Hepatitis B virus mutants in HBsAg positive children

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Genetic variations in the genome of the hepatitis B virus are quite frequent and well documented in patients with chronic hepatitis B. One reason is the replication strategy of the virus using reverse transcription to produce functional mRNAs before synthesizing the corresponding DNA. Reverse transcription is a highly error-prone process leading to a considerable number of variants (1). Another factor inducing genetic variations is the high viral replication. The changes of the nucleotide sequence may be silent, lethal or they might be associated with different phases of disease activity or the replication level. Mutations include base exchanges, deletions, insertions or rearrangements of viral sequences. Since the immunocompetence of the host is supposed to be responsible for the destruction of HBV infected liver cells and for the clearance of the virus, mutated proteins or mutations which hamper the expression of viral proteins could lead to an altered immune response resulting in immune escape of the hepatitis B virus and different clinical courses of the disease (2).

If there exist various viral populations of the hepatitis B virus simultaneously in different amounts, an increased immune response may lead to a competition between the variants in terms of virus maturation and spread in each cell of the liver tissue. The result will be the dominance of a certain genotype or variant which is most suitable and best adapted to the present immunological conditions (3).

During the course of chronic hepatitis B infection the change of the HBeAg positive phase with a high inflammatory activity and high viral replication to the anti-HBe positive stadium represents a striking immune-mediated process. The anti-HBe seroconversion is associated with a relatively low inflammatory activity and low viral replication. The process of seroconversion, however, is characterized by a strong immunologic activity, frequently associated with a flare-up of the serum liver enzymes. The seroconversion from HBeAg to anti-HBe either occurs spontaneously or is induced by alpha interferon treatment. In few patients the immune response can heighten dramatically even resulting in a fulminant liver disease (2,3,4).

The hepatitis B virus has a partially double stranded genome, which is 3.2 kb long. There are four open reading frames (ORF) encoding the three surface proteins (preS1, preS2, and S) the core proteins (preC/C and C), the transactivating X-protein (X-gene) and the DNA polymerase (P-gene). One translational product of the pre C/C-gene ist secreted as HBeAg. The second product is the hepatitis B core protein (HBcAg) which remains in the liver cell and is a target for the immune response at the surface of the hepatocyte. HBeAg has been proposed to be a tolerogen, especially in infants. HBcAg and HBeAg share humoral and T-cell epitopes (1,5).

Because of relevant immunological effects the preS/S region and preC/C gene are of particular interest for mutation studies. Furthermore, mutations in the core promotor which is in part covered by the X-gene may be involved in more severe clinical courses.

Precore and core promotor mutants

The preC region is highly conserved and a functional preC is only necessary for HBeAg production but not for virus replication. In the early 90's genetic investigations were performed in patients with anti-HBe chronic hepatitis and high viral replication, which is unusual after loss of HBeAg. A single base substitution (G-A) at the nucleotide position 1896 was identified. It was assumed that this particular mutation which introduces a stop codon in the preC gene was associated with more severe courses of chronic hepatitis B (6,7). Some more variants have been characterized. A quite frequent association with the G-A transition at 1896 is a further G-A exchange at position 1899. Very soon some more mutations were reported. A base exchange at nucleotide position 1858 is of special interest, since this base opposites nucleotide 1896 in the stem of the pregenomic RNA loop. Thus, the mutant 1896-A is frequently associated with a T-1858 strain (8).

It was tried to correlate the different types of HBV variants with the severity of chronic hepatitis and with a fulminant course of the disease. Furthermore the role of interferon treatment in the emergence of mutants was subject of numerous reports.

According to the present data the 1896-precore mutant seems to be very common in patients with anti-HBe seroconversion independent on the level of viral replication. Patients with hepatitis B virus genotype D are more likely to have a persistent infection by selection of precore mutants. In adults an increase of

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precore mutants could be detected after anti-HBe seroconversion to more than 50%. There seemed to be no difference between spontaneous and interferon induced seroconversion (4,7,9). The role of the precore mutant in fulminant hepatitis B is discussed controversially and may depend on geographic distribution. There are lines of evidence that fulminant hepatitis B in adults may not be caused by a specific genomic mutation (10). But mutations clustering in the core promotor region may contribute to a fulminant disease course possibly by increasing the viral replication through the enhanced promotor activity. The most important base exchanges are located in the T-A rich region of the core promotor at position 1762 and 1764 (11,12).

S gene mutants

A number of mutants in the preS1 and preS2 gene have been described. They comprise missense and silent mutations but include also deletions and initiation codon mutants which may affect immune epitopes and alter the expression of the preS proteins (13,14). But the most important mutations in the S gene refer to the a-determinant of the HBsAg major protein which is spanned from aminoacid 124 to 147. It is the major B-cell epitope recognized by the neutralizing antibody to HBsAg. HBV sequence mutational changes in the antigenic loop lead to the failure of the anti-HBs effectivity and the mutated virus escapes the immune response. The first detected variant was the result of a point mutation changing glycin to arginine at aminoacid 145 of the a-determinant. Escape mutants are of clinical significance in the diagnosis of HBV infection in HBsAg negative individuals, in case of vaccine failure in infants born to HBsAg positive mothers and in the immunoprophylaxis failure in liver transplant recipients (15).

HBV mutants in HBsAg positive children

Precore and core promotor

Compared with the numbers of reports about HBV mutants in adults with hepatitis B very little is known about the emergence of mutants in children with chronic infection or fulminant disease. The most important difference in acquiring chronic hepatitis B in children is the route and the age of infection. Mother-to-child transmission represents a particular form of infection resulting in chronic disease in more than 90% of infected babies. But also horizontal HBV infection in early childhood is associated with a considerable higher chronicity rate compared with adults. Since the level of viral replication and the activity of the immune response are important factors for the selection of HBV mutants it is a quite interesting issue at what time and under which conditions mutations will emerge during the chronic HBsAg carrier stage.

Two reports from the Mediterranean regions Italy and Spain investigated the HBV precore sequence in HBeAg positive children who in part underwent alpha interferon treatment. It was clearly shown that the precore stop mutant at nucleotide position 1896 as the single virus population could not be detected. Nevertheless, the prevalence of a mixed wildtype/precore mutant population ranged between 34% and 93%. Additionally a simultaneous 1899-G-A-mutation was observed in 43% (16,17). The reported frequency of infection with a mixed viral population in adults is below 50%. The reason for such a high mutant rate is both surprising and unclear. It may be due to geographical differences. No substantial changes in the prevalence of mutants were observed throughout the interferon treatment. The presence of HBeAg minus variants was associated with older age and a higher inflammatory activity. Own studies in Caucasian children did not confirm these findings. In the HBeAg positive phase only 5% showed a mixed wild-type/precore mutant population. After seroconversion to anti-HBe the proportion increased to 20%. However, in this phase in single cases the precore mutant may already be selected as the only detectable population. There was no difference between spontaneous or interferon induced seroconversion. It is likely that the immune pressure during the process of seroconversion plays a major role for selection.

The association of the precore mutants with fulminant hepatitis is discussed controversially (10). Several reports from infants and children try to elucidate the role of the precore mutants as well (18,19,20). There is no doubt that precore mutants could be detected in a considerable number of cases with fulminant hepatitis. Some of them were infected by their mothers, who showed the same mutation. But others demonstrated different HBV sequence changes. Thus, the precore mutations seem to be neither necessary nor sufficient to cause fulminant hepatitis B in childhood. It is likely that after transmission of a mixed virus population a selection process is started in the baby (21). Recently it was supposed that mutations in the core promotor region may have some additional influence (12). Own investigations in nine vertically infected infants with fulminant hepatitis confirmed these findings and showed a striking presence of core promotor and precore mutants (at positions 1762, 1764, 1896 and 1899) compared with clinically inapparent HBsAg carriers. In conclusion, despite the pathogenesis of fulminant hepatitis B is unclear, HBV mutants which modulate the viral replication may have a contributory role.

S gene

The dominant neutralizing epitopes of hepatitis B virus are contained within the envelope proteins. The smallest protein, HBsAg, is used for vaccination. As mentioned above the a-determinant represents the

major hydrophilic region between aminoacids 124 and 147. Most antibodies to HBsAg in sera of vaccinated children bind to aminoacids 139-147 (21). 5-6% of infants born to HBeAg seropositive mothers develop chronic HBV infection despite full passiv-active immunoprophylaxis (22,23,24). The reason is either "in utero" HBV infection or failure of immunisation. Mutations in the a-determinant were identified to result in conformational changes of the antibody binding site leading to a neutralization resistant escape HBV variant. Most common mutations were observed in codon 144 and 145 of the S gene (25,26,27,28). Meanwhile a lot of further variants from codon 126 to 159 were defined (22). Some of them are natural mutants and probably not neutralization escape mutants. Nevertheless, they might infect vaccinated individuals and become more common as vaccination coverage increases. Escape mutations in vaccinated children could be detected in 22% (26). Some of them persist over years. In cases with simultaneously investigated mothers in the majority of them corresponding mutations in HBsAg were not detected (27). Obviously S variants similar to precore stop mutants emerge or are selected under the immune pressure generated by the host. If selection is the most probable explanation, escape mutants have to preexist maternally as minor variants, even not detectable by molecular biological techniques.

Besides mutations in the S region HBV sequence changes may also occur in the preS1 and preS2 gene. Own investigations revealed a 183 bp deletion in a HBeAg positive boy. No further significant mutations were observed during the HBeAg positive phase. PreS2 start codon and missense mutations in the preS1 region could be identified after seroconversion to anti-HBe. But again no difference in interferon treated and patients without treatment was observed (29).

In conclusion the hepatitis B virus genome is continuously evolving in children with chronic hepatitis B or fulminant disease. A number of the reported variations have some clinical relevance, but others do not, and further study is necessary to reveal their importance and all their implications. The number of changes rises with an increased immune pressure which precedes the seroconversion from HBeAg to anti-HBe. In vertically transmitted hepatitis B immune pressure enhances the selection of variants which were harboured by the host in only a small proportion.

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