Hepatitis B virus S gene escape mutants

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Abstract

Hepatitis B virus (HBV) can be classified into nine immunological subtypes or eight genotypes. The most prevalent genotypes in Asia are genotypes B and C. HBV is transmitted parenterally and can produce either asymptomatic or symptomatic disease. Although the consequences of acute hepatitis B can be severe, serious sequelae are associated with chronic infections. HBV seroprevalence ranges from intermediate (2%-7%) to high (≥8%) levels in Asia. Several strategies for the control and prevention of HBV infection have been found to be efficacious. They include vaccination and the administration of HBIG, interferon-a and nucleoside/nucleotide analogues. However, these procedures also apply selective pressures on HBV in infected individuals leading to the generation and accumulation of mutations in the S gene. Most of these mutations occur in the major hydrophilic region (MHR) of the S gene. These mutations create public health concerns as they can be responsible for reactivation of hepatitis B and occult hepatitis B infection. The inability to detect occult infections means that these individuals may become blood donors. This suggests that new strategies for donor evaluation and selection may need to be developed to protect the blood supply.

Keywords: Escape mutants, genotype, hepatitis B virus, review, subtype, surface antigen

Introduction

Hepatitis B virus (HBV) is a member of the Hepadnaviridae family. HBV is transmitted parenterally and can produce either asymptomatic or symptomatic disease.[1,2] Although the consequences of acute hepatitis B can be severe, the majority of serious sequelae are associated with chronic infections. Worldwide, an estimated 360 million people are chronically infected with HBV.[3] Chronic infection occurs in about 90% of those infected perinatally, 30% in those infected in early childhood under five years of age and 6% when individuals are infected over five years of age.[4–7] Based on data from follow-up studies of individuals infected as infants or young children, about 25% of individuals with chronic hepatitis B die from cirrhosis or liver cancer; the majority remain asymptomatic for decades until the onset of cirrhosis or end-stage liver disease.[8–10] Chronically infected individuals are at increased lifetime risk for cirrhosis and hepatocellular carcinoma and serve as reservoirs for continued HBV transmission.

Estimates of chronic HBV infection are inferred from population sampling for the presence of hepatitis B surface antigen in the population. In Asia HBsAg seroprevalence ranges from intermediate (2%-7%) to high (≥8%) levels. In areas such as southern China, Korea, Melanesia, Micronesia, the Philippines and...
Hepatitis B virus S gene escape mutants have been shown to changes in HBV subtypes; however, this is not the case for genotypes. Because of ease of use, genotyping has become more widely accepted as the method for relating phenotype to genetics than serological subtyping. Also, single nucleotide mutations within the HBsAg revealed the importance of Lys122 for the expression of the α determinant.

HBV Subtypes

Blumberg et al. first described the Australian antigen, a, in 1965. Further research revealed the immunological heterogeneity of the Australian antigen. Two pairs of allelic variations, d/y and w/r, were discovered in 1971 and 1972, respectively. The discovery of additional determinants with the appearance of multiple naming conventions occurred at a rapid pace and an international synod was convened in Paris in 1975 to discuss a determinant heterogeneity and reach consensus. At this workshop, four sub-determinants of the a determinant were redefined as w sub-determinants (w1-w4) and the plethora of HBsAg determinants were found to be collapsible into eight subtypes; ayw1, ayw2, ayw3, ayw4, ayr, adw2, adw4 and adr. Following the discovery of the q determinant in 1975, it was determined that adr could be subdivided into q+ and q- categories. The development of monoclonal radioimmunoassays confirmed the existence of these nine subtypes. In 2002, Arauz-Ruiz et al. described a tenth subtype, adw3.

The development of DNA sequencing methodologies facilitated the elucidation of the specific amino acids in HBsAg responsible for the reactivity patterns with monoclonal antibodies. Chemical modification of HBsAg revealed the importance of Lys122 for the expression of the d determinant. Studies of two blood donors carrying compound subtypes, adyr and adwr, showed that only amino acid positions 122 and 160 are uniquely responsible for the expression of d/y and w/r specificity. These studies found that the d-to-y and w-to-r changes are mediated through a shift from Lys to Arg at positions 122 and 160, respectively. Site-directed mutagenesis later confirmed the specificity of Lys160 for w reactivity and delineated which amino acids at position 127 were responsible for w2-w4 reactivities. Norder et al. found that Phe134, Ala159 or both, are involved in the expression of w1 reactivity and recent research suggests position 140 may be more important in resolving w1 reactivity than position 134. Additional evidence suggested position 177 is involved in q reactivity of adr specimens and position 178 is involved in q reactivity of adw4 specimens. [Figure 1]

HBV Genotypes

Originally, genotypes were designated as sequences within a phylogenetic clade having no more than 4% sequence divergence between the members of the clade and more that 8% sequence divergence with extra-clade sequences. To date, eight genotypes, designated, A to H, have been described. Because of ease of use, genotyping has become more widely accepted as the method for relating phenotype to genetics than serological subtyping. Also, single nucleotide mutations within the a determinant may lead to changes in HBV subtypes; however, this is not the case for genotypes. Genotypes B and C are...
the most prevalent genotypes found in Asia; however, genotypes A and D are the most prevalent genotypes found in India.[11,12]

**HBV Subtypes and Subgenotypes**

DNA sequencing has led to the elucidation of associations between serologic subtypes and genotypes. Early research indicated an apparent association between genotype/subtype and geographic location.[45] Genotype A (subtypes adw2 and ayw1) is most prevalent in North America and northwestern Europe. Genotypes B (subtypes adw2, adw3 and ayw1) and C (subtypes adw2, adw3, adr and ayr) are highly prevalent in East Asia. Genotype D (subtypes adw3, ayw2, ayw3 and ayw4) is most prevalent in the Mediterranean and the Middle East. Genotype E (subtype ayw4) has been found in West Africa. Genotypes F (subtypes adw4 and ayw4) and H (subtype adw4) are found in Central and South America. Genotype G (subtype adw2) is found in the United States and Europe.[46,47] Indeed, a study conducted in Sweden concluded that the genotypes of HBV seen in patients at an outpatient clinic correlated more with the country of the patients' nativity than their current residency in Sweden.[48]

Genotypic sequences, which diverge by less than 4% but form distinct sub-clades within a genotypic clade in a phylogenetic tree, have been designated subgenotypes. Genotype A was found to segregate into two well-defined subgenotypes, Ae in Europe and Aa in Africa and Asia,[49] with a possible third subgenotype found in Cameroon.[50] Genotypes B and C each appear to have about four or five subgenotypes. [46,51–53] One of the B subgenotypes is due to recombination between genotypes B and C.[54] Genotype D segregates into four subgenotypes and genotype F forms two distinct subgenotypes. However, genotypes E, G and H do not appear to have subgenotypes.[46]

There was an indication that subgenotypes might correlate with serological subtypes as was seen with the Ae-adw2 association in northwest Europe and the Aa-ayw1 association in Central Africa.[45] Further research demonstrated this was not always the case.[46,48] Norder et al.[46] found that some subgenotypes were composed of sequences sharing a common serological subtype, while other subgenotypes were composed of sequences from multiple subtypes.

**Escape Mutants**

As stated above, vaccination with HBsAg has been efficacious in the pre-exposure setting; however, occult infections began to be noted in the late 1980's[55,56] and the first case of a vaccine-induced escape mutant, in a child in southern Italy, who received passive-active post-exposure immunization, was described in 1990.[57] Mutations within the S gene are known to be responsible for occult hepatitis B infections, reactivation of hepatitis B,[58,59] diagnostic assay failure[58,60–63] and reinfection in HBV-infected recipients of orthotopic liver transplantations.[17,64] Occult infections create public health concerns because asymptomatic carriers can be blood donors.[65–67] These mutations are stable and can be transmitted horizontally and vertically.[56,68–71]

HBV replicates to high titers in infected individuals. Because it replicates through an RNA intermediate synthesized by reverse transcriptase, mutant viral genomes[59,72] and quasi-species[71–77] are generated. This results in the production of viral mutants during naturally occurring infections.[78,79] Vaccination and the administration of HBIG and anti-viral drugs like luvamidine exert evolutionary pressures to select mutants.[70,80]

The a determinant is located on the major hydrophilic region (MHR) of the S gene, which is between amino acids 120 and 160. The MHR forms a two-loop structure. There are two alternative models for this double loop structure, which involve two pairs of cysteines forming disulfide bridges at the ends of each loop. The more generally accepted model envisions disulfide bridging between C124 and C137 and between C139 and C147.[58] The alternative model is built on disulfide bridging between C107 and C138.
and between C139 and C147.

Research with childhood vaccinations shows that mutations accumulate with higher frequency in vaccinated than unvaccinated children, with more mutations emerging in children vaccinated with plasma-derived vaccine than recombinant vaccine. Vaccinated children generated a preferential accumulation of mutations in the second loop of the MHR, while unvaccinated children generated random mutations. The prevalence of mutations increases over time and the frequency of amino acid variation per site increases with age. There is also an accumulation of S gene mutations in HBV related end-stage liver disease.

Mutations accumulate across the MHR. These mutations can disrupt the antigenicity of HBsAg in a number of ways. One way is by modifying amino acids directly involved in expression of the antigen, such as the amino acid specified by codon 122, which is responsible for the expression of d/y specificity. Alterations to the structure of HBsAg can also disrupt binding of polyclonal antibodies to it. These alterations include mutations to the cysteines involved in the formation of the disulfide bridges responsible for the two-loop structure of the MHR and modifications to protein hydrophilicity, electric charge and acidity. Some mutations involve amino acid insertions into the a determinant or the creation of stop codons. Other mutations eliminate the glycosylation site at residue 146 or create potential new glycosylation sites in the first loop of the MHR. However, not all mutations in the MHR lead to escape mutants.

While most research into HBsAg escape mutants has been directed specifically at the MHR, additional research has shown that mutations outside the MHR can also lead to the creation of escape mutants. Mutations near the MHR can alter group-specific antigenicity even though the mutations are not within the MHR. Monoclonal antibody binding assays have also shown that residues 178-186 are exposed on the surface of the 20 nm particle. This is the region which specifies the q sub-determinant.

Administration of nucleoside and nucleotide analogs, like lamivudine, which inhibit viral polymerase activity, can lead to the generation of mutants in the polymerase gene. Because the polymerase and S genes overlap any change in the polymerase has the potential for causing mutations in the S gene. Although many of these polymerase mutants are in the C domain near the YMDD motif, additional mutations are generated in the B Domain, which overlaps the a determinant. Indeed, lamivudine therapy is associated with the generation of escape mutants. Although polymerase mutants exhibit diminished replication activity, some polymerase/a determinant double mutants exhibit increased replication activity in the presence of lamivudine. It has also been found that recombinant HBV vaccine provided limited efficacy when given to post-liver transplant patients who have received prior lamivudine treatment for pre-existing chronic hepatitis B.

Besides escape mutants, nucleotide and nucleoside analogs can generate stop codons within the S gene.

Ultimately it would be beneficial to be able to correlate escape mutant phenotypes with a specific genetic sequence. Unfortunately, the multitude of mutations does not lend itself to easy categorization. While studies using synthetic peptides and analysis of point mutations allow examinations of individual positions, they do not help in investigating the presence of conformational epitopes. To further confound any analysis of escape mutants, multiple mutations within the MHR lead to generate revertants masking the effects of escape mutants. Nonetheless, it is possible to make some generalizations about HBsAg mutants. Patients with first loop mutants tend to have higher levels of HBV DNA than patients with second loop mutants. G145R, the prototypic HBsAg mutant, is the most stable mutant. In patients with the G145R mutant, a gradual decline in the HBV viral load has been observed. Patients with hepatocellular carcinoma or chronic hepatitis can have a higher frequency of mutations in the class I HLA-A2-restricted CTL epitope between residues 29 to 53 than the a determinant. The G145R and M133T mutations have also been implicated in the development of hepatocellular carcinoma.
As stated earlier, there is an accumulation of S gene mutations in HBV related end-stage liver disease.\[84]\n
The Future

HBV escape mutants cause a public health concern through reinfection and occult infection. This is especially true in Asia with its intermediate to high rates of chronic infection.\[12,14]\n
The health problems associated with HBV infection are well-documented with the majority of serious sequelae being associated with chronic infections. These consequences are amplified in infected infants and young children.\[8–10]\n
Depending on how blood donors are selected and screened the intermediate to high levels of circulating HBV in Asia increase the probability of contaminated blood entering the blood supply. Although prevention and control strategies have proven to be efficacious, escape mutants are generated in response to these immune pressures.\[75,79,106]\n
The large population of infected individuals also creates an environment in which evolutionary processes can lead to the creation of new HBV isolates able to escape detection and enter the blood supply.\[33,65,66]\n
Evaluation of donor selection and screening procedures may yield insights into strategies that could potentially safeguard the blood supply. To decrease the levels of post-transfusion hepatitis the feasibility and efficacy of using nucleic acid amplification testing, DNA dot blot hybridisation assays or multiple immunoassays may need to be examined.

A debate has begun about whether or not the development of vaccines, which protect against mutant strains of HBV, need to be used in conjunction with the wild-type vaccine.\[98,107]\n
No matter how this debate is resolved in the future, research has already shown that mutant strain vaccines can be protective against mutant strains.\[32,107]\n
If it is decided that mutant strain vaccines would have public health potential, a big part of this discussion will be about which mutants need to be targeted. As far as diagnostics is concerned, there is a concerted effort to understand how mutants affect a determinant antigenicity in order that assays, which can detect mutant strains, particularly in blood donors and liver transplant recipients, can be developed. However, the necessity for these assays has been questioned because while one diagnostic assay may fail to detect a particular mutant HBsAg, another assay might detect it, albeit at reduced reactivity.\[93,110]\n
A better understanding of the exact structural and physiochemical changes caused by mutations within the S gene is needed to determine which mutants modulate S gene antigenicity. At present, the best visualization of HBsAg structure is obtained from a 12.5Å resolution cryo-EM reconstruction of a 22-nm noninfectious viral particle.\[109]\n
With a higher resolution structure it would be possible to map precisely the residues involved in specifying the a determinant and determine their spatial relationship.\[111]\n
This would further allow investigation into the effect of single residue changes in the S gene with respect to changes in HBsAg protein folding and the $a$ determinant structure.\[109,110,153]\n
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**Figures and Tables**
Figure 1

Algorithms for determining HBV subtype from the primary structure of the HBV S gene. A. The decision tree for the ad subtypes. B. The original decision tree for determining the ay subtypes. C. A newly developed decision tree for Hepatitis B virus S gene escape mutants.
determining the ay subtypes.[39] Each box in each decision tree contains a number representing an amino acid position in the S gene. The amino acids listed below each number represent the permitted wild type residues at that position. The downward pointing triangles denote paths through each decision tree. The final designation at the end of each possible path is the subtype for that path.
Mutations in the HBsAg α determinant. The numbers above the line represent amino acid positions in HBsAg. The bold i's between the numbers 120 and 125 represent known insertion points in the surface antigen. Above the numbers is the wild type sequence for HBsAg, with multiple letters in a column denoting alternative amino acids seen in various subtypes. Emboldened positions denote putative residues that participate in the α determinant. The lower case n at position 146 is the site of N-linked glycosylation. Boxed positions show the positions at which mutations are most frequently seen. Below the line the columns of letters represent the mutant residues seen at each position. Asterisks denote stop codons. Numbers below the bold i's denote the oligopeptides inserted at these two positions. These oligopeptides are listed in the box at the bottom. The data for this figure come from several sources.\[85,86,89,91,93–95,98,108,110–153\]

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