**Name of Journal: *World Journal of Gastroenterology***

**Manuscript NO: 39576**

**Manuscript Type: REVIEW**

**Hepatitis B virus infection: defective surface antigen expression and pathogenesis**

Wu CC *et al*. Defective HBsAg expression and pathogenesis

Chun-chen Wu, Ying-shan Chen, Liang Cao, Xin-wen Chen, Meng-ji Lu

**Chun-chen Wu, Ying-shan Chen, Liang Cao,Xin-wen Chen,** State Key Laboratory of Virology, Wuhan Institute of Virology, Chinese Academy of Sciences, Wuhan 430071, Hubei Province, China

**Liang Cao,** Department of Microbiology and Immunology, Feinberg School of Medicine Northwestern University, Chicago, IL 60611, United States

**Meng-ji Lu,** Institute of Virology, University Hospital of Essen, Essen 45122, Germany

**ORCID number:** Chun-chen Wu (0000-0002-6888-213X); Ying-shan Chen (0000-0002-2276-4496); Liang Cao (0000-0001-5974-9815); Xin-wen Chen (0000-0002-4052-8155); Meng-ji Lu (0000-0003-4287-9941).

**Author contributions:** Wu CC contributed to analysis and interpretation of the data and drafting the article; Chen YS and Cao L contributed to revising the article for important intellectual content; Chen XW and Lu MJ contributed to conception and design, analysis and interpretation of data, and drafting and revising the article for important intellectual content.

**Supported by** the National Nature Science Foundation of China, No. 31770180; and the Youth Innovation Promotion Association CAS, No. 2016303.

**Conflict-of-interest statement:** The authors have declared that no potential conflict of interest exists.

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**Manuscript source:** Invited manuscript

**Correspondence to:** **Meng-ji Lu, PhD, Professor,** Institute of Virology, University Hospital Essen, Hufelandstrasse 55, Essen 45122, Germany. mengji.lu@uni-due.de

**Telephone:** +49-201-7233530

**Fax:** +49-201-7235929

**Received:** April 27, 2018

**Peer-review started:** May 4, 2018

**First decision:** May 23, 2018

**Revised:** June 1, 2018

**Accepted:** June 25, 2018

**Article in press:**

**Published online:**

**Abstract**

Hepatitis B virus (HBV) infection is a global public health concern. HBV causes chronic infection in patients and can lead to liver cirrhosis, hepatocellular carcinoma, and other severe liver diseases. Thus, understanding HBV-related pathogenesis is of particular importance for prevention and clinical intervention. HBV surface antigens are indispensable for HBV virion formation and are useful viral markers for diagnosis and clinical assessment. During chronic HBV infection, HBV genomes may acquire and accumulate mutations and deletions, leading to the expression of defective HBV surface antigens. These defective HBV surface antigens have been found to play important roles in the progression of HBV-associated liver diseases. In this review, we focus our discussion on the nature of defective HBV surface antigen mutations and their contribution to the pathogenesis of fulminant hepatitis B. The relationship between defective surface antigens and occult HBV infection are also discussed.

**Key words:** Hepatitis B surface protein; Defective surface antigen mutants; Endoplasmic reticulum stress; Fulminant hepatitis B; Occult hepatitis B virus infection; Pathogenesis

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**C****ore tip:** Defective surface antigen mutation is a type of mutation with great clinical relevance. Many previous publications have explored the association of defective surface antigen mutation with the development of hepatitis B virus (HBV)-associated hepatocellular carcinoma. However, there are no reviews available that elaborate on the relationship between defective surface antigen mutation and HBV-associated fulminant hepatitis (FH), as well as occult hepatitis B virus infection (OBI). This review will focus on these two aspects to discuss the nature of defective HBV surface antigen mutations and their contribution to the pathogenesis of FH. The relationship between defective surface antigens and OBI are also discussed.

Wu CC, Chen YS, Cao L, Chen XW, Lu MJ. Hepatitis B virus infection: defective surface antigen expression and pathogenesis. *World J Gastroenterol* 2018; In press

**INTRODUCTION**

Hepatitis B virus (HBV) is an important human pathogen that has caused chronic infections worldwide[1]. Recent data obtained from a modeling study has shown that the global prevalence of hepatitis B surface antigen (HBsAg) was 3.9% in 2016, corresponding to an estimated 290 million infections worldwide[2]. HBV mainly infects hepatocytes and causes a wide spectrum of clinical manifestations, ranging from an asymptomatic carrier state to acute or chronic hepatitis, with progression to liver cirrhosis, hepatocellular carcinoma (HCC), and other severe liver diseases[3,4]. Currently, interferon-α and nucleotide analogs are used to treat chronic HBV (CHB) infections; however, the outcome is far from satisfactory[5,6]. Prophylaxis using the current HBV vaccines has no impact on existing infections. Therapeutic vaccines of chronic HBV infection are under investigation, but further development is still required[7]. Therefore, understanding the molecular pathogenesis of HBV infection will provide opportunities for the development of better therapies and vaccines.

HBV belongs to the family *Hepadnaviridae* and is a small, enveloped virus with a partially double-stranded DNA genome approximately 3.2 kb in size[8]. During the life cycle of HBV, pre-genomic RNA (pgRNA) is transcribed from covalently closed circular DNA (cccDNA) and serves as the template for HBV DNA replication through a viral polymerase-mediated reverse transcription[9,10]. Because viral polymerase lacks a proof-reading function, the HBV genome evolves with an estimated rate of nucleotide substitutions of 1 × 10-3 to 1 × 10-6 per replication cycle, according to various investigators[11]. Although HBV genome replication involves a step of reverse transcription, which is similar to retroviral replication, the complex HBV genome structure with overlapping open reading frames and regulatory sequences apparently limits the spectrum and rate of mutations[3,12]. Nevertheless, this unique replication strategy leads to the great diversity of HBV genomes, thus resulting in the occurrence of various genotypes, subtypes, mutants, recombinants, and even viral quasi-species in the context of long-term HBV evolution[13,14]. Several reports have suggested that the emergence of HBV variants plays important roles in the progression of HBV-associated liver diseases[11,15-18]. Defective surface antigen mutation is a type of mutation with great clinical relevance[11,15,19]. In this review, we report the current information on HBV surface antigen mutations. Further, we focus our discussion on the contribution of defective surface antigen mutations on the pathogenesis of HBV-associated liver diseases.

**Biology of HBV surface antigen**

Three viral envelope/surface proteins — large surface antigens (LHBs), middle surface antigens (MHBs), and small surface antigens (SHBs)—are expressed from a single open reading frame (S-ORFs)[20,21], but they are translated from two different mRNAs. LHBs are encoded by the 2.4 kb subgenomic RNA, and MHBs and SHBs are encoded by the 2.1 kb subgenomic RNA[3]. Subgenomic RNAs of 2.4 kb and 2.1 kb are driven by preS1 and preS2/S promoters, respectively, allowing variable regulation of protein expression[3]. The preS1 promoter is situated within the upstream region of the S-ORF, whereas the preS2 promoter corresponds to the preS1 domain[21]. Therefore, the transcription of the 2.1 kb subgenomic RNA is also regulated by the preS1 domain[11] (Figure 1).

The three surface proteins share the same carboxy-terminal region and only differ in length due to their amino-terminal regions. As a result, the LHBs contain the preS1+preS2+S (389 or 400 amino acid [aa] residues), MHBs contain the preS2+S (281 aa residues), and SHBs contain the S domain (226 aa residues) alone[3,20,22] (Figure 1). Additionally, a truncated and mutated preS2/S (the LHBs and truncated MHBs) can be produced by integrated viral sequences that are defective for replication[23,24]. LHBs, MHBs, and SHBs are important for HBV structure and life cycle. Besides mediating HBV entry through binding to HBV receptors, the sodium taurocholate co-transporting polypeptide (NTCP) on hepatocytes, via the preS1 2–48 aa domain (numbering for HBV-genotype D) and subsequent infection, LHBs are indispensable for the formation and budding of virions[3,25-29]. It has been proposed that LHBs rearrange their structure during the maturation of HBV virions and thereby regulate the release and infectivity of virions[30-32]. The exact role of MHBs in the HBV life cycle remains an enigma. Early reports indicated that MHBs might be dispensable for HBV replication and virion formation; however, our data and those of other groups have shown that MHBs play a role in virion secretion[33-36]. Recently, MHBs were found to interact with ceruloplasmin and influence the production of extracellular virions[34]. As the predominant component of viral particles, including infectious virions and noninfectious subviral particles, SHBs are necessary for the production of virions and subviral particles[35]. For mature/infectious virions, LHBs, MHBs, and SHBs are present in the envelopes at a ratio of approximately 1:1:4[20]. Disturbance of this proportion impairs the production and release of virions[33]. For subviral particles, their amount outnumbers virions by 10000- to 1000000-fold, and the particles are detected serologically as HBsAg[11,37]. The secretion of subviral particles can also be suppressed by LHBs in a dose-dependent manner[38-41], thus promoting the S protein toward virion formation.

In addition, preS1, preS2, and S domains contain various B- and T-cell epitopes, which play an important role in inducing the host immune response[42,43]. The major hydrophilic region (MHR) between aa 100–169 of SHBs, especially the a-determinant located at aa 124–147, serves as the most important antigenic determinant in HBV surface proteins and is essential for HBsAg detection and HBV vaccine development[44,45]. Plasma-derived and recombinant HBsAg have been used for vaccine preparations and have induced strong specific and protective antibody responses in vaccines[46-48]. The presence of anti-HBs antibodies is considered to confer immunity against HBV infection. In contrast, a high quantity of circulating HBsAg in chronically HBV-infected patients is proposed as a factor leading to immune disturbance. Defective peripheral HBsAg-specific T cell responses in chronically infected patients were found to be correlated with serum HBsAg titers[49,50], suggesting that HBsAg overproduction influences the host’s immune system in a way that is advantageous for the virus. *In vitro*, HBsAg can interfere with Toll-like receptor functions and trigger interleukin (IL)-10 production in Kuppfer cells[51-54]. Recently, published data has suggested that HBsAg may facilitate the induction of myeloid-derived suppressor cells in chronically HBV-infected patients[55]. HBsAg is also associated with the induction of regulatory T cells, as shown in HBV mouse models[56,57]. Thus, HBsAg is not only a structural component of virions and subviral particles, but it also serves as an important immune modulator.

**Defective surface antigen mutation and HBV biology**

HBsAg mutants were first identified in individuals vaccinated against HBV but who were infected despite the presence of protective anti-HBs antibodies[58]. Those “immune escape” mutants with aa substitutions within a-determinants were found in different clinical settings, including vaccines, transplant patients receiving hyperimmunoglobulins, and immunocompromised patients with HBV reactivation[59-61]. Such mutant HBsAg commonly showed reduced binding to anti-HBs antibodies and decreased reactivity in established HBsAg detection assays[59-65]. The most widely known mutation is the sG145R mutation, which has been shown to be replication competent, may persist stably over time, and may be transmitted vertically or horizontally[66-69]. The sG145R mutation induces a strongly impaired anti-HBs antibody response, which could not efficiently clear HBsAg in an HBV hydrodynamic injection mouse model[70]. A similar result was also observed for another immune escape mutation, sK122I, indicating that such a defective surface antigen mutation may impair HBsAg neutralization and clearance during HBV infection. In addition, sG145R, sK122I, and other immune escape mutants occurring in the a-determinant of SHBs, such as the sT123N mutation, could affect HBsAg secretion[70-73].

Recently, chronically HBV-infected patients routinely received antiviral therapy based on nucleotide analogs[74]. Treatment with first-generation drugs, such as famciclovir and adeforvir, resulted in the emergence of drug-resistant HBV mutants, with aa substitutions within the HBV polymerase domain[75]. Some drug-resistant mutations occurring in the viral polymerase may lead to a stop codon mutation in the overlapping surface gene, cause intracellular retention of surface proteins, and result in secretion defects of viral particles, such as rtA181T/sW172\*, rtM204I/sW196\*, and rtV191I/sW182\*, as shown in previous reports[76-78] and in our unpublished data. The primary sW182\* mutation has also been identified in CHB patients. It was found to induce retention of the truncated S protein in the perinuclear endoplasmic reticulum (ER) and was associated with lower HBV transcript levels owing to decreased stability, but without impact on HBV replication[79].

Defective surface antigen mutations have been frequently detected in chronic HBV infection[16,71-73,80,81], in which deletions in the preS domains are the most common mutations[80,81]. Deletions in the preS domains are often clustered at the 3’ end of preS1 and the 5’ end of preS2[11,19,81-83]. Given that the preS2/S promoter is situated within the preS1 domain[11], deletions at the 3’ end of the preS1 may reduce MHBs and SHBs expression at the transcriptional level. Deletions at the 5’ end of the preS2 may remove the N-terminal preS2 domain, including the start codon of preS2 in the MHBS protein, leading to an impaired or a complete loss of MHBs expression[84]. These changes may disrupt the proper LHBs, MHBs, and SHBs ratio in the envelopes of virions. In addition, the junction between the preS1 and preS2 domain is required for virion formation[32]. For these reasons, preS deletions may potentially affect virion assembly, stability, or infectivity.

A large amount of evidence has demonstrated that DHBV envelope proteins can regulate cccDNA formation and amplification[85,86]. Infection of envelope protein-deficient recombinant DHBV results in more cccDNA accumulation[85,87,88]. Similarly, deficiencies in HBV envelope proteins can modestly increase the cccDNA level and result in a dramatic accumulation of deproteinized rcDNA[89-91]. It has been demonstrated that preS/S mutants with surface antigen secretion deficiency isolated from patients can lead to an increased accumulation of cccDNA molecules in the nuclei[79]. Therefore, defective surface antigen mutation may affect cccDNA synthesis and amplification.

**Defective surface antigen mutations and the host**

Defective surface antigen mutations have been found in acute hepatitis B infection, chronic hepatitis B infection, and occult HBV infection and are associated with advanced liver disease, including liver cirrhosis, fulminant hepatitis B, and HCC[15,82,92-105]. It has been questioned whether HBV mutants arise due to viral adaptation to inflammation and decreased liver function or, alternatively, causally contribute to liver pathogenesis. The mechanism of defective surface antigen mutations contribution to HCC development has been widely elucidated[11,23,41,106-111]. Here, we will emphasize in our discussion the relationship between defective surface antigen mutations and fulminant hepatitis B, as well as occult HBV infection.

***Defective surface antigen expression and fulminant hepatitis***

There is increasing evidence that defective surface antigen expression may play a role in the pathogenesis of fulminant hepatitis (FH). preS deletions, particularly those unable to synthesize the MHBs protein, have reported associations with cases of fulminant hepatitis (FH)[16,71,112,113], suggesting the potential pathogenic role of preS deletions. A mutation in the CAAT element of the S promoter has been found in the HBV genome isolated from a FH patient. This mutation led to excessive LHBs expression over MHBs and SHBs proteins and resulted in virus retention and misassembly[114-116]. Obviously, the accumulation of LHBs may be due to hepatocyte injury, as shown in transgenic mice with LHBs expression[41]. One of our previous studies also identified deletions within the preS regions from HBV strains isolated from a patient with HBV-associated FH[84]. In addition, a hepatitis B immune globulin (HBIG)-escape mutant sG145R on the HBsAg, causing 30% inhibition of virion secretion, has been identified from a study on FH strains, suggesting the potential role of defective surface antigen expression in the fulminant clinical course of HBV infection[71].

Mechanistically, defective surface antigen expression, such as specific mutations in the preS/S gene, may lead to secretion defects of viral proteins and particles, resulting in an accumulation of viral products in the ER of hepatocytes and causing ER stress and hepatocyte injury[16]. Subsequently, autophagy may be triggered[117-125] and thus enhance HBV replication[126,127]. Consistent with this speculation, it has been demonstrated that defective surface antigen expression may increase the replication capability of HBV, albeit the mechanism is still undefined[71,84]. In addition, the deficiency of hepadnavirus envelope proteins can result in accumulation of cccDNA[85,87,88] or deproteinized rcDNA[89-91] and may ultimately cause death of the infected hepatocytes by a direct cytopathic effect[85,87,88]. Meanwhile, the increase of the cccDNA level may facilitate HBV replication. Both the defect in viral particle secretion and enhanced replication competence may contribute to the severity of fulminant hepatitis[128].

The adaptive immune response, particularly the cytotoxic T lymphocyte (CTL) response, plays a crucial role in viral clearance and disease pathogenesis of HBV infection[129-131]. Intracellular retention of HBV surface proteins was found to be associated with FH in a transgenic mouse model showing panlobular necrosis and hepatic failure by inducing the indirect cytotoxic activity of CTLs[132]. In this setting, intracellular accumulation of viral products due to defective surface antigen expression mutations may cause liver damage through abnormal activation of the CTL response. Consistently, we also observed significantly stronger intrahepatic CTL responses and antibody responses specific to secretion-deficient HBsAg due to preS deletions[84]. A preS deletion mutant was found in a patient with acute exacerbation of liver diseases, along with wild-type HBV genomes. The co-existence of deletion mutants and wild-type HBV apparently allows the complementation and enhancement of HBV genome replication in hepatocytes. In an HBV mouse model, co-replication of a deletion mutant and wild-type HBV induced higher cellular and humoral immunity. Our findings further suggested the proposed role of HBV variants in the immunopathogenesis of HBV infection. Moreover, the mutations associated with defective surface antigen expression, such as deletion or missense mutation of the PreS2 ATG codon, can cause deletions or alterations of B- and T-cell epitopes located in preS1 and preS2 proteins. Considering that M protein-specific T- and B-cell immunities are important early events in the host immune response to HBV infection[43], these mutations may lead to an immune evasion and thus likely favor a more severe clinical course of infection[14,133]. In chronic HBV infection, high HBV replication levels were found to be associated with lower cellular immune responses to HBV; however, massive infiltration of unspecific immune cells occurred within the liver, accompanied by severe liver damage[134-136]. Thus, the presence of these mutations, including aa substitutions at the immunodominant epitopes for B or T cell recognition, may contribute to the spread of highly replicative escape mutants. It may also facilitate the development of fulminant hepatitis in chronically HBV-infected patients and heavily immunocompromised patients, like those with human immunodeficiency virus (HIV) co-infection[137] (Figure 2).

***Defective surface antigen expression and occult hepatitis B virus infection***

Occult HBV infection (OBI) is characterized by the presence of very low levels of HBV DNA in the plasma and/or liver of individuals negative for HBV surface antigen (HBsAg) and positive/negative for antibodies to the hepatitis core antigen (anti-HBc)[45,138,139]. OBI harbors the potential risk of HBV transmission through blood transfusion, organ transplantation, and hemodialysis as well as from occult infection or HBsAg-positive mothers to newborns[45]. The persistence of OBI may lead to the development of cirrhosis and HCC[45,140-145]. The reactivation of OBI can occur in patients following chemotherapy, immunosuppressive therapy, and after transplantation as well as in patients co-infected with HIV or hepatitis C virus (HCV)[45,146,147], which can result in the development of fulminant hepatitis and death[139,148-153].

Defective surface antigen expression mutations may be associated with OBI. Point mutations and deletions as well as insertion mutations are commonly encountered in OBI, in which mutations in the Pre-S/S gene are the most extensively studied[45]. High frequencies of MHR mutations, including those mutations within and outside of the a-determinant, have been observed in OBI strains of individuals[154-158]. *In vitro* and *in vivo* experiments have demonstrated that these MHR mutations can significantly decrease the detection sensitivity of commercial HBsAg immunoassays and impair virion and/or S protein secretion[156]. PreS/S mutations with deletions covering the preS1 and preS2/S promoters, PreS1 region, and PreS2 region have been frequently reported in OBI, which can alter the transcription of 2.4 kb and 2.1 kb HBV RNAs, expression of three envelope proteins, and the ratio of LHBs/MHBs/SHBs proteins[45]. PreS/S insertions, such as 2–8 aa insertions between codons 121 and 124 located upstream of the a-determinant, have also been observed in OBI patients[159].

On one hand, these mutations associated with defective surface antigen expression can directly decrease the levels of surface antigens. On the other hand, these mutations can cause the retention and accumulation of HBsAg within cells and impair the secretion of HBsAg by altering the ratio of LHBs/MHBs/SHBs proteins[72,73,160,161]. Therefore, circulating HBsAg levels are low in the peripheral blood. Moreover, it is well documented that neutralizing antibodies produced during natural infection, or following active or passive immunization against HBV, are targeted to the conformational epitopes of the a-determinant[162]. Hence, single or multiple mutations occurring within this region can lead to conformational changes with impaired antigenicity[72,160]. A recent report has identified novel SHBs mutations outside the MHR from untreated CHB patients. These mutations impaired virion secretion and caused lower binding affinity to antibodies used for HBsAg immunoassays[163]. For these reasons, the mutations can render HBsAg undetectable or poorly detected by immunoassays based on monoclonal antibodies against wild-type virus[60,62,65,164], contributing to some cases of OBI[165-170] (Figure 3).

**Perspectives**

Defective surface antigen expression has been well documented to be relevant for the progression of HBV-associated liver diseases, such as HCC. However, the role of defective surface antigen expression in FH still needs to be clarified in future research, particularly, using *in vivo* models and in patients. The exact molecular mechanisms of how defective HBV surface antigens cause damage to hepatocytes and induce liver injury and subsequent pathogenic processes should be investigated. A deep understanding of the molecular mechanisms of HBV pathogenesis related to defective surface antigens is crucial to designing future therapeutic approaches. A critical question would be whether currently used nucleotide analogues (NAs) and inferno-based therapies can prevent such pathogenic processes. NAs are able to efficiently inhibit HBV DNA synthesis but not gene expression. Thus, HBV proteins, including surface antigens, are continuously produced under NA therapies. Another problem is the production of mutated HBV proteins from integrated HBV DNA, which are not controlled by NA therapies at all. Thus, specific interventions may be required to block the pathogenic potential of HBV proteins, besides efficient inhibition of HBV DNA synthesis. RNA silencing may be a suitable choice to achieve this goal[5,6,171,172].

An additional issue to be addressed is whether the defective surface antigen-related mutations may represent novel biomarkers of OBI. With improvement of HBV antigen and DNA detection assays, OBI will likely be easier to diagnose in the future. However, the question remains whether OBI may be related to significant HBV pathogenesis and require therapeutic interventions, such as prophylaxis and antiviral therapy, to prevent HBV reactivation[173].

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**P-Reviewer:** Farshadpour F, Zhao HT **S-Editor:** Gong ZM

**L-Editor:** **E-Editor:**

**Specialty type:** Gastroenterology and hepatology

**Country of origin:** China

**Peer-review report classification**

Grade A (Excellent): 0

Grade B (Very good): B

Grade C (Good): C

Grade D (Fair): 0

Grade E (Poor): 0

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**Figure 1 The transcription and expression of hepatitis B virus surface proteins.** The three HBV surface proteins, LHBs, MHBs, and SHBs, are translated from two different mRNAs: LHBs are encoded by the preS1 promoter-initiated 2.4 kb subgenomic RNA; MHBs and SHBs are encoded by the preS2 promoter-initiated 2.1 kb subgenomic RNA. The 2.4 and 2.1 kb subgenomic RNAs share the same 3’ end and only differ in length due to differences at the 5’ end, which lead to different amino-terminal but identical carboxy-terminal regions of the three surface antigens. Therefore, LHBs contain preS1+preS2+S (389 or 400 aa residues), MHBs contain preS2+S (281 aa residues), and SHBs contain the S domain (226 aa residues) alone. For mature/infectious virions, LHBs, MHBs, and SHBs are present in the envelopes at a ratio of approximately 1:1:4. In addition, the major fraction of SHBs forms subviral particles (filaments and spheres) together with the minor parts of LHBs and/or MHBs. HBV: hepatitis B virus; LHBs: large surface antigens; MHBs: middle surface antigens; SHBs: small surface antigens.



**Figure 2 The proposed pathogenic role of mutated secretion-defective hepatitis B surface antigen in fulminant hepatitis.** Defective surface antigens, such as preS deletions and mutations within the “a” determinant, may lead to secretion deficiency of HBsAg. Defective HBsAg can covalently promote closed circular DNA (cccDNA) synthesis and amplification, thus facilitating HBV replication. The intracellular accumulation of defective HBsAg can cause endoplasmic reticulum (ER) stress, subsequently trigger autophagy, and may further enhance HBV replication. The enhanced HBV replication, in turn, leads to accumulation of more defective HBsAg, possibly resulting in a positive feedback with unfavorable outcomes and hepatocyte damages. Inflammation may occur in the liver by recruiting immune cells. Cytotoxic T lymphocyte (CTL) response may be abnormally activated and damage infected hepatocytes, contributing to the progression of fulminant hepatitis. HBsAg: hepatitis B surface antigen.

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**Figure 3 The relationship between the expression of defective surface antigens and occult hepatitis B virus infection.** Surface antigen mutations, such as preS deletions, can impair the transcription of 2.4 and 2.1 kb HBV RNAs, leading to decreased levels of three HBV surface proteins. In addition, defective surface antigens with preS deletions and mutations within the “a” determinant are secretion deficient. Single or multiple mutations occurring within MHR between the aa residues 99–169 of SHBs, especially those within the “a” determinant between aa 124–147, can lead to conformational changes of HBsAg. Mutated HBsAg is poorly detected by immunoassays based on monoclonal antibodies, contributing to some cases of OBI. OBI: occult hepatitis B virus infection; HBsAg: hepatitis B surface antigen.