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Mirjam B. Zeisel, Thomas F. Baumert

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Mirjam B. Zeisel and Thomas F. Baumert

Dept. of Medicine II, University of Freiburg, Freiburg, Germany

Corresponding author:
Thomas F. Baumert, M. D.
Dept. of Medicine II
University of Freiburg
Hugstetter Strasse 55
D-79106 Freiburg
Germany
Phone: (++49-761) 270-3401
Fax: (++49-761) 270-3259
e-mail: Thomas.Baumert@uniklinik-freiburg.de

Abbreviations

HCV: hepatitis C virus

HCV-LP: HCV-like particle

HCVpp: HCV pseudoparticle

HCVcc: cell culture-derived HCV

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Hepatitis C Virus: The Need for Model Systems

Hepatitis C virus (HCV) is a major cause of posttransfusion and community-acquired hepatitis in the world [1]. The majority of HCV-infected individuals develop chronic hepatitis that may progress to liver cirrhosis and hepatocellular carcinoma. Treatment options for chronic HCV infection are limited and a vaccine to prevent HCV infection is not available [1]. The development of efficient antivirals and a vaccine has been hampered by the lack of an efficient tissue culture model for HCV infection.

Several *in vitro* models for the study of HCV infection have been described. These models include human lymphocytic cell lines, human biliary epithelial cells, and primary hepatocytes from humans, chimpanzees or tree shrews (*Tupaia belangeri*). Primary hepatocytes can be successfully infected by serum-derived HCV [2, 3]. Limitations of these systems are the low level of HCV replication requiring RT-PCR for detection of viral infection, the variable quality of host cells such as human hepatocytes obtained from surgical specimens and the lack of a well-defined viral inoculum.

Alternative model systems have been developed for the study of defined aspects of the HCV life cycle such as viral entry, replication, assembly and release (Fig. 1). Recombinant HCV envelope glycoproteins [4], HCV-like particles (HCV-LPs) [3, 5] and retroviral HCV pseudotypes (HCVpp) [6, 7] have been successfully used to analyze virus attachment and entry. These systems allowed the isolation and functional analysis of CD81, scavenger receptor BI and highly sulfated heparan sulfate as cell surface molecules involved in viral attachment and entry [3-7]. A major step forward towards the study of viral replication was the development of HCV replicons, based on the self-replication of engineered minigenomes in human hepatoma cell lines [8]. Replicon-based-systems have allowed the elucidation and dissection of the viral replication complex, the study of mechanisms of viral resistance and the

exploration of novel antiviral approaches. Although extremely useful in the study of HCV genomic replication and screening for antiviral drugs, this system does not yet enable the study of viral infection or virus particle production. Finally, a novel model system for the efficient production of viral particles has been reported very recently [9]. Whether particles synthesized in this system are infectious is currently under investigation [9].

Production of Infectious HCV in Tissue Culture

In a collaborative approach, several groups now succeeded in establishing the long-sought-after cell culture system for HCV [10-12]. The first step towards this breakthrough was the development of a unique HCV genotype 2a replicon (JFH1) - derived from a viral isolate of a patient with fulminant hepatitis C - that replicates efficiently in human hepatoma Huh7 cells without adaptive mutations [13]. Independently, three papers published almost at the same time showed that transfection of full-length JFH1 or chimeric J6/JFH1 genomes into Huh7 or Huh7-derived cells results in secretion of infectious virus. Successful infection of naïve hepatoma cells with recombinant virions was demonstrated by detection of viral proteins and a highly reproducible time-dependent increase of viral RNA in infected cells [10-12]. Using control clones containing a mutation of the NS5B polymerase active site or a deletion of viral envelope glycoproteins, the authors elegantly demonstrated that the synthesis of infectious viral particles was dependent on an active viral polymerase and expression of a functional viral envelope containing HCV envelope glycoproteins E1 and E2 [10-12].

Using an antibody directed against an epitope of the HCV envelope glycoprotein E2, the Bartenschlager group was able for the first time to visualize cell culture-derived JFH1 virions by immunoelectron microscopy [10]. HCV particles were 50-65 nm in diameter, the predicted size of HCV. Secreted virions were further characterized biophysically by sucrose density gradient centrifugation: the peak HCV RNA fraction which also contained HCV core,

E1 and E2 proteins had a density of about 1.15-1.17 g/ml. Interestingly, viral particles exhibiting the highest infection efficiency had an apparent density of about 1.09-1.10 g/ml and 1.105 g/ml as reported by the Rice and Chisari labs, respectively [11, 12]. By inoculating supernatant from transfected cells into a chimpanzee, the Liang and Wakita lab investigated the infectivity of this JFH1 clone *in vivo* [10]. In deed, the inoculated chimpanzee developed a mild and transient HCV infection, which proved that the recombinant virions were infective *in vivo*.

Although all three groups used JFH1-derived HCV full-length clones for synthesis of viral particles, the studies differed in the hepatoma cell lines used. Whereas Wakita and colleagues transfected and infected native Huh7 cells as well as a highly permissive Huh7-derived cell clone, the Rice and Chisari labs used Huh7.5 and Huh7.5.1 cells for the generation of virions and infectivity studies. Huh7.5 and Huh7.5.1 cells are HCV-adapted cell lines generated by curing replicon expressing-Huh7 and Huh7.5 cells with IFN-α and IFN-γ, respectively [11, 12]. Infection efficiencies seemed to be comparable between these different cell lines, although Huh7.5 cells showed faster infection kinetics [11, 12]. Interestingly, the authors reported that virus titers decrease upon serial passages in Huh7 cells [10], whereas no detectable loss in infectivity was observed for Huh7.5.1 cells [12]. These findings suggest that Huh7.5-derived hepatoma cells may be superior for the efficient production of infectious virions and may allow to define cellular factors modulating HCV infection. In fact, Huh7.5 cells carry a point mutation in the retinoic acid-inducible gene-I (RIG-I) which reduces the cell's innate immune defenses and may contribute to the higher permissiveness. Other, to date unknown, factors may also contribute to favor HCV life cycle in these cells [14].

A current limitation of this new HCV model is its dependence on the JFH1 nonstructural genes. Nevertheless, the possibility to create chimeric infectious viruses

broadens the scope of this system. The Bartenschlager group recently reported that chimeric viruses composed of the core to NS2 region of the Con1 isolate (genotype 1) and the JFH1 replicase (genotype 2a) are also infectious (T. Pietschmann, G. Koutsoudakis, S. Kallis, T. Kato, S. Foung, T. Wakita and R. Bartenschlager, 11th international symposium on hepatitis C virus and related viruses, Heidelberg, October 2004). Similarly, the analogous chimera composed of the genotype 2a J6 and the JFH1 replicase also supports production of infectious HCV particles [11]. However, there seem to be some limitations in creating infectious chimera, as full-length chimeric genomes composed of the core-NS2 region from the infectious H77 (genotype 1a) and JFH1 NS3-NS5B genes are not infectious [11]. Further studies are needed to elucidate viral and host factors required for production of infectious particles and for generating of infectious viruses of other genotypes.

Impact for the Study of Viral Life Cycle and the Development of Antivirals and Vaccines

This cell culture-based model finally allows the study of all aspects of the HCV life cycle including viral attachment, entry, trafficking, replication, assembly and egress (Fig. 1). In a first approach to identify viral and cellular components involved in viral entry, the groups independently showed that viral envelope glycoprotein E2 as well as cellular CD81 appear to be essential for viral infection [10-12]. Anti-HCV antibodies in sera from HCV-infected patients were able to neutralize JFH1 viruses in a dose-dependent manner [10], suggesting that this cell culture model also provides a very valuable tool to study the role of neutralizing antibodies in HCV infection. In addition, this system may be extremely useful for screening and development of antiviral drugs. In fact, Lindenbach and colleagues reported that *in vitro* HCV infection can be efficiently inhibited by IFN-α and several HCV-specific antiviral compounds, such as NS3 serine protease-inhibitors and a nucleoside analogue targeting the NS5B polymerase [11]. Finally, this new HCV infection model may also contribute to HCV

vaccine development by gaining novel insights into antiviral innate and adaptive immune responses such as the molecular analysis of antibody-mediated virus neutralization. The future applications of this system clearly demonstrate that the development of this model marks a breakthrough for HCV research.

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Figure Legend

Fig. 1. *Putative HCV life cycle and HCV model systems.* HCV binding to a cell surface receptor complex leads to viral entry into host cell. In the cytoplasm, HCV genomic RNA functions as a template for HCV polyprotein translation. This polyprotein is then processed into structural (core (C), envelope proteins E1 and E2) and non-structural proteins (NS2 to NS5B). RNA-dependent RNA polymerase (NS5B) allows HCV genome replication. Replication occurs within cytoplasmic, membrane-associated replication complexes in a perinuclear membranous web (not shown). Progeny genomes assemble with structural proteins into HCV particles which are subsequently released from the cell. HCV-like particles (HCV-LPs) [3, 5] and retroviral HCV pseudotypes (HCVpp) [6, 7] have been used to analyze virus binding and entry. Replicon-based-systems [8] have allowed the elucidation and dissection of the viral replication complex. The new HCV tissue culture model system [10-12] resulting in the production of cell culture-derived HCV (HCVcc) finally allows the study of all aspects of the HCV life cycle including viral attachment, entry, trafficking, replication, assembly and release.