Review

EASL EUROPEAN ASSOCIATION FOR THE STUDY OF THE LIVER | JOURNAL OF HEPATOLOGY

Hepatitis C virus entry into hepatocytes: Molecular mechanisms and targets for antiviral therapies

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Hepatitis C virus (HCV) is a major cause of liver cirrhosis and hepatocellular carcinoma. Preventive modalities are absent and the current antiviral treatment is limited by resistance, toxicity, and high costs. Viral entry is required for initiation, spread, and maintenance of infection, and thus is a promising target for antiviral therapy. HCV entry is a highly orchestrated process involving viral and host cell factors. These include the viral envelope glycoproteins E1 and E2, CD81, scavenger receptor BI, and tight junction proteins claudin-1 and occludin. Recent studies in preclinical models and HCV-infected patients have demonstrated that the virus has developed multiple strategies to escape host immune responses during viral entry. These include evasion from neutralizing antibodies and viral spread by cell–cell transmission. These challenges have to be taken into account for the design of efficient antiviral strategies. Thus, a detailed understanding of the mechanisms of viral entry and escape is a prerequisite to define viral and cellular targets and develop novel preventive and therapeutic antivirals. This review summarizes the current knowledge about the molecular mechanisms of HCV entry into hepatocytes, highlights novel targets and reviews the current preclinical and clinical development of compounds targeting entry. Proof-of-concept studies suggest that HCV entry inhibitors are a novel and promising class of antivirals widening the preventive and therapeutic arsenal against HCV infection.

Introduction

Hepatitis C virus (HCV) is a major cause of chronic hepatitis worldwide. The current therapy against HCV infection, consisting of an association of pegylated interferon alpha (PEG-IFN) and ribavirin, is limited by resistance, adverse effects, and high costs. Although the clinical development of novel antivirals targeting HCV protein processing has been shown to improve sustained virological response, toxicity of the individual compounds and development of viral resistance remain major challenges [1–3]. To date, a vaccine is not available. The absence of preventive strategies is a major limitation for patients undergoing liver transplantation (LT) for HCV-related end-stage liver disease. Reinfection of the graft is universal and characterized by accelerated progression of liver disease [4]. Moreover, treatment of recurrent HCV infection after LT is challenging due to enhanced adverse effects and limited efficacy of IFN-based therapies in LT recipients [4,5]. Recurrent HCV liver disease in the graft with poor outcome has become an increasing problem faced by hepatologists and transplant surgeons. Thus, novel antiviral preventive and therapeutic strategies are urgently needed.

Viral entry is the first step of virus–host cell interactions leading to productive infection and thus represents an interesting target for antiviral therapy. HCV entry is believed to be a highly orchestrated process involving several viral and host cell factors, thereby offering multiple novel targets for antiviral therapy. However, multiple strategies evolved by the virus in order to escape the host immune system, such as escape from neutralizing antibodies and direct cell–cell transmission, have to be taken into account for the design of efficient novel antiviral strategies. Understanding the mechanisms of viral entry and escape is thus a prerequisite to define the viral and cellular targets that will give broad protection against HCV infection.

HCV is an enveloped single-strand RNA virus that mainly targets hepatocytes. Due to the difficulty to grow HCV *in vitro* and the species specificity of this virus, surrogate model systems have been developed to study HCV entry into hepatocytes: recombinant envelope glycoproteins [6], HCV-like particles (HCV-LP) [7], HCV pseudo-particles (HCVpp) [8,9] and recombinant infectious HCV (HCVcc) [10–12] have been used to study the interactions of the viral envelope with human hepatoma cells or primary human hepatocytes. Moreover, the use of transgenic immunodeficient mice with hepatocyte-lethal phenotype (Alb-uPA/SCID [13] and Fah/Rag2/IL2rγ mice [14]), that can be successfully transplanted with primary human hepatocytes, allowed to establish a small animal model to study certain aspects of HCV infection *in vivo* [15,16].

Using the above described model systems, tremendous progress has been made over the past years in deciphering the

Keywords: Hepatitis C virus; Entry; Antivirals.
Received 9 August 2010; received in revised form 12 October 2010; accepted 14 October 2010
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Abbreviations: CLDN1, claudin-1; HCV, hepatitis C virus; HCVcc, cell culture-derived HCV; HCVpp, HCV pseudo - particle; HCV-LP, HCV-like particle; JFH1, Japanese fulminant hepatitis 1; OCLN, occludin; SR-BI, scavenger receptor class B type I.

mechanisms of HCV-host interactions leading to viral entry. The understanding of these mechanisms has allowed researchers to identify novel targets for antivirals, and several compounds are reaching early clinical development. The aim of this review is to summarize the current knowledge on the complex mechanisms of HCV entry into host cells, as well as to highlight the antiviral targets and to review the current development of HCV entry inhibitors that represent a novel important class of antivirals. Developing efficient HCV entry inhibitors may hold great promises to improve the sustained virological response in chronic HCV-infected patients and thus prevent HCV re-infection during LT.

Hepatitis C virus evades host immune responses to enter the hepatocyte

Viral entry is the first step of HCV infection that requires interaction of the HCV envelope glycoproteins E1 and E2 and the host cell membrane. E1 and E2 are type I transmembrane proteins with an N-terminal ectodomain and a short C-terminal transmembrane domain (TMD). Functional virion-associated E1E2 envelope glycoproteins mediating viral entry form large covalent complexes stabilized by disulfide bridges [17]. The TMD plays a major role in the biogenesis of the E1E2 complexes and membrane fusion process [18]. The N-terminal ectodomains of E1 and E2 are heavily glycosylated. The glycans play a major role in E1E2 folding as well as HCV entry [19] and are of crucial importance for the evasion from the host immune responses by masking immunogenic envelope epitopes [20]. Moreover, HCV exists in heterogenous forms in human serum and may be associated with VLDL, LDL, and HDL [21–24] also shielding the virus from neutralizing antibodies targeting the HCV envelope glycoproteins.

Key points 1

- HCV entry into hepatocytes is a highly coordinated and multistep process requiring viral and host cell factors.
- The viral envelope glycoproteins E1 and E2 are essential for HCV entry.
- Lipoproteins have been shown to associate with the viral particle and interfere with viral entry.
- Host factors mediating viral attachment and binding to hepatocytes include highly sulfated heparan sulfate and the low-density lipoprotein receptor.
- CD81, scavenger receptor BI and the tight junction proteins caudin-1 and occludin act on a post-binding step and are essential for HCV entry.
- Host factors such as CD81 and CLDN1 form co-receptor complexes.
- HCV entry into hepatocytes depends on clathrin-mediated endocytosis.
- HCV appears to be delivered to early endosomes where the acidic pH provides an essential cue that triggers penetration and uncoating.
- An alternative route of viral entry is direct cell–cell transmission which is resistant to neutralizing antibodies.

Both E1 and E2 contain putative fusion domains [25,26]. While the role of E1 in HCV entry is not completely understood, several E2 domains play pivotal roles in viral entry, i.e. putative domain binding to two HCV entry factors, CD81 and scavenger receptor class B type I (SR-BI), and escape from host immune responses. Hypervariable regions (HVR) have been identified in E2. The first 27 amino acids of E2 called hypervariable region 1 (HVR1), are the most divergent among HCV isolates. HVR1 plays an important role in viral fitness, likely due to an involvement in SR-BI-mediated entry [27], assembly and release of virus particles [28] as well as HCV membrane fusion process [28]. HVR1 is a target for neutralizing antibodies. However, due to its high variability, antibodies targeting HVR1 exhibit poor cross-neutralization potency across different HCV isolates [29]. Broadly neutralizing antibodies are directed against conserved conformational epitopes within E2 [30,31] and mostly inhibit E2–CD81 interaction [32]. The region located immediately downstream of HVR1 contains a potent and highly conserved epitope. This epitope defined by the mouse monoclonal antibody (mAb) AP33 and a rat mAb 3/11, is involved in E2–CD81 [33] and E2-heparan sulfate interaction [34]. Importantly, mutated variants that escape from AP33 neutralization show very low infectivity [35]. Recently, new conformational and conserved epitopes were identified in the N-terminal part of E2. Antibodies targeting these epitopes neutralize genetically diverse HCV isolates and protect against heterologous HCV quasispecies challenge in the human liver-chimeric AlbuPA/SCID mouse model [31]. Since these epitopes are thought to be involved in HCV entry, viral mutation could induce escape from broadly neutralizing antibodies but at a substantial cost in viral fitness [35]. The conserved nature of these epitopes makes them of interest for vaccine and immunotherapeutic development.

In vivo, humoral responses are thought to play an important role in controlling HCV infection. Indeed, spontaneous resolvers tend to have an early induction of neutralizing antibody responses, whereas chronically evolving subjects have a delayed initiation of neutralizing antibody responses [36–38]. Furthermore, the generation of cross-reactive humoral responses is associated with protection against HCV re-infection [39]. These data suggest that protective immunity following HCV infection is possible and highlights the plausibility of preventive antiviral strategies including a vaccine [39]. However, the accelerated evolution [40] and the diversity of HCV, as well as the variety of strategies the virus evolved to escape antibody-mediated neutralization (reviewed in [41]), are a major challenge. Indeed, due to its very high replication rate and the highly error prone viral polymerase, HCV circulates as a pool of genetically distinct but closely related variants known as viral quasi-species. The capacity of HCV to mutate continuously allows a high plasticity, an ability of the virus to adapt to variable environmental conditions and escape the host's immune responses leading to HCV persistence [42,43]. Noteworthy, a recent longitudinal analysis of six HCV-infected patients undergoing LT suggests that efficient entry and escape from host neutralizing antibodies represent important mechanisms for the selection of HCV during LT [43]. As strains selected during LT could be neutralized by broadly neutralizing antibodies, the major challenge for developing efficient antiviral strategies targeting the HCV envelope glycoproteins will be to identify epitopes largely conserved among genotypes and selected isolates.