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Mutations of the surface protein of hepatitis B virus

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Abstract

Neutralizing antibodies induced by immunization against hepatitis B infection are targeted to the conformational epitopes of the common *a* determinant of the surface antigen. However, amino acid substitutions within this region of the surface protein of the virus, particularly in the region of amino acid 137–147 allow replication of hepatitis B virus in vaccinated subjects, since antibodies induced by current vaccines do not recognize crucial changes in the surface antigen domain. The G145R mutant is replication competent and is stable, and it appears to be the most common variant. There is evidence that these mutants may not be detected by current screening tests and diagnostic reagents. Epidemiological monitoring of hepatitis B virus surface mutants is essential.

Keywords: Hepatitis B virus; Surface antigen mutants; Vaccines

1. Introduction

More than a third of the world's population has been infected with hepatitis B virus and it is estimated that there are about 350 million carriers of the virus. Approximately 20–25% of carriers progress to chronic liver disease, including cirrhosis and hepatocellular carcinoma. The World Health Organization estimates that hepatitis B virus infection results in 1–2 million deaths every year.

Hepatitis B virus is a double-standard DNA virus which replicates by a process that involves an RNA intermediate and reverse transcription. The vision is a 42 nm particle comprising an electron dense nucleocapsid 27 nm in diameter surrounded by an outer envelope of the surface protein, hepatitis B surface antigen (HBsAg) embedded in a membranous lipid derived from the host cell. The nucleocapsid of the vision consists of the viral genome surrounded by the core antigen (HBcAg). The genome is approximately 3.2 kb in length. One of the two strands is incomplete and is associated with a DNA polymerase which is able to complete that strand in the presence of deoxynucleoside triphosphates.

Analysis of the coding potential of the genome reveals four open reading frames (ORFs). The first ORF encodes the various forms of the surface protein S, pre-S1 and pre-S2. The core ORF has two in-phase initiation codons. The "pre-core" region is highly conserved, has the properties of a signal sequence and is responsible for the secretion of HBeAg.

The third ORF, which is the largest and overlaps the other three, encodes the viral polymerase.

The fourth ORF was designated X because the function of its small gene product was not know. However, X has now been demonstrated to be a transcriptional transactivator (Fig. 1).

Mutations have been described in the S, pre-S1 and pre-S2 genome, in the core and pre-core region, the X reading frame, and more recently in the polymerase gene. Mutations in the polymerase gene have followed antiviral therapy with nucleoside analogues, including lamivudine and famciclovir.

2. Hepatitis B surface antigen mutants

Hepatitis B vaccine has been classified into six genotypes, designated A–F, based on phylogenetic analysis of complete viral genomes. Genotypes A and D are disseminated widely throughout the Old World, while genotypes B and C are confined to the east Asian populations, and genotype E to Sub-Saharan Africa. Genotype F is more divergent from the

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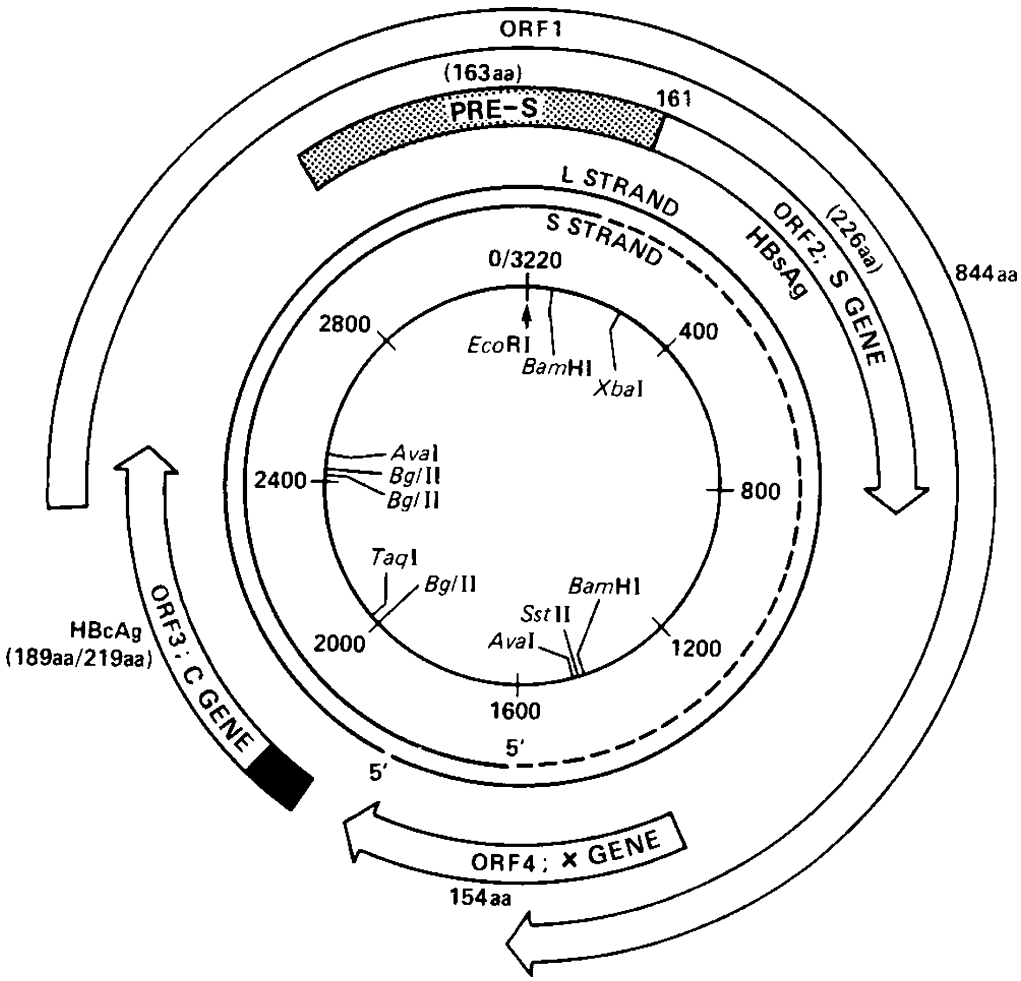

Fig. 1. Molecular structure of hepatitis B virus.

other genotypes and is found in aboriginal American populations. All six genotypes share a common immunodominant region on the surface antigen, termed the *a* determinant, which spans amino acids 124–147.

Neutralizing antibodies induced by immunization are targeted principally to the conformational epitopes of the *a* determinant, and there is evidence (reviewed below) that amino acid substitutions within this region of the surface antigen can allow replication of HBV in vaccinated persons, since antibodies induced by current vaccines do not recognize critical changes in the surface antigen domain (Fig. 2).

The emergence of variants of hepatitis B virus, possibly due to selection pressure associated with extensive immunization in an endemic area, was suggested by the findings of hepatitis B infection in individuals immunized successfully (Zanetti et al., 1988). These studies were extended subsequently by the finding on non-complexed HBsAg and anti-HBs and other markers of hepatitis B infection 32 or 44 vaccinated subjects, and sequence analysis from one of these cases revealed a mutation in the nucleotide encoding the *a* determinant, the consequence of which was a substitution from glycine to arginine at amino acid position 145 (G145R) (Carman et al., 1990).

Various mutations and variants of HBsAg have since been reported from many countries including Italy, the UK, Holland, Germany, the USA, Brazil, Singapore, Taiwan, China, Japan, Thailand, India, West and South Africa and elsewhere (reviewed by Zuckerman and Zuckeman, 1999; Francois et al., 2001). However, the most frequent and stable mutation was reported in the G145R variant. A large study in Singapore of 345 infants born to carrier mothers with HBsAg and HBeAg, who received hepatitis B immunoglobulin at birth and plasma-derived hepatitis B vaccine within 24 h of birth and then 1 and 2 months later, revealed 41 breakthrough infections with HBV despite the presence of anti-HBs. Tähere was no evidence of infection among 670 immunized children born to carrier mothers with HBsAg and anti-HBe, nor in any of 107 immunized infants born to mothers without HBsAg.

The most frequent variant was a virus with the G145R mutation in the *a* determinant. Another study in the USA of serum samples collected between 1981 and 1993 showed

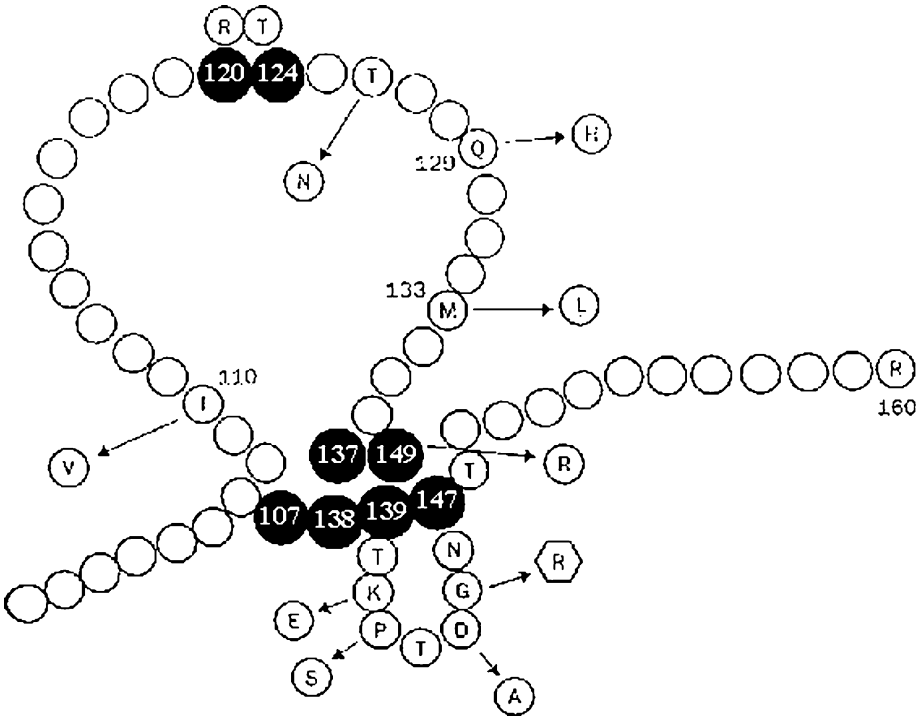

Fig. 2. Molecular structure of the major hydrophilic region of it the *a* determinant of HBsAg between the region of amino acids 100–160. Letters in circles are viral variants associated with vaccination; most are clustered in reguon of aminoacids 137–147. Commonest and most stable mutation is G145R. Shaded areas indicate disulfide bonds.

that 94 (8.6%) of 1092 infants born to carrier mothers became HBsAg positive despite post-exposure prophylaxis with hepatitis B immunoglobulin and hepatitis B vaccine. Following amplification of HBV DNA, 22 children were found with mutations of the surface antigen, most being in amino acids 142–145; five had a mixture of wild-type HBV and variants and 17 had only the 145 variant (Nainan et al., 1997). In a more recent study, Nainan et al. (2002) compared direct sequencing of amplified or cloned PCR products, solid phase detection of sequence-specific PCR products (SP-PCR), and limiting dilution cloning PCR (LDC-PCR), in order to determine their sensitivity in detecting differing concentration of HBsAg variants in the same population of the infants studied in the 1981–1993 post-exposure rophylaxis of hepatitis B.

3. Surface antigen mutants and blood donor screening

Another important aspect of the identification of HBsAg variants is the evidence that these mutants may not be detected by all of the blood donor screening tests and by existing diagnostic reagents (Carman et al., 1989; Coleman et al., 1999; Cooreman et al., 1999; Ireland et al., 2000; Jolivet-Reynaud et al., 2001; Baz et al., 2001 and review by Francois et al., 2001).

This is emphasized by the finding in Singapore, between 1990 and 1992, of 0.8% of carriers of HBV variants in a random population survey of 2001 people (Oon et al., 1995, 1996). These findings add to the concern expressed in a study of mathematical models of HBV vaccination, which predict, on the assumption of no cross-immunity against the variant by current vaccines, that the variant will not become dominant over the wild-type virus for at least 50 years—but the G145R mutant may emerge as the common HBV in 100 (or more) years' time (Wilson et al., 1999) in infants born to carrier mothers. LDC-PCR had the greatest sensitivity and could defect HBsAg variants at a concentration of 0.1% of the total viral population. HBsAg variants were detected in 47 of 93 (51%) of infants with chronic HBV infection acquired after post-exposure prophylaxis, and more than half of the variants were detected only by the most sensitive methods. The G145R variant (glycine to anginine at aa145) was identified most frequently.

A report from Taiwan (Hsu et al., 1999) noted the increase in immunized children in the prevalence of mutants of the *a* determinant of HBV over a period of 10 years, from 8 of 103 (7.8%) in 1984 to 10 of 51 (19.6%) in 1989, and 9 of 32 (28.1%) in 1994, is of particular concern. The prevalence of HBsAg mutants among those fully immunized was higher than among those not vaccinated (12/33 versus 15/153, $P = 0.0003$). In all 27 children with detectable mutants, the mean age of those vaccinated was lower than of those not vaccinated, and mutation occurred in a region with the greatest hydrophilicity of the surface antigen (amino acids 140–149) and more frequently among

those vaccinated than among those not vaccinated. More mutations to the neutralizing epitopes were found in the 1994 survey in Taiwan (Hsu et al., 1999).

4. Conclusions

- Variants of hepatitis B virus surface antigen proteins were identified over a decade ago and may have a potential impact on immunization against this important infection and on public health (Zuckerman, 2000).
- The G145R mutant is replication competent and is stable. It appears to be the most common variant and may persist in the host for at least 14 years.
- There is evidence that sera of 10% (up to 40% in high-risk groups) of individuals with antibodies to hepatitis B core antigen (anti-HBC) as the only marker of HBV infection may contain HBV DNA. At least some of the chronic low level carriers of HBV, where surface antigen is not detected and anti-HBe is the only serological marker of HBV infection, are infected with surface mutants (Weinberger et al., 2000, and others). Further studies are required.
- Epidemiological monitoring of HBV surface mutants is essential employing test reagents which have been validated for detection of the predominant mutations.
- Urgent consideration should be given to the introduction of routine screening for hepatitis B by nucleic acid based technology of blood donors and tissue and organ donors for transplantation.
- Consideration should be given to incorporating into the current hepatitis B vaccine of additional antigenic components which will confer protection against infection by the predominant *a* determinant mutation(s), if dictated by epidemiological findings.

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