HEPATITIS C VIRUS ENTRY: MOLECULAR MECHANISMS AND TARGETS FOR ANTIVIRAL THERAPY

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1. ABSTRACT

With an estimated 170 million infected individuals, hepatitis C virus (HCV) has a major impact on public health. The liver is the primary target organ of HCV, and the hepatocyte is its primary target cell. Attachment of the virus to the cell surface followed by viral entry is the first step in a cascade of interactions between the virus and the target cell that is required for successful entry into the cell and initiation of infection. Using recombinant HCV envelope glycoproteins and HCV pseudotype particles, several cell surface molecules have been identified interacting with HCV during viral binding and entry. These include CD81, highly sulfated heparan sulphate, the low-density lipoprotein receptor, scavenger receptor class B type I and claudin-1. Treatment options for chronic HCV infection are limited and a vaccine to prevent HCV infection is not available. Interfering with HCV entry holds promise for drug design and discovery as the understanding of molecular mechanisms underlying HCV interaction with the host cell is advancing. The complexity of the virus entry process offers several therapeutic targets.

2. INTRODUCTION

Hepatitis C virus (HCV) is a major cause of chronic hepatitis worldwide (1). Treatment options for chronic HCV infection are limited and a vaccine to prevent HCV infection is not available (2). Moreover, chronic hepatitis C may progress to liver cirrhosis and ultimatively lead to hepatocellular carcinoma. HCV is a small enveloped positive-strand RNA virus that has been classified in the genus *Hepacivirus* of the *Flaviviridae* family. *In vivo*, HCV infects only humans and chimpanzees (3). The liver is the primary target organ of HCV, and the hepatocyte is its primary target cell. Replication of the HCV genome has been demonstrated *in vivo* and *in vitro* in liver hepatocytes, and hematopoietic cells including dendritic cells and B lymphocytes (4, 5). Attachment of the virus to the cell surface followed by viral entry is the first step in a cascade of interactions between the virus and the target cell that is required for successful entry into the cell and initiation of infection (6). This step is an important determinant of tissue tropism and pathogenesis, it thus represents a major target for antiviral host cell responses, such as antibody-mediated virus neutralization, and antiviral therapy.

3. MOLECULAR MECHANISMS OF HCV ENTRY INTO TARGET CELLS

3.1. Viral determinants: envelope glycoproteins E1 and E2

The HCV genome encodes a single precursor polyprotein of about 3,000 amino acids that is cleaved co- and post-translationally into functional structural and non-structural proteins by host and viral proteases including three structural proteins: the core protein forming the viral nucleocapsid and two envelope glycoproteins, E1 and E2. HCV particles have a size of about 55-60 nm in diameter (7-9). In analogy to other members of the *Flaviviridae* family, HCV is thought to adopt a classical icosahedral scaffold in which the two envelope glycoproteins E1 and E2 are anchored to the host cell-derived double-layer lipid envelope (10). E1 and E2 are type I transmembrane glycoproteins containing up to 6 and 11 potential glycosylation sites, respectively (11) and forming noncovalent heterodimers. The nucleocapsid is probably composed of multiple copies of the core protein in complex with the viral genome and lies underneath the envelope (10).

Studies of the HCV life cycle have long been hampered by the lack of an efficient cell culture system to generate infectious virus *in vitro*. Several model systems have thus been developed for the study of defined aspects of the HCV life cycle such as viral entry, replication, assembly and release (for review see (12)). Recombinant HCV envelope glycoproteins (13), HCV-like particles (HCV-LPs) (14-16) and retroviral HCV pseudotypes (HCVpp) (17, 18) have been successfully used to analyze virus attachment and entry. Most recently, efficient *in vitro* model systems for the production of infectious recombinant virions (HCVcc) have been described (9, 19, 20). Using these model systems, it could be demonstrated that envelope glycoproteins E1 and E2 are crititcal for host cell entry. In fact, HCVpp assembled with either E1 or E2 glycoproteins were significantly less infective than HCVpp containing both envelope glycoproteins (17). Furthermore, HCVcc generated from a construct with an in-frame-deletion of the HCV envelope protein coding sequence are not infectious (9).

Monoclonal or polyclonal antibodies targeting both linear and conformational epitopes of envelope glycoprotein E2 have been shown to inhibit cellular binding of HCV-LP binding, entry of HCVpp and infection of HCVcc (9, 14-20) suggesting that envelope glycoprotein E2 plays a key role for host cell surface interaction. Within the E2 envelope glycoprotein sequence hypervariable regions have been identified. These amino acids differ by up to 80% among HCV genotypes, and even among different subtypes of the same genotype. The N-terminal 27 residues of E2 (aa 384-410) show a very high degree of variation and this portion of the sequence has been termed hypervariable region 1 (HVR-1) (21). This region plays a critical role in HCV interaction with host cells as HVR-1-delted HCVpp demonstrate reduced infectivity (22, 23). The important role of HVR-1 in HCV infectivity is further supported by studies demonstrating that antibodies targeting regions within HVR-1 inhibit cellular recombinant E2 (24, 25) and HCV-LP binding (15, 26) as well as HCVpp entry into target cells (18, 27). The exact role of E1 still remains unknown. E1 may directly interact with cell

surface molecules and/or contribute to proper folding and processing of E2. Interestingly, antibodies targeting the N-terminal region of E1 have been shown to inhibit HCV-LP binding (15, 28) as well as HCV infection of a B-cell-derived cell line (29) suggesting that E1-cell surface interaction may contribute to viral binding and entry. In addition, HCV envelope proteins E1 and E2 are thought to induce fusion between the viral envelope and host cell membranes (30).

3.2. Cellular determinants

Using recombinant HCV envelope glycoproteins and HCV-LPs, several cell surface molecules have been identified interacting with HCV during viral binding and entry. These include CD81 (13), the low-density lipoprotein (LDL) receptor (31), highly sulfated heparan sulfate (32), scavenger receptor class B type I (SR-BI) (24) and DC-SIGN (dendritic cell-specific intercellular adhesion molecule 3 grabbing non integrin)/L-SIGN (DC-SIGNr, liver and lymph node specific) (33, 34). Experimental data using HCVpp and HCVcc have confirmed functional roles for heparan sulfate (35) as well as CD81 (9, 19, 35) and SR-BI (22, 36-38) in HCV binding and entry, respectively. Most recently, an additional entry factor, claudin-1, has been identified (39).

3.2.1. Capture molecules

3.2.1.1. Glycosaminoglycans: heparan sulfate

Glycosaminoglycan (GAG) chains on cell surface proteoglycans provide primary docking sites for the binding of various viruses and other microorganisms to eukaryotic cells. The GAG heparan sulfate is an important cellular binding molecule for several viruses and may serve as the initial docking site for HCV attachment before the virus is transfered to high-affinity entry receptors. In fact, incubation of HCV-LPs with heparin - a structural homolog of highly sulfated heparan sulfate – reduced HCV-LP binding to human hepatoma cells resulting in a decreased internalization of theses particles (32). In addition, incubation of serum-derived HCV or VSV/HCV pseudotypes with heparin markedly inhibited virus binding to target cells (40, 41). Most recently, using the HCVcc system, it could be confirmed that heparan sulfate plays an important role for HCV binding as heparin and pre-treatment of cells with heparinases reduced HCVcc infectivity (35).

3.2.1.2. Lectins: DC-SIGN/L-SIGN

C-type lectins may act both as adhesion molecules and as pathogen recognition receptors. The mannose binding C-type lectins DC-SIGN and L-SIGN serve as adhesion receptors to mediate contact between dendritic cells, T lymphocytes and endothelial cells. Both lectins are not expressed in hepatocytes. DC-SIGN is expressed in Kupffer cells, which are immobile liver macrophages localized close to LSEC and hepatocytes (42). L-SIGN is highly expressed in liver sinusoidal endothelial cells. DC-SIGN and L-SIGN recognize carbohydrate structures on pathogens (43) and have been shown to bind envelope glycoprotein E2 with high affinity (33, 44). L-SIGN and DC-SIGN expressed on human Radji B cells or HeLa cells have also been shown to capture and transmit HCVpp to human hepatoma Huh7 cells in coculture model systems (34, 45). Capture of circulating HCV particles by liver sinusoidal cells may thus facilitate viral infection of neighbouring hepatocytes which are not in direct contact with circulating blood and may, therefore, contribute to the pathogenesis of viral infection.

3.2.2. Entry receptors

3.2.2.1. Tetraspanins: CD81 and claudin-1

Tetraspanins are widely expressed proteins that regulate cell morphology, motility, invasion, fusion and signalling (46). These proteins contain four transmembrane domains, short intracellular domains and two extracellular loops, namely the small extracellular loop and the large extracelullar loop (LEL). CD81 has been identified as an HCV E2 binding molecule by expression cloning (13) using a cDNA library derived from a subclone of the human T cell lymphoma cell line Molt-4, which exhibits a high E2-binding capacity (25). Anti-CD81 antibodies as well as a soluble form of CD81 LEL have been shown to inhibit HCVpp and HCVcc entry into Huh-7 hepatoma cells and human heptocytes (9, 17-20, 45, 47, 48). Furthermore, silencing of CD81 expression in hepatoma cells by small interfering RNAs inhibited HCVpp entry as well as HCVcc infectivity and expression of CD81 in hepatoma cell lines that are resistant to HCVpp and HCVcc infection conferred

susceptibility to HCV infection (22, 45, 47, 49). In addition, it has been demonstrated that CD81 expression levels on hepatoma cells correlate with HCV infectivity (49, 50). These results suggest that susceptibility to HCV infection may be linked to CD81 density on the cell surface and thus provide an explanation for HCV tissue tropism in vivo. Interestingly, CD81 from HCV refractory species are able to bind HCV E2 (51) and CD81 of various species may confer susceptibility to HCV infection (52) suggesting that CD81 is not the determinant for the species specificity of HCV. Recent studies using the HCVpp and HCVcc model system demonstrated the ability of anti-CD81 antibodies to inhibit HCV entry at a step post binding (35, 53) suggesting that CD81 functions as an HCV entry co-receptor after docking of the virus to attachment molecules. Most recently, another member of the tetraspanin family claudin-1 (CLDN1), has been identified as an HCV co- entry factor by expression cloning (39). CLDN1 is highly expressed in the liver but also in other tissues (54). However, CLDN1 expression correlates with HCV permissiveness and expression of CLDN1 in non-hepatic 293T cells renders them susceptible to HCVpp entry (39). In addition, overexpression of this molecule in CD81-deficient HepG2 hepatoma cells did increase their HCV permissivity, suggesting that CLDN1 is not an alternative entry pathway to CD81 (39). In addition, kinetic studies showed that CLDN1 acts at a post binding step after HCV interaction with CD81 (39). As tetraspanins are able to form associations with a wide variety of proteins as well as cholesterol and gangliosides (46), this suggests that several HCV co-receptors may be recruited to discrete membrane domains allowing the formation of an HCV entry receptor complex. Interestingly, murine CLDN1 also supports HCVpp entry, demonstrating that CLDN1, as CD81, is not a determinant for species host range (39).

3.2.2.2. Scavenger receptor SR-BI

SR-BI or CLA-1 (CD36 and LIMPII Analogous-1) is a 509 amino acid glycoprotein with a large extracellular loop anchored to the plasma membrane at both the N- and C- termini by transmembrane domains with short extensions into the cytoplasm (55). SR-BI is involved in bidirectional cholesterol transport at the cell membrane and is a multiligand receptor as it can bind both native high-density lipoprotein (HDL) and low density lipoproteins (LDL) as well as modified lipoproteins such as oxidized LDL (oxLDL). SR-BI is highly expressed in liver and steroidogenic tissues (55) as well as human monocyte-derived dendritic cells but not on any other peripheral blood mononuclear cells (56). Furthermore, SR-BI and its splicing variant SR-BII, have been found to mediate binding and uptake of a broad range of bacteria into nonphagocytic human epithelial cells overexpressing SR-BI and SR-BII (57, 58) suggesting that class B scavenger receptors may serve as pattern recognition receptors for bacteria. Cross-linking studies using recombinant C-terminally truncated HCV envelope glycoprotein E2 isolated SR-BI on HepG2 cells as a cell surface protein binding HCV envelope glycoprotein E2 (24). SR-BI recognition by HCV E2 required the hypervariable region HVR-1 (24). Moreover, antibodies directed against cell surface expressed SR-BI inhibited binding of recombinant envelope glycoproteins and HCV-like particles to primary hepatocytes (15) as well as HCVpp entry (22, 59, 60). In addition, it has been shown that physiological SR-BI ligands, such as HDL or oxLDL, can modulate HCV infection: HDL and oxLDL have been shown to enhance and inhibit HCVpp entry, respectively (61-63), whereas both HDL and LDL inhibited HCV replication in human hepatocytes infected with serum-derived HCV (64). These results suggest the existence of a complex interplay between lipoproteins, SR-BI and HCV envelope glycoproteins for HCV entry. Most recently, the important role of SR-BI in productive HCV infection has been confirmed using the HCVcc system. Overexpression of SR-BI and SR-BII was able to increase HCVcc infectivity (36) while down-regulation of this receptor by SR-BI specific siRNA markedly reduced the susceptibility of human hepatoma cells to HCVcc infection (Zeisel MB, Schnober EK, Haberstroh A, Lavillette D, Cosset FL, Wakita T, Jaeck D, Royer C, Schuster C, Stoll-Keller F, Blum H, Barth H, Baumert TF. 13th International Meeting on Hepatitis C Virus & Related Viruses 2006, Cairns, Australia). Moreover, anti-SR-BI antibodies directed against epitopes of the SR-BI extracellular loop specifically inhibited HCVcc infection and kinetic studies demonstrated that SR-BI acts predominately following binding of HCV at an entry step

occurring at a similar time point as CD81-HCV interaction (38).

3.2.2.3. LDL receptor

The LDL receptor (LDLR) transports cholesterol-containing lipoproteins into the cell by endocytosis *via* clathrin-coated pits. Receptor-ligand complexes are delivered into endosomes where low pH induces the release of lipoproteins which then proceed to lysosomes where free cholesterol is generated by cholesterol ester hydrolysis (65). The apolipoprotein B (apoB)-containing LDL and apolipoprotein E (apoE)-containing very low-density lipoproteins (VLDL) are the major LDLR ligands. As HCV is able to associate with LDL and VLDL in serum (66, 67), the LDLR was suggested to be a putative HCV receptor candidate. The LDLR has been shown to mediate internalize serum-derived HCV by binding virus-LDL particles (31). Anti-LDLR antibodies as well as anti-apoB and apoE antibodies were able to inhibit HCV endocytosis (17, 31, 40, 68). It could also be demonstrated that LDLR plays a role in an early step of serum-derived HCV infection of primary human hepatocytes (64). However, studies using the HCVpp system where HCV is not associated with lipoproteins suggest that LDLR does not appear to play a role for infection of Huh7 cells with HCVpp (17). Further studies using HCVcc and human hepatocytes will allow gaining more insight into the role of LDLR in HCV infection.

Despite the numerous experimental data demonstrating the importance of the above described receptors in HCV infection, none of these molecules has a liver-specific expression profile as it would be expected for receptors of a hepatotropic virus. Moreover, all HCV permissive cell lines identified so far express CD81, SR-BI and CLDN1 and are of hepatic origin but various cell lines of non-hepatic origin expressing these receptors are non permissive for HCV (17, 22, 27, 47), suggesting that additional liver specific factor(s) which are still to be discovered are required for HCV infection.

3.3. Mechanisms of HCV internalization and fusion

To multiply, viruses must deliver their genome into host cells. The critical step subsequently leading to viral replication is the penetration of the viral genome through a host cell membrane. Cell attachment of other members of the *Flaviviridae* family such as flaviviruses leads to endocytosis of bound virions (69). Clathrin-mediated endocytosis is the most commonly route of endocytosis for viruses that require internalization. It transports incoming viruses together with their receptors into early and late endosomes (6). It has been shown that early and late endosomes constitute distinct entry sites depending on the pH threshold of viral proteins. The acidic pH in endosomes provides an essential cue that triggers penetration and uncoating. Penetration of enveloped virus occurs by membrane fusion catalyzed by fusion peptides embedded in the viral envelope glycoproteins (70). In some cases, acidic pH is not sufficient to induce fusion and viral proteins need to be cleaved by endosomal proteases to become fusion competent. Two classes of viral fusion proteins (class I and II) mediating entry of enveloped viruses have been defined. Type II fusion proteins, occuring in flaviviruses and alpha viruses, are synthezised as heterodimers with other proteins that dissociate at the acidic pH in the endosome and assemble into more stable homotrimers that destabilizes the target cell membrane and then leads to the formation of a fusion pore (6, 71). Recent studies using HCVpp and HCVcc have demonstrated that HCV entry into both hepatoma cells and primary human hepatocytes depends on clathrin-mediated endocytosis (72-74). Structural homology with fusion proteins from flaviviruses suggests that HCV envelope glycoproteins may belong to class II fusions proteins (10, 75-77). Although – in contrast to flaviviruses- HCV glycoproteins are not matured by a cellular endoprotease during their transport through the secretory pathway (77), similar membrane fusion mechanisms may operate in HCV. This hypothesis is supported by the observation that HCVpp entry into Huh-7 cells is pH-dependent (17, 18, 78). In addition, HCVcc infection was markedly inhibited by agents preventing the acidification of endosomal compartments, suggesting that a pH-dependent membrane fusion process may be required for delivery of the HCV genome into the host cell cytosol (72, 79). Finally, it has been shown that HCVpp are delivered to early but not late endosomes (73). As HCV fusion kinetics are delayed as compared to other viruses, it has been suggested that after internalization, HCVpp entry necessitates additional, low-pH-dependent

interactions, modifications, or trafficking (73). However, neither HCVpp nor HCVcc require cleavage by endosomal proteases for fusion (73, 79).

4. VIRAL ENTRY: TARGET FOR ANTIVIRAL THERAPY

Current approaches for HCV antiviral drug development targeting viral enzymes comprise protease inhibitors, inhibitors of protein translation and polymerase inhibitors (for review see (80)). Interfering with HCV entry also holds promise for drug design and discovery as the understanding of molecular mechanisms underlying HCV interaction with the host cell is growing. Viral entry may be inhibited (i) by blocking interaction between the virus and the target cell, (ii) by interfering with post binding events, and (iii) by interfering with viral fusion (Fig. 1).

4.1. Neutralizing antibodies

Viral attachment and entry is a major target of adaptive host cell defences. Viral proteins are recognized as non-self by the host's immune system and induce the production of antibodies. A small proportion of these antibodies exhibit antiviral activity *in vitro* and are defined as virus-neutralizing antibodies. These antibodies render virions non-infectious by interfering with receptor binding and cell entry. Many successful antiviral vaccines are based on the induction of neutralizing antibodies. Isolation and characterization of antibodies targeting distinct steps of HCV entry is an important strategy for protection against this virus and provide a rational basis for HCV vaccine development. Antibody-mediated neutralization occurs during HCV infection *in vivo* but the role of antibodies for the control of HCV infection is still unclear. Antibodies with HCV neutralizing properties have been first described in experimental infection of chimpanzees (81, 82). These antibodies were directed against epitopes in the hypervariable region (HVR-1) of HCV envelope glycoprotein E2 and appeared to be isolate-specific (81, 82). The presence of antibodies directed against HVR-1 has also been associated with viral clearance in HCV-infected humans (83) and HCV-infected patients with primary antibody deficiencies have been reported to have accelerated rates of disease progression (84, 85). However, HCV infection is established despite the induction of an humoral immune response that targets various epitopes of the HCV envelope glycoproteins (26, 27, 86-88). Until recently, functional studies analyzing the neutralizing antibody response during acute and chronic HCV infection using HCV model systems demonstrated a lack of neutralizing antibodies in the majority of patients with acute HCV infection (26, 27, 86, 89). These studies were limited by the fact that the viral surrogate ligand was derived from a different isolate than the virus present in the infected patient thus precluding the detection of isolate-specific antibodies. Most recently, studies using well defined nosocomial or single-source HCV outbreaks with a defined inoculum enabled for the first time to study the role of isolate-specific neutralizing antibodies for HCV clearance in humans. Using the HCVpp model system, two studies demonstrated that neutralizing antibodies are induced in the early phase of infection by patients who subsequently clear the virus (90) or control viral infection (60). These results suggest that a strong early neutralizing antibody response may play a role in the outcome of HCV infection. Patients who do not clear the virus develop high-titer and even cross-neutralizing antibodies during the chronic phase of infection (9, 26, 27, 86, 90). Viral escape from antibody-mediated neutralization in these individuals may occur on several levels: (i) HCV exists as a quasispecies with distinct viral variants in infected individuals changing constantly over time and his variability has been shown to represent a mechanism of escape from antibody-mediated neutralization in the chimpanzee model (27); (ii) the interplay of HCV glycoproteins with high-density lipoprotein and the scavenger receptor BI has been shown to mediate protection from neutralizing antibodies present in sera of acute and chronic HCV-infected patients (61, 91); and (iii) as shown for other viruses such as HIV, escape from neutralizing antibodies may occur through a combination of different mechanisms, for instance point mutations, insertions/deletions or changes in glycosylation patterns of the viral envelope (92) or conformational masking of receptor binding sites following envelope-antibody interaction (93) preventing neutralizing antibody binding. The HCV tissue culture model systems and patient cohorts with well defined viral isolates will now allow to adress these questions and

define therapeutic strategies based on neutralizing antibodies. Several viral epitopes targeted by neutralizing antibodies have already been identified: epitopes of the E2 HVR-1 region (aa 384-410) (18, 25, 61), an epitope adjacent to the N-terminal region HVR-1 region (aa 408-422) (26, 94), the E2 CD81 binding region (aa 474-494 and aa 522-551) (18, 47, 94) and conformational epitopes within glycoprotein E2 (95, 96). These epitopes may represent potential candidate targets for antibodies in passive immunoprophylaxis. Indeed, two studies have demonstrated that monoclonal antibodies directed against conformational epitopes (95) or epitope aa 412-423 exhibited broad cross-neutralizing activity among all major genotypes of HCVpp entry (94) as well as HCVcc infectivity (97). Future *in vivo* studies are required to study the relevance of these findings for antibody-mediated prevention of HCV infection. This may have important implications for the development of novel preventive and curative antiviral strategies, e. g. passive immunoprophylaxis after accidental exposure to HCV and prevention of reinfection of liver grafts after liver transplantation.

4.2. Entry inhibitors

4.2.1. Inhibitors of viral attachment

The lectin cyanovirin-N (CV-N) has initially been discovered as an active compound against HIV and was then shown to present antiviral activity against other enveloped viruses (98, 99). This antiviral activity results from interactions between CV-N and high-mannose oligosaccharides on viral envelope glycoproteins (100). HCV envelope glycoproteins are highly glycosylated and contain oligomannose glycans. It has been shown that these oligomannose glycans interact with CV-N resulting in HCV antiviral activity by blocking HCV entry into target cell (101). As most of the HCV glycosylation sites are highly conserved, drugs that target glycans on HCV glycoproteins may not lead so rapidly viral escape/resistance as it is the case for HIV (92). Other carbohydrate-binding agents, such as plant lectins, monoclonal antibodies and the mannose-specific non-peptidic antibiotic Pradimicin A have been shown to prevent HIV infectivity (102). Such substances might also be efficient against other viruses that require a glycosylated envelope for entry into target cells.

Another approach to target HCV attachment might be the development of heparin-derived molecules, as heparin has been shown to potently inhibit HCV E2, HCVpp, HCV-LP as well as HCVcc binding to hepatoma cells (32, 35, 53, 103). The systematic generation and screening of heparan sulfate-like molecules and semisynthetic derivatives is already explored as an antiviral approach against dengue virus infection (104).

4.2.2. Inhibitors of post binding steps

Antiviral compounds targeting viral entry may either act on conserved mechanisms or target specific cell surface molecules. Recent studies have shown that long phosphorothioate oligonucleotides (PS-ON), that are amphipathic DNA polymers, displays a sequence independent antiviral activity against HIV by blocking virus-cell fusion (105). Most recently, it could be demonstrated that PS-ON inhibit HCV fusion and entry (Matsumara T, Kato K, Hu Z, Juteau JM, Vaillant A, Liang JT. The 57th Annual Meeting of the American Association for the Study of Liver Diseases, 2006, Boston, USA). The PS-ON are a promising new class of antiviral compounds that may have a broad spectrum in all families of enveloped viruses.

Structural information of HCV envelope glycoprotein E2 and CD81 was used to identify imidazole based compounds that mimic an alpha helix in the LEL of CD81 and compete for the binding of HCV E2 to CD81 expressed on target cells. These drugs bind HCV E2 in a reversible manner and block HCV E2 interaction with CD81 while having no effect on CD81 expression nor on CD81-interaction with physiological partner molecules (106). However, data of the effect of these drugs on HCVcc infection are not yet available. Recently, SR-BI has been demonstrated to bind and internalize serum amyloid A (SAA), an acute phase protein mainly produced in the liver and known to mediate pro-inflammatory cellular responses (107, 108). SAA was shown to inhibit HCV entry by interacting with the virus thereby reducing its infectivity (109). Thus, SAA analogues might present potent anti-HCV activity. In addition to small molecule inhibitors, peptides that mimic conserved regions of HCV E2 interacting with cell entry receptors may also provide an interesting approach to prevent HCV infection but have weaknesses as drugs

because they are not orally available and expensive to produce.

4.2.3. Fusion inhibitors

Insights into the molecular mechanisms of HCV fusion are just about to arise and molecules likely to interfere with HCV penetration have not yet been described. As HCV enters the host cell through endocytosis and requires low pH for delivery of HCV genome, agents preventing the acidification of endosomal compartments, such as chloroquine, are able to prevent infection (72, 79). Potential targets for anti-fusion drugs might arise in the next few years while insights into HCV fusion process are growing. Peptide-based fusion inhibitors have already been established for the treatment of other viral infections such as HIV infection. Enfuvirtide which blocks HIV fusion to host cells is the first compound of this family approved for clinical use (110).

5. PERSPECTIVES

The development of novel HCV model systems allowed to increase the understanding of the complex viral entry process thereby offering new therapeutic targets to prevent the virus to reach its site of replication. Major progress has been made over the past few years in the characterization of host cell molecules involved in virus entry and the sequence of events ultimatively leading to viral replication. Both virus and host cell components involved in virus entry may serve as targets for the development of HCV entry inhibitors (Table 1). As for other infectious diseases, it might be preferable to target viral proteins than host cell components because of potential adverse effects resulting form interference with normal cell functions. The optimal entry inhibitor would block viral binding sites on receptors without affecting functional physiological ligand binding. Future anti-HCV therapies might be combinations of drugs targeting distinct steps of HCV infection, i. e. entry inhibitors, protease inhibitors and polymerase inhibitors that might have complementary effects and delay the emergence of drug resistance.

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Abbreviations: aa: amino acid; apo: apolipoprotein; CLDN1: claudin-1; DC-SIGN: dendritic cell-specific intercellular adhesion molecule 3 grabbing non integrin; HCV: hepatitis C virus; HCVcc: cell culture-derived HCV; HCV-LP: HCV-like particles; HCVpp: HCV pseudoparticles; HDL: high-density lipoprotein; HIV: human immunodeficiency virus; HVR-11: hypervariable region-1; LDL: low-density lipoprotein; LDLR: LDL receptor; L-SIGN: DC-SIGNr, liver and lymph node specific; siRNA: small interfering RNA; SR-BI: scavenger receptor class B type I; VLDL: very low-density lipoprotein

Keywords: hepatitis C virus, viral entry, entry inhibitor, neutralizing antibodies

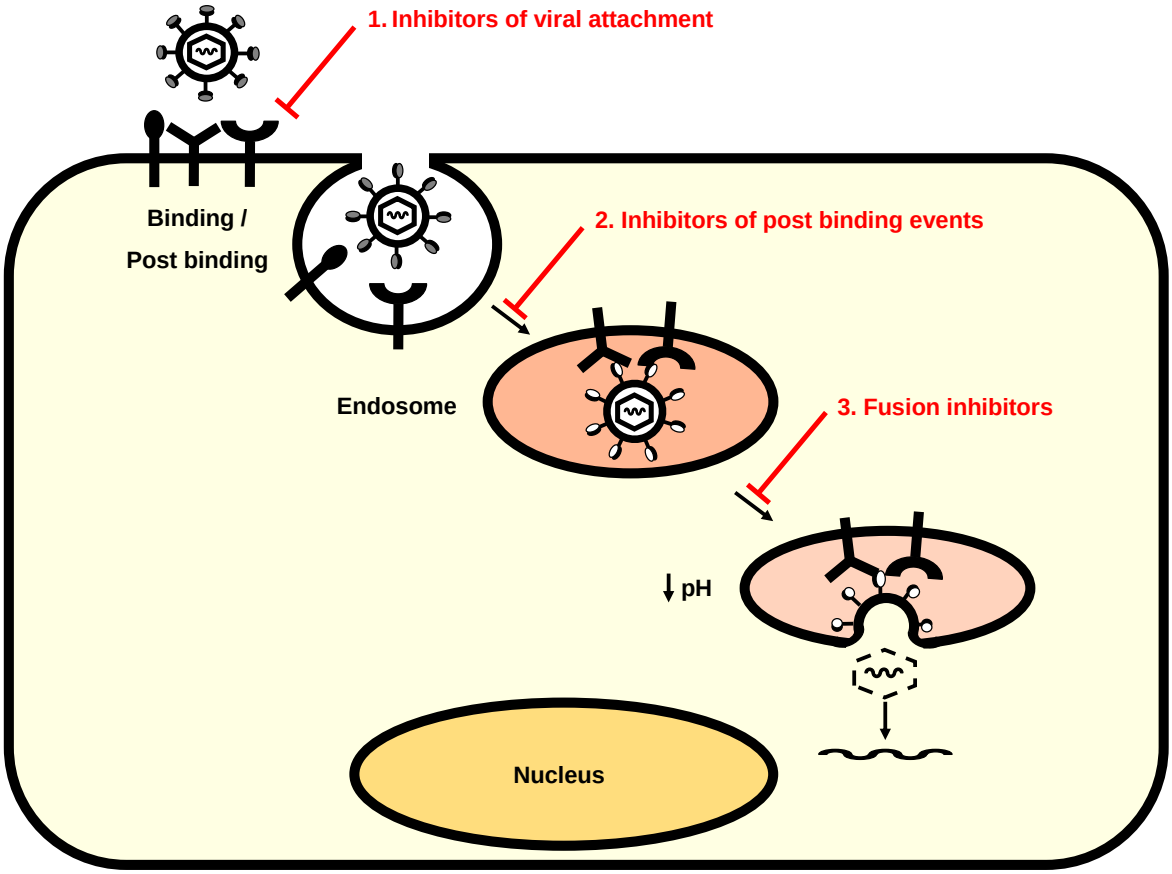
Fig. 1. HCV entry: targets for antiviral therapy. Both virus and host cell components involved in virus entry may serve as targets for the development of HCV entry inhibitors. Viral entry may be inhibited (1) by blocking interaction between the virus and the target cell, (2) by interfering with post binding events, and (3) by interfering with viral fusion. Future anti-HCV therapies might be combinations of drugs targeting distinct steps of HCV infection, i. e. entry inhibitors, protease inhibitors and polymerase inhibitors that might have complementary effects and delay the emergence of drug resistance.

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Table 1. HCV entry: viral and cellular targets for antiviral therapy

Antiviral target	**Antiviral compounds**
Viral targets	
HCV envelope glycoproteins	Neutralizing antibodies
	Carbohydrate binding agents, e. g. cyanovirin
	Heparin-derived molecules
	Imidazole-based compounds
	Serum amyloid A
Cellular targets	
HCV entry molecules	HCV E2 peptides
	Phosphorothioate oligonucleotides
Viral fusion mechanisms	Endososomal acidification inhibitors, e. g. chloroquine
	Peptide-based fusion inhibitors

Fig. 1


1. Inhibitors of viral attachment
Binding /
Post binding
2. Inhibitors of post binding events
Endosome
3. Fusion inhibitors
pH
Nucleus