Review

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Hepatitis B virus PreS/S gene variants: Pathobiology and clinical implications

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Summary

The emergence and takeover of hepatitis B virus (HBV) variants carrying mutation(s) in the preS/S genomic region is a fairly frequent event that may occur spontaneously or may be the consequence of immunoprophylaxis or antiviral treatments. Selection of preS/S mutants may have relevant pathobiological and clinical implications. Both experimental data and studies in humans show that several specific mutations in the preS/S gene may induce an imbalance in the synthesis of the surface proteins and their consequent retention within the endoplasmic reticulum (ER) of the hepatocytes. The accumulation of mutated surface proteins may cause ER stress with the consequent induction of oxidative DNA damage and genomic instability. Viral mutants with antigenically modified surface antigen may be potentially infectious to immune-prophylaxed patients and may account for cases of occult HBV infection. In addition, preS/S variants were reported to be associated with cases of fulminant hepatitis as well as of fibrosing cholestatic hepatitis, and they are associated with cirrhosis and hepatocellular carcinoma development.

Introduction

Despite the availability of an effective vaccine, hepatitis B virus (HBV) infection remains a major health problem worldwide with estimates of nearly 400 million chronic HBV surface antigen (HBsAg) carriers. HBV infection may be associated with a large spectrum of clinical manifestations, ranging from very mild and asymptomatic clinical forms to the most severe liver diseases including fulminant hepatitis, cirrhosis, and hepatocellular carcinoma (HCC) [1]. The variation of the natural course of the infection and related disease is determined by the interaction between virus and host factors. The HBV replication cycle is not directly cytopathic and host immune responses against viral antigens is considered the main cause of hepatocellular injury [2]. However, several lines of evidence indicate that a certain number of HBV genetic variants, apparently provided with higher pathogenicity, may emerge during the course of the infection under endogenous (host immunity) and/or exogenous (immunoprophylaxis and antiviral therapies) selection pressures [3]. In this context, a growing number of studies performed in different geographic areas – thus evaluating different HBV genotypes – are pointing out the considerable importance of HBV envelope protein mutants (preS/S variants) including those able to escape the vaccine-induced anti-HBV neutralizing antibodies as well as those frequently associated with severe forms of acute and chronic liver disease and hepatocellular carcinoma (HCC) development [4–16]. Aim of this review was to revise the collection of data on the biological and clinical impacts of preS/S HBV variants, also stressing the aspects that are widely accepted by the scientific community and those that are still debated.

Key Points

- Selection and emergence of naturally occurring or therapeutic induced HBV variants with mutations in the preS/S genomic region is a frequent event in chronically HBV infected patients
- S-escape variants may be undetectable by the commercially available HBsAg assays and are potentially capable of infecting properly immune-prophylaxed patients, and they may also account for cases of occult HBV infection
- Several specific mutations in the preS/S gene may induce an unbalanced production of envelope proteins that accumulate in the endoplasmic reticulum (ER) of the hepatocytes, and may activate the ER stress-signaling pathways with consequent induction of oxidative DNA damage and genomic instability
- The cytotoxic effects exerted by the intracellular accumulation of surface proteins can contribute to liver damage, favoring the progression of the disease toward cirrhosis and the development of HCC

Keywords: HBV preS/S genetic variability; Vaccine escape variants; Occult HBV infection; Endoplasmic reticulum stress signaling pathway; Ground glass hepatocytes; Hepatocellular carcinoma.
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HBV virology

HBV is one of the smallest viruses in nature and its genome presents a highly compact genetic organization. It consists of a relaxed circular partially double-stranded DNA (rcDNA) of approximately 3200 nucleotides and comprises four partially overlapping open-reading frames (ORF): preS/S, preCore/Core, Pol, and X. There are no non-coding sequences in the viral genome and all regulatory regions (enhancer II/basal core promoter, preS1 promoter, preS2/S promoter, enhancer I/X promoter) are also part of protein-encoding sequences. The PreS/S ORF encodes the three different, structurally related envelope proteins, which are synthesized from alternative initiation codons and are termed Large (L), Middle (M), and Small (S) protein, respectively. The three proteins share the same carboxy-terminus part but have different amino-terminal extensions. In particular, the S-protein – corresponding to the HBsAg – consists of only 226 amino acids (aa), the M-protein contains an extra N-terminal extension of 55 aa, whereas the L-protein has a further N-terminal sequence of 108 or 119 aa – depending on the genotype – compared to the M-protein [17] (Fig. 1).

Envelope proteins contain the major viral antigenic domains. In particular, the immunodominant determinant bearing the anti-HBs neutralization domain, termed "a" determinant, has been mapped to amino acids 99–170 of the S-protein [17,18]. The preS/S ORF completely overlaps with the Pol ORF, which encodes the viral polymerase, a multifunctional protein that possesses reverse transcriptase, DNA-dependent DNA polymerase, and RNase H activities, and also functions as a terminal protein for priming. The pre-Core/Core and X ORFs overlap with the Pol ORF only in part. The pre-Core/Core region encodes the structural protein of the viral nucleocapsid (the hepatitis B core antigen, HBcAg) and the non-structural secreted hepatitis B e protein (HBeAg). These two viral proteins also derive from alternative initiation of translation at two in-frame initiation codons. The X region encodes the small regulatory X protein, which is essential for viral replication, and can directly and indirectly modulate host and viral gene expression [17,19].

Upon infection of hepatocytes, the HBV rcDNA is converted by cellular enzymes into a covalently closed circular DNA (cccDNA) inside the cells nuclei. Episomal HBV cccDNA persists in the hepatocyte as a stable minichromosome organized by histone and non-histone proteins. The viral minichromosome utilizes the cellular transcriptional machinery to produce all viral RNAs necessary for protein production and viral replication, which requires reverse transcription of the pregenomic RNA (pgRNA) [20]. Unlike retroviruses, HBV does not need to integrate its DNA into the host genome to replicate. Nonetheless, HBV DNA integration occurs frequently during the course of viral infection, and the integrated viral sequences, being usually deleted or rearranged, are replication-incompetent and differ from each other in size and structure [21]. Following transcription, all viral RNAs are transported to and translated in the cytoplasm. The nucleocapsid protein HBcAg, the soluble secreted HBeAg and the Pol protein are produced from the 3.5-kb pgRNA/pre-Core RNAs; the L envelope protein from the 2.4-kb RNA; the M and S envelope proteins from the 2.1-kb RNA; the X-protein from the 0.7 kb RNA. In the cytosol, the 3.5 kb pgRNA – apart from being transcribed – is selectively incorporated into progeny nucleocapsids and reverse transcribed by the co-assembled viral polymerase into new HBV genomes [19]. The mature rcDNA-containing nucleocapsids can then either re-deliver their genomes to the nucleus to build up a pool of around 10–100 copies cccDNA [22] or can interact with the envelope proteins at ER/Golgi apparatus and be secreted as new infectious virions [23]. Envelope protein synthesis follows a pathway that is distinct from viral replication. It occurs in the ER and leads to amounts of proteins that far exceed those required for virion assembly. Excess envelope proteins undergo dimerization and multimerization resulting in their budding from the ER/Golgi compartment as both non-infectious spherical and filamentous subviral particles (SVP) or as virions. The SVPs typically outnumber the virions by a factor of 1,000- to 10,000-fold, they may be components of circulating immune complexes [24], and may induce immune tolerance by a mechanism of "viral apoptotic-like mimicry" [25]. HBsAg is the most abundant protein in SVPs as well as in virions, whilst the M- and L-protein constitute approximately 20% of the total envelope proteins present in the HBV particles [26]. Of note, the commercial HBsAg quantification assays available target all forms of circulating envelope proteins, since the antibodies used in the quantitative immunoassays identify epitopes in the S domain, and are not capable of distinguishing between virion-associated HBsAg and subviral particles or HBsAg produced by possible integrated HBV sequences [27].

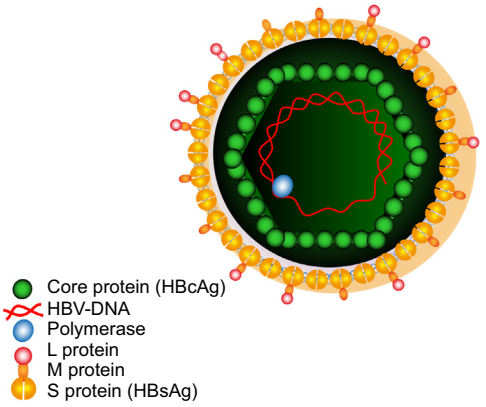

Fig. 1. Schematic representation of the hepatitis B virion. The virion consists of an envelope containing three related surface proteins (S-, M-, and L-proteins) and lipids, an icosahedral nucleocapsid that is constituted by the core protein (HBcAg) and that encloses the viral DNA genome covalently linked to the terminal protein of the viral polymerase.

The L, M, and S proteins perform different functions during viral morphogenesis and release, and their specific roles appear to be strictly related to their transmembrane topology. In particular, the L envelope protein shows two transmembrane topologies. In fact, the preS1 domain of the L protein can be either projected towards the cytoplasm or oriented towards the ER lumen. This is consistent with the different and essential functions fulfilled by the preS1 domain in the HBV life cycle, given that the cytoplasmic fraction performs a matrix-like function in nucleocapsid envelopment, and the fraction that faces the ER lumen ends up exposed on the viral surface of mature viral particles and is involved in the attachment of HBV to hepatocytes [23,28]. HBV polymerase lacks a proofreading function, thus the reverse transcription step results in the selection of HBV quasispecies containing several mutations within their viral genome. Indeed, HBV exhibits a mutation rate more than 10-fold higher than other DNA viruses. Mutations accumulating in individual genomes reflect both the duration of active HBV infection and the strength of the immune response. Moreover, apart from viral and host factors, exogenously induced selection