Hepatitis C virus entry: potential receptors and their biological functions

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Several cellular molecules have been identified as putative receptors for Hepatitis C virus (HCV): CD81 tetraspanin, scavenger receptor class B type I (SR-BI), mannose-binding lectins DC-SIGN and L-SIGN, low-density lipoprotein receptor, heparan sulphate proteoglycans and the asialoglycoprotein receptor. Due to difficulties in propagating HCV in cell culture, most of these molecules have been identified by analysing their interaction with a soluble, truncated form of HCV glycoprotein E2. A recent major step in investigating HCV entry was the development of pseudoparticles (HCVpp), consisting of unmodified HCV envelope glycoproteins assembled onto retroviral core particles. This system has allowed the investigation of the role of candidate receptors in the early steps of the HCV life cycle and the data obtained can now be confirmed with the help of a newly developed cell-culture system that allows efficient amplification of HCV (HCVcc). Interestingly, CD81 and SR-BI have been shown to play direct roles in HCVpp and/or HCVcc entry. However, co-expression of CD81 and SR-BI in non-hepatic cell lines does not lead to HCVpp entry, indicating that other molecule(s), expressed only in hepatic cells, are necessary for HCV entry. In this review, the molecules that have been proposed as potential HCV receptors are described and the experimental data indicating that CD81 and SR-BI are potentially involved in HCV entry are presented.

Introduction

To initiate its life cycle, a virus must bind to the host cell and cross the plasma membrane to gain access to the inner contents of the cell. Attachment is mediated by the binding of a protein present at the surface of the virion to a molecule on the cell surface, acting as a virus receptor. Viral attachment can occur as a multistep process, involving more than one type of receptor or co-receptor. After binding to the host cell, enveloped viruses gain entry to the cytoplasm by fusing their lipid envelope with the plasma membrane or an endosomal membrane.

Hepatitis C virus (HCV) is a small, enveloped virus that belongs to the genus Hepacivirus in the family Flaviviridae (Lindenbach & Rice, 2001). Its genome encodes a single polyprotein precursor of about 3000 amino acid residues, which is cleaved co- and post-translationally by cellular and viral proteases to yield at least 10 mature products. The HCV genome encodes three structural proteins: a capsid protein and two envelope glycoproteins, E1 and E2. The envelope glycoproteins interact with each other to form a non-covalent heterodimer (reviewed by Op De Beeck et al., 2001). This glycoprotein complex is the viral component present at the surface of HCV particles (Wakita et al., 2005) and it is therefore the obvious candidate ligand for cellular receptor(s). In addition, glycoprotein E2 is the subunit involved in interactions with putative receptor(s) (Rosa et al., 1996).

Due to difficulties in propagating HCV in cell culture, a soluble, truncated form of HCV glycoprotein E2 (sE2) (Fig. 1) has been used by several laboratories as a tool to search for cell-surface proteins potentially involved in HCV entry.

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Fig. 1. Schematic representation of HCV glycoprotein E2 and the truncated soluble form sE2. Numbers correspond to the positions in the HCV polyprotein of reference strain H (GenBank accession no. AF009606). Hypervariable regions 1 (HVR1) and 2 (HVR2) and the transmembrane domain (TMD) are indicated. Arrows show the position of amino acid residues 420, 527, 529, 530 and 535, which are potentially involved in binding to CD81 (Patel et al., 2005).
entry. This approach has allowed the identification of a series of molecules proposed as candidate receptors for HCV. However, validation of a viral receptor requires proof that the putative receptor is essential for infection. Until recently, this was not easy for HCV, due to the absence of a robust cell-culture system to amplify this virus. In this review, we present the model systems developed recently for studying HCV entry and we describe the molecules that have been proposed as potential HCV receptors. We also present the experimental data indicating that some of these molecules are potentially involved in HCV entry. Finally, we discuss the potential role of the most promising candidates.

**Model systems to study the relevance of potential HCV receptors**

During the past decade, several laboratories have tried to develop surrogate models to study HCV entry (reviewed by Op De Beeck & Dubuisson, 2003). Most of these models are based on the expression of HCV envelope glycoproteins. However, many difficulties have been encountered by using these approaches, because HCV envelope glycoproteins are mainly located in the endoplasmic reticulum and their folding and assembly are very sensitive to mutations or deletions affecting the endoplasmic reticulum-retention domains (reviewed by Op De Beeck et al., 2001). As a first approach, virus-like particles (VLPs) have been produced in insect cells infected by a recombinant baculovirus containing the cDNA of HCV structural proteins (Baumert et al., 1998). However these VLPs are not infectious and they are retained in an intracellular compartment. It is therefore difficult to evaluate how close these VLPs are to native virions. In addition, due to the absence of infectivity, these VLPs cannot be used for functional studies.

A major advance in investigating HCV entry has been achieved by the development of pseudoparticles (HCVpp), which consist of unmodified HCV envelope glycoproteins assembled onto retroviral core particles (Bartosch et al., 2003a; Drummer et al., 2003; Hsu et al., 2003). Extensive characterization of HCVpp has shown that these mimic the early steps of the HCV life cycle. Indeed, they exhibit a preferential tropism for liver cells and they are neutralized specifically by anti-E2 monoclonal antibodies (mAbs), as well as sera from HCV-infected patients (Bartosch et al., 2003a; Hsu et al., 2003; Op De Beeck et al., 2004). Studying entry with HCVpp has shed some light on the role of some cell-surface molecules involved in the early steps of the HCV life cycle.

More recently, several laboratories have also reported the development of a cell-culture system that allows a relatively efficient amplification of HCV (HCVcc) (Lindenbach et al., 2005; Wakita et al., 2005; Zhong et al., 2005). This system is based on the transfection of the human hepatoma cell line Huh-7 with genomic HCV RNA derived from a cloned viral genome (Wakita et al., 2005). This cell-culture system permits, for the first time, the study of the whole life cycle of HCV and it is also playing an important role in the validation of molecules involved in HCV entry.

**CD81 and HCV entry**

**Identification of CD81 as a molecule involved in HCV entry**

By using sE2 as a probe to identify cell-surface proteins potentially involved in HCV entry, Pileri et al. (1998) have identified a cell-surface molecule, called CD81, as a first putative receptor for HCV. CD81 is a member of the tetraspanin family, containing four transmembrane domains, short intracellular domains and two extracellular loops called the small extracellular loop (SEL) and the large extracellular loop (LEL). The LEL contains characteristic motifs, especially the conserved cysteine–cysteine–glycine (CCG) motif involved in the formation of disulphide bridges (Seigneuret, 2006) (Fig. 2a).

Studies with HCVpp and HCVcc have confirmed the involvement of CD81 in HCV entry. Indeed, HCVpp show a restricted tropism for human hepatic cell lines expressing CD81 (Bartosch et al., 2003a, b; Hsu et al., 2003; Cormier et al., 2004a; Zhang et al., 2004). Anti-CD81 mAbs, as well as a recombinant, soluble form of CD81 LEL, inhibit the entry of both HCVpp and HCVcc into hepatoma cell lines (Bartosch et al., 2003a; Hsu et al., 2003; Cormier et al., 2004a; Zhang et al., 2004; Lavillette et al., 2005; Wakita et al., 2005; Zhong et al., 2005). In addition, it has also been shown that a threefold reduction in CD81 cell-surface expression by small interfering RNAs abolishes infection of Huh-7 hepatoma cells by HCVpp (Zhang et al., 2004). Although CD81 is normally expressed at the surface of primary hepatocytes and potentially in hepatoma cell lines, it has been observed that HepG2 cells do not express CD81. Interestingly, the ectopic expression of CD81 in non-permissive HepG2 cells confers susceptibility to infection by HCVpp and HCVcc (Bartosch et al., 2003b; Cormier et al., 2004a; Zhang et al., 2004; Lavillette et al., 2005; Lindenbach et al., 2005), providing additional evidence of the involvement of CD81 in HCV entry. A chimeric molecule between CD81 and the closely related tetraspanin CD9 has been made to map the CD81 domain functionally involved in HCVpp entry. This approach has confirmed that the LEL domain of CD81 is a determinant for viral entry (Zhang et al., 2004). Altogether, these data demonstrate that CD81 plays a critical role in HCV entry.

**Regions of interaction between E2 and CD81**

The CD81 binding site for E2 has been localized within the LEL (Pileri et al., 1998) and specific LEL amino acid residues essential for this interaction have been identified (Higginbottom et al., 2000; Drummer et al., 2002). Recognition of CD81 by sE2 requires intact disulphide bonds in CD81 (Petracca et al., 2000) and the binding site for E2 has been mapped to the variable double-helix subdomain (Fig. 2b) (Kitadokoro et al., 2001; Drummer et al., 2002). Interestingly, the homologous region in the tetraspanin...
CD9 is important for egg–sperm fusion (Zhu et al., 2002) and the same region in the tetraspanin CD151 is required for its lateral interaction with α3 integrin (Kazarov et al., 2002). It is important to note that the identification of CD81 residues involved in interactions with E2 was done with sE2. However, structural differences exist between truncated forms of E2 and the full-length E1E2 complex. Differences in the efficiency of binding to CD81 have indeed been observed between sE2 and E1E2 (Cocquerel et al., 2003). In addition, differences have been observed when comparing the sE2–CD81 interaction and HCVpp infectivity (Zhang et al., 2004). Indeed, a CD81 mutation, which has previously been reported to disrupt the sE2–CD81 interaction, has been shown to confer susceptibility to HCVpp infection in HepG2 cells. As the presence of E1 might potentially affect the properties of E2 (Cocquerel et al., 2003; Brazzoli et al., 2005), it would be interesting to reanalyse the E2–CD81 interaction in the context of the E1E2 heterodimer, which is a more physiological ligand. It is also worth noting that differences in sE2 binding can be observed between LEL and the full-length CD81 (Drummer et al., 2005). Altogether, these data suggest that biochemical studies with sE2 and CD81 LEL do not necessarily reflect the reality of E2–CD81 interaction as it occurs during virus entry.

Despite numerous studies using different forms of E2 and blocking mAbs as tools to identify amino acid residues critical for CD81 binding, the E2 regions involved in CD81 interaction have not yet been well defined (Table 1). This is partly due to the use of different forms of recombinant E2 protein and to indirect approaches, such as binding inhibition by mAbs. A more recent analysis using alanine-scanning mutagenesis in the context of HCVpp is potentially providing more accurate data on E2 residues involved in contacts with CD81. Indeed, this approach has shown that residues at positions 420, 527, 529, 530 and 535 are potentially involved in binding to CD81 (Patel et al., 2005).

Several studies have suggested that HCV glycoproteins might display genotype-specific differences in CD81-binding affinity (Yagnik et al., 2000; Roccasecca et al., 2003; Shaw et al., 2003). For instance, sE2 from genotype 3 failed to bind to CD81 (Shaw et al., 2003). However, as discussed above, biochemical studies with sE2 do not necessarily reflect the reality of E2–CD81 interaction as it occurs during virus entry and studies using HCVpp have shown that CD81 is necessary for all HCV genotypes to enter host cells (McKeating et al., 2004; Lavillette et al., 2005). However, McKeating et al. (2004) have highlighted subtle differences
in CD81 residues that can lead to modulation of HCVpp entry for different HCV subtypes.

Role of CD81 in HCV entry

The exact role of CD81 in HCV entry is not well understood. Although its role in virus entry has been confirmed with the HCVpp and HCVcc systems, ectopic expression of the human CD81 in non-hepatic cell lines does not lead to HCVpp entry, suggesting that additional molecule(s) is/are needed for HCV entry (Bartosch et al., 2003). Cormier et al. (2004a) have proposed that CD81 may function as a post-attachment entry co-receptor, suggesting that CD81 might play a role after binding of the particle to a first receptor. However, additional experimental evidence is needed to confirm such a role. Interestingly, members of the tetraspanin superfamily are able to form lateral associations with multiple partner proteins and with each other in a dynamic assembly, described as the 'tetraspanin web' (Boucheix & Rubinstein, 2001; Levy & Shoham, 2005). As members of the tetraspanin family are known to be involved in membrane-fusion processes, our current knowledge cannot exclude a role for CD81 in the fusion step. Indeed, CD81 and CD9 have been shown to be involved in egg–sperm fusion (Kaji et al., 2002; Higginbottom et al., 2003; Rubinstein et al., 2006) and mice that are deficient in these tetraspanins develop multinucleated giant cells spontaneously in the lung (Takeda et al., 2003). In addition, CD81 has been shown to be involved in human T-cell leukemia virus type I-induced syncytium formation (Imai & Yoshie, 1993). HCV is not the only pathogen dependent on CD81 for its infectious cycle. It has recently been observed that CD81 is required on hepatocytes for human Plasmodium falciparum and rodent Plasmodium yoelii sporozoite infectivity (Silvie et al., 2003). P. yoelii sporozoite fails to infect CD81-deficient mouse hepatocytes and antibodies against human and mouse CD81 inhibit the hepatic development of P. falciparum and P. yoelii, respectively. Although the mechanism is still unclear, CD81 may be involved directly or indirectly in the formation of a parasitophorous vacuole that is essential for parasite differentiation (Silvie et al., 2004). Whether HCV and Plasmodium spp. use CD81 in a similar way for infectivity remains, however, to be determined.

SR-BI and HCV entry

Identification of SR-BI as a molecule involved in HCV entry

By using sE2 as a probe to identify cell-surface proteins potentially involved in HCV entry, Scarselli et al. (2002) have identified the human scavenger receptor class B type I (SR-BI) as another putative receptor for HCV. Although the

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**Table 1. Regions of E2 potentially involved in CD81 interaction**

<table>
<thead>
<tr>
<th>E2 residues/regions*</th>
<th>Form†</th>
<th>Assay</th>
<th>Reference</th>
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<tbody>
<tr>
<td>517–535</td>
<td>Cell surface-expressed E2-715</td>
<td>Blocking antibodies‡</td>
<td>Forns et al. (2000)</td>
</tr>
<tr>
<td>412–423</td>
<td>E2-660</td>
<td>Blocking antibodies</td>
<td>Owsianka et al. (2001)</td>
</tr>
<tr>
<td>HVRs/613–618</td>
<td>E2-661</td>
<td>Blocking antibodies</td>
<td>Owsianka et al. (2003)</td>
</tr>
<tr>
<td>HVR1</td>
<td>HCVpp</td>
<td>Deletion</td>
<td>Callens et al. (2005)</td>
</tr>
<tr>
<td>420, 527, 529, 530 and 535</td>
<td>HCVpp</td>
<td>Ala scanning</td>
<td>Patel et al. (2005)</td>
</tr>
</tbody>
</table>

*Positions of amino acids in the polyprotein of reference strain H (GenBank accession no. AF009606).
†E2-661, E2-715, E2-683 and E2-660 are for sE2 ending at indicated positions.
‡Indicated E2 regions correspond to the epitopes of blocking antibodies.
§Virus-like particles produced in insect cells.

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human homologue of SR-BI is called CLA-1 (for CD36 and LIMPII Analogous-1) (Calvo & Vega, 1993), the name human SR-BI is generally used in HCV studies. SR-BI is a 509 aa cell-surface glycoprotein (Calvo & Vega, 1993; Acton et al., 1994) that contains two short cytoplasmic domains and two membrane-spanning domains, separated by a large extracellular domain of which the structure is currently unknown (Fig. 3a) (Rhainds & Brissette, 2004). The human SR-BI contains nine potential N-glycosylation sites (Viñals et al., 2003; Rhainds & Brissette, 2004). In addition, it has been shown to be palmitoylated (Babitt et al., 1997; Gu et al., 1998).

Although the interaction between sE2 and SR-BI has been shown to be specific (Scarselli et al., 2002; Barth et al., 2005), no direct interaction between SR-BI and the E1E2 heterodimer has yet been reported. Attempts to demonstrate such an interaction by using E1E2 isolated from a cell lysate have failed in our laboratory (M. Lavie & J. Dubuisson, unpublished data). However, we cannot exclude the possibility that, in the presence of detergent, SR-BI is in a conformation that is not compatible with E2 binding. Alternatively, due to structural differences between truncated forms of E2 and the full-length E1E2 complex (Cocquerel et al., 2003), the SR-BI binding domain might not be accessible to E2 in the context of the E1E2 heterodimer.

Studies with HCVpp are also in agreement with the involvement of SR-BI in HCV entry (Bartosch et al., 2003b, 2005; Lavillette et al., 2005; Voisset et al., 2005). Indeed, pre-incubation of Huh-7 with a polyclonal antibody to SR-BI has been shown to reduce HCVpp infectivity (Bartosch et al., 2003b). However, reduction in SR-BI expression by small interfering RNAs was not as conclusive as in the case of CD81. Indeed, in a recent study, silencing of SR-BI expression reduced HCVpp infectivity (Lavillette et al., 2005), but this was not confirmed in our laboratory (Voisset et al., 2005). The discrepancy between these data may potentially be explained by differences in the levels of downregulation of SR-BI expression in these experiments. Indeed, one cannot exclude the possibility that HCV requires only very small amounts of SR-BI to enter target cells, which is consistent with observations made on the entry of some other enveloped viruses (Kuhmann et al., 2000; Tailor et al., 2000, 2003).

SR-BI is expressed in a large variety of mammalian tissues and cell types (Bartosch et al., 2003b; Rhainds & Brissette, 2004; Yamada et al., 2005), but its expression is particularly high in the liver and in steroidogenic tissues (adrenal gland and ovary) (Acton et al., 1996; Landschulz et al., 1996; Cao et al., 1997; Rhainds & Brissette, 2004). SR-BI was first
identified as a binding receptor for acetylated low-density lipoproteins (LDL) and oxidized LDL and was subsequently shown to bind to high-density lipoproteins (HDL) (Acton et al., 1994, 1996) (Fig. 3b). It has since been shown to be a multiligand receptor (Rigotti et al., 1997; Krieger & Kozarsky, 1999; Trigatti et al., 2000). Intriguingly, HDL have been shown to facilitate HCVpp entry into host cells in an SR-BI-dependent manner (Bartosch et al., 2005; Meunier et al., 2005; Voisset et al., 2005). This is surprising, as both HCVpp and HDL would have been expected to compete for the same receptor. In addition, no interaction between HCVpp and SR-BI has been identified, indicating that HCVpp do not enter into target cells by using HDL as a carrier to bind to SR-BI (Voisset et al., 2005). Interestingly, HDL-mediated facilitation of HCVpp entry depends on the lipid-transfer properties of SR-BI (Bartosch et al., 2005; Voisset et al., 2005), suggesting that HCV exploits the physiological activity of SR-BI for promoting its entry into target cells. In the presence of HDL, SR-BI can therefore be considered as a booster for HCV entry.

**Regions of interaction between E2 and SR-BI**

Residues involved in the E2–SR-BI interaction have not yet been identified. However, several reports suggest that SR-BI may interact with E2 via its hypervariable region 1 (HVR1) segment (Fig. 1). Indeed, deletion of HVR1 has also been shown to impair the sE2–SR-BI interaction (Scarselli et al., 2002) and to reduce HCVpp infectivity (Bartosch et al., 2003a; Callens et al., 2005). In addition, HDL-mediated facilitation of HCVpp entry is abolished in the absence of HVR1 (Bartosch et al., 2005; Voisset et al., 2005). However, one cannot exclude the possibility that the deletion of HVR1 has an indirect effect on the E2–SR-BI interaction. Although an HCV clone lacking HVR1 was shown to be infectious in chimpanzee, this mutant virus was attenuated, suggesting that HVR1 plays a facilitating role in HCV infectivity (Forns et al., 2000). Together, these data suggest that HVR1 might potentially modulate HCV infectivity by helping E2–SR-BI interaction directly or indirectly.

**Role of SR-BI in HCV entry**

The exact role of SR-BI in HCV entry is far from understood. Although recent data favour a role for both CD81 and SR-BI in HCV entry, the ectopic expression of these molecules in non-liver cell lines does not lead to HCVpp entry, suggesting that additional molecule(s) is/are required for HCV entry (Bartosch et al., 2003b). In addition, it is not known at what entry stage SR-BI is required. Interestingly, SR-BI has been shown to internalize some of its ligands (Rhainds & Brissette, 2004). This suggests that SR-BI may have the capacity to traffic HCV virions to low-pH compartments where the fusion of HCV may occur. Anti-SR-BI antibodies would therefore prevent an HCV–SR-BI interaction and, consequently, virus internalization. This hypothesis fits with a recent report showing that human SR-BI enhances the uptake of several bacteria when transfected into non-phagocytic cells (Philips et al., 2005). Although this hypothesis looks very attractive, the involvement of SR-BI in HCV endocytosis remains to be demonstrated.

Several reports also indicate that SR-BI is able to modulate the plasma-membrane composition (Rigotti et al., 1996; Reaven et al., 1998, 2000, 2001; Kellner-Weibel et al., 2000; Williams et al., 2002; Huang et al., 2003; Peng et al., 2004). The relationship between SR-BI and HCV infection may thus be based on the capacity of SR-BI to modulate the lipid composition of the plasma membrane to render the membrane permissive to HCV entry. In agreement with this hypothesis, the SR-BI-mediated selective uptake of lipids from HDL has been shown to markedly enhance HCVpp entry (Bartosch et al., 2005; Voisset et al., 2005). It is thus tempting to speculate that SR-BI is not a classical receptor to which the virus binds. It might rather be a molecule that modulates the plasma-membrane composition, which might be essential at some stage of HCV entry.

**Other molecules potentially involved in HCV entry**

**C-type lectins**

As observed for the envelope glycoproteins of several viruses (van Kooyk & Geijtenbeek, 2003), it has also been shown that sE2 binds specifically to L-SIGN and DC-SIGN (Gardner et al., 2003; Lozach et al., 2003; Pöhlmann et al., 2003). These molecules are homotetrameric type II membrane proteins belonging to the C-type lectin family (van Kooyk & Geijtenbeek, 2003). They both have an extracellular C-terminal region that contains a calcium-dependent carbohydrate-recognition domain and a membrane-proximal heptad-repeat region important for oligomerization.

DC-SIGN serves as an adhesion receptor to establish cellular interactions between dendritic cells and T cells or endothelial cells. In addition, DC-SIGN is also an antigen receptor that internalizes upon binding to antigens and targets bound antigen to the late endosomal/lysosomal compartment for processing and presentation to T cells (Engering et al., 2002). Similar to DC-SIGN, L-SIGN may establish cellular interactions with T cells (Bashirova et al., 2001). This may enable activated T cells to recirculate to the liver and to the lymph nodes through interactions with L-SIGN. Furthermore, L-SIGN, like DC-SIGN, recognizes carbohydrate structures on pathogens (Koppel et al., 2005). HCVpp, as well as native HCV particles, have also been shown to bind cells expressing L-SIGN and DC-SIGN (Gardner et al., 2003; Pöhlmann et al., 2003; Lozach et al., 2004). However, these lectins are not expressed on hepatocytes and are therefore not receptors for these cells. DC-SIGN is expressed on dendritic cells, whereas L-SIGN is mainly expressed on sinusoidal endothelial cells in the liver and lymph nodes (Koppel et al., 2005). L-SIGN and DC-SIGN may rather contribute to the establishment or persistence of infection, both by the capture and delivery of virus to the liver and by modulating dendritic-cell functions.
as suggested by Cormier et al. (2004b) and Lozach et al. (2004). This, however, remains to be demonstrated in vivo.

An approach using virus-like particles produced in insect cells has also led to the identification of the asialoglycoprotein receptor as another candidate receptor for HCV (Saunier et al., 2003). This receptor is a C-type lectin that is found most commonly in the liver (Stockert, 1995). However, further investigations with HCVpp and HCVcc systems are needed to determine the potential role of this molecule in HCV entry.

**LDL receptor**

Because of the physical association of HCV with LDL or very-low-density lipoproteins (VLDL) in serum (Thomssen et al., 1992; André et al., 2002), the LDL receptor has also been proposed as a putative candidate receptor for HCV (Agnello et al., 1999; Monazahian et al., 1999; Wünschmann et al., 2000). In the context of native particles isolated from HCV-infected patients, the LDL receptor has been shown to mediate HCV internalization by binding to virion-associated LDL particles (Agnello et al., 1999). However, a role for the LDL receptor in virus entry has not been confirmed with HCVpp (Bartosch et al., 2003a), but we cannot exclude the possibility that HCVpp do not associate with LDL and VLDL, as these are produced in a non-hepatic cell line. Indeed, VLDL, which are assembled in the endoplasmic reticulum of the hepatocyte, might associate with HCV particles during viral morphogenesis. HCVcc, which are produced in hepatic cells, should therefore allow the reinvestigation of the potential role of the LDL receptor in HCV entry.

**Glycosaminoglycans**

Glycosaminoglycan chains on cell-surface proteoglycans provide primary docking sites for the binding of various viruses to host cells (Villanueva et al., 2005). Based on this observation and on a study of interactions between the HCV envelope glycoprotein E2 and heparin, it has been postulated that highly sulphated heparan sulphate may serve as the initial docking site for HCV attachment (Barth et al., 2003). After binding to heparan sulphate, HCV might be transferred to a second high-affinity receptor, triggering entry. However, it has recently been shown that the E2 glycoprotein associated with HCVpp does not interact with heparin, suggesting that the heparin-binding domain of E2 is not accessible on the mature form of E2 associated with HCVpp (Callens et al., 2005). These data indicate that, in the context of HCVpp, heparin-like proteoglycans are not involved in virus entry. Further studies with the HCVcc system should clarify the role of glycosaminoglycans in HCV entry.

**Conclusions and future directions**

Although CD81 is essential for HCV entry and SR-BI is at least a modulator of entry, expression of these molecules in non-liver cell lines does not lead to HCVpp entry (Bartosch et al., 2003b; Hsu et al., 2003; Zhang et al., 2004). This suggests that additional molecule(s), expressed in hepatic cells only, is/are necessary for HCV entry. As CD81 interacts with other molecules to form a tetraspanin web, it would be interesting to investigate the role of other molecules of the CD81-associated tetraspanin web present in hepatic cells. Besides finding additional molecule(s) involved in HCV entry, the precise functions of CD81 and SR-BI in virus entry also need to be determined. HCVpp remain the best tool available for functional studies of the early steps of the HCV life cycle and the recent development of the HCVcc system provides an additional opportunity to confirm the relevance of candidate receptors. In addition, even if some doubts have been raised on the relevance of studying the interactions between sE2 and recombinant candidate receptors, analysis of the interactions between native candidate receptors and native E1E2 complexes, which are more physiologically relevant molecules, is still needed to understand the detailed mechanisms leading to HCV entry. In conclusion, major progress has recently been made in the characterization of cell-surface molecules involved in HCV entry and further studies with recently developed tools should rapidly provide a mechanistic view of the early steps of HCV entry.

**REFERENCES**


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Imai, T. & Yoshie, O. (1993). C33 antigen and M38 antigen recognized by monoclonal antibodies inhibitory to syncytium formation by human T cell leukemia virus type 1 are both members of the transmembrane 4 superfamily and associate with each other and with CD4 or CD8 in T cells. J Immunol 151, 6470–6481.


Roccasecca, R., Ansuini, H., Vitelli, A. & 11 other authors (2003). Binding of the hepatitis C virus E2 glycoprotein to CD81 is strain specific and is modulated by a complex interplay between hypervariable regions 1 and 2. *J Virol* 77, 1856–1867.


