

HEPATITIS B SURFACE GENE MUTANTS AND THEIR EMERGING ROLE IN THE EFFICACY OF HBV VACCINATION PROGRAMS

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The advances in molecular biology techniques in the past decade have led to the clarification of hepatitis B viral DNA sequences. However, much remains to be established regarding the emerging hepatitis B mutants. Several putative forms of hepatitis B virus have been isolated, but their epidemiology, natural course of infection and clinical significance remain sketchy. Compounding these problems are factors created by human interventions, such as HBsAg mass immunization, chemotherapy of chronic HBV patients and HBIG prophylaxis of orthotopic liver transplant (OLT) patients, which tend to encourage new mutants to emerge.

Despite the availability of HBsAg vaccines and the mass immunization schemes implemented in over 80 countries worldwide, HBV infection continues to be a major public health problem. There are over 350 million chronic carriers worldwide, out of which about 10% die of cirrhosis and hepatocellular carcinoma.¹ A wide geographical variation exists for HBV, ranging from low, through intermediate, to high and very high endemicity. The low prevalence rates in developed countries sharply contrast with the high infection rates of Southeast Asia, Africa, China and the Middle East. Perinatal transmission and childhood horizontal infections are known to be very common in endemic areas of the world.

Although the efficacy of HBV immunization is well proven, cases of vaccine failure leading to chronicity, acute self-limited symptomatic hepatitis and, in some rare cases, fulminant hepatitis, have been reported.² In some of these cases, anti-HBs neutralization-resistant mutants or naturally occurring escape mutants have been identified.^{3,4} It is, therefore, paramount to establish not only prevalences of HBsAg in endemic areas, but also to focus on the type of mutants emerging. This will help future policy decisions relating to vaccine components, as well as diagnostic assay design.

Hepatitis B Virus Genome

Hepatitis B infection is caused by a Hepadnavirus. The HBV genome of 3.2 kilobase pairs makes it the smallest DNA virus known to man. The DNA is circular, partially double-stranded, containing a longer (–) and a shorter (+) strand that encodes viral proteins from four overlapping transcription open-reading frames (ORF), as shown in

Figure 1. These ORFs encode the pre-S/S gene coding for the envelope protein, the C gene for the core protein, the P gene for the DNA polymerase/reverse transcriptase, and the X gene coding for a small protein X, whose function remains obscure.

The surface (S) gene codes for the envelope (HBsAg) of the virus. HBsAg appears as small spherical (22 nm) or filamentous noninfective particles in the serum. HBsAg carries a group-specific *a* determinant, which is common to all subtypes of the virus. It is this determinant that forms the basis for anti-HBs response during HBsAg immunization or HBV wild type virus infection. Several mutants from the S gene have been identified worldwide among vaccinees, patients receiving anti-HBs treatment, and also among naturally infected HBsAg carriers.

The core gene consists of 549 nucleotides and codes for 183 amino acids. It is preceded by a highly conserved pre-core region made up of 87 nucleotides encoding 29 amino acids, with properties of a signal sequence that codes for HBeAg, a cleavage product of HBcAg. The pre-core product of the core gene allows HBcAg to be targeted to the endoplasmic reticulum, where HBeAg is cleaved and secreted.⁵ The presence of HBeAg indicates a high level of viral replication.

The polymerase gene, the largest of the four ORFs, overlaps the envelope gene and as a result, mutations in the P gene can affect the amino acid sequence of the envelope HBsAg protein. The polymerase gene codes for the replicative proteins. The enzymes fulfill several functions, such as reverse transcription, DNA polymerase activity, priming of DNA synthesis, RNase-H activity, and so on. Mutations in the polymerase gene may occur as a result of long-term nucleoside analogue therapy. Drugs, such as Foscarnet and Lamivudine, normally used to maintain suppression of viral replication, have led to emergence of mutants resistant to therapy in certain cases.^{6,7}

The X gene encodes a small protein (HBX) consisting of 154 amino acid residues, with a molecular weight of 17 kd. This protein has broad transactivator properties in which it acts as a co-factor in hepatocarcinogenesis by preventing DNA repair leading to mutant emergence.^{8,9} The X gene seems to play a role in the neoplastic transformation of hepatocytes in HBV-infected liver.¹⁰ Mutants in this region have been found to be associated with a significant number of diseases.

Hepatitis B Surface Proteins

The surface proteins consist of a 2.4 kilobase transcript coding for three alternative translation products of the surface (S) gene, namely the pre-surface 1 (pre-S1), pre-surface 2 (pre-S2) and the surface (S) antigen protein.¹¹ The protein is found at the carboxyl terminal end, and is the principal component of the virus envelope. It is also the primary target for neutralizing antibodies. Although the conformational structure and the relative antigenicity of HBsAg is still unclear, there is evidence that some epitopes of the surface proteins are hidden by the pre-S1 component of the transmembrane protein. The tertiary structure of the surface protein shows five sub-domains (HBs 1-5) in which HBV variants are generally found in antigenic regions HBs 2 and HBs 3 of the major hydrophilic region (MHR). HBV mutants, especially those emerging in vaccinees, are located in the HBs 4 region.

The surface proteins have many functions, including attachment and penetration of the virus into hepatocytes at the beginning of the infection process. Since HBV infections depend on persistence of viremia for transmission from person to person, both the surface proteins and the intact virus co-exist in the infected host.

The *a* determinant is a common constituent of each pair of mutually exclusive determinants, *d* or *y* and *w* or *r*, resulting in four major antigenic subtypes, namely, *adw*, *ayw*, *adr* and *ayr*. Additional minor subspecificities based on subtypic determinants have also been described.¹² The group-specific *a* determinant encompassing codon 122 to 147 is within the major hydrophilic region^{13,14} (Figure 2). This area is highly immunogenic and forms the basis of HBsAg vaccines. The major B cell epitopes of HBsAg have been shown to reside in the *a* determinant at amino acid positions 139-147,^{15,16} and elicit anti-HBs capable of cross-protection among the different subtypes. However, selective pressures, such as anti-HBs response as a result of immunization or administration of monoclonal antibodies during prophylaxis, are capable of inducing an environment that may encourage "escape mutants" to emerge. These mutants may not be neutralized by anti-HBs.¹⁷

Interestingly, a single point mutation in the S gene can induce either a complete switch from one subtype to another, or a loss of expression of an epitope against which the vaccine-induced anti-HBs response is directed. Two good examples are point mutations at *aa* positions 122 (*d/y*) and 160 (*w/r*), involving lysine or arginine.³ These mutants may not interact with the B cell epitopes of HBsAg. Of clinical significance is the fact that the variation does not alter the ability of the virus to be attached to the hepatocyte and replicate, leading to chronic liver disease. For example, the substitution of arginine at

position 145 by glycine causes a vaccine escape mutant to emerge.^{3,17,18} A loss of antigenicity at the *a* determinant, leading to lack of detection of these mutants by some monoclonal HBsAg immunoassay kits, has been reported.^{19,20} Thus, there is a potential threat to the success of vaccination programs and the safety of blood supply in endemic areas of the world where these mutants are prevalent. The antigenic spectrum of the present hepatitis B vaccine may not be adequate to produce antibodies capable of neutralizing the emerging escape mutants and may need to be modified.

HBV Variants and Mutants

Hepatitis B variants are naturally occurring, and may have been selected over a long period of time, probably centuries, whereas hepatitis B mutants are variants which have evolved over a shorter period of time. Variants may also be referred to as subtypes. A wide geographical variation is known to exist for HB variants worldwide, making it essential for each region to define its own variants and mutants.

HBV endemic areas in Far Eastern countries have reduced their carrier rates by means of universal vaccination programs. However, there is evidence of mutant infections among HBsAg vaccinated individuals.²¹ The clinical significance of such mutants cannot be ignored, especially since these mutants replicate with normal efficiency and can infect vaccinees. It remains essential for HBV endemic areas to determine the role of mass vaccination and the effect of local mutants which may not be protected by vaccine-induced antibodies. Policy decisions in the future regarding vaccination and HBsAg testing stand to benefit from such results.

The G145R mutant is well recognized worldwide. It has commonly been found among vaccinees,^{2,22,23} liver transplant patients on HBIG prophylaxis,²⁴⁻²⁶ chronic HBV patients with or without immunosuppression,^{24,27} and patients infected with hepatitis B virus serologically non-reactive to available commercial kits.²⁸⁻³⁰ It has also been found, to a lesser extent, in natural isolates. This substitution of glycine by arginine at position 145 seems to lead to a mutant with complete antigenic variation. The position is within the *aa* 139-147 crucial epitope to which vaccine-induced neutralizing antibody binds. It is also the target for detection by specific antibody of most commercially available HBsAg kits. Such findings are bound to question the efficacy of vaccination programs and the sensitivity of HBsAg screening kits for blood donors.

Other mutants, such as T/I,126N and K141E, have been isolated in Japan³¹ and Gambia,²² respectively. In Papua New Guinea alone, as many as nine variants were found among 13 PCR-positive samples that gave discordant results with monoclonal and polyclonal HBsAg ELISA kits.²⁸ In South Africa, subtype *ayw* was more difficult to detect with available commercial kits, as

compared to subtype *adv*. Three percent of vaccinated health care workers in Sardinia were still found to be anti-HBc positive, an indication of possible infection with mutants. In most of these cases, diagnostic failures may occur depending on the ELISA kit used.²⁸ These results have wider implications in the clinical and blood transfusion settings.³² As mass immunization takes effect in Saudi Arabia, it will be expected that “escape mutants” will emerge. It is vital to define and establish the prevalence of such mutants in our general population and establish the clinical role they play in our community.

HBIG Therapy

Long-term therapy with HBIG antibodies may exert immunological pressure on the HB virus, inducing the S gene to mutate. This is usually more prevalent among orthotopic liver transplant (OLT) patients who need to combine immunosuppression with long-term immunotherapy to reduce HBV infection.^{24,33} A strong correlation between emergence of escape mutants, high-dose prophylaxis, and duration of therapy has been reported.^{34,35} In most cases, the emerging mutants tend to have a decrease-binding capacity for HBIG, leading to escape of neutralization and breakthrough infections. The possible use of combination therapy with antiviral compounds to reduce mutation of the S gene remains to be explored.

Clinical Significance of HBV Mutants

HBV Pre-Core Mutants

There is evidence that HBV mutants can influence not only the course of infection but also the clinical manifestation of the disease. Most of our knowledge of the clinical significance of HBV mutants is based on patients with mutation in the pre-core gene. By far, the most common mutation in the pre-core gene is a single base change of *G* to *A* at nucleotide 1896. This point mutation has provided the stabilizing effect needed for the emergence of a new mutant.³⁶ The single-base change at codon 28 is known to prevent transcription of the pre-core gene, resulting in the failure to encode the infected cell to secrete HBeAg. These patients, though lacking HBeAg, still show persistent HBV-DNA positivity and active viral replication.³⁷ Recently, four HBsAg carrier surgeons were found to have transmitted the virus to their patients despite their anti-HBe status.³⁸

A wide geographical variation seems to emerge for the pre-core mutants. As high as 25% of chronic HBV infections in patients in Delhi, India, were found to be caused by mutants.³⁹ Similarly, high prevalence rates have been reported in HBV-endemic areas in the Mediterranean basin,⁴⁰ including Italy³⁷ and Greece,⁴¹ and Far Eastern countries, such as China and Japan.^{17,23} In contrast, the mutant is less dominant in non-endemic areas, such as Europe and North America.^{42,43} A high association of

fulminant hepatitis has been reported with this mutant in Japan.⁴⁴ In general, patients infected with this mutant are more likely to progress to cirrhosis and hepatic insufficiency, compared to those infected by the wild virus.⁴⁵ In a study of chronic hepatitis patients in France, Zarski et al. found a significant increase in cirrhosis among pre-core mutant infections. This was attributed to patients having early childhood infections.⁴⁶ There are reports that these pre-core mutants could induce resistance to interferon therapy,⁴⁷ increase incidence of fibrosing cholestatic hepatitis in liver transplant patients,⁴⁸ increase susceptibility to hepatitis D co-infection⁴⁹ and cause fulminant hepatic failure.⁵⁰ Despite the high HBV endemicity in Saudi Arabia and the reported high prevalence of the pre-core mutant in the Mediterranean basin, very little is known about HBV pre-core mutants in this country.

HBV Surface Mutants

HBV surface mutants have been identified more commonly among HBsAg-negative patients due to diagnostic kit failures, in vaccinated infants born to HBsAg positive mothers due to vaccine failures, or in OLT recipients due to immunoprophylaxis failure. Unlike the pre-core mutants, very little is known about the clinical significance of the HBV surface mutants, except that the disease they cause seems to develop earlier, and the infection is quite often asymptomatic and has strong association with active HBV replication. In certain cases of mutant-related hepatocellular carcinoma, only HBV-DNA positivity is observed, HBsAg remaining seronegative.⁵¹ In a recent study, Protzer-Knolle et al. found 44% of OLT patients with mutant infections to have a worse clinical outcome, compared to 23% of controls.³⁵

Hepatitis B Vaccination Program in Saudi Arabia

Saudi Arabia and neighboring Gulf States have been considered to be an area of high endemicity for HBV in the past. A survey of 50,000 Saudis showed a prevalence of 8.3% HBsAg carrier rate.⁵² However, other studies, mainly in the 1980s, based on smaller sample numbers have shown higher prevalences, ranging from 9% to an astronomical 25% in certain regions of Saudi Arabia.^{53,54} Apart from the high chronicity among adults in the general population, HBV has been known to play an important etiological role in acute and chronic cases of liver diseases throughout the Kingdom.^{55,56} Perinatal and childhood infections caused by horizontal transmission of the virus are a major mode of transmission in this country.^{57,58}

To interrupt this mode of transmission and to ensure restriction of the reservoir of HBV carriers which serves as a source of future infections, a Royal decree (No 8111 dated 1/6/1408), establishing a committee of specialists, led to an order (No 133140 dated 1/9/1408), making it

mandatory for all newborns in Saudi Arabia to be immunized against hepatitis B virus. This order has been in force for nearly 10 years. The program, which consists of three doses of HBsAg vaccine given at birth, at one, and at six months of life, respectively, has been integrated into the Expanded Program of Immunization in the country. In addition, schoolchildren were also targeted between 1990 and 1996. It is expected that new infections in this age group will be drastically reduced.

Al-Faleh et al. have recently confirmed a remarkable reduction of HBV prevalence from 8% in 1989 to a mere 0.3% in 1998 among vaccinated Saudi children.⁵⁸ Unfortunately, efficacy studies do not take into account the emergence of new HBV mutants which may not be neutralized by vaccine-induced antibodies or detected by ELISA diagnostic kits, which are usually based on the wild type viral antigens. Despite these deficiencies, a sharp drop in HBsAg prevalences among adult blood donors has been observed in recent years. Data compiled throughout the Kingdom over the past 10 years show prevalence rates of 4.4% (82,317 out of 1,888,205) in 1997. This present figure compared to the over 10% prevalence rates reported in the 1980s is very encouraging. The impact of the socioeconomic improvement and the contribution of the mass immunization during the past 10 years must have played a favorable role in the reduction of HBsAg prevalence among Saudis.

The success of the HBV vaccination program has been achieved at a great cost. A maximum impact on public health must be anticipated. Our present knowledge about increasing emergence of hepatitis B mutants which may evolve in the presence of vaccine-induced anti-HBs antibodies poses a serious threat to the objectives of the immunization program.

The 10 years of mandatory vaccination of all newborns, pre-school and schoolchildren must have effectively provided a population of children and young adults immunized against hepatitis B wild virus infection. However, the possibility of immunological selection pressure on the virus due to the anti-HBs immune environment cannot be discounted.

The most significant mutants in this region must be clearly defined and incorporated into present vaccine regimens to ensure higher efficacy for both natural isolates and mutants. It is also vital for HBV diagnosis and blood donor screening to ensure that the present assay kits are sensitive not only to the wild type virus but also to emerging mutants. Awareness of the medical community and commercial institutions about emerging mutants may be the first step to total eradication of all types of HBV. The clinical significance of these mutants continues to emerge, but it is in areas such as sensitivity of diagnostic kits, efficacy of HBsAg vaccines, and effectiveness of HBIg treatment that the subject of HBV mutant research has received the urgency that it deserves. The need for the identification of new mutants in our population and the

altering of the vaccine antigens to reflect on mutants cannot be underestimated. Diagnostic kits may have to be customized to include local mutants, and HBIg antibodies for prophylaxis may need to be based on viruses (wild type and mutants) prevalent in Saudi Arabia.

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