Hepatitis B virus receptor : the role of human annexin V

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Despite the development and commercialization of plasma derived and recombinant vaccines, hepatitis B virus (HBV) infection is still a worldwide health problem, especially in African and Asian countries. At present there are still more than 350 million carriers of hepatitis B surface antigen (HBsAg) with a mortality of 1 to 2 million per year due to variceal bleeding, decompensated liver cirrhosis or hepatocellular carcinoma. Although much information about the molecular biology of HBV has been gained in the last decades, still little is known about the mechanism of the initial steps of infection, namely the attachment and penetration of HBV into human hepatocytes, the natural host cells of HBV. In addition, the molecular mechanism of species and organ specificity of HBV infection is still unknown. Only humans, chimpanzees, gibbons (Robinson *et al.*, 1982), certain macaca and tupaia (Walter *et al.*, 1996) are so far known to be susceptible to HBV infection. Rats and mice are not infectable by HBV (unpublished observations). However, a successful production of HBV was detected after *in vitro* transfection of a rat hepatoma cell line (Shih *et al.*, 1989) and after *in vivo* transfection of rat hepatocytes (Takahashi *et al.*, 1995) with a construct containing the HBV genome. In addition, cross species replication of HBV has been obtained in hepatocytes of HBV transgenic mice (Guidotti *et al.*, 1995). These findings suggest that the species barrier of HBV infection and replication may be located at the early steps of viral infection, namely the adsorption or penetration of HBV into hepatocytes.

Specific attachment of HBV particles to the plasma membrane of their host cells is considered to be an important initial step in HBV infection. In this process the HBV envelope is thought to play a crucial role in the interaction with the hepatocyte plasma membrane. The HBV envelope is composed of lipids and envelope proteins. Three related envelope proteins are known, termed small, middle, and large HBsAg. Small HBsAg (SHBsAg) contains 226 amino acids, middle HBsAg (MHBsAg) has an additional preS2 peptide (55 amino acids), while large HBsAg (LHBsAg) has an additional preS2 and preSI (119 amino acids) extension. Initially it was suggested that the preS2 domain could act, via polymerized human serum albumin, as an attachment site to human hepatocytes. In recent years other observations suggested that the preS1 domain and not the preS2 domain, is probably the most important attachment site to human hepatocytes. However, controversial findings with regard to cellular proteins for binding to the preS 1 domain have been described. Also SHBsAg was found to be important for binding to hepatocytes by binding to apolipoprotein H (for a detailed review see De Meyer *et al.*, 1997).

In a previous study we have demonstrated that SHBsAg is able to bind specifically to intact human hepatocytes (Leenders *et al.*, 1990 ; de Bruin *et al.*, 1 995). Furthermore, human Annexin V (hAV), a Ca^{2+}-dependent phospholipid binding protein present on the plasma membrane of human hepatocytes, was identified as a specific SHBsAg binding protein. In this regard, it is important to note that rat Annexin V does not bind to SHBsAg (Hertogs *et al.*, 1993). In addition, we have reported the spontaneous development of anti-HBs antibodies (anti-idiotypic antibodies, Ab2) in rabbits immunized with hAV, but not in rabbits immunized with rat AV (Hertogs *et al.*, 1994), suggesting that there is a receptor-ligand relationship between hAV and SHBsAg.

In addition, we have determined that the presence of a species-specific type of AV is correlated with the species tropism of HBV infection. By Western blotting using U4C8, a monoclonal antibody that detects specifically human AV, AV was detectable in liver extracts from species susceptible to HBV infection. No AV reacting with U4C8 was found in liver extracts from species non-susceptible to HBV infection (de Bruin *et al.*, 1996 and own unpublished observations). Moreover, we have demonstrated the involvement of hAV in the initial steps of HBV infection. We showed that hAV expressing cells such as human hepatocytes are susceptible to HBV infection, while cells that do not express hAV such as rat hepatocytes or rat hepatoma cells are not infectable by HBV. Subsequently, we have transfected rat hepatoma FT02B cells with a construct containing the hAV gene. These transfected cells, assigned as FTO9.1 cells, expressed hAV and were susceptible to HBV infection (Gong *et al.*, 1999). Namely, after *in vitro* infection of these FTO9.1 cells, HBV mRNA, covalently closed circular (ccc) DNA, replicative intermediates, HBsAg and HBcAg and secreted HBV DNA could be detected, while none of these markers could be found after *in vitro* infection of non- transfected FT02B cells. Moreover, we

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have demonstrated the infectivity of this *in vitro* propagated HBV by infection of naive FTO9.1 cells with the culture supernatant of infected FTO9.1 cells.

Despite these findings, we have demonstrated that the administration of an excess hAV could not prevent HBV infection in primary cultures of human hepatocytes. Replicative markers of HBV infection such as mRNA and ccc DNA could still be detected after *in vitro* infection in the presence of an excess hAV. The failure of hAV to block HBV infection in primary cultures of human hepatocytes may be explained by the fact that hAV, which is able to bind to SHBsAg, has also the capacity to bind to human hepatocytes. Further binding experiments and immunostaining showed that hAV is able to bind to rat hepatocytes. This binding enables rat hepatocytes to bind to SHBsAg and makes them in this way susceptible to HBV infection. After *in vitro* infection of primary cultures of rat hepatocytes in the presence of hAV, HBV mRNA, ccc DNA, replicative intermediates, HBsAg and HBcAg and secreted HBV DNA could be detected. None of these markers could be detected in primary cultures of rat hepatocytes infected without addition of hAV. (unpublished observations) These findings further support the involvement of hAV in the initial steps of HBV infection.

Recently, in order to get a better insight into the hAV-HBV interaction, we have determined more specifically the hAV-SHBsAg binding epitopes. Using binding experiments we have demonstrated that at least two epitopes on hAV are involved in the binding to SHBsAg. One epitope binds to phosphatidylserine, a phospholipid component of the SHBsAg envelope, whereas the other epitope binds to a non-phospholipid component of the SHBsAg envelope. The importance of phosphatidylserine was further evidenced by the fact that phosphatidylserine is able to directly block the binding of hAV to SHBsAg and that it could even prevent HBV infection *in vitro*. In contrast, phosphatidylcholine, the major phospholipid component of the HBV envelope, has no effect on the binding of hAV to SHBsAg and could not prevent HBV infection *in vitro* (De Meyer *et al.*, 1999b). Using a panel of synthetic peptides covering the regions of SHBsAg located on the outer surface of the virus, we have found that the regions covering amino acid 125 to 131 and amino acid 158 to 169 of SHBsAg are involved in the binding of SHBsAg to hAV. (De Meyer *et al.*, 1999a) Since these regions are located in the structural vicinity according to the topological model of SHBsAg proposed by Chen *et al.* (Chen *et al.*, 1996), these findings suggest that these SHBsAg regions are part of a conformational epitope of SHBsAg for binding to hAV. This insight may be useful for the development of new generation vaccines or drugs for prevention or for treatment of patients with HBV infection.

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