"Receptor-ligand" based new strategy for treatment of patients with chronic hepatitis B

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Currently available approaches to the treatment of patients with chronic hepatitis B are : 1) modulation of cellular and/or humoral immunity by interferon-alpha or beta, thymosin or by vaccination ; 2) suppression of viral replication by interferon-alpha or beta or by other antiviral agents, such as arabinosides, lamivudine, ganciclovir and famcyclovir. Although in clinical trials most of these treatment modalities have been shown to be effective in certain extends, no drug has been considered to be the treatment of choice in clinical practice, because of the toxicity, duration of treatment, costs, effectiveness, relapse rate and/or the development of resistant mutants.

Because of the limitations of success in treating patients with chronic hepatitis B and despite the development and commercialization of effective vaccines for prevention, hepatitis B virus (HBV) infection is currently still a major public health problem in the world, especially in African and Asian countries. It has been estimated that today more than 2 billion people worldwide have been infected with the HBV and about 350 million are HBsAg carriers with a mortality rate of 1 or 2 million per year.

Treatment of chronic hepatitis B patients is recommended, since persistent viral replication often shows rapid progression of liver cell damage with an unfavorable prognosis and spontaneous cessation of viral replication occurs only in a small minority of patients. In addition, HBV carriers are potential sources of infection.

In the initial event of HBV infection, the attachment of viral particles onto the plasma membrane of their host cells, namely the human hepatocytes is considered as an important step. The HBV lipid and protein containing envelope of the HBV particle is thought to be involved in this attachment process. The HBV envelope proteins consist of small HBsAg, middle HBsAg and large HBsAg. Small HBsAg is encoded by the S region of the S gene, while middle HBsAg is encoded by the S region with an additional preS2 extension and large HBsAg with an additional preS2 and preS1 extension. Although many studies have indicated the preS1 and the preS2 region as a potential attachment site of HBV, we have demonstrated that small HBsAg binds directly and specifically to intact human hepatocytes and to the plasma membrane of human hepatocytes.

Subsequently, we have identified human liver Annexin V, a Ca-dependent phospholipid binding protein present on human hepatocyte plasma membranes, as a specific small HBsAg binding protein. Moreover, we have observed the spontaneous development of anti-idiotypic antibodies in rabbits immunized with human AV, but not in rabbits immunized with rat AV and in chickens immunized with the F(ab')2 fragment of rabbit anti-AV IgGs. This finding suggests the existence of a receptor ligand relationship between hA-V and small HBsAg. Recently, we demonstrated the involvement of human AV in the initial event of HBV infection. In these experiments, we showed that human AV expressing cells are infectable by HBV and that cells not infectable by HBV become infectable after transfection with the human AV gene leading to human AV expression. These findings indicate that human AV is indeed involved in the initial step of HBV infection and plays a key role in the cell susceptibility to HBV infection.

Although currently available vaccines contain the small HBsAg, after vaccination, antibodies are produced mainly against the region of aa 124 to aa 147, the so called "alpha determinant" epitopes. These antibodies are able to neutralize HBV particles. However, these epitopes are not involved in the interaction between HBsAg and human AV and in the attachment of HBV particles to the plasma membrane of human hepatocytes. Antibodies targeted to these epitopes are also not effective for treatment of patients with chronic hepatitis B. In addition, these antibodies could not prevent HBV infection when there is an amino acid change in this "alpha determinant" region as in the case of "vaccine escape" mutants.

Our present aim is to develop a new strategy for treatment of patients with chronic hepatitis B based on antibodies or molecules which are able to block the interaction between small HBsAg of the HBV envelope and AV on the human hepatocytes. Experiments are currently still in progress to test whether these antibodies and molecules are able to prevent HBV infection in an *in vitro* system and whether they subsequently can be used for treatment of patients with chronic hepatitis B.