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Review

Advances in experimental systems to study hepatitis C virus *in vitro* and *in vivo*

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ABSTRACT

Hepatitis C virus (HCV) represents a global health concern affecting over 185 million people worldwide. Chronic HCV infection causes liver fibrosis and cirrhosis and is the leading indication for liver transplantation. Recent advances in the field of direct-acting antiviral drugs (DAAs) promise a cure for HCV in over 90% of cases that will get access to these expensive treatments. Nevertheless, the lack of a protective vaccine and likely emergence of drug-resistant viral variants call for further studies of HCV biology. With chimpanzees being for a long time the only non-human *in vivo* model of HCV infection, strong efforts were put into establishing *in vitro* experimental systems. The initial models only enabled to study specific aspects of the HCV life cycle, such as viral replication with the subgenomic replicon and entry using HCV pseudotyped particles (HCVpp). Subsequent development of protocols to grow infectious HCV particles in cell-culture (HCVcc) ignited investigations on the full cycle of HCV infection and the virus–host interactions required for virus propagation.

More recently, small animal models permissive to HCV were generated that allowed *in vivo* testing of novel antiviral therapies as well as vaccine candidates. This review provides an overview of the currently available *in vitro* and *in vivo* experimental systems to study HCV biology. Particular emphasis is given to how these model systems furthered our understanding of virus–host interactions, viral pathogenesis and immunological responses to HCV infection, as well as drug and vaccine development.

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Introduction

Hepatitis C virus (HCV) is a (+)-sense, single-stranded RNA virus of the *Flaviviridae* family that infects humans and chimpanzees via direct blood contact (e.g. intravenous drug use or contaminated blood supplies) and targets primarily hepatocytes. Current estimates indicate that more than 185 million people (approximately 3% of the world's population) have been infected with the virus (Mohd Hanafiah et al., 2013). HCV stains are classified into 7 genotypes based on phylogenetic and sequence analyses (>30% divergence at nucleotide level). Genotypes 1 and 3 are the most prevalent worldwide, accounting together for 137.7 million cases, while genotypes 4 and 5 are mostly distributed in lower-income countries (Messina et al., 2015). More than 70% of the individuals contracting HCV progresses on developing a chronic infection that often remains asymptomatic for decades. This suggests that the virus has evolved successful strategies to overcome antiviral cellular defences and coexist with its host. Ultimately, however, chronic HCV infection leads to the development of liver fibrosis, cirrhosis, hepatocellular carcinoma (HCC), and end-stage liver disease.

For twenty years, HCV has been treated with interferon (IFN)-based regimens that present important side effects and are ineffective in at least 50% of cases (Manns et al., 2006). Exciting progress on the HCV therapy front were made in 2011, when the first direct-acting antivirals (DAAs) received approval from the US Food and Drug Administration. DAAs target the NS3/4A protease, the NS5A protein, or the NS5B polymerase and when used in appropriate combinations they achieve very high cure rates, with sustained virological responses (SVR) above 90% of cases (Aghemo and De Francesco, 2013).

The recent successes stem from two decades of efforts in building the necessary experimental systems that led to crucial breakthroughs on various aspects of HCV infection. The unique species and tissue tropism of HCV and the difficulty of generating suitable cell culture and small animal models has posed a great challenge to investigate HCV biology and the contribution of host responses to HCV persistence or clearance. On the 25th anniversary of the discovery of HCV, we can look back to assess the progress made and acknowledge that much has been learned about this pathogen. We can now study all the steps of its life cycle *in vitro* and in small animal models, dissect the virus–host interactions that mediate infection, and can begin to understand how the host contributes to establishment of chronicity, and progression of HCV-mediated disease.

HCV represents a peculiar and puzzling example of a virion, in that it circulates in the bloodstream of the infected individuals associated with host lipoproteins in a complex structure called the lipoviral particle (LVP) (Andre et al., 2002). HCV reaches its target organ, the liver, by crossing the fenestrated endothelium and interacting initially with attachment factors like heparan sulfate proteoglycans (HSPGs) and receptors such as the tetraspanin CD81 on the basolateral side of hepatocytes (Barth et al., 2003; Pileri et al., 1998). The interaction of HCV virions with endogenous lipoproteins represents an efficient mode of entry into the liver cells of a new host. In fact, the list of HCV receptors includes two lipoprotein receptors: the scavenger receptor class B, type I (SR-BI)(Scarselli et al., 2002a) and the low-density lipoprotein receptor (LDLR)(Agnello et al., 1999). The model of HCV entry has become increasingly complicated with six novel entry factors identified over the last eight years only: the tight junction molecules claudin-1 (CLDN1) (Evans et al., 2007) and occludin (OCLN) (Ploss et al., 2009), the epidermal growth factor receptor (EGFR) and the ephrin type-A receptor 2 (EphA2) (Lupberger et al., 2011), the cholesterol uptake molecule Niemann–Pick C1-like 1 (NPC1L1) (Sainz et al., 2012) and the transferrin receptor 1 (TFR1) (Martin and Uprichard, 2013). So far, direct proof of interaction with the virus exists for CD81 and SR-BI only, which were identified as candidate receptors precisely for their ability to bind the HCV envelope glycoprotein, E2 (Pileri et al., 1998; Scarselli et al., 2002a). Because of the spatial segregation of HCV receptors into different subcellular domains, active transport towards the apical side is thought to occur, bringing the virus in close proximity of additional entry factors, such as CLDN1 and OCLN. EGFR-mediated signaling via Ras, as well as Rho GTPase, have been proposed to induce lateral movement of CD81-bound virions to the cell-cell contact sites where CD81 would then engage CLDN1. HCV internalization then occurs by clathrin-mediated endocytosis. Finally, delivery of the virus to Rab5a-positive early endosomes should provide the acidic environment necessary to induce fusion (Zeisel et al., 2013). Fusion of the viral envelope with the endosome subsequently releases the viral capsid into the cytoplasm where the viral RNA genome is immediately translated to produce the HCV polyprotein. The concerted action of host- and viral-encoded proteases leads to the production of ten viral proteins. The first three proteins are structural components of the virion: Core, which forms the nucleocapsid, and the viral envelope glycoproteins, E1 and E2. The non-structural (NS) proteins include the ion channel p7, the auto-protease NS2, the protease/helicase NS3/4A, NS4B, NS5A and the viral RNA-dependent RNA polymerase NS5B. The replicase complexes assemble on the endoplasmatic reticulum (ER), forming invaginations of ER membranes called the “membranous web”. It is within these nuclease-resistant compartments that HCV RNA is transcribed. The resulting progeny HCV RNA is then packaged into viral capsids, decorated with viral glycoproteins and secreted from the cells via the secretory pathway. Although the exact mechanism of LVP formation is not fully elucidated, at some point during the maturation process, HCV virions acquire endogenous lipids and a thick shell of host-derived apolipoproteins coating the viral envelope that presumably aid both release and entry of the virus. In fact, the secretion of nascent virions from infected cells is tightly linked to the very-low density lipoprotein (VLDL) biosynthetic pathway, with the strongest evidence being that apoE is essential for particle release (Bartenschlager et al., 2011).

In addition to infecting hepatocytes from the bloodstream, an entry route that is termed “cell-free”, HCV particles can be directly transmitted between neighboring cells, so called “cell-to-cell” spread. The lateral movement of HCV without diffusion through the extracellular environment could facilitate viral dissemination, especially since two of its co-receptors, CLDN1 and OCLN, are recruited to the intercellular interface. The extent to which cell-free versus cell-to-cell transmission contribute to HCV persistence is unknown, but the latter route provides potential advantages in terms of infection efficiency and immune evasion and, as such, may be more relevant for maintaining infection over the course of the years.

In this review, we highlight the main *in vitro* and *in vivo* experimental systems that have enabled studies of HCV so far and provide an outlook on further developments that can improve our ability to understand and treat this virus.

In vitro systems

Since the discovery of HCV in 1989 (Choo et al., 1989), the lack of a cell culture system for the production of infectious HCV virions