MINIREVIEW

Hepatitis B virus vaccine breakthrough infection: surveillance of S gene mutants of HBV

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Summary. – Hepatitis B (HB) is a worldwide public health problem, closely related with liver cirrhosis and hepatocellular carcinoma (HCC). The implementation of universal hepatitis B virus (HBV) vaccination programs has led to significant reduction in incidence of acute and chronic HB, liver cirrhosis and HCC. However, this success is now being threatened by the discovery of HBV vaccine breakthrough infection caused by the S gene mutants of HBV, high maternal viral load and virus-induced immunosuppression. An alteration in the antigenicity and immunogenicity of hepatitis B surface antigen (HBsAg) due to S gene mutations may compromise detection of HBsAg (diagnosis-escape mutants), treatment with hepatitis B-specific immunoglobulin (HBIG), and even cause infections in individuals who are antihepatitis B surface antigen (anti-HBs) antibody-positive after immunization (vaccine-escape mutants). By surveilling for S gene mutants of HBV among vaccinated population, we will have a better understanding of the mechanism of HBV vaccine breakthrough infection; potentially providing new ideas for designing better diagnostic assays and effective vaccines for prevention and treatment of HBV. This review attempts to briefly summarize the status and role of S gene mutations, B-cell epitopes and T-cell epitope mutants, and surveillance of mutant HBV variants in a hospital setting.

Contents

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1. Introduction

Chronic HBV infection continues to be a major public health concern worldwide despite the availability of an effective vaccine and potent antiviral treatments (Beasley, 2009). A global systematic review showed that in 2010, about 248 million individuals in the general population were chronically infected with HBV worldwide. China has been classified as the higher intermediate-endemic country because

HBsAg-positive population of China is 74 million, i.e., 5.49% (5.47–5.50) of the total Chinese population (Schweitzer *et al.*, 2015), which is lower than the epidemiological data of China in 2006 (93 million HBsAg-positive carriers). HBV is mainly transmitted via mother-to-infant and household contact in endemic countries (Edmunds *et al.*, 1996), and through intravenous drug usage and high-risk sexual behavior in more industrialized countries (Mast *et al.*, 2006). However, the patterns of HBV infection in vaccinated population are unknown. HBV, has a unique life cycle including the activity of error-prone reverse transcriptase, high virion production per day and the ease with which it can mutate, resulting in different genetic variants. S gene mutants have important clinical significance in vaccinated population as they may infect vaccinated individuals because anti-HBs antibodies induced by the current vaccine may not recognize changes in HBsAg caused by S gene mutations. Thus, S mutant breakthrough infections may occur in vaccinated people, putting the whole population at risk (Hudu *et al.*, 2015). There are significant differences in the distribution of S gene mutations in various genotypes (Ma *et al.*, 2012). Universal HBV vaccination programs has been implemented for all neonates in China since 1992, effectively preventing the occurrence of chronic liver disease and HCC (Li *et al.*, 2004) Nevertheless, HBV vaccine breakthrough infection and occult hepatitis B infection (OBI) have been found in vaccinated subjects and blood donors, which may be associated with S gene mutants of HBV. Thus, extensive molecular characterization of vaccine-escaped hepatitis B strains needs to be performed.

For this review, we identified studies by searching PubMed for articles published from January 2006 to December 2016 using the keywords "HBV vaccine breakthrough infection", "HBV" and "Vaccine-Escape Mutant". Additional papers were identified by a manual search of references from key articles.

2. Definition of HBV vaccine breakthrough infection

HBV vaccine breakthrough infection is an infection caused by HBV in an individual with validated history of full primary hepatitis B vaccination and who is HBsAg-, anti-hepatitis B core antigen (anti-HBc)- or HBV DNA-positive regardless of the serostatus of anti-HBs. There is no standard definition of HBV vaccine breakthrough infection. In an international meeting organized by the Viral Hepatitis Prevention Board in Milan, Italy, participants expressed the need for further clarification and standardization of the definition of breakthrough infection, since the term covered situations ranging from infection with HBV in a person unresponsive to primary hepatitis B vaccination to infection because of an unknown vaccine failure (FitzSimons *et al.*, 2013). The

prevalence of S gene mutants varies among HBV genotypes. There was a prevalence of 5.5% for hepatitis B-escaped mutants among blood donors and vaccinated undergraduates, with the most common mutation being found at amino acid position 16 (G16L) of S gene (Hudu *et al.*, 2015). The Chinese prevalence is 7.7%; among them 1% were found to be HBsAg-positive and 6.7% were HBsAg-negative and anti-HBc-positive (Zhu *et al.*, 2011).

3. Causes of vaccine breakthrough infection

The main reasons for the hepatitis B vaccine breakthrough infection are not very clear as very little research has been carried out in this area. Studies have shown that this may be associated with the emergence of S gene mutants, high maternal viral load, immunosuppression, and intrauterine infection (Chang, 2010). A ten-year surveillance study of breakthrough HBV infections in vaccinated people in Italy showed that out of seven correctly vaccinated and HBV DNA-positive subjects, four cases were attributable to a lack of immune response and other three to viral mutants able to evade the immune response. The main risk factors of infection include household HBsAg-positive, intravenous drug use, and hemodialysis (FitzSimons *et al.*, 2013). Besides the opinions above, there is also opinion that the HBV vaccine breakthrough infection is mainly caused by the wild type genotype E strain and that immune escape mutants are uncommon (Mendy *et al.*, 2008). However, HBV mutants may play a role in establishing infection later in life when anti-HBs antibody concentration has begun to decline. At the same time, a study in Taiwan showed that most HBV breakthrough infections are due to maternal transmission and immunized children born to genotype C mothers may have a higher rate of breakthrough infections than those born to genotype B mothers (Wen *et al.*, 2011). Another research found that although non-responders to HBV vaccination (anti-HBs <10 mUI/ml in serum) have a suboptimal B cell response, nonetheless it may be enough to protect against clinically significant breakthrough hepatitis B infection (Valats *et al.*, 2010).

4. S gene mutants of HBV

The HBV genome has four open reading frames (ORFs) named X, C, P and S. Surface protein, including T-cell and B-cell antigenic epitopes (Table 1), is coded by S ORF, which is the major HBV antigen that mediates virus attachment and entry and determines virus genotype. HBV mutants may be selected under immune pressure or therapy with antiviral drugs or naturally occur and accumulate during chronic infection. Immune-escape mutants that emerge

Table 1. The epitopes and corresponding mutant sites of HBsAg

HBsAg epitopes	Amino acid position	Mutations
B-cell	100-160 (Gerlich et al., 2006) (exclusion124-147)	P120T/S/Q, T123A/N (Hudu et al., 2015); S114T/F (Ziaee et al., 2016; Mizukoshi et al., 2004); K160R, I110L, S113T (Ziaee et al., 2016); V106A, T118V (Larralde et al., 2013).
	124-147("a" determinant region)	G145A/R (Ma et al., 2012; Lin et al., 2013; Ziaee et al., 2016); D144A/E (Ma et al., 2012; Lin et al., 2013; Ye et al., 2015); N131T (Ma et al., 2012; Lin et al., 2013; Ye et al., 2015); T126S/I, Q129H/R, S143L, M133L (Honorati et al., 1997; Lin et al., 2013); G130N, S140T (Ma et al., 2012).
T-helper	19-28 (Larralde et al., 2013)	F20S, T23A (Larralde et al., 2013).
	80-98 (Bauer et al., 2002)	F85C (Lin et al., 2013).
	186-197 (Mancini-Bourgine et al., 2006)	I194V (Lin et al., 2013); L186P, I195M (Ziaee et al., 2016).
	215-223 (Bauer et al., 2002)	L216*, W223*(Ziaee et al., 2016).
CTL	28-51(Tai et al., 1997)	S45T, V47T, L49P, L42S (Lin et al., 2013); P29S, S34L (Larralde et al., 2013).
	171-179 (Shahmoradi et al., 2012)	S173F(Ziaee et al., 2016); S171F, S174N (Ye et al., 2015).
	175-184 (Mancini-Bourgine et al., 2006)	Q181R (Ye et al., 2015).
	206-215 (Mancini-Bourgine et al., 2006)	S207N (Lin et al., 2013).
TH/CTL Inter-epitopes	–	Y200H/F (Ziaee et al., 2016; Lin et al., 2013); E164G/V (Ziaee et al., 2016; Bauer et al., 2002); P56Q, T57N, N59S, S64C (Lin et al., 2013); R78Q (Ziaee et al., 2016).

under active and/or passive vaccination and are responsible for HBV vaccine breakthrough infection are of particular clinical relevance (Gerlich, 2006).

Mutation in the surface protein is one of the most powerful viral strategies for escaping recognition by both the B- and T-cell-mediated immune responses. As a structural protein, HBsAg serves as an immune target. Mutations in B-cell and T-cell epitopes are likely to significantly alter the immunological characteristics of HBsAg, rendering MHC and MHC class II-restricted T-helper (TH) cells to recognize B-cell and T-cell, restrictively. In addition, these mutations also result in decreased binding affinity by MHC class I-mediated presentation of modified oligopeptides on the cell surface of hepatocytes and enable HBV to escape immune surveillance and immune clearance (Bauer et al., 2002; Mizukoshi et al., 2004).

5. Mutations in surface protein B-cell epitopes

B-cell epitopes are clustered within the major hydrophilic region (MHR; aa 100–160) (Honorati et al., 1997). The region of amino acids 124-149 within MHR, known as the "a" determinant gene, is the major region for mutation. The "a" determinant region of HBsAg is the main target for vaccine development, including antibody production, which can protect against infection by all HBV genotypes (from A to J). Mutations in the surface protein, a result of amino acid deletions or substitutions, may lead to the conformational change

of epitopes affecting the antigenicity and immunogenicity particularly in the "a" determinant gene, resulting in immune escape (Wu et al., 2010). Thus, the mutant virus cannot be identified and removed by the immune system of the host and can infect both vaccinated and unvaccinated individuals. Escape mutations in surface protein B-cell epitopes are reported widely worldwide. Generally, mutations were more likely to be discovered at amino acid positions 120, 123, 126, 129, 130, 133, 134, 137, 139, 140, and 143–145, and a subject generally exhibits multiple mutations. Most of the mutations were located at the "a" determinant region (Ma et al., 2012; Lin et al., 2013; Ye et al., 2015; Ziaee et al., 2016), indicating the region is more likely to be affected by immune selection. G145A appears to be the most common escape mutation induced by anti-HBs immune pressure (Lin et al., 2013; Ma et al., 2012; Ziaee et al., 2016). A study found that eight important mutations associated with diagnostic failure are P120T, T126S, Q129H, G130N, S143L, D144A, and G145A/R; whereas the mutations related to vaccine escape and HBIG therapy failure were located mainly at positions 120, 126, 129, 130, 133, 134, 137,140, 143, 144 and 145 (Ma et al., 2012). Further, there are differences in the position of mutations among vaccinated and unvaccinated subjects. Hsu et al. found that vaccinated subjects had a tendency of higher mutation rates in "a" determinant of HBsAg than unvaccinated subjects (Hsu et al., 2015). Mutations occur more frequently in the second loop (aa124–137) than in the first loop (aa139–149) of the "a" determinant region (Hsu et al., 2004; Michler et al., 2014).

6. Mutations in surface protein T-cell epitopes

Mutations in T-cell epitopes may in theory influence the HBsAb profile through altered interaction between CD4+ TH cells and B-cells (Cooreman *et al.*, 2001). Suitable T-cell response is a prerequisite to producing enough antibodies after HBV infection and vaccination. The humoral response to HBsAg depends on T cells, since four regions within HBsAg, which contain epitopes for MHC class II-restricted CD4+ T-cells have been reported (Larralde *et al.*, 2013; Mancini-Bourgine *et al.*, 2006). Thus, mutations in TH-cell epitopes will affect humoral response and anti-HBs antibody production (Shi *et al.*, 2014). Mutants of HBsAg also have the potential to escape cellular immune response of individuals vaccinated against HBV due to the loss of immune recognition of TH-cell epitopes. Bauer *et al.* (2002) had a study on 30 vaccinated volunteers to test T-cell reactivity toward 23 reported mutants at T-cell epitopes of HBsAg. These mutations mainly occurred in P1 (aa16–33) and P4 (aa213–216), and six of them resulted in complete or significant loss of T-cell reactivity because these mutated epitopes could not activate T cells. In addition, it has been experimentally proved that adaptive immune response mediated by MHC class I-restricted cytotoxic CD8+ lymphocytes (CTLs) is necessary for controlling HBV infection (Zhang *et al.*, 2012). In H-2b mice, even small changes in amino acid residues within two different CTL epitopes (aa208–215, aa190–197) completely eliminated the immunogenicity of each epitope (Schirmbeck *et al.*, 2003). Ye *et al.* (2015) showed that there were three new mutations (S171F, S174N, Q181R) at the position of the MHC class -restricted CTL epitopes of HBsAg in patients with chronic hepatitis B, suggesting that these mutations might contribute to chronic infections. This may be because mutations in CTL epitopes can evade cellular immunity and contribute to persistency (Franzese *et al.*, 2005; Zuckerman AJ, 2000). In summary, a number of mutations upstream and downstream of MHR were found to be located within the known T cell epitopes of HBsAg. These mutations lead to amino acid changes, which may contribute to T-cell non-responsiveness or an inappropriate T-cell response, and are potentially responsible for vaccine breakthrough infection and HBsAg undetectability (Ma *et al.*, 2012; Lin *et al.*, 2013; Ye *et al.*, 2015; Ziaee *et al.*, 2016).

7. S gene mutations in patients with HBV vaccine breakthrough infection

The mechanisms by which mutated virus causes HBV vaccine breakthrough infection include: 1) altered conformational structure and/or T-cell epitope structure allowing mutated virus to evade recognition and clearance by the immune system; 2) altered antigenicity of HBsAg through changes in hydrophobicity profile and putative glycosylation site, which consequently decrease or abolish affinity for anti-HBs, offering lower protection from vaccination (Luongo *et al.*, 2015) and permitting infection with HBV isolates of different genotypes or subtypes in patients with a normal anti-HBs response (Wu *et al.*, 2012); and 3) the accumulation of aa substitutions because of mutations in and around the "a" determinant region may change immunogenicity of HBsAg, producing less effective neutralizing anti-HBs response and enabling the mutated virus evade clearance and survive long terms (Kfoury *et al.*, 2001). S gene mutations of HBV can occur in vaccinated population with fulminant, acute and chronic HB. Similar HBsAg escape mutations in 2nd loop of the "a" determinant (S143L; S143 M) were observed in two virus strains causing fulminant hepatitis, but no such mutations were detected in asymptomatic and chronic hepatitis B patients (T. Michler *et al.*, 2014). Luongo *et al.* also reported a male patient with high seroprotective anti-HBs titer (>200 IU/l) and acute HB with three mutations (M125T, T127P and Q129H) in the virus strain (Kfoury *et al.*, 2001). S gene mutants are the dominant HBV variants in immunized population with fulminant and acute HB (Hsu *et al.*, 1997), because these mutations allow the virus to escape neutralization by antibodies and inefficient CTL-mediated control. HBsAg escape mutations have mostly been described in the context of chronic infections. There was a research that found five of 8 completely vaccinated individuals, who were seropositive for HBV DNA, carried variants with mutations in the S gene. The localization of mutations spread over the entire surface protein, including S114T, T131N, G145A, E164V, R78Q, L173F, L186P, I195M, Y200H, L216*, W223 (Lai *et al.*, 2012). Other researchers have also found some meaningful mutations, such as P120T, T126S, Q129H, S143L/M, D144A, G145A/R and more (Ma *et al.*, 2012; Lin *et al.*, 2013; Ye *et al.*, 2015; Ziaee *et al.*, 2016). More often, there are more than one concurrent mutations of HBsAg and they may have cumulative effects on the conformation of HBsAg (Wu *et al.*, 2010). In recent studies, significant differences were found in the distribution of escape mutants in various genotypes (Ma *et al.*, 2012). Genotypes display a certain geographical distribution and have different clinical significance in diagnosis of mutated virus, vaccine design and immunoglobulin treatment (Chotiyaputta *et al.*, 2009). Therefore, it is meaningful to systematically study the frequency of HBsAg mutations in different geographical areas and different genotypes. Since HBV genotypes A-D are more prone to harbor escape mutants, they should be monitored more closely (Ma *et al.*, 2012).
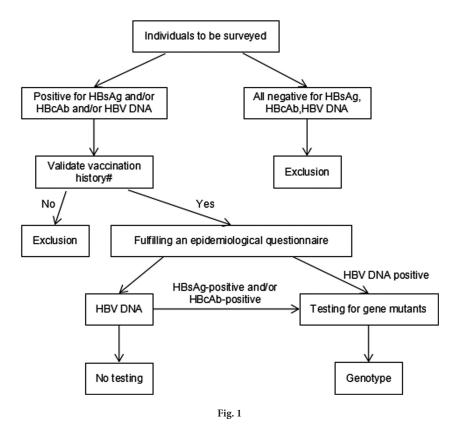
8. Surveillance for S gene mutants of HBV vaccine breakthrough infection patients
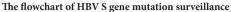
Even though effective vaccine and potent antiviral treatments are available to control HBV infections, there are still

problems hindering full control of HB and to completely clear HBV and HBV escape mutants. In the last 20 years, numerous studies have shown that S gene mutations have impact on the progress of the disease, the reliability of diagnostic method and success of vaccination and antiviral therapy. Furthermore, anti-HBs immune pressure-induced HBV escape mutants in vaccinated people lead to the spread of mutated HBV. Besides the vaccinated population, the surveillance of S gene mutants is also very important to the children whose households have HBsAg carriers, drug-resistant patients, or other high-risk groups, such as blood donors, intravenous drug users, and adolescents with sexual promiscuity. Thus, surveillance of the molecular epidemiology of hepatitis B mutants is very important. We recommend the following roadmap for surveillance of hepatitis B mutants in a hospital setting (Fig. 1). On encountering a subject who has completed the primary vaccination, HBsAg-, anti-HBc- and HBV DNA-positive should be checked no matter his or her anti-HBs status to understand whether breakthrough infection occurs. Then, sequencing of the surface gene as

well as genotyping should be done to clarify whether S gene mutant is present in those, whose HBV DNA is positive.

9. Conclusion

Numerous studies have confirmed that the implementation of universal HBV vaccination programs has significantly reduced HBsAg carriage and infection rates worldwide. Nevertheless, an increased seroprevalence and a high percentage of HBsAg mutants, especially G145R/A and T126S have been found in individuals with HBV vaccine breakthrough infection. Although present vaccines are capable of inducing immunity against the mutants; long-term surveillance and more research on S gene mutants is required for identification of additional mutant variants with significant effect on B and T-cell levels, their frequencies and geographical distribution. The information obtained from these studies would provide new insight into the pathogenesis of HBV vaccine breakthrough infection and occult hepatitis B infection to reduce

Fig. 1

The flowchart of HBV S gene mutation surveillance
First to determine research objects who are seropositive for HBsAg or anti-HBc or HBV DNA, regardless of their serostatus for anti-HBs. And a subject should fulfill an epidemiological questionnaire (including basic information, HB vaccination history, risk factors and so on). We will check the serum for HBV DNA if one has accomplished vaccination. Serum found to be positive for HBV DNA are then processed for sequencing to identify S gene mutation and genotype. #Validate vaccination history: Firstly, there are three separate doses of vaccination following the immunization schedules. Secondly, HBV infection occurred after completing the vaccination.

HBsAg carrier rate and control of HBV. Furthermore, the meaningful mutants may be used for improving diagnostic assays and treatment or designing new vaccines for HBV.

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