Hepatitis B Virus (HBV) and S-Escape Mutants: From the Beginning until Now

Abstract

Despite the progress made in vaccine and antiviral therapy development, hepatitis B virus (HBV) infection remains a major health care problem. More than 240 million people are chronically infected worldwide showing differences in the severity of liver disease, clinical outcome and response to immune- and antiviral-therapy. Parameters associated with the host immune system (HBV specific T- and/or B-cell repertoires, defective antigen presentation and diminished Th1/Th2 response ratio) and viral factors such as the HBV genotypes and their evolving variants/mutants, have largely contributed to explaining such differences. The unique genomic structure and replication cycle of HBV provide much opportunity for mutations to occur in any of its genes undergoing selection pressures, such as those associated with the host immune system, the hepatitis B vaccine and/or hepatitis B immune globulin and the antiviral therapy with nucleos(t)ide analogues. Firstly, this review describes the current prevalence of S-escape mutants worldwide. Secondly, the clinical implications of such surface gene variants and the impact of universal hepatitis B vaccination on HBV mutations and genotypes are discussed. Finally, the fact that the immune escape process also extends well beyond HBV is addressed.

Keywords: HBV; HBsAg; S-immune escape mutants; Hepatitis B vaccine; OBI, HBIG

Abbreviations: WHO: World Health Organization; HBV: Hepatitis B Virus; CHB: Chronic Hepatitis B; HCC: Hepatocellular Carcinoma; HIV: Human Immunodeficiency Virus; ART: Antiretroviral Therapy; OBI: Occult HBV Infection; HDV: Hepatitis Delta Virus; HBIG: Hepatitis B Immune Globulin; ORF: Open Reading Frame; aa: Amino Acids; HBsAg: Hepatitis B Surface Antigen; cccDNA: Covalently Closed Circular DNA; MHC: Major Histocompatibility Complex Class; OBI: Occult Hepatitis B

Introduction

In accordance with data provided by the World Health Organization (WHO), hepatitis B virus (HBV) chronically infects almost 240 million people worldwide despite the availability of the hepatitis B vaccine since 1982 [1]. Chronic hepatitis B (CHB) is an important global health concern due to its significant morbidity and mortality. It is the cause of approximately 50% of the world's cases of hepatocellular carcinoma (HCC) and about 30% of all cases of liver cirrhosis [2], leading to over 780,000 annual deaths [3].

In the last few years, considerable progress has been made in the construction and development of effective hepatitis B vaccines and in the implementation of childhood immunization programs so as to protect future generations. In this regard, in 1992 the WHO passed a resolution which recommended universal infant vaccination against hepatitis B. As a result, 183 countries around the world have incorporated the hepatitis B vaccine into their corresponding national childhood immunization schedules [1]. However, several gaps remain to be solved. Firstly, the implementation of hepatitis B vaccination is still considerably low where it is most needed, especially in Central Africa, where the prevalence rates of HBV infection are the highest to be reported [4,5]. Secondly, some of the vaccinated infants still sometimes get infected due to vaccine-escape mutants [6-9].

Thirdly, in the era of dual active antiretroviral therapy (ART) used in HBV/human immunodeficiency virus (HIV) co-infected patients ART-induced vaccine-escape HBV mutants emerged as a new hazard. In this case, vaccine-escape HBV mutants do not arise from attempts to escape selection resulting from the hosts immune surveillance or exposure to hepatitis B immuno prophylaxis, but from treatment-induced mutations from overlapping genes (i.e. viral polymerase mutations induce both drug resistance and vaccine-escape S gene mutants) [10]. Lastly, vaccine-escape mutants may also cause occult HBV infection (OBI), that may lead to false negative results in diagnosis (diagnostic-escape mutants), a problem that may also extend to hepatitis delta virus (HDV)/HBV co-infected patients, since the first step in the diagnosis of HDV infection is testing HBsAg-positive individuals for the antibody to the HD antigen [11].

Consequently, HBV vaccine-escape mutants still constitute a matter of public health concern.

In this manuscript, the terms HBV vaccine-escape mutants, hepatitis B immune globulin (HBIG)-escape mutants, HBV immune-escape mutants and S-mutants will be used indistinctly. Their impact on diagnosis, universal hepatitis B vaccination, and the effectiveness of the current genotype-A vaccine to induce cross protection against the other genotypes are discussed. Finally, some fresh insights into the growing problem of HBV...
immune- and/or therapy-escape variants and the finding of an increasing detection of these variants in HIV co-infected patients are addressed.

The structure of hepatitis B virus

HBV is a species of the genus Orthohepadnavirus that is part of the Hepadnaviridae family, whose genome consists of a 3.2-kb-long partially double-stranded relaxed-circular DNA molecule containing four overlapping open reading frames (ORFs): the surface (S-), the core (C-), the polymerase (P-) and the X- genes codifying for all viral proteins [12]. The S-ORF includes the sequence assumed to codify the binding sites for the hepatocyte and has three in-frame AUG codons dividing this ORF into three regions, known as Pre-S1, Pre-S2, and S, encoding for these homonym viral envelope proteins [13]. The C-ORF has two initiation codons encoding for the structural protein of the nucleocapsid, which plays an important role in viral particle assembly (hepatitis B core antigen [HBcAg]), and a soluble antigen secreted by infected cells (hepatitis B e antigen [HBeAg]) [14]. The X-ORF encodes for the hepatitis B x antigen [HBxAg], which displays trans-activating activity and other regulatory functions [15]. Finally, the P-ORF encodes for a multifunctional enzyme, the polymerase, which constitutes the current target for the nucleos(t)ide analogues used in the treatment of CHB [14].

The lack of proof-reading activity of HBV polymerase results in a high mutation rate of 1.4 to 3.2 x 10⁻⁵ nucleotide substitutions per site per year [16]. This figure is one to two orders of magnitude lower than in other viruses lacking polymerase-associated proof-reading functions, such as RNA viruses [17], and four orders of magnitude greater than in “common” (without reverse transcriptase) DNA viruses [18]. Therefore, HBV populations may evolve at a faster rate than most DNA viruses as a result of environmental factors; hence, viral HBV mutants may likely emerge in response to those factors. Lately, an estimated rate of 7.9 x 10⁻⁵ nucleotide substitutions per site per year has been recorded after analyzing the evolution of B full-length genotype B DNA genomes from untreated patients (HBeAg minus) during a 25-yr period [19]. It is interesting to note that a rate of 2.7 x 10⁻³ nucleotide substitutions per site per year was recorded when such rate was studied during a 3-yr period in a patient infected with an HBeAg minus genotype F strain in the presence of anti-HBs antibodies –that is, with HBeAg and HBsAg mutants [20,21].

Additionally to the well known T-cell epitopes found within the C and S proteins, highly conserved amino acid sequences within the viral polymerase, were also described. These sequences are CD8+ and/or T-cell epitopes involved in viral clearance and even-in some cases—in the development of subclinical forms of hepatitis B disease [22]. Therefore, there is a seeming paradox in that natural infection progresses efficiently in spite of the production of highly immunogenic particles. This region is characterized by high immunogenicity and is potentially under selective pressure of the immune system [24].

The a determinant is a complex conformational region and disulfide bridges among highly conserved cystein-residues play a significant role in the preservation of the native tertiary structure of this important HBsAg determinant [24,25,27]. Of the 14 cystein-residues in the 226-aa-sequence common to all three HBV envelope proteins, 8 are found within the MRH of HBsAg, being important in the preserving of the structural conformation and therefore the antigenicity of the a determinant [25,27]. It is thought that the a determinant is made up of two loops bound together by disulfide bridges between C107 and C138, and between C139 and C147 [25,27,28]. This important determinant is considered the main neutralization epitope and is common to all HBV genotypes. Thus, antibodies targeting this determinant are supposed to protect against re-infection with any of the HBV genotypes [29]. Changes in the conformation which can successively alter the binding of neutralizing antibodies can occur due to amino acid substitutions within the a determinant [30].

HBsAg mutants

An HBV mutant is defined as a variant that arises as a result of specific selection, conferring a specific phenotype [31]. In the case of vaccine-escape mutants this specific selection is conferred by forces such as the host immune system that leads to mutations in the S-ORF or the antiviral therapy leading to mutations in the overlapped P-ORF. HBV mutant viruses are allowed to escape from both the humoral and cellular host’s immune response by mutated S-genes, thus reducing diagnostic and Immuno prophylaxis efficacy. Many of these S-immune-escape...
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HBsAg-immune-escape mutants may be found both within and without the a determinant [30]. In this connection, it was suggested that areas located upstream and downstream of the MHR play an important role in neutralization [33]. Mutations occurring outside the MHR are frequently seen, having a tendency to cluster in two regions: around codons 44 to 49 and 152 to 213. The first region contains both a major histocompatibility complex class I (MHC-I)-restricted T-cell epitope and a B-cell epitope, while the second region, at least up to aa 207, displays both MHC-II T helper epitopes and B-cell epitopes. It was also reported that changes within this second region, located immediately downstream of the a determinant, may cause alterations in the structural component of such immunogenic determinant [30,34].

In accord with this notion, many researchers have demonstrated that the binding to anti-HBs antibodies is annullled by aa insertions and deletions in this region [35,36]. Consequently, HBsAg mutations may occur in aa substitutions, insertions, and/or deletions. Ogura et al. noted that during the natural course of infection a high number of mutations arise within the a determinant within the first loop (aa 107 to 138), whereas those mutations induced under immune pressure as a result of active and/or passive immunization most frequently occur within the second loop (aa 139 to 147) [37].

Discussion

Current prevalence of S-escape mutants

HBsAg escape mutants may develop in HBV infected individuals after vaccination with hepatitis B vaccine and/or HBIG administration, in chronic HBV patients during the natural course of HBV infection, and/or in chronic HBV patients receiving antiviral therapy with nucleos(t)ide analogues as a result of the selective pressure exerted by the host immune system or by the antiviral drugs currently used as therapy. These selective pressures allow the survival of the fittest variant [38].

The first report mentioning the emergence of vaccine-escape mutants dates back to 1988 in an Italian boy [39]. Since then, these mutants have been reported by several countries worldwide. Among them, G145R is considered to be the most relevant and best-documented mutation as well as the most commonly found. Such mutant is also stable over time, can replicate to high titers even outside the MHR.

These mutants can be found both in vaccinated or unvaccinated individuals and may also fail to bind properly to anti-HBs antibodies in many commercial diagnostic kits used for HBsAg detection during hepatitis B disease screening, and also during hepatitis D screening [29,42]. Mutations that only change the subtype alleles w to r (K 160R) but do not modify the a determinant are not clinically relevant. Nevertheless, there could be a correlation between the subtype allelic mutation d to y (K122R) and a higher failure rate of passive-active Immuno prophylaxis in infants of HBeAg positive mothers [44]. Mutations between codons 40 and 49, and between codons 198 and 208 not altering the a determinant were identified in patients having HBIG prophylaxis following orthotopic liver transplantation [45]. Those mutations occurring within the first region could be selected by the immune pressure since this region bears both a MHC-I-restricted T-cell epitope and a B-cell epitope. On the other hand, the second region displays both MHC-II T helper epitopes and B-cell epitopes [41].

Ma & Wang assessed 11,221 non-redundant HBV sequences of eight genotypes (from A to H), recovered from the National Center for Biotechnology Information (NCBI) and established the prevalence of S-immune escape mutants in the different HBV genotypes [46]. On the one hand, they reported the prevalence of S-immune escape mutants P120T, T126S, Q129H, G130N, S143L, D144A and G145A/R, which are related to diagnostic failure in one or more genotypes, exhibiting a frequency of no less than 1%. On the other hand, they reported the prevalence of S-escape mutants associated with vaccine or HBIG failure at positions 120, 126, 129, 130, 133, 134, 137, 140, 143, 144 and 145; however, they observed great heterogeneity among the different HBV genotypes [46]. A cross-sectional study carried out in adult patients with liver disease in India, showed that the prevalence of S-immune escape mutants was 0.7% [47]. In Italy, the prevalence of the G145R mutant was 3.1%. In that study, authors reported that in 62.5% of cases, the G145R mutant was alone, whereas in 37.5% of cases, it was accompanied by multiple mutations (T126I-T131A-C139Y-E/D144G, T126I-M133L, and P120Q-T126I) [48].

In a large study carried out in Singapore, about 12% of infants born to HBsAg (+) and HBsAg (+) carrier mothers were infected with HBV despite receiving hepatitis B vaccination and HBIG at birth [49].

The authors of a research paper from China reported that there was a 3.4% failure rate in the HBV vaccine evaluated in the Chinese adult population involved in the HBV vaccination program [50]. Another study also conducted in China, compared the prevalence of HBV mutants in children and adults between 1992 – the year of nationwide HBV vaccine program implementation in China- and 2005 [51]. The study showed that the prevalence of S-immune escape mutants in children increased from 6.5% in 1992 to 14.8% in 2005, where the G145R mutant was found to occur more frequently.

In contrast, there was less difference in mutation frequencies between 1992 (9.4%) and 2005 (9.9%) in adults [51]. In that study, authors concluded that immunization in China increased HBsAg mutation frequencies and the prevalence of Pre-S1 mutants, particularly disease-related mutants [51]. Moreover, in a study carried out in Taiwan no increase was reported in the prevalence of S-immune escape mutants.
of HBsAg-immune escape mutants in children and adolescents that had been fully covered by universal infant immunization [52]. However, when authors compared the prevalence of S-escape mutants between immunized and non-immunized children, aged 1-4 years, they observed that more S-mutants were found in the first group (31% vs 4%, respectively) [52]. It is interesting to note that there were higher frequencies in the emergence of S-escape mutants in those children immunized with plasma-derived vaccines rather than in those immunized with recombinant vaccines (0.3% vs 0.06%) [52]. In this connection, it was previously demonstrated that the levels and durability of the anti-HBs antibodies elicited by second-generation vaccines or recombinant yeast-derived vaccines are lower than those achieved within the HBsAg vaccine derived from the plasma of infected individuals (first-generation vaccines) [53].

Third-generation vaccines or mammalian cells-derived vaccines display a potential superiority over yeast-derived vaccines in helping to avoid vaccine induced escape mutants. This superiority is mainly related to the lack of Pre-S antigens (Pre-S1 and Pre-S2) that carry neutralizing epitopes, and to the presence of misfolded HBsAg in yeast-derived vaccines, whereas mammalian cells-derived vaccines contain correctly folded HBsAg and the neutralizing epitopes of the Pre-S antigens (Pre-S1 and Pre-S2) [54]. Another difference is that human plasma- and recombinant mammalian cell-derived vaccines are glycosylated, whereas yeast-derived HBsAg is not neither glycosylated from Saccharomyces cerevisiae nor from the methyloptropic yeasts Pichia pastoris and Hansenual polymorpha [55].

In other parts of the world, as for example in Latin America, the epidemiological information related to HBsAg mutants is scarce or practically non-existent. This kind of data is of vital importance in an increasingly immunized world against HBV due to the fact that HBsAg mutants may become set up and spread within populations that are assumed to be protected, and may not be detected by immunodiagnostic kits. Therefore, epidemiological research works from different parts of the world are really essential for gaining insight into the prevalence and characteristics of HBsAg mutants. Once available, such information will be extremely valuable both for producing new HBV vaccine(s) and designing new immunoassays for HBsAg detection as well as for avoiding false negative results when screening for HBsAg in blood donors.

Some reports from Argentina have recently documented the emergence of S-immune escape mutants in spite of the presence of usually protective anti-HBs antibodies [8,20,21,30,34,56,57] and of cytotoxic T lymphocyte-specific dones [30]. Some of them occurred naturally in HBV-chronically infected patients while others are the result of selected immunological pressures upon vaccination. To date, there are no reports referring to these mutants in other parts of the world. The following are among the S-mutants reported in Argentina C69Y, C90Y, L110I, T114A, L158F, A168T, C176R, N178S [34]; S54A, P46H, L49H, C107R, T125A, M133K, N152F, P153T, Y161S, G185E, A194TG202R, I213L [30]; D144A, L209V [8].

In general terms, the worldwide prevalence of S-immune escape mutants due to the selection pressure exerted by the hepatitis B vaccine is low, not yet constituting a threat to public health that would require the modification of the available hepatitis B immunization programs; however, this fact does not mean that improvement in current hepatitis B vaccines is unnecessary. Nevertheless, nowadays, there is worrying evidence related to P-mutants in ducing vaccine-escape S gene mutants due to treatment induced mutations. The overlapping nature of the S- and the P-ORFs in the HBV genome may lead almost every single mutation to exert an influence on more than one function of the corresponding nucleotide sequence.

Hence, mutations that occur in the S-ORF selected by forces such as the immune system may be translated as mutations in the P-ORF that eventually may result in resistance to the antiviral therapy. Likewise, those mutations occurring in the P-ORF selected by forces such as the antiviral therapy may be translated as mutations in the S-ORF that may eventually produce an S-immune escape mutant. The circulation and transmission of S-escape mutants resistant to the nucleos(t)ide analogues currently used as anti-HBV therapy and vice versa, may pose a significant risk for the community since these mutants may potentially infect both naïve and immunized individuals and negatively affect the efficacy of both the antiviral treatment and the vaccination programs [56,58].

In this regard, there is considerable concern of the increasing number of reports in the international literature referring to the detection of S-escape mutants in HIV co-infected patients during long-term ART [59,60]. Three major patterns of mutations in the HBV polymerase associated with lamivudine resistance were described in HBV/HIV co-infected patients receiving ART with lamivudine as the sole-anti-HBV drug: single rtM204V, double rLt180M+rtM204V and triple rtV173L+rtL180M+rtM204V. This triple mutant is related to a vaccine escape mutant due to the corresponding change in the overlapped S-ORF [61]. Thus, the use of lamivudine as the only active anti-HBV agent is discouraged [62]. It has been widely accepted that HIV therapy with nucleos(t)ide analogues active against HBV improves hepatitis outcomes in HIV/HBV co-infected patients, especially when tenofovir is used [62]. However, drug resistance, cross-resistance with other antiviral drugs and the occasionally selection for HBV vaccine-escape mutants are still the major drawbacks of the nucleos(t)ide analogues currently used in the ART regimen.

During an interesting follow-up study carried out by Lacomba et al. in HBV patients co-infected with HIV, authors observed a high frequency of S-immune escape mutants selected by the nucleos(t)ide analogues used as therapy [63]. Another study showed that anti-HBV drug resistant mutations were detected in 54% of HBV/HIV co-infected patients receiving dual active ART and 45% of whom had an immune escape mutation [64].

In summary, the prevalence of S-immune-escape mutants displays a great variability among the different group of patients analyzed: vaccinated infants, chronic patients, liver transplant recipients and HBV/HIV co-infected patients. Thus, S-mutants selected by the immune system are responsible for 0.2-4.6% of the cases of breakthrough infections in newborns that received the recommended immune prophylaxis at birth, and up to 40% of the liver transplant recipients that received HBIG as immune prophylaxis, develop S-escape mutants [29]. At present, the high and steadily increasing incidence of HBV vaccine-escape mutants among HIV co-infected patients receiving dual ART must be seriously considered and a judicious selection of antiviral agents
and vigilant monitoring of viral mutants during the course of therapy are mandatory.

**Pathobiology and clinical implications of S-escape mutants**

The clinical spectra of S-mutants in HBV-infected patients range from asymptomatic without any sign of hepatic injury to fulminating hepatitis, chronic hepatitis, fibrosing cholestatic hepatitis, cirrhosis and HCC [29]. Experimental data and studies conducted in humans have demonstrated that some mutations in the S protein may be cytotoxic to hepatocytes. Therefore, mutations related to viral retention and the characteristic appearance of ground-glass cells may contribute to a poorer prognosis due to a more progressive liver disease [65,66]. Furthermore, Asahina et al have described some mutations in HBsAg associated to acute exacerbations of CHB such as amino acidic substitutions L95W, I126S/T, G130N, T131N, M133T/K y V173L [67].

Hence, it is of the utmost importance to detect S-mutants in CHB patients so as to identify those patients requiring a preventive and appropriate therapy as well as a closer follow-up strategy for early detection of HCC [66]. As mentioned above, due to the overlapping structure of the HBV genome, it was reported that the lamivudine-selected mutants E164D/I195M demonstrated a minimal binding of anti-HBs antibodies while the E164D, M198I, I195M and W196S lamivudine-selected mutants also showed reduced binding to anti-HBs antibodies [68]. This phenotype is currently observed in HIV co-infected patients receiving dual ART at a steadily increasing frequency. Moreover, few cases of reactivation of CHB in the absence of HBsAg have been reported in individuals with different immuno deficiencies and in those with HIV co-infection following ART discontinuation [60].

However data on clinical and virological outcomes in HBV and HIV co-infected patients receiving active ART are limited and further studies are needed to understand the complex molecular virology of HBV during HIV co-infection. Another interesting issue is related to HDV co-infected patients. HDV must either co-infect or super-infect with HBV and requires HBsAg and Pre-S antigens to assemble into a new HDV virion in the infected hepatocytes [69]. Thus S-immune escape mutants may also represent a problem of concern in the hepatitis D clinical setting due to their eventual effect in HDV pathogenicity. In this regards, it is widely well known that HDV co-infection is usually associated to a poorer prognosis in CHB patients (more frequent cirrhosis, increased risk of HCC and/or and fulminant hepatitis). In the hepatitis C virus (HCV) co-infection setting, the role of S-mutants and liver disease is also uncertain.

Finally, S-escape mutants have become an important clinical and public health concern because they might be associated with OBI (presence of viral DNA with undetectable HBsAg and low viral replication) [70,71]. Both anti-HBc and/or anti-HBs positive individuals and individuals negative for all serologic HBV infection may be harboring OBI [70,72]. The exact mechanism of OBI has still to be elucidated. However, such mechanism may be possibly explained by the intra hepatic persistence of cccDNA under the host’s vigorous immune suppression of viral replication and gene expression [71]. Additional mechanisms unrelated to the host’s response were also reported, including:

(i) The presence of defective particles or of mutations in transcription control regions within the viral polymerase causing low HBV replication and HBsAg expression

(ii) The circulation of S-escape mutants

(iii) Hepatitis C virus (HCV) or hepatitis D virus (HDV) co-infection, since these viruses down-regulate HBV replication, thus reducing HBsAg expression

(iv) abolished the HBsAg expression due to a RNA splicing event [72-75].

Several epidemiological and molecular studies demonstrated that OBI can lead to the development and exacerbation of HBV related diseases, such as liver fibrosis, cirrhosis and HCC [76]. Different virological characteristics were found in HCC patients with OBI, for example, higher frequencies of amino acidic mutations in Pre-S/S such as the presence of premature stop codons or some nucleotidic substitutions in the enhancer II region such as G1721A. The overall conclusion is that, despite the low viral load, the risk of HCC in OBI patients is high [77].

Another epidemiology study conducted in Taiwan demonstrated that 10.9% of HBsAg zero negative vaccinated children had OBI, and although the most documented mutation G145R was not found, other mutations inside the MHR were recorded (C139S and A159T) [78]. Deletions in the 3’ terminus of Pre-S1 led to the loss of immune epitopes and to a reduced secretion of HBsAg [78]. In immuno compromised patients, OBI may reactivate and cause acute hepatitis [71]. Although the relation between OBI and the development of chronic liver diseases and HCC is not well understood, it was reported that OBI contributes to the genesis of HCC directly by its own proto-oncogenic effect and indirectly by causing persistent liver inflammation and fibrosis [71].

As mentioned above, the presence of co-infections usually influences the natural evolution of each of the infectious agents present by either facilitating their virulence or by competing for their resources. Many studies have focused on the clinical impact of HIV, HCV and HDV on the natural evolution of HBV infection, finding that co-infection carries an increased risk of progressive liver disease and HCC. A persisting risk of repeated flares of hepatitis and progressive liver disease is even more frequent when co-infections are present in patients with OBI [79].

**Diagnostic escape mutants**

Today, in many countries around the world, HBV is still one of the serious infectious risks to blood transfusion safety [46]. One of reasons is the worldwide circulation of the S-immune escape mutants that pose a challenge not only to hepatitis B prophylactic tools but also to the diagnosis; issues that should also be extended to HDV since it requires coinfection with HBV for infection of hepatocytes, replication and formation of virions. In this regards, Delfino et al. provided valuable evidence of the presence of HDV infection in OBI patients due to S-escape mutants [80]. Hence, a re-evaluation of HDV diagnostic algorithms is mandatory.

Many current ELISA kits used may be unable to properly detect S-immune escape mutants [42]. Therefore, they may be potentially transmitted horizontally by blood transusions of an asymptomatic carrier having no known risk factors concerning HBV infection, rendering a false negative result in HBsAg serology for harboring an HBsAg mutant virus that commercial HBsAg kits

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have failed to detect [72,81].

The introduction of novel HBsAg assays able to detect these S-gene mutants permanently improves the sensitivity of such assays. Full sensitivity is not guaranteed by polyclonal capture antibody-based assays, despite performing much better than most commercially available double monoclonal (capture and tracer) assays, which employ antibodies directed against the second loop of the a determinant. The major disadvantage of employing polyclonal antibody immunoassays is that their specificity is lower than that of monoclonal assays. Therefore, the use of mixtures of monoclonal antibodies that are able to recognize both wild-type and S-mutants strains is advised for HBsAg screening. With the purpose of reducing failure in the diagnosis associated with HBsAg-negative mutants, tracer and capture antibodies against the Pre-S region were recommended for inclusion in diagnostic immunoassays. Nevertheless, if a mixture of antibodies is used, there may be a decrease in the analytical sensitivity for HBsAg of different genotypes even of wild-type virus. Antibodies directed against the second loop of the a determinant should not be used for immunoassays since they are not able to detect G145R mutants [29].

Weber also suggested that anti-HBc testing should be used in combination with HBsAg for blood donor screening, particularly in those countries where there is no mandatory HBV DNA detection [29] by nucleic acid amplification technology (NAT), such as in many regions of Africa and Latin America. For many years, HBV DNA tests were not very sensitive. Nowadays, new generation DNA detection technologies such as real-time PCR and transcription-based mediated amplification (TMA) allowed a decrease in the lower detection limit (< 5 IU/mL of HBV DNA), which is particularly important in OBI since DNA levels vary at -5-10 IU/mL (range < 10 to 425 copies/mL) [79].

HBV Genotypes and hepatitis B vaccine

Phylogenetic analysis based on the comparison of complete human HBV genomes has defined at least nine genotypes (A-H, and J) and 34 sub genotypes [82]. A putative genotype I has been proposed [83,84] but its existence is still controversial. HBV genotypes have been shown to have a distinct geographic distribution and seem to have differences in biological properties that may account for differences in the clinical outcome of the infection or in the antiviral treatment response [29].

Genotype E from Africa is the most predominant genotype in Western and Central Africa. Genotype E strains isolated in Europe and their exceptional detection in America have been derived from HBV carriers of African origin, regardless of their country of residence [30,85]. Noteworthy, genotype E shows a marked genotypic divergence with respect to all genotypes within the a determinant which seems to account for vaccination failure and also appears to be an influencing factor for HBsAg screening by diagnostic assays [86]. Genotype F is the most predominant genotype in Central and South America and is recognized as the most divergent of all the genotypes.

It is extremely important to note that the current available hepatitis B vaccine has the HBsAg genotype A2, subtype adw2, whereas genotype E isolates have subtype ayw4, and genotypes F isolates have subtypes adw4q- and/or ayw4. The fact that subtype-epitopes are very immunogenic, together with the emergence of S-escape mutants, has raised questions about the effectiveness of the current vaccine in Africa where genotype E is the most predominant HBV genotype, as well as in Central and South America where F genotype is frequent [8,87]. Finally, in some Asian countries, such as in Japan and Korea, where genotype C is the most prevalent one, vaccines generated from genotype C are used. Interestingly, in these areas acute hepatitis B due to genotype A2 has increased [87]. Therefore, further studies are needed, since little is known about whether vaccination against one genotype can induce effective cross protection against the others.

Conclusion

HBsAg mutants, either naturally occurring or selected through immunological or antiviral therapy pressure are of clinical significance due to the role they play in active and/or passive HBV immuno prophylaxis failures and loss of diagnostic accuracy not only in patients HBV-infected patients, but also in those with HDV. Therefore, it is advisable and of great significance to adequately the currently available commercial immunoassays kits used in the diagnosis of hepatitis B for the detection of individuals harboring such emerging viruses, and to reevaluate HDV diagnostic algorithms.

Due to the high incidence of nucleos(t)ide analogue resistance associated vaccine-escape HBV mutants among HIV-co infected patients undergoing dual ART, hepatitis B treatment options that aim to reduce the risk of HBV mutations from emerging should be seriously considered, not only from clinical but also from public health perspectives. From a Public Health point of view, it is important to understand the distinct traits of these S-escape mutants in relation to their prevalence and heterogeneity since this knowledge could be of great help for improving diagnostic assays, designing new vaccines, preventing HBIG therapy failure, and reevaluating hepatitis B treatment options among HIV-co infected patients.

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References

1. WHO (2015) Hepatitis B. World Health Organization Fact sheet N°204.
2. El-Seraq HB (2011) Hepatocellular carcinoma. N Engl J Med 365(12): 1118-1127.
3. Gish RG, Locarnini S (2007) Genotyping and genomic sequencing in clinical practice. Clin Liver Dis 11(4): 761-795.
4. Komas NP, Vickos U, Hübschen JM, Béreé A, Manirakiza A, et al. (2013) Cross-sectional study of hepatitis B virus infection in rural communities, Central African Republic. BMC Infect Dis 13: 286.
5. Dussurget A, Abeguiguen P, Girugul J, Mansour W, Pivert A, et al. (2013) High endemicity and low molecular diversity of hepatitis B virus infections in pregnant women in a rural district of North Cameroon. PLoS One 8(11):e000346.
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6. Lee KM, Kim YS, Ko Y, Yoo BM, Lee KJ, et al. (2001) Emergence of vaccine-induced escape mutant of hepatitis B virus with multiple surface gene mutations in a Korean child. J Korean Med Sci 16(3): 359-362.

7. Basuni AA, Butterworth L, Cocksley G, Locarnini S, Carman WF (2004) Prevalence of HBsAg mutants and impact of hepatitis B infant immunization in four Pacific Island countries. Vaccine 22(21-22): 2791-2799.

8. Mathet VL, Cuestas ML, Ruiz V, Minassian ML, Rivero C, et al. (2006) Detection of hepatitis B virus (HBV) genotype C carried –even in the presence of high titters of anti-HBs antibodies- by an Argentinean patient of African descent who had received vaccination against HBV. J Clin Microbiol 44(9): 3435-3439.

9. Lin YM, Jow GM, Mu SC, Chen BF (2013) Naturally occurring hepatitis B virus B-cell and T-cell epitope mutants in hepatitis B vaccinated children. Scientific World Journal 2013: 571875.

10. Lacombe K, Boyd A, Gojdan J, Lavocat F, Girard PM, et al. (2010) Drug-resistant and immune-escape HBV mutants in HIV-infected hosts. Antivir Ther 15(3PtB): 493-497.

11. Olivero A, Smellie A (2012) Hepatitis delta virus diagnosis. Seminar Liver Dis 32(3): 220-227.

12. Shen T, Yan XM (2014) Hepatitis B virus genetic mutations and evolution in liver diseases. World J Gastroenterol 20(18): 5435-5441.

13. Price PM, Mohamad A, Zelent A, Neurath AR, Acs G (1988) Translational selection in the expression of the hepatitis B virus envelope proteins. DNA 7(6): 417-422.

14. Cuestas ML, Mathet VL, Oubiña JR, Sosnik A (2010) Drug delivery systems and liver targeting for the improved pharmacotherapy of the hepatitis B virus (HBV) infection. Pharm Res 27(7): 1184-1202.

15. Bouchard MJ, Schneider RJ (2004) The enigmatic X gene of hepatitis B virus. J Virol 78(23): 12725-12734.

16. Gamem D, Prince AM (2004) Hepatitis B virus infection:natural history and clinical consequences. N Engl J Med 350(11): 1118-1129.

17. Brunetto MR, Rodríguez UA, Bonino F (1999) Hepatitis B virus mutants. Intervirology 42(2-3): 69-80.

18. Pumpens P,rens E, Nassal M (2002) Molecular epidemiology and immunology of hepatitis B virus infection – an update. Intervirology 45(4-6): 218-232.

19. Osiowy C, Giles E, Tanaka Y, Mizo-Kami M, Minuk GY (2006) Molecular evolution of hepatitis B virus over 25 years. J Virol 80(21): 10307-10314.

20. López JL, Mathet VL, Oubiña JR, Campos RH (2007) Intrahost evolution of HBs antigen-negative hepatitis B virus genomes ascribed to the F genotype: a longitudinal 3 year retrospective study. J Gen Virol 88(Pt F): 86-91.

21. Mathet VL, López JL, Ruiz V, Sánchez DO, Carbalal G, et al. (2007) Dynamics of a hepatitis B virus e antigen minus population ascribed to genotype F during the course of a chronic infection despite the presence of anti-HBs antibodies. Virus Res 123(1): 72-85.

22. Wai CT, Fontana RJ (2004) Clinical significance of hepatitis B virus genotypes, variants and mutants. Clin Liver Dis 8(2): 321-352.

23. Antoni BA, Rodríguez-Crespo I, Gómez-Gutiérrez J, Nieto M, Peterson D, et al. (1994) Site-directed mutagenesis of cysteine residues of hepatitis B surface antigen. Analysis of two single mutants and double mutants. Eur J Biochem 222(1): 121-127.

24. Seddigh-Tonakaboni S, Waters JA, Jeffers S, Gehrke R, Ofenloch B, et al. (2000) Effect of variation in the common “a” determinant on the antigenicity of hepatitis B surface antigen. J Med Virol 60(2): 113-121.

25. Mangold CM, Streeck RE (1993) Mutational analysis of the cysteine residues in the hepatitis B virus small envelope protein. J Virol 67(8): 4568-4597.

26. Chen YC, Delbrook K, Dealwis C, Mimms L, Mushahwar IK, et al. (1996) Discontinuous epitopes of hepatitis B surface antigen derived from a filamentous phage peptide library. Proc Natl Acad SCI USA 93(5): 1997-2001.

27. Mangold CM, Uncellk F, Herr W, Streeck RE (1997) Analysis of intermolecular disulphide bonds and free sulphhydryl groups in hepatitis B surface antigen particles. Arch Virol 142(171): 2257-2267.

28. Carman WF (1998) Infections associated with medical intervention: hepatitis viruses and HGV. Br Med Bull 54(3): 731-748.

29. Weber B (2005) Genetic variability of the S gene of hepatitis B virus: clinical and diagnostic impact. J Clin Virol 32(2): 102-112.

30. Cuestas ML, Mathet VL, Ruiz V, Minassian ML, Rivero C, et al. (2006) Unusual naturally occurring humoral and cellular mutated epitopes of hepatitis B virus in a chronically infected argentene patient with anti-HBs antibodies. J Clin Microbiol 44(6): 2191-2198.

31. de Franchis R, Hadengue A, Lau G, Lavanchy D, Lok A, et al. (2003) EASL International Consensus Conference on Hepatitis B. 13-14 September, 2002 Geneva, Switzerland. Consensus statement (long version). J Hepatol 39 Suppl 1: S3-25.

32. Dindost P, Jazayeri SM, Karimzadeh H, Saberfar E, Miri SM, et al. (2012) HBsAg variants: common escape issues. Jundishapur J Microbiol (4(4): 521-527.

33. Yamamoto K, Horikita M, Tsuda F, Itoh K, Akahane Y, et al. (1994) Naturally occurring escape mutants of hepatitis B virus with various mutations in the S gene in carriers seropositive for antibody to hepatitis B surface antigen. J Virol 168(4): 2671-2676.

34. Mathet VL, Feld M, Espinola L, Sánchez DO, Ruiz V, et al. (2003) Hepatitis B virus S gene mutants in a patient with chronic active hepatitis with circulating anti-HBs antibodies. J Med Virol 69(1): 18-26.

35. Hou J, Kajiyannis P, Waters J, Luo K, Liang C, Thomas HC (1995) A unique insertion in the S gene of surface antigen-negative hepatitis B virus Chinese carriers. Hepatology 21(2): 273-278.

36. Shields PL, Owsianka A, Carman WF, Boxall E, Hubscher SG, et al. (1999) Selection of hepatitis B virus surface “escape” mutants during passive immune prophylaxis following liver transplantation: potential impact of genetic changes on polymerase protein function. Gut 45(2): 306-309.

37. Ogura Y, Kurosaki M, Asahina Y, Enomoto N, Marumo F, et al. (1999) Prevalence and significance of naturally occurring mutations in the surface and polymerase genes of hepatitis B virus. J Infect Dis 180(5): 1444-1451.

38. Kaymakoglu S, Baran B, Onel D, Badur S, Atamer T, et al. (2014) Acute hepatitis B due to immune-escape mutations in a naturally immune patient. Acta Gastroenterol Belg 77(2): 262-265.

39. Zanetti AR, Tani E, Mazzullo G, Maio G, Sbreglia C, et al. (1988) Naturally occurring escape mutants of hepatitis B virus with various mutations in the S gene in carriers seropositive for antibody to hepatitis B surface antigen. J Virol 62(4): 2191-2198.

40. Zuckerman JN, Zuckerman AJ (2003) Mutations of the surface protein of hepatitis B virus. Antiviral Res 60(2): 75-78.
Hepatitis B Virus (HBV) and S-Escape Mutants: From the Beginning until Now

41. Kreutz C (2002) Molecular, immunological and clinical properties of mutated hepatitis B viruses. J Cell Mol Med 6(1): 113-143.

42. Coleman PF (2006) Surveillance for hepatitis B surface antigen mutations. J Med Virol 78(5): 56-58.

43. Weinberger KM, Bauer T, Böhm S, Jilg W (2000) High genetic variability of the group –specific a-determinant of hepatitis B virus surface antigen (HBsAg) and the corresponding fragment of the viral polymerase in chronic virus carriers lacking detectable HBsAg in serum. J Gen Virol 81(Pt5): 1165-1174.

44. Hsu HY, Chang MH, Ni YH, Jeng YM, Chiang CL, et al. (2013) Long-term follow-up of children with postnatal immunoprophylaxis failure who were infected with hepatitis B virus surface antigen gene mutants. J Infect Dis 207(7): 1047-1057.

45. Ghany MG, Ayola B, Villamil FG, Gish RG, Rotter S, et al. (1998) Hepatitis B virus S mutants in liver transplant recipients who were reinfected despite hepatitis B immune globulin prophylaxis. Hepatology 27(1): 213-222.

46. Ma Q, Wang Y (2012) Comprehensive analysis of the prevalence of hepatitis B virus escape mutants in the major hydrophilic region of surface antigen. J Med Virol 84(2): 198-206.

47. Kamat SP, Mehta PR, paranje SM, Ingle NA (2014) Hepatitis B virus (HBV) infection in liver disease patients in Mumbai, India with special reference to hepatitis B surface antigen (HBsAg) mutant detection. J Clin Diagn Res 8(3): 19-21.

48. Stich L, Coligiarri P, Caccini R, Alicko C, Bruzzone B (2013) Epidemiology of HBV S-gene mutants in the Liguria Region, Italy: implications for surveillance and detection of new escape variants. Hum Vaccin Immunother 9(3): 568-571.

49. Oon CJ, Chen WN (1998) Current aspects of hepatitis B surface antigen mutants in Singapore. J Viral Hepat Suppl 2: 17-23.

50. He C, Nomura F, Iisago S, Isobe K, Nakai T (2001) Prevalence of vaccine-induced escape mutants of hepatitis B virus in the adult population in China: a prospective study in 176 restaurant employees. J Gastroenterol Hepatol 16(12): 1373-1377.

51. Bian T, Yan H, Shen L, Wang F, Zhang S, et al. (2013) Change in hepatitis B virus large surface antigen variant prevalence 13 years after the implementation of a universal vaccination program in China. J Virol 87(22): 12196-12206.

52. Hsu HY, Chang MH, Ni YH, Chiang CL, Chen HL, et al. (2010) No increase in prevalence of hepatitis B surface antigen mutant in a population of children and adolescents who were fully covered by universal infant immunization. J Infect Dis 201(8): 1192-1200.

53. Jilg W, Schmidt M, Deinhardt F (1988) Prolonged immunity after late booster doses of hepatitis B vaccine. The Journal of Infectious Diseases 157(6): 1267-1269.

54. Gerlich WH (2015) Prophylactic vaccination against hepatitis B: achievements, challenges and perspectives. Med Microbiol Immunol 204(1): 39-55.

55. Lündorf H, Gurrumkonda C, Adnan A, Khanna N, Rinas U (2011) Virus-like particle production with yeast. Ultrastructural and immunocytological insights into Pichia pastoris producing high levels of the hepatitis B surface antigen. Microbial cell Factories 10:48.

56. Cuestas ML, Rivero CW, Minassian ML, Castillo AL, Gentile EA, et al. (2010) Naturally occurring hepatitis B virus (HBV) variants with primary resistance to antiviral therapy and S-mutants with potential primary resistance to adefovir in Argentina. Antiviral Res 87(1): 74-77.

57. Defino CM, Gentile EA, Castillo AL, Cuestas ML, Patacinni G, et al. (2014) Hepatitis B virus and hepatitis D virus in blood donors from Argentina: circulation of HBsAg and reverse transcriptase mutants. Arch Virol 159(5): 1109-1117.

58. Romano L, Paladini S, Galli C, Raimondo G, Pollicino T, et al. (2015) Hepatitis B virus vaccination. Hum Vaccin Immunother 11(1): 53-57.

59. Milazzo L, Ebranatti E, Cattaneo D, Gabanelli E, La L, et al. (2014) Recurrence of another hepatitis B virus escape mutants comes back in a patient infected with HIV and low CD4+ count. J Med Virol 86(1): 97-101.

60. Costantini A, Marinelli K, Bagionti G, Monachetti A, Ferreri ML, et al. (2011) Molecular analysis of hepatitis B virus (HBV) in an HIV co-infected patient with reactivation of occult HBV infection following discontinuation of lamivudine including antiretroviral therapy. BMC Infect Dis 11: 310.

61. Pal A, Sarkar N, Saha D, Guha SK, Saha B, et al. (2015) High incidence of lamivudine-resistance associated vaccine-escape HBV mutants among HIV-coinfected patients on prolonged antiretroviral therapy. Antivir Ther Epub ahead of print.

62. Soriano V, de Mendoza C, Peña JM, Barreiro P (2015) Advances in treating drug-resistant hepatitis B virus in HIV-infected patients. Expert Opin Pharmacother 16(2): 179-1.

63. Lacombe K, Boyd A, Lavocat F, Pichoud C, Godan J, et al. (2013) High incidence of treatment-induced and vaccine-escape hepatitis B virus mutants among human immunodeficiency virus/hepatitis B-infected patients. Hepatology 58(3): 912-922.

64. Coffin CS, Osiowy C, Myers RP, Gill MJ (2013) Virology and clinical sequelae of long term antiviral therapy in a North American cohort of hepatitis B virus (HBV)/human immunodeficiency virus type 1 (HBV/HIV-1) co-infected patients. J Clin Virol 57(2): 103-108.

65. Chiari FV, Klopchin K, Moriyma T, PasquNeillini C, Dunsford HA, et al. (1989) Molecular pathogenesis of hepatocellular carcinoma in hepatitis B virus transgenic mice. Cell 59(6): 1145-1156.

66. Pollicino T, Cacciola I, Saffioti F, Raimondo G (2014) Hepatitis B virus PreS/S gene variants: pathobiology and clinical implications. J Hepatol 61(2): 408-417.

67. Ashina Y, Enomoto N, Ogura Y, Kuroski M, Sakuma I, et al. (1996) Sequential changes in full-length genomes of hepatitis B virus accompanying acute exacerbation of chronic hepatitis B. J Hepatol 25(6): 787-794.

68. Torresi J (2002) The virological and clinical significance of mutations in the overlapping envelope and polymerase genes of hepatitis B virus. J Gastroenterol Hepatol 20(40): 14589-14597.

69. Huang CR, Szeheng JL (2014) Hepatitis B virus infection, replication and cross-talk with the hepatitis B virus. World J Gastroenterol 20(25): 97-106.

70. Said ZN (2011) An overview of occult hepatitis B virus infection. World J Gastroenterol 17(15): 1927-1938.

71. Kwak MS, Kim YJ (2014) Occult hepatitis B virus infection. World J Hepatol 6(2): 860-869.

72. Thakur V, Kazim SN, Guptan RC, Hasnain SE, Bartholomeusz A, et al. (2005) Transmission of G145R mutants of HBV to an unrelated contact. J Med Virol 76(1): 40-46.

73. Chevrier MC, St-Louis M, Perreault J, Canón B, Castillo ML, et al. (2007) Detection and characterization of hepatitis B virus of anti-hepatitis B core antigen-reactive blood donors in Quebec with an

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in-house nucleic acid testing assay. Transfusion 47(10): 1794-1802.

74. Gerlich WH, Bremer L, Saniewski M, Schüttler CG, Wen UC, et al. (2010) Occult hepatitis B virus infection: detection and significance. Dig Dis 28(1): 116-125.

75. Ozaslan E, Purnak T (2010) Is it a case of transfusion-transmitted acute HBV or reactivation of occult hepatitis B virus infection? Transfus Med 20(4): 275.

76. Raimondo G, Caccamo G, Filomia R, Pollicino T (2013) Occult HBV infection. Semin Immunopathol 35(1): 39-52.

77. Huang X, Hollinger FB (2014) Occult hepatitis B virus infection and hepatocellular carcinoma: a systemic review. J Viral Hepat 21(3): 153-162.

78. Mu SC, Lin YM, Jow GM, Chen BF (2009) Occult hepatitis B virus infection in hepatitis B vaccinated children in Taiwan. J Hepatol 50(2): 264-272.

79. Maldonado-Rodriguez A, Cevallos AM, Rojas-Montes O, Enríquez-Navarro K, Alvaréz-Muñoz MT, et al. (2015) Occult hepatitis B virus co-infection in human immunodeficiency virus-positive patients: A review of prevalence, diagnosis and clinical significance. World J Hepatol 7(2): 253-260.

80. Delfino CM, Eirin ME, Malan R, Gentle E, Castillo A, et al. (2012) HDAg-L variants in covert hepatitis D and HBV occult infection among amerindians of Argentina: new insights. J Clin Virol 54(3): 223-228.

81. Levický-Steziára S (2004) Hepatitis B surface antigen escape mutant in a first time blood donor potentially missed by routine screening assay. Clin Lab 50(1-2): 49-51.

82. Cao GW (2009) Clinical relevance and public health significance of hepatitis B virus genomic variations. World J Gastroenterol 15(46): 5761-5769.

83. Huy TT, Sall AA, Reynes JM, Abe K (2008) Complete genome sequence and phylogenetic relatedness of hepatitis B virus isolates in Cambodia. Virus Genes 36(2): 299-305.

84. Olinger CM, Lazouskaya NV, Eremin VE, Muller CP (2008) Multiple genotypes and subtypes of hepatitis B and C viruses in Belarus: similarities with Russia and western European influences. Clin Microbiol Infect 14(6): 575-581.

85. Kramvis A, Kew MC (2007) Epidemiology of hepatitis B virus in Africa, its genotypes and clinical associations of genotypes. Hepatol Res 37(s1): S9-S19.

86. Karthigesu VD, Allison LM, Ferguson M, Howard CR (1999) A hepatitis B virus variant found in the sera of immunized children induces a conformational change in the HBsAg "a" determinant. J Med Virol 58(4): 346-352.

87. Ogata N (2009) Comparison of antibody response to hepatitis B surface antigen among four recipients groups of hepatitis B vaccines that have been approved in Japan: evaluation using passive hemagglutination assay and chemiluminiscent immunoassay. Rinsho Byori 57(10): 954-960.