Hepatitis B virus escape mutants induced by antiviral therapy

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The hepatitis B virus (HBV) polymerase and envelope genes overlap in such a way that resistance mutations to antiviral agents in the reverse transcriptase gene may affect the antigenicity of the HBV surface antigen. Mutant viruses may escape serological diagnosis using specific anti-HBV surface antigen antibodies, causing occult forms of chronic hepatitis B. Moreover, these HBV strains may evade vaccine protection, representing a public health challenge. Thus, the circulation of HBVs encoding envelope mutations selected by antiviral agents requires close monitoring.

Keywords: hepatitis B virus, HBV, drug resistance, lamivudine, HBsAg, occult hepatitis, vaccine

Since the implementation of infant hepatitis B vaccination programmes, the prevalence of chronic hepatitis B has dramatically declined in the Western world. The hepatitis B virus (HBV) vaccine consists of a yeast-derived recombinant hepatitis B surface antigen (HBsAg) protein and is effective at producing protection in up to 95% of immunocompetent recipients. Antibodies elicited by the vaccine are predominantly directed towards a highly conformational and cysteine-rich domain; the ‘a’ determinant. An alteration of the antigenicity of the HBsAg protein and subsequent failure of anti-HBs antibodies to neutralize HBV may occur as a consequence of mutations in and around the ‘a’ determinant. Several implications may follow this phenomenon: first, HBV reactivations by escape mutants in previously anti-HBs immune persons, as already reported in lymphoma patients receiving chemotherapy; secondly, possibly HBV vaccine failures; lastly, misdiagnosis of chronic hepatitis B at least using some antibody tests, as specific antibodies have to bind to a conserved region of the HBsAg. The presence of HBV replication in the absence of detectable serum HBsAg may be associated with liver damage, and transmission of hepatitis B from ‘occult’ infected blood donors has already been described.

The first description of an HBsAg mutant was made in a child born to an HBsAg-positive mother who developed acute hepatitis B despite being vaccinated and passively immunized against HBV. The breakthrough viral strain was shown to contain a substitution of a glycine to an arginine at position 145 (sG145R) in the HBsAg. Since then, several other studies have documented the presence of HBV replication despite vaccination as a result of vaccine escape mutations. Although the G145R mutation remains by far the predominant HBsAg mutant responsible for this phenomenon, a wide range of other HBV envelope changes have more recently been described that may cause a similar effect, including amino acid substitutions, deletions or insertions across the whole ‘a’ determinant.

To complicate things further, the HBV genome is organized in such a way that the envelope (S) gene is completely overlapped by the polymerase gene. Therefore, viruses encoding changes associated with antiviral resistance in the polymerase may have consequent changes in the envelope gene. Some of these overlapping mutations have already been shown to produce functional changes in the surface antigen (Figure 1). A triple mutational pattern causing lamivudine resistance (rtV173L+rtL180M+rtM204V) has recently been shown to enhance HBV replication, compared with rtL180M+rtM204V alone. This triple HBV mutant causes two amino acid changes in the overlapping surface gene (sE164D+sI195M), which reduce anti-HBs binding to levels seen only with the vaccine escape mutant sG145R. In agreement with this observation, some patients treated with lamivudine and apparently showing clearance of the HBsAg actually remain with detectable circulating plasma HBV-DNA. Selection of an sP120A mutation in these patients is associated with the apparent HBsAg seroconversion. This mutation produces a reduced anti-HBs binding, which explains the failure to detect HBsAg.

Vaccine/hepatitis B immunoglobulin (HBIG)-escape mutations sP120T and sG145R in combination with lamivudine-associated resistance mutations are often seen in HBV mono-infected patients following lamivudine or HBIG treatment. They produce changes, rtT128N and rtW153Q, respectively, in the polymerase protein and have been found to partially restore the in vitro replicative capacity of lamivudine-resistant HBV. In an investigation of the virological events that follow the development of lamivudine-resistant mutants, Yeh et al. found four patients who developed an rtA181T mutation that in an in vitro phenotypic assay was confirmed to be responsible for the lamivudine resistance. This mutation concomitantly generates a stop codon in the surface antigen (sW172stop), which results in impaired secretion of the HBsAg and reduced viral fitness. However, the replicative capacity of this mutant virus is

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Amino acid changes in the envelope and polymerase genes as a consequence of antiviral therapy or immune selection pressures. Mutations within the HBV polymerase are listed on the left-hand side (within the virion), whereas changes in the HBV envelope are listed on the right-hand side (at the surface of the virion). The former may be induced by antiviral drugs and the latter may be selected by antibodies. Each change in the polymerase has a ‘mirror’ change indicated on the same line in the envelope and vice versa.

The impact of HBV genotype variation on HBsAg detection and anti-HBs binding needs to be further investigated. In a recent study, we have demonstrated that mutations in the HBV surface antigen selected by lamivudine therapy develop more frequently in HBV genotype A when compared with genotype D. It would be interesting to find out whether other genotypes are more prone to select these HBV escape mutations, especially in regions where the HBV vaccine and/or HBIG are widely used. Interestingly, neither the adenosine-associated resistance mutation rtN236I nor the tenofovir-associated resistance mutation rtA194T causes changes in the HBV surface gene. However, further investigation is needed to determine the effect on vaccine escape of other mutational changes selected by newer antiviral agents.

In summary, HBV genotyping and polymerase/HBsAg gene sequencing may be helpful to appropriately manage patients undergoing antiviral therapy for chronic hepatitis B. This may be particularly helpful in patients co-infected with HIV, as most of them carry HBV genotype A, which is more prone to selection of the lamivudine-resistant triple mutant rtV173L+rtI180M+rtM204V, and may cause vaccine escape or hepatitis B misdiagnosis. As yet, there has been no evidence of HBV surface antigen mutants being transmitted to vaccinated individuals, but transmission of hepatitis B from ‘occult’ infected blood donors has already been described. However, the circulation of HBVs encoding envelope mutations selected by antiviral agents requires further investigation, especially those with replication capacities similar to wild-type viruses. If transmissible, they may represent an important public health threat.

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Transparency declarations

None to declare.

References


