

A Model for the Study of Hepatitis C Virus Entry

Bartosch B, Dubuisson J, Cosset F-L. Infectious hepatitis C virus pseudo-particles containing functional E1-E2 envelope protein complexes. *J Exp Med* 2003;197:633-642. (Reprinted with permission.)

Abstract

The study of hepatitis C virus (HCV), a major cause of chronic liver disease, has been hampered by the lack of a cell culture system

supporting its replication. Here, we have successfully generated infectious pseudo-particles that were assembled by displaying unmodified and functional HCV glycoproteins onto retroviral and lentiviral core particles. The presence of a green fluorescent protein marker gene packaged within these HCV pseudo-particles allowed reliable and fast determination of infectivity mediated by the HCV glycoproteins. Primary hepatocytes as well as hepatocarcinoma cells were found to be the major targets of infection *in vitro*. High infectivity of the pseudo-particles required both E1 and E2 HCV glycoproteins, and was neutralized by sera from HCV-infected patients and by some anti-E2 monoclonal antibodies. In addition, these pseudo-particles allowed investigation of the role of putative HCV receptors. Although our results tend to confirm their involvement, they provide evidence that neither LDLr nor CD81 is sufficient to mediate HCV cell entry. Altogether, these studies indicate that these pseudo-particles may mimic the early infection steps of parental HCV and will be suitable for the development of much needed new antiviral therapies.

Comments

Despite recent advances in the study of hepatitis C virus (HCV) replication, based largely on the development of subgenomic and genomic length replicons,¹⁻⁴ little is known about the early steps of HCV infection. This is mainly because of the difficulties in obtaining sufficient quantities of native HCV virions from serum and the lack of an efficient cell culture system permissive for HCV infection. Cultured primary human hepatocytes may most closely reflect the natural situation, but even in this *in vitro* model, the level of HCV replication is very low, requiring quantitative reverse transcription polymerase chain reaction (RT-PCR) techniques as a read-out for successful infection.⁵

Given the lack of native HCV particles and efficient cell culture systems, various alternatives have been explored to study the early steps of HCV infection. Soluble C-terminal truncated versions of HCV envelope glycoprotein E2,⁶⁻¹⁰ liposomes reconstituted with HCV E1 and E2,¹¹ and virus-like particles expressed in insect cells^{12,13} have been used to study HCV glycoprotein interactions with the cell surface. Moreover, pseudotyped vesicular stomatitis virus (VSV) or influenza virus particles have been reported incorporating chimeric E1 and/or E2 glycoproteins whose C-terminal transmembrane domains were modified to allow transport to the cell surface.^{7,14-16} However, such modifications may interfere with the multiple and complex roles of the E1 and E2 transmembrane domains¹⁷ and may perturb the conformation and functions of E1-E2 complexes. Therefore, the use of such pseudotypes as a tool to study HCV assembly and entry remains controversial.¹⁶

With this background, the article by Bartosch et al.¹⁸ and a more recent independent report by Hsu et al.¹⁹ represent a major breakthrough in HCV research. These

investigators took advantage of two remarkable properties of retroviruses, namely the capacity to incorporate heterologous glycoproteins and their capacity to express reporter genes from replication-defective viral particles, to establish a specific, convenient, and highly reproducible infection assay based on pseudoparticles carrying functional HCV glycoproteins.

In the study by Bartosch et al., the investigators generated infectious pseudoparticles bearing unmodified HCV glycoproteins on retroviral core particles derived from murine leukemia virus (MLV) or HIV-1.¹⁸ This was achieved by cotransfection of 293T human embryo kidney cells with expression vectors coding for (1) HCV genotype 1a or 1b E1-E2 glycoproteins with their genuine transmembrane domains, (2) a *gag-pol* packaging construct encoding retroviral core proteins, and (3) packaging-competent green fluorescent protein (GFP) or *lacZ* (the gene coding for β -galactosidase) retroviral transfer vectors (Fig. 1). Pseudoparticles carrying HCV envelope glycoproteins instead of the retroviral envelope protein on their surface and harboring a conveniently and rapidly measurable reporter gene were secreted into the supernatant and could be purified by centrifugation through sucrose cushions.

Infectivity of these pseudoparticles was examined on various cultured cell lines and on primary human hepatocytes using flow cytometry or X-gal staining as a read-out for GFP or β -galactosidase, respectively. Interestingly, there was a clear tropism for liver-derived cell lines (Huh-7, PLC/PRF/5, and Hep3B cells) and primary human hepatocytes, whereas a number of other cell lines, not derived from liver, were not or only weakly infectable. Experiments performed with pseudotypes displaying E1 and/or E2 glycoproteins showed that both glycoproteins were needed to allow efficient infection, but that they could be expressed independently from separate plasmids. No difference was observed with glycoproteins derived from HCV genotype 1a and 1b. A number of controls, including pseudoparticles without HCV E1-E2, without retroviral core proteins, with assembly defective core proteins, or with E1 or E2 alone, did not result in infection. Moreover, infectivity of pseudoparticles could be specifically neutralized by sera from HCV-infected individuals and by certain monoclonal antibodies specific for E2. Taken together, the preferential tropism for hepatic cells and the specific neutralization provide convincing evidence that these pseudoparticles closely mimic the HCV infection process.

Hsu et al. generated and characterized HIV-1-based retroviral particles pseudotyped with unmodified HCV genotype 1a or 1b envelope glycoproteins.¹⁹ In this case, luciferase was used as a reporter gene allowing quantita-

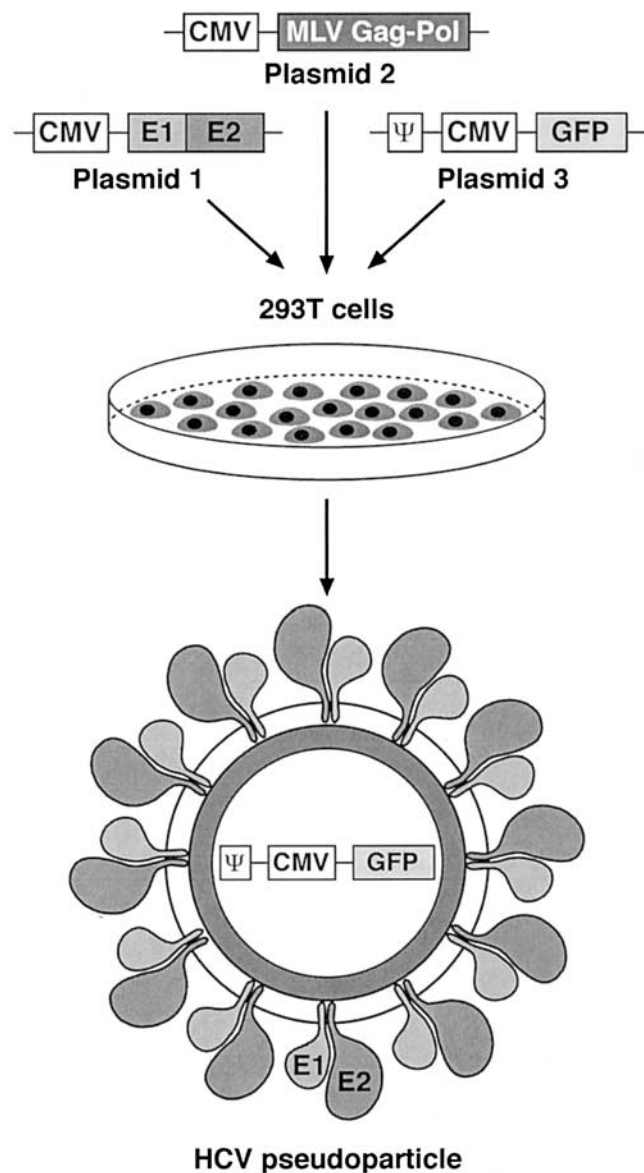


Fig. 1. Generation of infectious HCV pseudoparticles. Cotransfection of 293T human embryo kidney cells with plasmids allowing expression of (1) unmodified HCV E1-E2 glycoproteins, (2) retroviral core proteins, and (3) a packaging-competent green fluorescent protein (GFP) expression construct leads to secretion into the supernatant of pseudoparticles bearing HCV envelope glycoproteins instead of the retroviral envelope protein on their surface. CMV, cytomegalovirus promoter; Ψ, retroviral packaging sequence.

tion of successful infection with a high dynamic range. Similar to the observations by Bartosch et al., these investigators found a strong preference of their pseudoparticles for liver-derived cell lines, and infection could be neutralized by anti-E2 monoclonal antibodies. Interestingly, among different controls Hsu et al. also generated pseudoparticles bearing chimeric E1-E2 molecules with heterologous C-terminal transmembrane domains, and they found that these particles were not infectious. This observation suggests that the transmembrane domains of E1

and E2 are essential for the formation of functional E1-E2 complexes and, as discussed above, raises doubt about the usefulness of VSV and influenza virus pseudotypes bearing chimeric glycoproteins as a model to study HCV entry.

The fact that pseudoparticles bearing HCV glycoproteins were secreted from transfected 293T cells is in itself rather unexpected. E1 and E2 have been shown to be retained in the endoplasmic reticulum (ER),¹⁷ whereas retroviruses assemble and bud from the plasma membrane. However, flow cytometry studies of transfected 293T cells showed that, although the majority of HCV E1 and E2 glycoproteins were retained intracellularly, a small but significant proportion of the glycoproteins could be detected at the cell surface.^{18,19} This was independent of retroviral protein expression¹⁹ and may be due to a leakiness of the ER retention machinery in cells overexpressing E1-E2 glycoproteins. It will be interesting to examine whether this is a general phenomenon in various cell types or whether this is restricted to 293T cells.

An obvious application of infectious HCV pseudoparticles is the validation of HCV receptor candidates and the discovery of novel receptor molecules. In this context, CD81,⁶ the low density lipoprotein receptor (LDL-R),²⁰ scavenger receptor class B type 1 (SR-B1),⁸ and dendritic cell-specific intercellular adhesion molecule 3 grabbing nonintegrin (DC-SIGN) as well as its closely related homolog DC-SIGNR/L-SIGN^{9,10} have recently attracted considerable interest as candidate receptors for HCV.

The role of CD81 was addressed both by Bartosch et al. and by Hsu et al. Both teams quite conclusively showed that HCV pseudoparticle infection can be blocked by a recombinant soluble form of CD81 or the preincubation of Huh-7 cells or primary human hepatocytes with anti-CD81 monoclonal antibodies. However, expression of CD81 on otherwise nonpermissive cell types did not confer permissivity to HCV pseudoparticles. Based on these observations, the authors concluded that CD81 is a necessary component of a receptor complex, but is not sufficient alone to confer infection. This is in line with recent results obtained with infection of primary human hepatocytes using HCV-containing serum.²¹

The potential role of the LDL-R was examined by competition experiments using purified LDL or VLDL as well as anti-LDL-R antibodies. In this case, infection by HCV pseudoparticles was not or only weakly inhibited. However, it is important to note that HCV virions in the plasma of infected patients are associated with LDL and VLDL.^{20,22} This may not be the case with pseudoparticles secreted from nonhepatic cells in tissue culture. Therefore, these results do not exclude a role of the LDL-R in HCV entry, possibly *via* association of LDL and/or VLDL with native viral particles. Indeed, the LDL-R

could be involved in the early steps of *in vitro* infection of primary human hepatocytes by HCV positive human serum samples (Castet V et al., unpublished data, July 2002).

The potential role of SR-B1 was studied by Hsu et al.¹⁹ These investigators found that infection of Huh-7 cells was not blocked by a monoclonal antibody against SR-B1. In addition, PLC/PRF/5, a human hepatocellular carcinoma line, was infectable despite having undetectable levels of SR-B1 at the cell surface and a number of non-hepatic cell lines failed to support HCV pseudotype infection despite cell surface expression of SR-B1. Similarly, expression of DC-SIGN in a nonhepatic cell background was not sufficient to confer permissivity to HCV pseudotype infection.

A number of exciting applications of this model system can be envisioned apart from the validation of existing and the discovery of novel HCV receptor candidates. For example, it will be very interesting to further characterize the glycosylation pattern and antigenic conformation of E1-E2 complexes in their functional state. Moreover, the early steps of the HCV life cycle, including internalization and fusion, are now amenable to systematic study. In this context, Hsu et al. found that pseudoparticle infection is pH dependent, suggesting entry *via* receptor-mediated endocytosis and release from the endosome by a low pH-triggered fusion process. It will be very interesting to study the activation of the HCV fusion machinery by low pH. Perhaps most important, this model system will allow us to develop and evaluate novel antiviral strategies targeting HCV entry. Finally, it represents the basis for robust and reproducible neutralization assays, which may be critical for the development of HCV vaccines as well as for studies on the role of humoral immunity in hepatitis C protection and pathogenesis.

In conclusion, the studies by Bartosch et al. and by Hsu et al. remove a major roadblock in HCV research, allowing, for the first time, studies on the early steps of the HCV life cycle in a robust and reproducible setting. A number of exciting studies utilizing this system can be expected in the near future. These will, in all likelihood, tell how closely HCV-retroviral pseudoparticles reflect authentic HCV virions.

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