus are unable to curtail replication and thus develop a chronic infection.

Licensing of direct-acting antivirals (DAAs) has revolutionized HCV patient care, offering a cure for many patients worldwide.[2] However, providing the drugs to more than 140 million chronically infected patients is a major public health challenge. Moreover, treatment-induced cure does not protect from HCV re-infection. Thus, global control of HCV-associated disease burden would benefit from a prophylactic vaccine. However, a

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