Manuscript title:

Antibodies to the high density lipoprotein receptor SR-B1 potently inhibit hepatitis C virus replication in vivo: New avenues for preventing reinfection of the liver following transplantation.

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Direct acting antivirals (DAAs), together with pegylated interferon and ribavirin, have shown great promise in the successful treatment of chronic hepatitis C virus (HCV) infections. However, the use of DAAs in a setting of liver transplantation remains problematic due to side effects, reduced efficacy and the possibility of selecting a resistant virus subpopulation. An alternative treatment strategy is to target cellular proteins essential for viral replication.

Hepatitis C virus uniquely exploits the physiological pathways of the liver to complete its viral replication cycle. Unlike other viruses, HCV exists as a lipoviral particle with similar lipid and cholesterol content to that of the serum lipoproteins. Decorating the surface of HCV are two virus-encoded glycoproteins, E1 and E2, that function together to allow entry into the hepatocyte. Productive entry of the HCV lipoviral particle into hepatocytes occurs in a coordinated manner and involves initial binding to glycosaminoglycans and the high-density lipoprotein receptor, scavenger receptor class B type 1 (SR-B1). At this stage, HCV entry depends on the lipid exchange properties of SR-B1 that modifies the lipoviral-particle such that glycoprotein E2 can now bind the cell surface receptor CD81. This binding event triggers lateral movement of CD81-bound particles to tight junctions where they encounter claudin-1 and occludin. Following clathrin dependent endocytosis, the lipid transfer properties of Niemann-Pick C1-like 1 (NPC1L1) and SR-B1 further remodel the lipoviral particle, priming the viral glycoproteins such that low pH dependent fusion of the viral and endosomal membrane occurs. These events ultimately result in the delivery of the nucleocapsid to the cytoplasm of the cell [for review see (1)]. In addition to this cell-free virus entry pathway, HCV can be transmitted between adjoining cells via cell-cell transmission. Cell-cell transmission of HCV is more resistant to viral glycoprotein-directed antibody-mediated neutralization and may confound antibody-based therapeutic approaches targeting the viral glycoproteins (2).

The role of SR-B1 in HCV entry is multifaceted, being dependent on both its physiological role of binding lipoproteins and bidirectional cholesterol exchange as well as direct binding to the viral glycoprotein E2. SR-B1 is highly expressed in the liver and the level of SR-B1 in liver grafts correlates with viral load decay suggesting it plays a major role in uptake of HCV during liver transplantation (3). Furthermore, SR-B1 plays a major role in both cell-free and cell-cell transmission of HCV. As such, SR-B1 is an attractive antiviral target as it provides multiple opportunities to block virus entry and spread. The small molecule SR-B1 inhibitor ITX5061 inhibits HDL lipid transfer, blocks E2 binding to SR-B1 and potently inhibits HCV replication in cell culture (4). As a result of these promising studies, ITX5061 has entered clinical development and was shown to be safe and well tolerated in a Phase Ib study conducted in treatment naïve Genotype 1a infected adults, however viral loads were not significantly reduced (5). In the setting of liver transplantation, more promising results were achieved with a $2Log_{10}$ reduction in viral load observed in 6/6 treated patients compared with 0/6 in the control group in the first seven days after transplantation with no adverse events reported (6).

Alternatives to small molecule inhibitors are monoclonal antibodies (mAb) that block SR-B1 function. One such antibody, mAb1671, has previously been shown to potently inhibit both cell-free and cell-cell infection of hepatocytes with cell culture-derived HCV. The mAb can prevent infection of

human liver uPA-SCID mice when given prior to challenge with HCV, and can inhibit the spread of virus in an established productive infection (7). However, HCV has the capacity to acquire mutations that can alter its dependence on cellular receptors involved in viral entry. Indeed, numerous mutations within the viral glycoproteins have been shown to reduce the dependence of HCV to utilize SR-B1 as an entry receptor thereby making them more resistant to anti-SR-B1 therapy in cell culture (8-11). Such anti-SR-B1 resistant variants could potentially confound the use of therapeutic agents that target SR-B1.

In this issue of Hepatology, Vercauteren et al examine a panel of these HCV variants for their susceptibility to mAb1671. In vitro assays employing cell culture-derived HCV and its anti-SR-B1 resistant variants show that mAb1671 and ITX5061 equally inhibited infection of hepatoma cells with wild-type HCV but in both cases, a less pronounced inhibition was observed against the anti-SR-B1 resistant viruses. Similarly, cell-cell spread of wild-type HCV was efficiently inhibited by mAb1671 and ITX5061, but resistant virus was able to spread in the presence of either inhibitor. However, a different effect was observed in vivo when mAb1671 was examined in human liver uPA-SCID mice challenged with HCV. Mice were administered 6 doses of mAb1671, 3 days after challenge with either wild-type virus or an anti-SR-B1 resistant virus. Surprisingly, RNA could not be detected in any of the mice challenged with either the wild-type or anti-SR-B1 resistant viruses, while 7/8 mice in the control group had detectable RNA. In the pre-exposure group, one dose of antibody was given one day before challenge, followed by 5 doses after challenge. Two out of three animals had no detectable RNA while one appeared to control viraemia for a limited time and then had rebound after cessation of therapy. It is rare that an antiviral agent is more effective in vivo than in vitro.

To explain the enhanced in vivo efficacy of mAb1671, the authors proceeded to examine the composition of the virions and the role of lipoproteins in the entry process. By comparing cell culture-derived HCV and virions produced from infected human liver uPA-SCID mice, the authors were able to exclude differences in virion density and mAb1671 sensitivity suggesting a similar overall lipoprotein composition and that the passaged viruses do not acquire adaptive mutations. As HCV infectivity is affected by lipoproteins, the authors examined the hypothesis that the human liver tissue transplanted onto the mice produced sufficient levels of serum HDL and VLDL to affect the infectivity of HCV, possibility modulating the anti-viral activity of mAb1671. Firstly, they show important differences in the susceptibility of wild-type and anti-SR-B1 resistant viruses to physiologically relevant levels of lipoproteins. While wild-type virus is only sensitive to inhibition by VLDL and not HDL, the anti-SR-B1 resistant viruses are sensitive to both. When HDL or VLDL is combined with mAb1671, a synergistic enhancement of the anti-viral activity of mAb1671 is observed against both wild-type and anti-SR-B1 resistant viruses, almost completely abolishing infectivity in cell culture. These findings unveil a dual role for HDL in HCV entry. In wild-type virus, where the SR-B1 pathway of entry dominates, HDL supports or enhances viral entry. However, when the SR-B1 pathway is blocked, either through mutations in the virus or antibody-mediated blockade of SR-B1, HDL becomes a potent inhibitor of HCV infection.

Previous studies have shown that HDL enhances HCV infectivity and as a result makes HCV more resistant to the effects of neutralizing antibodies directed towards the viral glycoproteins (12-14). Agents that block the lipid transfer activity of SR-B1 reverse the ability of HDL to enhance HCV infectivity (15). Interestingly, anti-SR-B1 resistant viruses are more sensitive to neutralization with anti-E2 antibodies (8, 16). Together with the findings of this study, it is possible that viral mutations that confer anti-SR-B1 resistance compromise the integrity of the lipoviral particle in such a way that it disrupts the coordinated sequence of events involving SR-B1, thereby prematurely exposing the virion to agents that can inhibit entry (HDL or anti-glycoprotein antibodies). Further detailed analysis of the apolipoprotein content of anti-SR-B1 resistant HCV and the use of anti-glycoprotein antibodies to probe conformation of the glycoproteins may reveal subtle differences in lipoviral particle integrity. Nevertheless, adaptions of the virus to reduce SR-B1 dependency are likely to confer a significant loss of fitness in the presence of an adaptive immune response. As such, therapies targeting SR-B1 may be less likely to select for resistant viruses, further supporting their therapeutic potential.

The findings here that anti-SR-B1 antibody mAb1671 potently inhibits HCV replication and acts synergistically with HDL in vivo opens up new opportunities for the development of antibody-based therapies for chronic HCV. Such a therapeutic antibody could be used in conjunction with neutralizing antibodies directed towards the viral envelope proteins to provide a potent therapeutic cocktail with the ability to prevent both cell-free and cell-cell spread of HCV and prevent reinfection of livers following transplant.

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