Hepatitis B Virus Cellular Entry – Erecting a Barrier

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The identity of the cell surface receptor (or receptors) that mediates infection of human hepatocytes by Hepatitis B virus (HBV) has proven elusive. A number of candidates have been proffered over the years, but the lack of good cell culture-based infectivity model systems has tended to frustrate researchers in the field. A recent report [1] using the HepaRG human hepatoma cell line [2] offers insights into cellular barriers to viral entry. The data suggest that the susceptible cells must adopt a polarized architecture similar to hepatocytes within the normal liver, including the formation of bile canalicular structures. Unlike most epithelial cells, which form sheet-like structures, hepatocytes polarize in a distinctive, three-dimensional array. Tight junctions form between adjacent cells so as to create distinct basolateral (sinusoidal blood-facing) and apical (bile-facing) compartments. Interestingly, hepatic cell polarity has also been implicated in Hepatitis C virus (HCV) infection; consideration of what is known about HCV infection is worth considering as the viruses are share the same cell tropism.

Most current models of HCV entry recognize a role for four distinct putative cellular co-receptors – the tight junction proteins claudin-1 and occludin, as well as the scavenger receptor-B1 (SR-B1) and the tetraspanin family protein CD81 [3]. All four proteins seem to contribute to enhancing viral entry in a transgenic mouse model [4]. SR-B1 on the basolateral side of the hepatocytes may interact initially with the viral glycoprotein E2. CD81 also can interact with E2, and CD81 has been reported to associate with claudin-1 when the latter is not localized to tight junctions, but they do not associate at tight junctions [5,6]. Thus, these proteins also have the potential to interact with HCV particles on the basolateral surfaces of cells. Occludin, however, is largely absent from that surface of the hepatocytes, raising the possible involvement of tight junctions in mediating virus entry. Overall, one model consistent with most data suggests that the virus might attach initially via SR-B1, subsequently interacting with a complex of claudin-1/CD81, all at the basolateral surface of the cell. Recent data analyzing the kinetics of virus neutralization by antibodies suggests that the claudin-1/CD81 complex is involved in endocytosis of HCV [7]. Occludin involvement could suggest a transition of this putative entry complex to tight junctions prior to internalization [8]; alternatively, occludin may play a role in an endocytic compartment [3].

The report by Schulze et al. [1] regarding the association of HBV entry into hepatocytic cells with proper cell polarization invites comparison of their results with what is known about HCV. Although increased frequency of HepaRG cell polarization was associated with increased virus infection, disruption of tight junctions also lead to increased levels of HBV. The authors interpret their results as allowing the virus access to receptors that are sequestered on the basolateral surface of polarized cells. This situation might indeed parallel that for HCV entry, where the key molecules SR-B1, CD81 and claudin-1 all are available and able to interact on the basolateral cell surface. Schulze et al. [9,12] also performed experiments with primary human hepatocytes (PHH); virtually all cells could be infected if the number of genome equivalents present in the viral inoculum was sufficiently high. The authors postulate that all cultured PHH adopt a polarized architecture, with formation of distinct bile canalicular apical structures, whereas only a subset of HepaRG cells form such structures. Although use of PHH would seem preferable, access to these cells is limited and variable. The HepaRG cells present a potentially valuable alternative for delineation of the nature of the cellular receptor(s) for HBV. Further exploration of culture conditions that promote more efficient cell polarization is likely to be of value.

The possibility that viral infection influences the polarized state of the cell should also be considered. We have obtained preliminary data comparing the hepatoma cell line HepG2 to a derivative that harbors HBV, HepG2.2.15; the virus-containing cells appear to be more highly polarized (H. Liang and P.A. Norton, unpublished results).

The findings of Schulze et al. [9,12], suggest that, in vivo, the virus receptor would be accessible to the bloodstream. More importantly, they suggest that interventions to disrupt virus entry into hepatocytes could target either the virus or the cell surface receptor(s), which leads us to consider these targets. As mentioned above, progress in identifying the cellular receptor(s) for HBV has been slow, hampered in large part by limited cell culture model systems. Heparan sulfate proteoglycans may provide an initial, low affinity interaction with the virus [9], but this interaction seems unlikely to account for the cell specificity of infection, suggesting the existence of other receptors. Various surface protein candidates have not withstood scrutiny, and receptors identified for the duck HBV do not appear to be shared [10]. There is no basis on which to predict whether any of the proteins that mediate HCV entry play a role in HBV entry, but a basolateral distribution would be predicted.

More progress has been made on identifying the viral components required for attachment to cells [10]. Most critical residues appear to be confined to the pre-S1 domain, which is present in the longest envelope protein, LHBs, but is absent from the more abundant MHBs and HBs. Specifically, the acylated N-terminal 48 amino acids of pre-S1 have been shown to block infection of susceptible cells [11,12], including primary hepatocytes [1]. These discoveries have led to further preclinical testing of pre-S1 derivatives. One, termed Myrcludex-B, has been tested in an innovative mouse-human chimeric model, and shown effective in inhibiting both HBV and HDV infection [13,14]. Evaluation of this peptide-based therapeutic in the clinic is a likely next step.
Delineation of the viral envelope protein sequences involved in virus-cell interactions has lead to the development of a new agent that can reduce spread of HBV in vivo in an animal model. Identification of the cellular partner, or more likely partners, could lead to additional therapeutic interventions to reduce virus spread. Small molecules that disrupt virus-host cell interactions can now be sought. Such compounds would have the potential to be used in combination with approved nucleoside antivirals that inhibit the viral polymerase [15], or perhaps with newer compounds that reduce secretion of virions and/or the viral envelope proteins [16,17]. It is hoped that by elucidating the mechanism by which the virus manages to enter the human hepatocyte, this knowledge can be exploited in the future to keep HBV out.

References