Cell Entry of Hepatitis C Virus Requires a Set of Co-receptors That Include the CD81 Tetraspanin and the SR-B1 Scavenger Receptor

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Several cell surface molecules have been proposed as receptor candidates, mediating cell entry of hepatitis C virus (HCV) on the basis of their physical association with virions or with soluble HCV E2 glycoproteins. However, due to the lack of infectious HCV particles, evidence that these receptor candidates support infection was missing. Using our recently described infectious HCV pseudotype particles (HCVpp) that display functional E1E2 glycoprotein complexes, here we show that HCV is a pH-dependent virus, implying that its receptor component(s) mediate virion internalization by endocytosis. Expression of the CD81 tetraspanin in non-permissive CD81-negative hepato-carcinoma cells was sufficient to restore susceptibility to HCVpp infection, confirming its critical role as a cell attachment factor. As a cell surface molecule likely to mediate endosomal trafficking, we demonstrate that the human scavenger receptor class B type 1 (SR-B1), a high-density lipoprotein–internalization molecule that we previously proposed as a novel HCV receptor candidate due to its affinity with E2 glycoproteins, is required for infection of CD81-expressing hepatic cells. By receptor competition assays, we found that SR-B1 antibodies that blocked binding of soluble E2 could prevent HCVpp infectivity. Furthermore, we establish that the hyper-variable region 1 of the HCV E2 glycoprotein is a critical determinant mediating entry in SR-B1-positive cells. Finally, by correlating expression of HCV receptors and infectivity, we suggest that, besides CD81 and SR-B1, additional hepatocyte-specific co-factor(s) are necessary for HCV entry.

Hepatitis C virus (HCV)1 is a major cause of chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma worldwide (1).

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1 The abbreviations used are: HCV, hepatitis C virus; HCVpp, HCV pseudotype particles; E1, HCV glycoprotein 1; E2, HCV glycoprotein 2; SR-B1, scavenger receptor class B type 1; LDL, low density lipoprotein; LDLr, LDL receptor; MLV, murine leukemia virus; GFP, green fluorescent protein; VSV, vesicular stomatitis virus; VSV-G, VSV glycoprotein; CHO, Chinese hamster ovary; TU, transducing units; FACS, fluorescence-activated cell sorter; HVR1, hyper-variable region 1.
sent the envelope glycoproteins of wild-type HCV and are both required for infection (14). The infectivity of E1E2-pseudotyped retroviruses for similar cells was confirmed by others, as was the ability of monoclonal antibodies to neutralize the viral particles (15, 16). These pseudotype particles may therefore allow for the first time to decipher the mechanisms of HCV entry into cells.

We recently identified the human scavenger receptor class B type 1 (SR-B1), a high-density lipoprotein-binding molecule, as a putative HCV receptor because of its affinity to E2 glycoproteins (17). Here, we used HCVpp to determine the role of SR-B1 in HCV entry. We show that SR-B1 is required for infection of cells that also express the CD81 tetraspanin and the density lipoprotein receptor (LDLR), two other previously proposed HCV receptor candidates (18, 19). Furthermore, we establish that the hyper-variable region 1 of the HCV E2 glycoprotein is a critical determinant mediating entry in SR-B1-positive cells. Finally, our data indicate that, in addition to LDLR, CD81, and SR-B1, liver-specific co-factor(s) are necessary for HCV entry.

EXPERIMENTAL PROCEDURES

Expression Constructs and Production of HCV Pseudotype Particles—Expression vectors for E1E2 glycoproteins of 1a and 1b genotypes have been described previously (14). They were used to construct by PCR-mediated and oligonucleotide site-directed mutagenesis (details and sequences available upon request) the expression vectors for the mutant E2 glycoproteins that harbor the deletion of the hyper-variable region 1 (del384–410) and/or the V514M and L615H point mutations. The CMV-Gag-Pol murine leukemia virus (MLV) packaging construct, encoding the MLV gag and pol genes, and the MLV-GFP plasmid, encoding an MLV-based transfer vector containing the GFP marker gene, have been described previously (20). The phCMV-G, pCMV-4070A, phCMV-HA, and phCMV-RD (21) expression vectors encode the VSV G protein, the amphotropic MLV glycoprotein, the fowl plague virus hemagglutinin, and the feline endogenous virus RD14 glycoprotein, respectively.

Production of HCV pseudotype particles was carried out as described previously (14). Briefly, 293T cells were transfected with expression vectors encoding the viral components, i.e. E1E2 glycoproteins, retroviral core proteins, and packaging-competent GFP-containing retroviral transfer vectors, using a calcium-phosphate transfection protocol (Clontech, Le Pont de Claux, France). Supernatants containing the pseudotype particles were harvested 24 h later, filtered through 0.45-μm pore-sized membranes, and used in infection assays.

Cells and Infection Assays—Huh-7 human hepatocellular carcinoma (22), PLC/PRF/5 human hepatoma (CRL-8024), Hep2B human hepatocellular carcinoma (HB-8065), 293 human embryonic kidney (CRL-1573), and HepG2 human hepatocellular carcinoma (CRL-2257), were used as target cells. These cells were grown as recommended by the ATCC (American Type Culture Collection, Rockville, MD). 293-SR-B1 cells were derived from 293 cells by transfection with pcDNA3-SR-B1, an expression vector encoding SR-B1 (or C-LA) and the human scavenger receptor class B type 1 (GenBank™ accession number Z22555) (23). CHO-SR-B1 cells were described previously (17). CHO-CD81/SR-B1 and HepG2/SR-B1 cells were obtained by infection of CHO-SR-B1 and HepG2 cells, respectively, with a retroviral vector containing the human CD81 gene (GenBank™ accession number NM_004356). Construct details are available upon request.

Infection assays were performed as described previously (14). Unless otherwise indicated, dilutions of viral supernatants containing the pseudotype particles were added to the cells seeded the day before and plates were incubated for 3 h. The supernatants were then removed and the cells incubated in regular medium for 72 h at 37 °C. The infectious titers, expressed as transducing units (TU)/ml, were deduced from the transduction efficiencies, determined as the percentage of GFP-positive cells measured by FACS analysis (21).

Antibodies—E1 and E2 glycoproteins were detected with the A4 (24) and H52 (25) monoclonal antibodies, respectively. HCVpp core proteins were detected with an anti-capsid (MLV CA) antiserum (ViroMed Bio-safety Laboratories). These reagents were used in Western blot analysis of purified pseudotype particles as previously described (14).

RESULTS

Cell Entry of HCVpp Is pH-dependent—Envelope viruses penetrate the host cells by a process of fusion between the viral and cell membranes that is catalyzed by a fusogenic activity harbored by viral surface glycoproteins. Activation of such fusion properties occurs in an acid pH-dependent manner, via acidified endosomal vesicles into which the viros are routed following receptor binding (28), or, alternatively, in a pH-independent manner via direct interaction of the viral glycoproteins with their receptors. These two cell entry pathways can be distinguished by assessing the effect in infection assays of drugs that inhibit endosomal-pH acidification, such as weak bases (e.g. chloroquine), and vacuolar H+-ATPase inhibitors (e.g. bafilomycin A1) (28). We determined whether entry of HCVpp in Huh-7 hepatocarcinoma cells is likely to proceed via internalization by treating target cells before and during infection with bafilomycin A1. Infectivity of control pseudotype particles generated with pH-independent glycoproteins from MLV-A or RD114 was not affected by bafilomycin A1, at concentrations of up to 50 nM (Fig. 1), consistent with the pH-independent entry route adopted by these retroviruses (29). That bafilomycin A1 seemingly reduced the infectivity of these
two control viruses at 100 nM could be clearly attributed to cell toxicity of the drug (data not shown). In contrast, bafilomycin A1 reduced in a dose-dependent manner the infectious titers of HCVpp, as well as the infectivity of pseudotype particles generated with the VSV-G glycoprotein, an avian influenza virus, or from VSV. At 100 nM of bafilomycin A1, the titers of the HCVpp and the pseudotype particles coated with FPV hemagglutinin were reduced by a factor of 30–50-fold (Fig. 1). Similar results were obtained with chloroquine and when using Hep3B as alternative target cells (data not shown). These data indicated that the fusion-activation of HCV E1E2 glycoproteins is pH-dependent and that cell entry of HCVpp most likely occurs by endocytosis. Thus some of the receptors used by HCV to infect the target cells may be responsible for virion internalization.

**CD81 and SR-B1 Co-expression Is Not Sufficient for HCV Cell Entry**—The LDLr, the CD81 tetraspanin, and the SR-B1 have been proposed as putative HCV receptors mediating entry into hepatic cells (17–19, 30) on the basis of their physical association with virions or with soluble E2 glycoproteins. Although virion association to LDLr is most likely due to indirect interaction, *via* virion-bound apolipoproteins (31), direct interaction between soluble E2 and CD81 or SR-B1 could be demonstrated (17, 27). However, due to the lack of infectious HCV particles, evidence that these receptor candidates could support infection was missing. By receptor competition assays, we recently provided evidence that CD81 was involved in the early steps of infection, but was not sufficient to permit infection (14). Indeed, non-hepatic human cells that express CD81 were found to be not or very poorly permissive to infection (Table I), with infectious titers of HCVpp more than 100-fold lower than those measured on Hep-7 cells. Furthermore, ectopic expression of human CD81 in non-permissive murine cells did not restore infection (14). Thus, hepato-carcinoma cells, which co-express LDLr, CD81, and SR-B1, exhibited the maximal levels of infectivity. Therefore, because SR-B1 is expressed in most cells types, though at highly variable levels (Table I), non-permissiveness to infection may be due to lack of sufficient levels of SR-B1 expression, or, alternatively, to lack of a critical hepatocyte factor(s). To address either of these possibilities, we performed infection assays in CHO cells and 293 human embryonic kidney cells that expressed no and low levels of SR-B1, respectively (Table I). CHO cells, which are not permissive to HCVpp infection (14), were engineered to allow stable and constitutive expression of CD81 alone, SR-B1 alone, or both molecules. Expression of either molecule was sufficient to allow binding of E2 soluble glycoproteins (17, 27). All these CHO-derived cells were highly permissive to infection with control pseudotype particles generated with the VSV-G glycoprotein, which binds a non-related receptor. However, despite densities of CD81 and/or SR-B1 molecules similar if not higher than that of the highly permissive Huh-7 cells (Table I), we found no evidence for infection with HCVpp based on genotype 1a (H77 strain) and 1b (J strain) (Fig. 2). In contrast, over-expression of SR-B1 in the poorly permissive 293 cells (14), which naturally express abundant levels of CD81 but very low levels of SR-B1 (Table I), resulted in increased levels of infection for both HCVpp-1a and -1b. Using HCVpp harboring a lacZ marker gene, titers in 293 cells: (2.6 ± 0.5) × 10^4 TU/ml; titers in 293-SR-B1 cells: (4.9 ± 1.5) × 10^4 TU/ml.

| Cell lines          | Tissue                             | HLDLρ⁺  | hCD81⁺  | hSR-B1⁺  | Infectivity⁺  |
|---------------------|------------------------------------|---------|---------|---------|---------------|
| Huh-7               | Hepatocellular carcinoma           | +       | +       | +       | +             |
| PLC/PRF/S           | Hepatoma                           | +       | +       | +       | +             |
| Hep3B               | Hepatocellular carcinoma           | +/-     | +       | +       | +/-           |
| HepG2-CD81          | Hepatocellular carcinoma           | +/-     | +       | +       | +/-           |
| HepG2               | Hepatocellular carcinoma           | +       | +       | +/-     | +/-           |
| SW-13               | Adrenocortical carcinoma           | +       | +       | +       | +/-           |
| 293-SR-B1           | Embryonic kidney                   | +/-     | +       | +       | +/-           |
| 293                 | Embryonic kidney                   | +/-     | +       | +       | +/-           |
| HOS                 | Osteosarcoma                       | +/-     | +       | +       | +/-           |
| HeLa                | Cervix adenocarcinoma              | +       | +       | +       | +             |
| TE671               | Rhabdomyosarcoma                   | +/-     | +       | +       | +/-           |
| Molt-4              | T lymphoblastic leukemia            | +/-     | +       | +       | +/-           |
| CHO                 | Chinese hamster ovary              | +/-     | +       | +       | +/-           |
| CHO-CD81/SR-B1      | Chinese hamster ovary              | +/-     | +       | +       | +/-           |

Detection of human SR-B1 using mouse SR-B1 serum diluted 1/100 on the surface of the indicated cells by flow cytometry. –, MFI (mean fluorescent intensity) shift of 1; +/-, MFI shift between 2 and 4; +, MFI shift between 4 and 10; +++, MFI shift over 10.

Detection of human CD81 using JS-81 antibodies on the surface of the indicated cells by flow cytometry. –, MFI shift of 1; +, MFI shift between 1 and 50; +++, MFI shift over 50.

Detection of human SR-B1 using mouse SR-B1 serum diluted 1/100 on the surface of the indicated cells by flow cytometry. –, MFI shift of 1; +/-, MFI shift between 2 and 6; +, MFI shift between 6 and 20; +++, MFI shift over 20.

* Infectivity of HCVpp of genotype 1a and 1b harboring the GFP marker gene. +, titers higher than 10^5 TU/ml; +, titers between 10^4 and 10^5 TU/ml; +/−, titers between 10^3 and 10^4 TU/ml; −, titers lower than 10^3 TU/ml, which corresponds to the threshold of detection of infected cells by FACS analysis.

* Overexpression of SR-B1 in 293 cells resulted in ca. 10-fold increased infectious titers of HCVpp harbouring a lacZ marker gene. Titers in 293 cells: (2.6 ± 0.5) × 10^4 TU/ml; titers in 293-SR-B1 cells: (4.9 ± 1.5) × 10^4 TU/ml.
sivity to HCVpp was a result of this defect. Interestingly, infection assays performed in HepG2-CD81 cells revealed that expression of CD81 was sufficient to restore HCVpp entry in HepG2 cells by factors of 100- and 30-fold for HCVpp of genotype 1a and 1b, respectively (Fig. 2). Altogether, these results pointed out the involvement of several cell surface molecules in HCV entry in cells of hepatic origin, among which CD81 and SR-B1 seem important, at least to bind E2 glycoproteins.

Thus to specifically investigate a functional role played by SR-B1 in mediating HCVpp cell entry, we designed receptor competition assays using polyclonal antibodies reactive with the SR-B1 ectodomain (Table I). Pre-incubation of target cells with an SR-B1 anti-serum, which blocked binding of soluble E2 glycoproteins from the 1a and 1b HCV strains (Fig. 3A), diminished the infectivity of HCVpp of both genotypes 1a and 1b in Huh-7 (Fig. 3B) and HepG2-CD81 (data not shown) target cells, in a dose-dependent manner. Particularly, at a 1:10 serum dilution, an 80% inhibition of infection on Huh-7 could be obtained for HCVpp based on genotype 1a in a manner correlated with inhibition of soluble E2 binding. No reduction of infectivity was found for control pseudotype particles generated with a non-relevant glycoprotein, derived from RD114 cat endogenous virus (Fig. 3B), demonstrating the specificity of inhibition mediated by the SR-B1 antibodies. Likewise, pre-incubation of target cells with a pre-immune serum or with non-relevant monoclonal antibodies inhibited neither binding of recombinant E2 (Fig. 3A) nor HCVpp infection (data not shown). Altogether, these results established that SR-B1 is involved in cell entry of HCVpp, perhaps by being part of an HCV receptor complex with CD81 and/or LDLr.

Hyper-variable Region 1 (HVR1) Is Involved in SR-B1-mediated HCVpp Cell Entry—Studies of epitopes that neutralize E2-binding to CD81 have raised the possibility that the CD81-binding domain consists of three discrete segments at both ends of E2 that may join together in the head-to-tail model of E2 glycoprotein homodimer (32). In this putative structure, the CD81-binding domain may be located in close proximity to HVR1, a 27-amino acid long segment found at the amino-terminal end of E2 that has been proposed to play a role in cell attachment (33). Interestingly, in studies with recombinant soluble E2, we found that deletion of HVR1 increased binding to CD81 (27) but decreased binding to SR-B1 (17). Furthermore, this latter phenotype was partially compensated by either the V514M or L615H compensatory mutations (17), which

**Fig. 2.** Results of infection assays in CHO and HepG2 cells expressing CD81 and/or SR-B1. The results of experiments performed with CHO-derived and with HepG2-CD81 target cells are displayed as TU/ml of supernatant (mean ± standard deviations) for HCVpp of genotypes 1a and 1b. Control experiments were performed using pseudotype particles generated with VSV-G (VSV-Gpp) or lacking glycoproteins (pp cores). The infectious titers of the latter viral particles were lower than 10^2 TU/ml.

**Fig. 3.** Inhibition of SR-B1-mediated HCV-pp infection. A, binding assays on HepG2 and Huh-7 cells were performed with soluble E2 glycoproteins of genotype 1a and 1b in the presence of SR-B1 serum, as indicated. Control experiments were performed with the pre-immune serum. The results are expressed as percentages of binding relative to binding determined in the absence of antibodies. B, infection of Huh-7 target cells with HCVpp-1a and 1b were performed in the presence of the SR-B1 serum at the indicated dilution. Control experiments were performed using pseudotype particles generated with RD114 glycoproteins (RD114pp), rather than with VSV-G that exhibits sensitivity to human complement (21). The results are expressed as percentages of the average infectious titers ± standard deviations relative to titers determined in the absence of antibodies.
have been selected in the E2 glycoproteins of a HVR1-deleted HCV of genotype 1a following inoculation in chimpanzees (34).

These previous results suggested that HVR1 represents an important determinant of SR-B1 binding and that the CD81-binding domain and HVR1 may compete for binding to these two HCV receptors. Thus to investigate the contribution of HVR1 and/or compensatory mutations in cell entry, we generated HCVpp with E1E2 glycoproteins harboring the deletion of HVR1 and/or the V514M and L615H mutations. As demonstrated by Western blot analysis of purified HCVpp (Fig. 4A), these pseudotype particles incorporated normal levels of mutated E1E2, compared with unmodified E1E2 glycoproteins. This indicated that the mutations harbored by the E2 glycoprotein did not impair its folding, maturation, and viral assembly, consistent with studies using soluble recombinant proteins (17). The HCVpp harboring the deletion of HVR1 were infectious (Fig. 4B); yet, compared with parental pseudotype particles, their infectivity was reduced by about 40-fold, on average, for both genotypes. Unexpectedly, the V514M and L615H mutations, introduced alone or together in the E2–1a glycoprotein, resulted in an even more dramatic loss of infectivity of the pseudotype particles harboring HVR1-deleted E2 glycoproteins (Fig. 4B), in contrast to their capacity to compensate an SR-B1-binding defect (17).

To further investigate the potential role of HVR1 in cell entry mediated by SR-B1, we performed competition assays of HVR1/SR-B1 interaction with HVR1 or with SR-B1 antibodies. The 9/27 monoclonal antibody, which binds the C-terminal part of the H77 HVR1 sequence (35) and specifically blocks binding of soluble genotype 1a E2 to SR-B1 (17), was found to efficiently neutralize infectivity of HCVpp of genotype 1a but not 1b in a dose-dependent manner (Fig. 4C). Furthermore, no significant neutralization of HVR1-deleted HCVpp-1a by the 9/27 antibody could be detected (Fig. 4C), indicating that neutralization of the parental pseudotype particles by these antibodies occurs via blocking of HVR1. In agreement with these findings, the SR-B1 antibodies were unable to inhibit the HVR1-deleted HCVpp, most likely because SR-B1-binding of the latter viruses was abolished, in contrast to pseudotype particles generated with unmodified E1E2 glycoproteins (Fig. 4D).

Altogether these results are consistent with a role for HVR1 in HCVpp entry via SR-B1. That the HVR1-deleted HCVpp were still infectious, albeit at significantly reduced levels, may be due to residual binding to SR-B1 or, alternatively, to increased binding to CD81.
**HCV Cell Entry Requires CD81 and SR-B1 Co-receptors**

**DISCUSSION**

Enveloped viruses enter into cells via a series of steps that involve, on the one hand, interactions between the viral surface glycoproteins and several cell surface molecules, called receptors and co-receptors, and, on the other hand, activation of cell entry pathways that lead to internalization of the viral particles. In the absence of a suitable cell culture system for HCV, an alternative approach to search for candidate receptors has been to use truncated soluble forms of HCV glycoproteins for binding studies to human cells. By using a soluble form of E2 as a probe, CD81 has been identified as a putative receptor for HCV (19). With a very similar approach, the SR-B1 scavenger receptor (17) and the mannose-binding lectins, DC-SIGN and L-SIGN (36–38), have also been proposed as candidate receptors for HCV. With several candidate receptors already identified and the current limitations to analyze their role in HCV entry, it is getting more and more difficult to propose a convincing model for HCV entry and explain its tropism to hepatic cells.

Upon appropriate activation of the viral surface glycoproteins occurs a dramatic refolding within the glycoproteins that results in the exposure of the fusion machinery. This conformational rearrangement is thought to release the energy necessary for the fusion process. Fusion activation of pH-independent viruses (e.g. most retroviruses) is triggered by highly ordered interactions of their glycoproteins with receptors to which they bind (39) and may occur at the cell surface. In contrast, the signal that initiates the conformational rearrangements of the pH-dependent viruses (e.g. influenza virus) is caused by acidification of the environment of the virion, implying that some cell surface receptors that mediate their binding internalize and traffic them to acid-pH cell compartments where membrane fusion occurs (28). Because of the structure of its genome and mechanisms of replication, HCV has been tentatively classified within its own genus, Hepacivirus, within the Flaviviridae family, which also comprises the Flavivirus and Pestivirus genera (40), and whose members, at least for the flaviviruses, exhibit pH-dependence in cell entry (41). Consistently, our results establish that inhibitors of endosomal-pH acidification reduce the infectivity of HCVpp to the same extent as that of pseudotype particles generated with glycoproteins from several pH-dependent viruses (Fig. 1), indicating that its cell surface receptor(s) should traffic cell-bound virions to endosomal compartments. Some of the different HCV receptor candidates are likely to fulfill this role. Although the LDL receptor was shown to mediate indirect HCV internalization, via binding to lipoproteins associated to virions, it is clear that alternative routes exist as suggested by studies with LDLr-deficient cells (18). The CD81 tetraspan is likely involved in HCVpp infection because competition assays strongly inhibited the infectivity of HCVpp (14) and because HepG2 cells become permissive to infection only when they were engineered to express CD81 (Fig. 2). This is supported by the fact that none of the permissive cell types was found negative for CD81 expression (Table I). However, CD81 may have a very poor capacity to mediate virion internalization (42). Thus, CD81 may allow HCV attachment to the cell surface and subsequent interaction with a cell entry receptor, among which SR-B1 stands as a strong candidate. Indeed this receptor mediates selective uptake of a variety of macro-molecules such as high-density lipoprotein, LDL exchangeable apolipoproteins, protein-free lipid vesicles, and lipopolysaccharides (LPS) and is predominantly expressed in hepatocytes and steroidogenic tissues like adrenal glands and ovaries (43). Co-localization experiments demonstrated that SR-B1-bound LPS enters different intracellular compartments such as the Golgi complex and the endocytic recycling compartments after rapid endocytosis (44). This indicates that SR-B1 may have the capacity to traffic cell-bound virions to endosomal compartments wherein low-pH could activate the fusion properties of the HCV glycoproteins. Strain-specific differences in binding to either receptor have been detected (17, 27, 42, 45). E2 glycoproteins of the H77 strain, of genotype 1a, were found to bind more efficiently CD81 than E2 glycoproteins of genotype 1b, such as the J strain used in this study (27). Consistent with this finding, our results suggest that infectivity of HCVpp-1a is more efficiently inhibited by CD81-blocking antibodies (data not shown) and is restored to a level higher than HCVpp-1b in HepG2 cells expressing CD81 (Fig. 2). Nevertheless, despite a lower affinity to E2-1b glycoproteins, it is important to note that CD81 is critical for the infectivity of HCVpp of both genotypes as shown by results of genetic complementation assays in CD81-deficient hepatoma cells (Fig. 2) and by results of receptor competition assays (14). Hence, functional data, obtained with infectious pseudotype particles, may not completely match the data of binding assays and caution should be observed when interpreting the latter results. In contrast to CD81, there are no SR-B1-deficient hepatoma-carcinoma cells, thus limiting the experimental approaches to decipher the role of SR-B1 in HCV cell entry. Although over-expression of SR-B1 in 293 target cells resulted in significantly increased infectivity of HCVpp, the absolute titers of the latter virions remained low (Table I), most likely because of lack or insufficient expression of a factor predominantly expressed in hepatic cells. Therefore, formal demonstration of the involvement of SR-B1 in infection of hepatoma-carcinoma cells essentially relied on receptor competition assays using SR-B1 antibodies (Fig. 3) and on results obtained with the study of HVRI-deletion mutants (Fig. 4). The HVRI segment, located at the amino terminus of the E2 glycoprotein, has been proposed to modulate accessibility to either CD81 or SR-B1 because its deletion from soluble E2 increased binding to CD81 (27) but abrogated binding to SR-B1 (17). Because infectivity of HVRI-deleted HCVpp was reduced by about 40-fold (Fig. 4B), these results indicate that loss of binding of E2 glycoproteins to either receptor can be compensated perhaps by interaction with another (co)-receptor. In agreement with this possibility, binding of soluble E2 glycoproteins could not be inhibited by more than 80% in Huh-7 or HepG2 cells, even at saturating concentration of SR-B1 antibodies (Fig. 3A), indicating interaction with other cell surface components. Alternatively and not exclusively, residual binding to SR-B1 may still occur with the HCVpp and may allow infection. That HCV may require very little amounts of receptors to mediate cell entry is consistent with recent results obtained with some other membrane-enveloped viruses (39, 46).

A growing body of evidence indicates that the DC-SIGN and L-SIGN lectins might play a role in HCV tropism. Both lectins, expressed in dendritic cells and in liver sinusoidal endothelial cells, bind high-mannose N-glycans of HCV E2 soluble glycoprotein, plasmatic HCV, and pseudotype particles (36–38). DC-SIGN has been shown to facilitate cell entry by enhancing cell attachment of several human pathogens like human immunodeficiency virus, Ebola virus, cytomegalovirus, and Dengue virus (47–51), thereby increasing the likelihood of interaction with specific entry receptors. Several studies have indeed reported alteration of dendritic cell functions in HCV-infected patients (52–54), and this may be a consequence of HCV interaction with DC-SIGN. Furthermore, because of its selective expression in liver sinusoidal endothelial cells, L-SIGN might have an important function in receptor-mediated targeting of HCV to the liver. Indeed, these cells play a key role in the initial uptake of viral pathogens into the liver, as shown for...
Hepatitis B virus (55). A general mechanism by which hepatitis viruses, initially scavenged by liver sinusoidal endothelial cells are thereafter released to infect adjacent hepatocytes, the only cells capable of replicating these viruses, has been proposed (55). Thus HCV might be captured by L-SIGN after contamination by infected blood, resulting in concentration of virions in the liver and subsequent trans-luminal presentation to hepatocytes when it enters via use of specific receptors, including CD81 and SR-B1.

Although the capacity to bind DC-SIGN and L-SIGN may explain many features of HCV pathogenesis, it is unlikely that lectin-mediated capture of HCV is the sole determinant of hepato-tropism. Indeed L-SIGN is expressed in lymph nodes but is not expressed on hepatocytes, which represent the major target of infection. Furthermore, as shown elsewhere (16) and by us, expression of DC-SIGN in several non-permissive cells did not allow infection by HCVpp. Of note, all cells permissive to HCVpp co-express LDLr, CD81, and SR-B1 and are of liver origin (Table I). However, several cell lines of non-hepatic origin express all three cell surface molecules but are not or poorly permissive to HCVpp infection (Table I). These results imply that additional liver specific co-factor(s) are necessary for HCV entry. The nature of such molecule(s) is currently unknown, and the functional cloning of these additional molecules will be essential to decipher the early steps of HCV lifecycle.

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