Review

Hepatitis C virus cell entry: role of lipoproteins and cellular receptors

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Hepatitis C virus (HCV), a major cause of chronic liver disease, is a single-stranded positive sense virus of the family *Flaviviridae*. HCV cell entry is a multi-step process, involving several viral and cellular factors that trigger virus uptake into the hepatocyte. Tetraspanin CD81, human scavenger receptor SR-BI, and tight junction molecules Claudin-1 and occludin are the main receptors that mediate HCV entry. In addition, the virus may use glycosaminoglycans and/or low density receptors on host cells as initial attachment factors. A unique feature of HCV is the dependence of virus replication and assembly on host cell lipid metabolism. Most notably, during HCV assembly and release from the infected cells, virus particles associate with lipids and very-low-density lipoproteins. Thus, infectious virus circulates in patient sera in the form of triglyceride-rich particles. Consequently, lipoproteins and lipoprotein receptors play an essential role in virus uptake and the initiation of infection. This review summarizes the current knowledge about HCV receptors, mechanisms of HCV cell entry and the role of lipoproteins in this process.

Introduction

Hepatitis C virus (HCV) infection is a major world health problem; it has a prevalence of about 2 %, representing 130–170 million infected people worldwide (Shepard *et al.*, 2005). In most cases (60–85 %), HCV infection progresses to chronic liver disease, which can lead to liver cirrhosis and hepatocarcinoma (Shepard *et al.*, 2005). The current therapy, based on the combination of pegylated interferon with ribavirin, is effective in only 50–80 % of the patients, depending on the virus genotype, and has several serious side effects (Fried *et al.*, 2002; Manns *et al.*, 2001; Keam & Cvetkovic, 2008; Pawlotsky, 2006). A better understanding of the mechanisms leading to the initiation of infection is essential for the development of new therapeutic approaches targeting the early stage of the HCV replication cycle.

HCV is a small, enveloped virus belonging to the family *Flaviviridae* (genus *Hepacivirus*). HCV has a single-stranded positive sense RNA genome of about 9.6 kb, encoding a polyprotein of about 3000 amino acids (aa), which is cleaved into structural (core, E1 and E2) and non-structural (p7, NS2, NS3, NS4A, NS4B, NS5A and NS5B) proteins by host and viral proteases. Structural proteins form viral particles with the help of p7 and NS2, whereas non-structural proteins NS3 to NS5B are involved in genome replication (reviewed by Bartenschlager *et al.*, 2004; Bartenschlager & Sparacio, 2007; Brass *et al.*, 2006; Penin *et al.*, 2004). The putative infectious virus particle is composed of a nucleocapsid or ribonucleoprotein complex bearing the HCV genome; this inner structure is surrounded by a phospholipid bilayer, into which E1 and E2 envelope glycoproteins are anchored. However, infectious HCV particles become tightly associated with very-low-density lipoproteins (VLDL) during virus assembly and are (co-)secreted with VLDL, although the exact nature of this association remains unclear (Gastaminza *et al.*, 2008; Ye, 2007).

Virus entry is defined as the steps from particle binding to the host cell up to the delivery of the viral genome to its replication site within the target cell, which, in the case of HCV, is the human hepatocyte. This process relies on specific interactions between virus components, mainly envelope proteins and multiple cellular factors.

Recent advances in cell culture models have significantly contributed to our understanding of the molecular virology of HCV infection, in particular the entry steps. Before discussing these observations, we will briefly summarize the main model systems used to study HCV cell entry.

Model systems for studies of HCV cell entry

Discerning the mechanisms of HCV cell entry was difficult for a long time due to the absence of an appropriate animal model and an efficient *in vitro* cell culture system supporting the complete HCV life cycle and enabling the

production of infectious virus particles. For unknown reasons, serum-derived HCV (sHCV) poorly replicates in primary human hepatocytes and hepatoma cells *in vitro*. Thus, several surrogate models were used in studies of virus entry before the development of an *in vitro* cell culture system, allowing the reproduction of all steps of the HCV replication cycle, including cell entry (Wakita *et al.*, 2005; Lindenbach *et al.*, 2005).

Plasma-derived HCV

The first approaches to studying HCV infection were based on the inoculation of primary human hepatocytes, T cells and B cells with sHCV (Shimizu *et al.*, 1992; Fournier *et al.*, 1998; Rumin *et al.*, 1999). The primary limits of this system were (i) the low level of HCV replication, which required the use of RT-PCR to detect viral RNA in infected cells, (ii) difficulties in discriminating between newly synthesized and input HCV RNA and (iii) the absence or very low levels of infectious virus particle production (reviewed by von Hahn & Rice, 2008). In addition, a homogeneous and well-characterized inoculum could not be obtained due to virus heterogeneity in the serum (see below) and the association of virus particles with plasma lipoproteins.

Recombinant E2 glycoprotein

In the absence of a reliable *in vitro* model for virus multiplication, a truncated, soluble form of recombinant E2 glycoprotein (sE2) was used to search for candidate receptors involved in HCV cell entry. These approaches allowed the identification of two major HCV receptors: tetraspanin CD81 (Pileri *et al.*, 1998) and human scavenger receptor class B (SR-BI/Cla1) (Scarselli *et al.*, 2002); this provided evidence for the interaction of E2 with heparan sulphate (HS) proteoglycans (Barth *et al.*, 2003). The limitation of this system was the fact that the viral glycoproteins E1 and E2 form a heterodimer on the viral envelope and thus isolated E2 may behave differently; indeed, in addition to hepatocytes, sE2 binds to various cell lines of human origin (Flint *et al.*, 1999; Michalak *et al.*, 1997).

HCV-like particles (HCV-LPs)

HCV-LPs, produced in baculovirus expression systems, bind and enter into hepatoma cells and human primary hepatocytes in a receptor-mediated manner (Barth *et al.*, 2006a). These particles were produced using recombinant baculovirus expression vectors and insect cell cultures (Baumert *et al.*, 1998). Although E1 and E2 form a heterodimeric complex in this system, glycosylation in insect cells does not properly mimic the situation in human cells. Moreover, the particles are not secreted but rather retained in intracellular vesicles making their preparation difficult. Finally, it is not clear how well these particles reflect the earliest stages of infection by authentic HCV.

HCV pseudoparticles (HCVpp)

An important breakthrough in getting access to a system that most closely mimics entry of authentic HCV cell entry was the development of HCV pseudotypes. This system is based on the production of lentiviral particles that incorporate unmodified HCV glycoproteins into the lipid envelope (Bartosch *et al.*, 2003a; Hsu *et al.*, 2003). Production of HCVpp is achieved by co-transfection of 293T cells with plasmids encoding three components: full-length E1 and E2 glycoproteins, retroviral or lentiviral core and polymerase proteins and a proviral genome carrying a marker gene, such as green fluorescent protein or luciferase. These particles are infectious and show a tropism for human liver cells. Moreover, cell entry of HCVpp is neutralized by antibodies directed against the E2 protein (Bartosch *et al.*, 2003a; Keck *et al.*, 2008). The main disadvantage of this system is that it mimics only the very early steps, from particle binding to liberation of the capsid. Most importantly, unlike the natural virus, HCVpp are not associated with lipoproteins, since they are produced in 293T kidney cells that do not synthesize lipoproteins.

Cell culture-produced HCV (HCVcc)

The development of the first *in vitro* model reproducing the complete viral replication cycle and supporting the production of authentic virus particles that are infectious *in vitro* and *in vivo* was an important milestone in the HCV field. This model is based on a particular genotype 2a virus strain, JFH-1, or chimeras of this, cloned from the serum of a patient with fulminant hepatitis C. Subclones of the human hepatoma cell line Huh-7 (Huh7.5 and Huh7-Lunet) transfected with the JFH1 genome efficiently replicate the virus and secrete infectious particles (Lindenbach *et al.*, 2005; Wakita *et al.*, 2005). Besides their capacity to infect naive Huh-7 cells, virus produced *in vitro* is infectious in chimpanzees and in uPA-SCID mice with human liver grafts (Lindenbach *et al.*, 2006; Wakita *et al.*, 2005), a model that has been used largely for the evaluation of antiviral compounds (Meuleman *et al.*, 2005; Meuleman & Leroux-Roels, 2008). Although this HCV *in vitro* replication model mimics a natural HCV infection, it has some important limitations: it is restricted to two particular cell lines (Huh-7 and LH86), which have abnormal lipoprotein metabolism, and essentially to the JFH-1 strain. Although a highly cell culture-adapted genotype 1a strain has been described that also supports production of infectious virus in Huh-7 cells, virus titres are very low and not robust enough to allow detailed studies of virus entry. Moreover, JFH-1 particles produced in hepatoma cells have a lower specific infectivity than virus produced in infected animals (Lindenbach *et al.*, 2006). This observation most likely reflects defective lipoprotein metabolism in Huh-7 cells and, thus, differences in the structure of virus particles produced *in vitro* and *in vivo*. Animal-derived viruses have a lower density but higher infectivity than cell culture-produced viruses, arguing that there is a more extensive or different

association with lipoproteins (Lindenbach *et al.*, 2006; Bukh & Purcell, 2006).

Virus proteins mediating cell entry

HCV envelope glycoproteins E1 and E2 are type I membrane proteins, with a large N-terminal ectodomain and a short C-terminal transmembrane domain. In their functional form, these proteins assemble into a non-covalent heterodimer (Deleersnyder *et al.*, 1997; Dubuisson, 2000). Their synthesis and glycosylation of E1 and E2 take place in the endoplasmic reticulum (ER), where the N-terminal domains of E1 and E2 are directed into the ER lumen (Cocquerel *et al.*, 2002). The transmembrane domains of E1 and E2 are multifunctional: they anchor the glycoproteins to the ER membrane and they contain sequences essential for heterodimerization and their retention in the ER (Cocquerel *et al.*, 2000).

Studies carried out using HCVpp provided evidence that the E1–E2 heterodimer is involved in virus entry, as HCVpp expressing E1 or E2 separately are non-infectious (Bartosch *et al.*, 2003a; Drummer *et al.*, 2003). E2 glycoprotein probably plays a major role in the interaction between the virus and its major cellular receptors CD81 and SR-BI/Cla1. The CD81 binding region of E2 requires correctly folded E2 (Flint *et al.*, 1999), as several E2 regions are engaged in CD81 binding. The first region is located between aa 480 and 493 (Clayton *et al.*, 2002; Flint *et al.*, 1999; Owsianka *et al.*, 2001), the second spans aa 528–535 (Clayton *et al.*, 2002; Owsianka *et al.*, 2001), whereas the third encompasses aa 544–551 (Flint *et al.*, 1999; Owsianka *et al.*, 2001). In addition, specific amino acid residues conserved across all HCV genotypes that are critical for CD81 binding have been identified (W420, Y527, W529, G530 and D535) (Owsianka *et al.*, 2006).

Antibodies directed to aa 412–423 and 432–447 block interaction of the receptor with virus-like particles (Owsianka *et al.*, 2001) and mutation of the region between aa 612 and 620 abolishes CD81 binding. Thus, it has been proposed that a complex interplay between several regions of E2 is responsible for modulating receptor binding, possibly through intramolecular interactions (Roccasecca *et al.*, 2003).

Hypervariable region 1 (HVR1) on E2 glycoprotein is involved in virus interaction with human SR-BI (SR-BI/Cla1) (Scarselli *et al.*, 2002). Due to its high variability, this region may contribute to virus escape from host immune responses (von Hahn *et al.*, 2007). Indeed, antibodies targeting HVR-1 inhibit cell entry of HCVpp and cellular binding of HCV-LPs (Barth *et al.*, 2005; Bartosch *et al.*, 2003b). In spite of the high sequence variability, the conformation and structural properties of HVR-1 are highly conserved, suggesting that HVR1 is indeed functional (Penin *et al.*, 2001); nevertheless, attempts to demonstrate a direct interaction of the receptor with recombinant E1–E2 were unsuccessful (Cocquerel *et al.*,

2006). Moreover, the role of HVR1 in HCV infection *in vivo* remains to be confirmed, as its deletion did not induce a loss of virus infectivity in experimentally infected chimpanzees, although infectivity of the mutant was attenuated (Forns *et al.*, 2000).

HCV envelope glycoproteins are highly glycosylated: 4 and 11 glycosylation sites are highly conserved in E1 and E2 proteins, respectively. Glycosylation may play a key role in the HCV life cycle, as deletion or mutation of some glycosylation sites reduces infectivity of HCVpp. Moreover, some glycans decrease the sensitivity of HCVpp to neutralization by envelope-specific antibodies, but simultaneously mask the CD81 binding site (Goffard *et al.*, 2005; Helle & Dubuisson, 2008; Helle *et al.*, 2007).

The role of E1 in HCV infection remains poorly understood, but it appears to be involved in the fusion process (Garry & Dash, 2003; Lavillette *et al.*, 2007). Moreover, antibodies directed against the N-terminal region of E1 reduce the binding of HCV-LPs to hepatoma cell lines and to dendritic cells (DCs), suggesting a role for this region in HCV cell entry (Barth *et al.*, 2005; Triyatni *et al.*, 2002).

Virus receptors on host cells

Several cell surface molecules, such as tetraspanin CD81 (Pileri *et al.*, 1998), SR-BI/Cla1 (Scarselli *et al.*, 2002), Claudin-1 (CLDN-1) (Evans *et al.*, 2007) and occludin (Liu *et al.*, 2009), are considered to be essential receptors or co-receptors for HCV cell entry. In addition, glycosaminoglycans, such as heparan sulfate, the lectins DC-specific intracellular adhesion molecule-3-grabbing non-integrin (SIGN) and liver-specific (L)-SIGN (Cormier *et al.*, 2004a), and low-density lipoprotein (LDL)-receptor (LDL-R) (Agnello *et al.*, 1999) have been implicated in HCV cell attachment and entry.

Tetraspanin CD81

CD81 is an unglycosylated membrane protein; it is an integral member of the tetraspanin family that is ubiquitously expressed. It contains four transmembrane domains, two small and one large extracellular loops (SEL and LEL, respectively) and N- and C-terminal intracellular domains. CD81 is a part of the B/T cell receptor complex and is involved in the fusion of vesicles (Hemler, 2003; Levy & Shoham, 2005). It is required for normal CD19 expression and plays multiple roles in the processing, intracellular trafficking and membrane functions of CD19 (Shoham *et al.*, 2006).

CD81 has been proposed as an HCV receptor molecule, based on CD81 LEL binding to sE2 (Pileri *et al.*, 1998). This binding appears to be species-specific, as sE2 does not bind mouse or rat CD81 (Flint *et al.*, 2006). Studies with sE2 have demonstrated that the two disulphide bonds, which stabilize the loop domain, are required for the

interaction between sE2 and CD81 (Pileri *et al.*, 1998; Drummer *et al.*, 2005). The role of CD81 in HCV infection was confirmed by several experimental approaches. Indeed, non-permissive human hepatoma cell lines such as HepG2 and HH29, which do not express CD81, become susceptible to HCVcc and HCVpp infection upon ectotopic expression of CD81 after transduction (Bartosch *et al.*, 2003b; Cormier *et al.*, 2004b; Lavillette *et al.*, 2005; Zhang *et al.*, 2004).

An HCV E2 binding region maps to the LEL of CD81 (Pileri *et al.*, 1998; Drummer *et al.*, 2002; Zhang *et al.*, 2004). The site is conformational and a number of specific amino acid residues were found to be critical for the interaction with E2 (Pileri *et al.*, 1998; Bertaux & Dragic, 2006; Drummer *et al.*, 2002; Higginbottom *et al.*, 2000; Zhang *et al.*, 2004).

Monoclonal antibodies (mAbs) directed against CD81 and a soluble form of CD81 LEL inhibited HCVpp and HCVcc infectivity *in vitro* (Bartosch *et al.*, 2003a; Cormier *et al.*, 2004b; Hsu *et al.*, 2003; Molina *et al.*, 2008a; Wakita *et al.*, 2005) and HCV infection *in vivo* (Meuleman *et al.*, 2008). CD81-specific antibodies or downregulation of CD81 expression using siRNA also inhibited infection with sHCV (Molina *et al.*, 2008a; Zhang *et al.*, 2004).

Several studies suggest that CD81 actually acts as a post-binding entry molecule. In fact, antibodies against CD81 and human recombinant CD81 LEL inhibit HCV infection only after virus attachment (Cormier *et al.*, 2004b; Flint *et al.*, 2006; Koutsoudakis *et al.*, 2006). Moreover, susceptibility to infection is related not only to the expression level of CD81 (Akazawa *et al.*, 2007; Koutsoudakis *et al.*, 2007) but also to the proportions of CD81 and SR-BI at the cell surface (Kapadia *et al.*, 2007). EWI-2wint, a cellular partner of CD81 expressed on the cell surface, could efficiently block viral entry by inhibiting viral interaction with CD81 (Rocha-Perugini *et al.*, 2008). The absence of this natural inhibitor of CD81 in hepatic cells may thus enable virus entry and contribute to the hepatotropism of HCV.

Recent studies elucidated the cellular pathways triggered by HCV binding to CD81. Indeed, engagement of CD81 plays a fundamental role in HCV infectivity through the activation of Rho GTP-ases and the actin-dependent relocalization of the E2/CD81 complex to cell–cell contact areas where CD81 comes into contact with the tight junction proteins occludin and CLDN-1 (Brazzoli *et al.*, 2008), molecules recently described as HCV co-receptors (see below). Finally, CD81 engagement activates the Raf/MEK/ERK signalling cascade, which affects post-entry steps of the virus life cycle (Brazzoli *et al.*, 2008). Thus, CD81 is not a mere attachment factor but actively promotes infection by triggering signalling cascades important for virus entry and more downstream events.

Finally, CD81 may also play a role in the modulation of the adaptive immune response through virus interaction with CD81 on T and B cells; this, in turn, could contribute to

virus persistence, liver pathogenesis and, via polyclonal activation of B cells, to extrahepatic manifestations frequently observed in chronic hepatitis C patients (Crotta *et al.*, 2002; Machida *et al.*, 2005; Wack *et al.*, 2001).

SR-BI/Cla1

SR-BI is expressed in various mammalian cells but is mostly expressed in the liver and steroidogenic tissues. It is a 509 aa glycoprotein with two cytoplasmic domains, two transmembrane domains and a large extracellular loop with nine potential *N*-glycosylation sites (Acton *et al.*, 1994; Calvo & Vega, 1993; Rhainds & Brissette, 2004). SR-BI is a 'multi-ligand' receptor for various classes of lipoproteins [high-, low- and very-low-density lipoproteins (HDL, LDL and VLDL, respectively)] as well as for chemically modified lipoproteins such as oxidized and acetylated LDL. Indeed, SR-BI contains distinct binding sites that can independently interact with their respective ligands (Rhainds & Brissette, 2004). In addition, SR-BI may play the role of an endocytic receptor (Rhainds & Brissette, 2004). The essential function of SR-BI is the selective cholesteryl ester (CE) uptake from HDL. During this process, the core of CEs from the HDL particle is delivered into the cell without degradation of the protein moiety. SR-BI-mediated cholesterol efflux to HDL plays an important role in reverse cholesterol transport and atherogenesis (Krieger, 1999; Swarnakar *et al.*, 1999).

In addition to its established function in HDL metabolism, SR-BI plays an important physiological role in the catabolism of VLDL and in the selective CE uptake from VLDL, as recently shown in a mouse model and studies on primary human hepatocytes (Van Eck *et al.*, 2008).

SR-BI/Cla1 has been proposed to act as a putative HCV entry molecule on the basis of its reactivity with sE2 (Scarselli *et al.*, 2002). SR-BI binding to sE2 appears to be species-specific, as mouse SR-BI does not bind sE2. The HVR-1 region of E2 is responsible for binding SR-BI, since deletion of HVR-1 impairs the interaction between SR-BI and sE2, and reduces HCVpp infectivity (Bartosch *et al.*, 2003a; Scarselli *et al.*, 2002). Furthermore, antibodies against SR-BI also significantly reduce HCVpp infectivity (Bartosch *et al.*, 2003b). Similar to CD81, SR-BI acts as a 'post-binding' receptor; indeed, antibodies against both receptors inhibited infection when added until 60 min after virus binding (Cormier *et al.*, 2004b; Zeisel *et al.*, 2007).

Interestingly, HDL, the main SR-BI ligand, facilitates HCVpp and HCVcc cell entry, with no evidence for a direct interaction between HDL and virus particles (Bartosch *et al.*, 2005; Dreux *et al.*, 2006; Voisset *et al.*, 2005). It has been postulated that the enhancing effect of HDL on HCVpp and HCVcc cell entry could be mediated through either an interaction between HDL and lipid membranes or the activation of SR-BI by HDL. HDL decreased the efficiency of HCV neutralizing antibodies by

mechanisms related to the stimulation of cell entry (Dreux *et al.*, 2006). In contrast with the enhancing effect of HDL, other natural SR-BI ligands, such as VLDL (Maillard *et al.*, 2006) and oxidized LDL (von Hahn *et al.*, 2006), had significant inhibitory effects on serum HCV and HCVpp cell entry via SR-BI, respectively.

Several studies suggest that SR-BI/Cla1 co-operatively interacts with CD81 in HCV cell entry. Indeed, HDL enhanced HCVcc infectivity only when CD81 was expressed (Zeisel *et al.*, 2007). Furthermore, the depletion of cholesterol from cholesterol-enriched plasma membrane micro-domains by treatment with methyl-β-cyclodextrin significantly reduced the expression of CD81 but not SR-BI on the plasma membrane, decreasing levels of cell infection with HCVcc and HCVpp (Kapadia *et al.*, 2007). Analogous to its role in the infection of hepatocytes by *Plasmodium falciparum* (Yalaoui *et al.*, 2008), SR-BI could mediate an arrangement of the plasma membrane, acting as a major cholesterol provider and regulating organization of CD81 at the plasma membrane, thus 'boosting' permissiveness of the cell to HCV infection. The role of SR-BI was confirmed by findings that high avidity mAbs directed against SR-BI efficiently block infection of hepatoma cells *in vitro* (even in the presence of HDL) and of chimpanzees with cell-derived HCVcc (Catanese *et al.*, 2007), and that the expression levels of SR-BI can modulate HCVcc infectivity (Grove *et al.*, 2007).

SR-BI binds serum amyloid A (SAA) (Cai *et al.*, 2005), an acute-phase protein produced primarily by the hepatocytes during infection (Uhlar & Whitehead, 1999). Human SAA inhibits HCV cell entry into Huh7.5 cells (Cai *et al.*, 2007). Interestingly, HDL reduces the antiviral effects of SAA, suggesting a relationship between SAA, HDL and HCV infectivity, probably due to the competition between HDL and SAA. However, the exact mechanisms by which SAA affects HCV infectivity require further investigation.

Recently, the antiviral action of interferon has been linked to a decrease in the expression levels of SR-BI on the cell surface, thereby restricting virus attachment and entry into hepatocytes (Murao *et al.*, 2008). These data underline the crucial role of SR-BI in cell infection by HCV.

Tight junction proteins: CLDN1 and occludin

Tight junctions are major components of cell–cell adhesion complexes that separate apical from basolateral membrane domains and maintain cell polarity by forming an intramembrane; this permits diffusion of certain molecules and limits others (Shin *et al.*, 2006). The tight junction multiprotein complex is composed of four types of transmembrane proteins: occludins, Claudins, junction–associated molecules (JAMs) and the coxsackie virus B adenovirus receptors (CARs) (Greber & Gastaldelli, 2007).

Hepatocyte tight junctions play key roles in several liver functions, including bile formation and secretion (Van Itallie & Anderson, 2004). The major transmembrane proteins of the tight junctions are targeted by several reoviruses, coxsackie virus B3 (CVB3), human adenoviruses Ad2/5 (Greber & Gastaldelli, 2007) and HCV (Evans *et al.*, 2007; Liu *et al.*, 2009; Ploss *et al.*, 2009).

CLDN1. Evans *et al.* (2007) have identified CLDN1, a member of the Claudin gene family, which is a new protein involved in HCV entry. CLDN1 is expressed in all epithelial tissues but predominantly in the liver, forming networks at tight junctions (Furuse *et al.*, 1998). The molecule is composed of 211 aa with two extracellular loops, four transmembrane segments and three intracellular domains (Van Itallie & Anderson, 2006). The highly conserved domain in the first extracellular loop (EL1) seems to be involved in HCV entry (Evans *et al.*, 2007).

The expression of CLDN1 confers susceptibility to HCVpp infection in non-hepatic cell lines such as 293T and SW13 (Evans *et al.*, 2007). Moreover, silencing of CLDN1 inhibits HCV infection in susceptible cells (Huh7.5) (Evans *et al.*, 2007). Nevertheless, there is still no evidence for a direct interaction between CLDN1 and the virus. By contrast, functional studies indicate that CLDN1 plays a role in the post-binding phase of infection, subsequent to HCV binding to CD81 and probably subsequent to HCV binding to SR-BI (Evans *et al.*, 2007). Analogous to other viruses (Coyne & Bergelson, 2006), interactions with CLDN1 are thought to take place after a lateral migration of the virus–SR-BI/CD81 complex to tight junctions. Indeed, the initial binding of the viral particle may cluster receptor molecules to activate signalling pathways that promote transfer of the bound virus from the basolateral sinusoidal surface to the tight junction facing the bile canaliculi, since a similar mechanism has been described for the human picornavirus CVB3, whose binding to the decay-accelerating factor on the apical surface of the Caco-2 cell triggers their movement to the tight junctions and their cell entry (Coyne *et al.*, 2007).

Two other members of the Claudin family, CLDN6 and CLDN9, also mediate HCV entry (Zheng *et al.*, 2007; Meertens *et al.*, 2008). As with CLDN1, these molecules are expressed in the liver; however, unlike CLDN1, they are also present in peripheral blood mononuclear cells, another possible HCV replication site in addition to the human hepatocyte. CLDN6 and CLDN9 have a highly conserved EL1 region and a significant sequence homology with CLDN1 (Zheng *et al.*, 2007).

Recent data indicate that distribution of CLDN1 in tight junctions correlates with permissiveness to HCV infection (Liu *et al.*, 2009; Yang *et al.*, 2008), thereby confirming that localization of CLDN1 tight junctions is critical for viral entry and cellular tropism of HCV.

Occludin. CLDN1 has been shown to be essential for HCV infection of human hepatoma cell lines, even though there is still no evidence that CLDN1 binds HCV directly. In addition, human cell lines such as HeLa and HepH

(CD81- and SR-BI-positive) remained HCV resistant when overexpressing CLDN1, suggesting that additional factors were needed for successful HCV cell entry (Evans *et al.*, 2007). Recent studies have provided evidence that another transmembrane component of the tight junctions that is structurally related to Claudins plays an important role in HCV cell entry and initiation of a productive HCV infection (Liu *et al.*, 2009). Occludin is a 60 kDa protein with four transmembrane regions, two extracellular loops and N- and C-terminal cytoplasmic regions (Furuse *et al.*, 1993). Occludin participates in both cell–cell adhesion in the paracellular space and anchoring of the junctional complex to the cytoskeleton. The latter function is accomplished through binding of the C-terminal cytoplasmic region of occludin to scaffolding zonula occludens proteins (ZO-1, -2 and -3) which mediate binding to cytoskeletal actin (Peng *et al.*, 2003). The occludin polypeptide is delivered to the plasma membrane in a microtubule- and temperature-dependent manner, whereas its steady state localization at the cell surface depends on intact microfilaments (Subramanian *et al.*, 2007). Targeting CLDN1 and occludin by siRNA and shRNA interference demonstrated that reduction of the expression of both of these molecules inhibited HCVpp and HCVcc cell entry (Liu *et al.*, 2009). Confocal microscopy analyses showed that occludin accumulates in the ER and co-localizes with the E2 HCV protein (Benedicto *et al.*, 2008). The E2–occludin association was further confirmed by co-immunoprecipitation and pull-down assays (Benedicto *et al.*, 2008; Liu *et al.*, 2009) using a JFH1 variant. Recently, it has been shown that human occludin renders murine cells infectable with HCVpp (Ploss *et al.*, 2009). Together, these data suggest that occludin interacts directly with E2, facilitating viral entry through hepatocyte tight junctions, and that this process may require a delicate molecular architecture of proteins occurring at tight junctions.

A striking observation, however, was that HCV infection may alter localization of tight junction proteins (Benedicto *et al.*, 2008; Liu *et al.*, 2009). Indeed, expression levels of CLDN1 and occludin were downregulated following infection, rendering the infected cells refractory to HCVpp superinfection (Liu *et al.*, 2009). Thus, it appears that HCV infection leads to global reduction of tight junction proteins in HCV infected cells. As tight junction proteins are critical in maintaining the polarity of hepatocytes and their essential functions, altered expression of tight junction proteins may lead to several reported symptoms, including cholestatic disorders.

Glycosaminoglycans (GAGs)

GAGs are linear polysaccharides expressed on the cell surface that act as binding sites for many viruses. GAGs may serve as 'primary', low affinity, but abundant receptors, involved in the initial interaction of the virus with the cell surface prior to binding to high affinity receptors (Barth *et al.*, 2003; Germi *et al.*, 2002). There are different types of GAGs, but highly sulphated GAGs in particular, such as HS, appear to be involved in interaction with several viruses, triggering their subsequent uptake. Likewise, docking between HCV and cellular GAGs has been observed (Barth *et al.*, 2006b; Cribier *et al.*, 1998; Germi *et al.*, 2002; Thomssen *et al.*, 1992). In addition, heparin, an HS analogue, and heparinase, an enzyme degrading HS, inhibit HCV attachment to cells and treating cells with glycosidases reduces HCV infectivity (Barth *et al.*, 2006b; Basu *et al.*, 2007; Koutsoudakis *et al.*, 2006; Morikawa *et al.*, 2007).

Attachment of the virus to HS may play an essential role in the early steps of HCV infection. However, the exact role of GAGs in HCV cell entry remains unclear. sE2 binds to heparin with high affinity, but studies with HCVpp, which carry E1–E2 heterodimers on their surface, failed to confirm these findings (Callens *et al.*, 2005). These observations suggest that either the HS binding site is not accessible on the functional E1–E2 heterodimer or the attachment of authentic HCV particles to cellular GAGs is mediated by lipoproteins associated with virus particles. Indeed, HCV can bind and enter the cell by the HS-dependent pathway due to the interaction of virus-associated lipoproteins with lipoprotein lipase (LPL), mediating HCV cell entry (Andréo *et al.*, 2007).

Lectins: DC-SIGN and L-SIGN

Lectins are another class of molecules involved in binding and cell entry of several viruses. DC-SIGN and L-SIGN are homotetrameric type II membrane proteins from the C-type lectin family. They contain a carbohydrate recognition domain in their extracellular C-terminal region; this domain binds virus carbohydrates in a calcium-dependent manner.

Both lectins are involved in binding, internalization and elimination of a variety of pathogens (Cambi *et al.*, 2005; Van Kooyk & Geijtenbeek, 2003). Some viruses bypass this mechanism and use C-lectins as cell attachment and entry factors. L-SIGN and DC-SIGN bind sE2 with high affinity through high mannose-type oligosaccharides, but also interact with HCVpp (Lozach *et al.*, 2004; Pöhlmann *et al.*, 2003) and natural viruses from sera of infected individuals (Gardner *et al.*, 2003). DC-SIGN is expressed on Kuppfer cells, DCs and lymphocytes. L-SIGN is expressed in liver sinusoidal endothelial cells. DC-SIGN and L-SIGN may thus function as capture receptors capable of transmitting the virus to permissive cells and may play a role in the initiation of HCV infection and tissue tropism (Cormier *et al.*, 2004a; Gardner *et al.*, 2003; Lozach *et al.*, 2004).

The asialoglycoprotein receptor (Stockert, 1995) has also been proposed as a cell surface molecule that might be involved in HCV cell entry (Saunier *et al.*, 2003). Indeed, it is expressed on the surface of liver cells and has the capacity

to bind and internalize HCV-LPs, but its relevance for HCV infection remains to be demonstrated.

Mechanism of HCV cell entry

After attachment to several cell surface molecules, HCV is directed to tight junctions where it interacts with CLDN1 and occludin, which may directly facilitate its cellular uptake. Similar to other flaviviruses, HCV entry is thought to be mediated by clathrin-mediated endocytosis, with delivery of the viral nucleocapsid via (early) endosomes (Fig. 1). This mechanism is pH-dependent, with an optimum at about pH 5.5; indeed, HCVpp and HCVcc entry can be blocked by substances that block acidification of early endosomes (Blanchard et al., 2006; Hsu et al., 2003; Koutsoudakis et al., 2006; Meertens et al., 2006).

E1 and E2 are class II fusion proteins. The HCV fusion process promotes the rearrangement of the E1–E2 hetero-dimer into its active form and leads to the formation of the fusion pore (Lavillette et al., 2006). Characterization of fusion determinants led to the conclusion that three discrete regions of both E1 and E2 proteins participate in the membrane fusion process (Lavillette et al., 2007); indeed, several hydrophobic patches have been identified in both E1 and E2 glycoproteins (Pérez-Berná et al., 2008). However, neither of the HCV receptors has yet been shown to mediate viral fusion. Recent data from our group suggest that HCV cell entry is also dependent on an intact microtubule network firstly for virus transport between its cell attachment site and the site where fusion takes place and also later after nucleocapsid release into the cytoplasm (Roohvand et al., 2009).

Role of lipoproteins in virus cell entry

The liver plays a key role in the metabolism of plasma apolipoproteins, endogenous lipids and lipoproteins. A unique feature of HCV is that both RNA replication and virion assembly depend on cholesterol metabolism and fatty acid biosynthetic pathways in host cells (Huang et al., 2007; Kapadia & Chisari, 2005; Ye et al., 2003; Ye, 2007). Moreover, HCV infection is known to induce large changes in cellular lipid metabolism, including abnormal levels of serum lipoproteins (reduced) in chronic HCV infection and an accumulation of lipids in liver parenchymal cells (steatosis) (André et al., 2005; Petit et al., 2003).

HCV particles in patient sera

Although detailed morphological analysis of authentic HCV particles is missing, we can assume that virus particles are most likely composed of the nucleocapsid surrounded by the lipid envelope into which E1 and E2 glycoproteins are embedded. However, HCV circulates in patient sera as a very heterogeneous population of particles with a density between 1.03 and 1.34 g ml^{-1} (André et al., 2005). Only a minor population of serum particles has properties corresponding to the canonical 'flavivirus-like' particles (Petit et al., 2005). Instead, various unconventional forms of HCV were detected in HCV-positive sera and characterized. These include lipo-viro particles (LVPs) (André et al., 2002; Nielsen et al., 2006), exosomes [membranous vesicles bearing HCV envelope proteins, viral RNA and CD81 (Masciopinto et al., 2004)] and non-enveloped ('naked') nucleocapsids (Maillard et al., 2001).
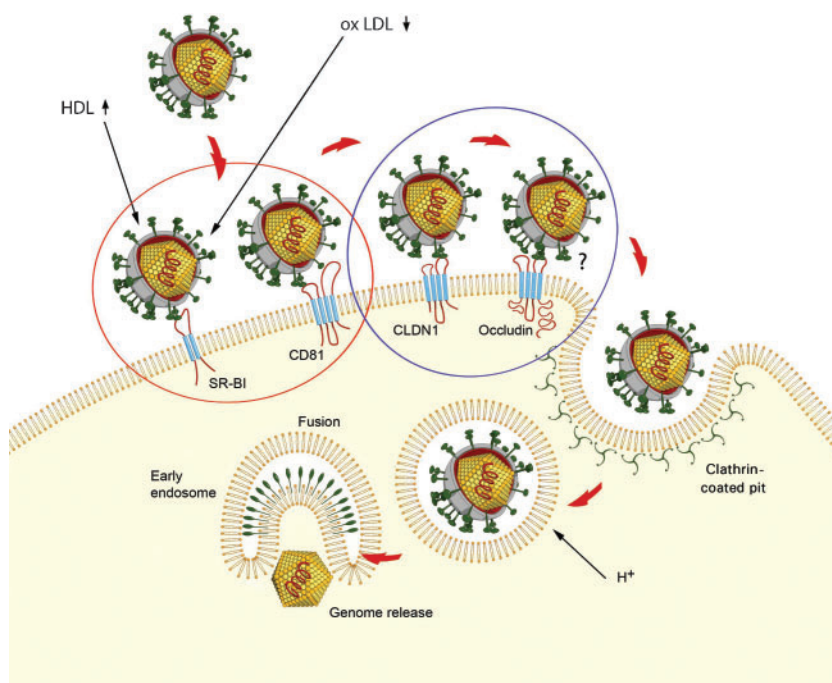
Fig. 1. Model for cellular uptake of HCV particles not associated with lipoproteins. Virus entry is mediated by the direct interaction of envelope glycoproteins with co-receptors. HCV particles are bound, presumably in a consecutive manner, by a complex formed by SR-BI and CD81. Virus associated to CD81 is subsequently transferred to tight junctions where it interacts with CLDN1 and occludin. HCV enters the cell by clathrin-dependent endocytosis and, upon acidification, fusion of the viral envelope, presumably with the membrane of an early endosome, leads to the release of the viral nucleocapsid into the cytoplasm. This model excludes the role of lipoproteins in HCV cell entry and corresponds to cell entry of HCVpp, which, unlike authentic HCV, are not associated with lipoproteins. However, the envelope-mediated HCV entry can be indirectly enhanced by HDL due to its action on the cholesterol transfer function of SR-BI and can be inhibited by oxidized LDL, one of the natural SR-BI ligands.

Most circulating HCV particles are of low density due to association with β-lipoproteins (Thomssen *et al.*, 1992; Agnello *et al.*, 1999; Prince *et al.*, 1996). Only a very low-density population of sHCV was highly infectious in chimpanzees (Beach *et al.*, 1992; Bradley *et al.*, 1991) and tissue culture cells, and LDL-R appeared to play an important role in infection (Agnello *et al.*, 1999; André *et al.*, 2002; Bradley *et al.*, 1991; Monazahian *et al.*, 1999). In contrast, high-density populations of HCV were poorly infectious (Bradley *et al.*, 1991; Agnello *et al.*, 1999). These studies suggest that lipoproteins play a critical role in the infectivity of natural HCV.

Low-density, highly infectious HCV in the serum primarily corresponds to LVPs, which are lipoprotein-like structures composed of triglyceride-rich lipoproteins containing apolipoprotein (Apo)B and ApoE, viral nucleocapsids and envelope glycoproteins (André *et al.*, 2002; Nielsen *et al.*, 2006; Diaz *et al.*, 2006). These particles contain ApoB100, which is of hepatic origin and an integral part of VLDL. However, the presence of ApoB48, normally produced by enterocytes, suggests that a part of circulating LVPs may originate not only from the liver but also from the intestine (Diaz *et al.*, 2006).

Recently, Lindenbach *et al.* (2006) provided evidence that the specific infectivity of HCVcc produced *in vitro* and recovered from experimentally infected chimpanzees or uPA-SCID mice with human liver grafts was higher and buoyant density was lower than those of the same virus strain produced in cell culture. Thus, the association between virus particles and lipoproteins may confer higher viral infectivity. In accordance with this notion, HCVcc infection *in vitro* could be efficiently inhibited not only by antibodies directed against the HCV envelope but also by antibodies against ApoB-containing lipoproteins (Andréo *et al.*, 2007), supporting the essential role of lipoproteins in virus cell entry and in the initiation of infection.

Role of lipoproteins in virus interaction with SR-BI/Cla1

No evidence for an interaction between SR-BI and the E1–E2 heterodimer is available (Cocquerel *et al.*, 2006), despite the direct interaction between sE2 and SR-BI (Scarselli *et al.*, 2002) and the unequivocal role of this receptor in HCVpp and HCVcc cell entry (Kapadia *et al.*, 2007; Bartosch *et al.*, 2003b). In addition, natural serum-derived HCV does not recognize SR-BI directly via viral envelope glycoproteins; instead, ApoB lipoproteins associated with virus particles play a key role in the initial interaction of the receptor by serum-derived authentic HCV (Maillard *et al.*, 2006). In fact, polyvalent high-affinity anti-E1 and -E2 antibodies, or antibodies directed against HVR-1 and inhibiting HCVpp infection, did not block virus–SR-BI interactions. In contrast, VLDL, a natural ligand of the receptor, and antibodies directed against β-lipoproteins were found to be extremely efficient inhibitors of SR-BI-mediated virus uptake. Thus, natural serum-derived HCV

recognizes SR-BI indirectly via ApoB-containing lipoproteins (mainly VLDL) associated with virus particles. VLDL promotes virus uptake and protects the virus against potentially neutralizing antibodies. These data corroborate *in vitro* studies showing that SR-BI facilitates the clearance of VLDL (Van Eck *et al.*, 2008) and clinical observations that genetic variation of the SR-BI gene locus is associated with alterations in the metabolism of ApoB-containing lipoproteins in humans (Peréz-Martínez *et al.*, 2003). These data could explain the co-existence of infectious virus particles with neutralizing antibodies in the sera of most HCV-infected individuals; the antibodies are potentially neutralizing but are not able to control chronic HCV infection (von Hahn *et al.*, 2007; Haberstroh *et al.*, 2008).
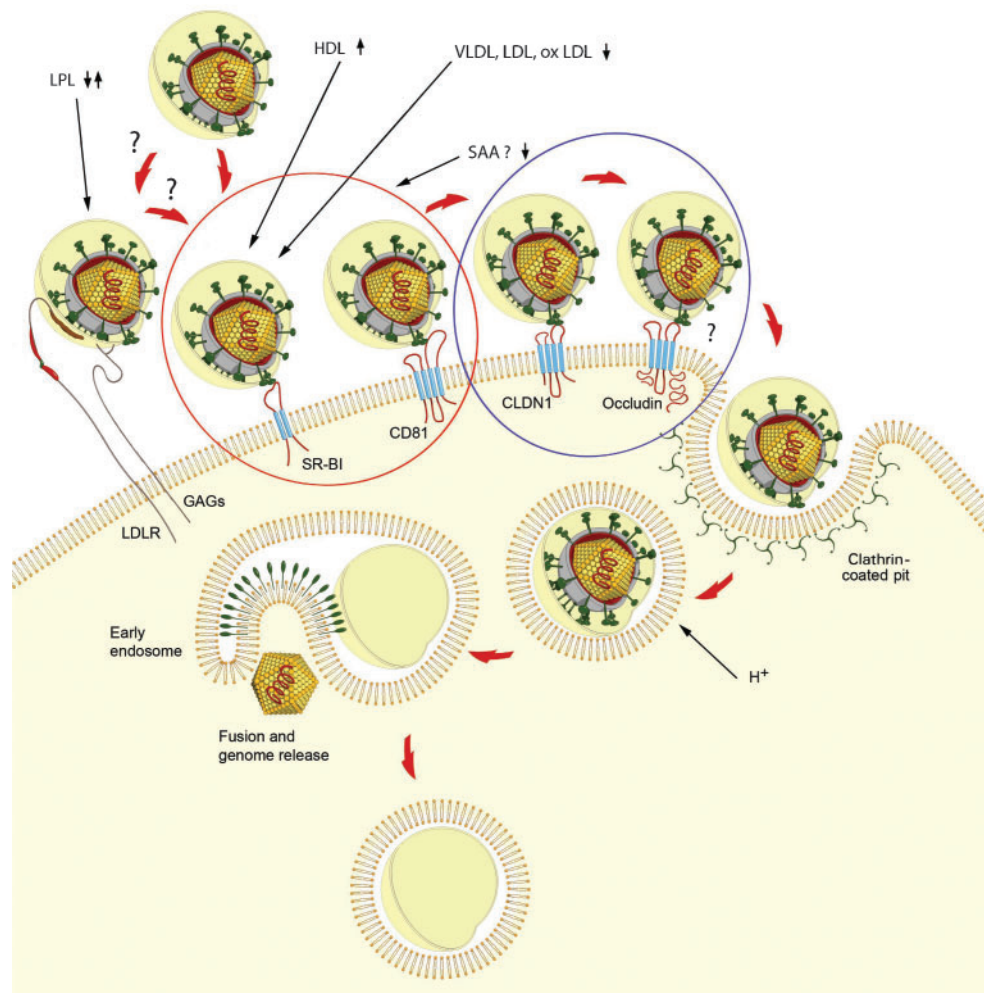
LDL-R

Another lipoprotein receptor potentially involved in the uptake of lipoprotein-associated HCV into hepatocytes is LDL-R. Hepatocytes acquire cholesterol via endocytosis involving LDL-R. The most important ligands for this receptor are LDLs, which are responsible for the transport of the majority of plasma cholesterol. After binding to the receptor, LDLs are internalized by clathrin-mediated endocytosis and are transported to endosomes, where the acidic pH induces the release of lipoprotein particles from LDL-R. Lipoproteins are subsequently degraded in lysosomes and cholesterol is released into the cells (Rudenko & Deisenhofer, 2003).

The role of LDL-R in HCV entry was first proposed by Agnello *et al.* (1999). The authors demonstrated that lipoprotein-associated HCV from patient sera and other viruses from the family *Flaviviridae* utilize LDL-R for cell entry. In accordance with these observations, LVPs isolated from patient sera were shown to infect hepatoma cells in an LDL-R-dependent manner (André *et al.*, 2002). Nevertheless, the function of LDL-R in HCV infection remains controversial, as the role of this receptor in the *in vitro* HCVcc infection model has not yet been demonstrated. More recently, the significance of LDL-R in HCV infection of primary human hepatocytes has been confirmed (Molina *et al.*, 2007). Moreover, the correlation of cell surface expression of LDL-R in patients with chronic HCV infection and with a high viral load implies that LDL-R is actually involved in the viral replication cycle *in vivo* (Petit *et al.*, 2007). It is conceivable that, similar to SR-BI, LDL-R may participate in virus cell entry via interaction with lipoproteins that are associated with HCV at an early step of infection, preceding virus interaction with CD81 (Fig. 2). However, one cannot exclude the possibility that LDL-R mediates an alternative pathway of HCV cell entry.

Role of LPL in HCV cell entry

Nascent VLDL particles released from the liver are substrates for LPL, a key enzyme in the metabolism of lipoproteins, which hydrolyses triglycerides in the core of

Fig. 2. Cell entry of natural, ApoB-associated HCV. Virus binding and internalization is initiated by the interaction between HCV-associated lipoproteins (mainly VLDL) with lipoprotein receptors SR-BI and/or LDL-R and/or GAGs. HCV cooperates with the SR-BI–CD81 complex and the virus is subsequently transferred by CD81 to tight junction proteins CLDN-1 and occludin. Virus enters the cell from the tight junction via endocytosis and fusion is mediated by envelope glycoproteins; this event permits the virus to escape the lipoprotein degradation pathway. Lipoprotein-mediated HCV cell entry is inhibited by natural ligands of lipoprotein receptors such as VLDL, LDL and oxidized LDL. Cell entry can also be inhibited by SAA, enhanced by HDL and regulated by LPL. This model corresponds to the cell entry of HCVcc and natural HCV from patient sera, which are associated to various extents with ApoB-containing lipoproteins.

lipoprotein particles and targets lipoproteins to the liver for hepatic uptake (Mead *et al.*, 2002). After digestion by LPL, VLDL particles are transformed into intermediate-density lipoproteins; these are efficiently removed from the plasma by the LDL-R family on hepatocytes or further transformed by hepatic lipase into LDL (Merkel *et al.*, 2002).

We have recently demonstrated that LPL mediates the binding and uptake of lipoprotein-associated HCV particles to different types of cells (Andréo *et al.*, 2007). The mechanism of action of LPL on natural HCV involves the formation of a bridge between virus-associated lipoproteins and HS at the cell surface (Andréo *et al.*, 2007). LPL-mediated cellular uptake of the virus may involve HCV receptors or receptors from the LDL-R family or the HS endocytic pathway, independent from other receptors. LPL mediates cellular uptake of HCV to human hepatoma cells, but has a potent inhibitory effect on HCVcc infection *in vitro* (JFH1 strain), probably leading to a non-productive pathway of virus cell entry in this experimental system (Andréo *et al.*, 2007). Since lipoproteins associated with virus particles are essential for LPL-mediated virus uptake, these observations suggest that LPL could be a natural modulator of HCV infectivity *in vivo* and that the LPL effect could depend on the composition of virus-associated lipoproteins.

Role of apolipoproteins in HCV cell entry

Human apolipoproteins are protein constituents of chylomicrons, VLDL and HDL. In particular, ApoCs have an inhibitory or stimulatory effect on a variety of receptors [such as LDL-R, VLDL-R and LDL-R-related protein (LRP)] and enzymes involved in lipoprotein metabolism (such as LPL and hepatic lipase). Indeed, ApoC2 is an important activator of LPL, whereas ApoC3 inhibits lipolysis of TG-rich lipoproteins, hampering the interaction of these lipoproteins with the HS–LPL complex (Jong *et al.*, 1999), and acts as an inhibitor of lipoprotein receptors (LDL-R and LRP).

HCV circulating in patient sera contains ApoE and ApoB as a part of VLDL and/or LDL (André *et al.*, 2002; Maillard *et al.*, 2006; Nielsen *et al.*, 2006). The association of ApoC1 with infectious particles in the serum of experimentally infected chimpanzees has also been documented (Meunier *et al.*, 2008).

Apolipoproteins are also efficient regulators of HCV infectivity. Indeed, ApoC1, an exchangeable apolipoprotein that predominantly resides in HDL, enhances HCVpp cell entry when exogenously supplied (Meunier *et al.*, 2005). When released from HDL, ApoC1 promotes HCV membrane fusion between viral and cellular membranes in the liposome system, suggesting interplay between the HVR1 of the HCV E2 glycoprotein, HDL and SR-BI (Dreux *et al.*, 2007). siRNAs targeting ApoB (Huang *et al.*, 2007) or ApoE (Chang *et al.*, 2007) efficiently inhibit release of HCV and HCV infectivity is positively correlated with levels of secreted ApoE (Chang *et al.*, 2007). As all these apolipoproteins (B, E and C1) are components of VLDL, these studies confirm that mechanisms of infectious HCV secretion follow the secretion pathway of VLDL, and underline the enhancing effect of lipoproteins (and their protein components) on the infectivity of the virus.

ApoC3 is a known inhibitor of lipoprotein receptors (LDL-R and LRP) and LPL activity (Jong *et al.*, 1999). Recent studies identified ApoC3 as a potential plasma biomarker associated with the resolution of acute HCV infection (Molina *et al.*, 2008b), suggesting an important role of this apolipoprotein in the outcome of HCV infection.

Role of lipoproteins in the formation and secretion of HCV particles

Triglyceride-rich VLDL, the main lipoprotein component of HCV in patient sera, is produced and secreted by hepatocytes. VLDL assembly requires microsomal triglyceride transfer protein (MTP), which transfers triglycerides from cytosolic lipid droplets or the ER to nascent ApoB100 (Shelness & Sellers, 2001). This ApoB-containing VLDL precursor (pre-VLDL) subsequently fuses with triglyceride droplets in the ER/golgi luminal compartment. This process is also mediated by MTPs (Shelness & Sellers, 2001). Incomplete VLDL is not secreted by hepatocytes, but is targeted for degradation by LDL-R (Larsson *et al.*, 2004).

Recent studies provided evidence that HCV-associated lipoproteins are not simply adsorbed at the surface of virus particles circulating in patient sera, but that VLDLs are an integral part of HCV particles. Indeed, Nielsen *et al.* (2006) have shown that ApoB remains associated with infectious HCV after treatment of the virus with either deoxycholic acid or NP-40. This indicates that a firm binding exists between HCV and ApoB-containing lipoproteins. In addition, the production and release of HCV by the human hepatoma cell line Huh-7 depends on the assembly and secretion of VLDL, and drugs that block VLDL assembly (such as MTP inhibitors or siRNA targeting ApoB or ApoE) also inhibit the secretion of HCV particles (Huang *et al.*, 2007). In accordance with these observations, the density of intracellular virus particles produced *in vitro* is much higher than that of secreted HCVcc (Gastaminza *et al.*, 2006, 2008). High-density (immature) HCV particles are actively degraded in a proteasome-independent manner, whereas low-density, i.e. VLDL-associated, HCV particles are efficiently secreted from infected cells. These findings provide an explanation for the presence of very-low-density, infectious virus particles circulating in patient sera and the role of lipoprotein receptors for virus cell entry.

The property that makes the virus unique is that the entire virion is not exposed to serum during circulation and virus particles have to escape from VLDL-derived lipoprotein particles in endocytic vesicles. Exactly what triggers the escape of the virus from VLDL particles during cell entry so that E2 glycoprotein can react with virus receptors and induce envelope fusion still remains to be elucidated.

Conclusions

Currently available data suggest that HCV cell entry is a multi-step process requiring a set of entry molecules. CD81, SR-BI/Cla1 and the tight junction proteins CLDN1 and occludin are essential (co-)receptors for HCV cell entry. Recent data support the model that HCV enters the cell from tight junctions, even if the exact sequence of events leading to infection still remains to be elucidated.

Recent studies underline the importance of VLDL in the assembly and secretion of infectious HCV particles, in accordance with the association of the majority of the circulating virus with ApoB- and ApoE-containing lipoproteins and their role in virus infectivity. Several observations suggest that lipoproteins play an important role in virus cell entry and initiation of infection. It is conceivable that HCV infection is initiated by the interaction between the lipoprotein-associated virus particle and lipoprotein receptors SR-BI/Cla1 and/or LDL-R. In addition, cell surface proteoglycans facilitate infection, probably in a lipoprotein-dependent manner (Fig. 2).

Apart from lipoproteins, other host molecules, such as LPL (Andréo *et al.*, 2007) or EWI-2wint (Rocha-Perugini *et al.*, 2008), may contribute to the hepatotropism of HCV.

Indeed, LPL, which targets lipoproteins to the liver, may be involved in the early steps of HCV cell entry. LPL may act as a natural modulator of HCV infectivity, affecting the lipoprotein composition of virus particles, which is apparently different for viruses produced *in vivo* and those produced in currently available *in vitro* cell culture models (Huh-7 and LH86 hepatoma cell lines). Additionally, EWI-2wint, a cellular partner and natural inhibitor of CD81, could also regulate cell invasion by HCV. The various forms of HCV circulating in patient sera could allow the virus to use different pathways of cell entry and thus different modes of infection.

The development of the HCVcc system is a major accomplishment, permitting important insights into virus–host cell interaction. However this system also has some limitations, including genetic defects of the cell lines used, which do not permit normal production of lipoproteins compared with primary human hepatocytes. These defects may affect virus composition; therefore, the results obtained in this culture system do not necessarily reflect the *in vivo* situation properly (Bukh & Purcell, 2006).

A detailed understanding of the mechanism of HCV entry is fundamental for the development of new therapeutic strategies to fight HCV infection. Indeed, molecules that target envelope glycans (such as inhibitors of glycosylation) or lectins (such as cyanovirin-N) to inhibit virus binding to cell surface receptors or imminosugars have been proposed as antiviral drugs (Helle *et al.*, 2006; Steinmann *et al.*, 2007). Likewise, drugs that downregulate expression of HCV receptors at the surface of hepatocytes may be used to block HCV cell entry. Indeed, alpha interferon, in addition to other effects, downregulates cell surface expression of SR-BI and LDL-R (Murao *et al.*, 2008). Finally, molecules targeting the microtubule network may prevent initiation of productive HCV infection and drugs downregulating hepatic VLDL production limit virus propagation by affecting morphogenesis and release of progeny virus from infected cells. In light of the currently limited therapeutic options, the need for more efficacious therapies is obvious.

Acknowledgements

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