Low frequency of mutations in the core promoter and precore regions of hepatitis B virus in anti-HBe positive Brazilian carriers

ABSTRACT

The data reported here are not in accordance with some reports from other parts of the world. In half of the isolates, none of the mutations previously described could explain the anti-HBe phenotype.

INTRODUCTION

Background Chronic hepatitis B virus (HBV) infection, defined by the persistence of the surface antigen (HBsAg) in serum for longer than six months, may lead to a wide spectrum of liver disease, including asymptomatic carrier state, chronic active hepatitis (CAH), cirrhosis, and hepatocellular carcinoma (HCC). Hepatitis B e antigen (HBeAg) is considered a marker for viral replication, whereas the presence of anti-HBe antibodies often indicates a low level of viral production. Seroconversion from HBeAg to anti-HBe normally correlates with improvement of liver disease. However, HBV variants have been described with mutations in the precore region that prevent HBeAg synthesis, despite continuing production of infectious virions. The most common of these mutations is a G to A substitution at nucleotide (nt) 1896, that prevents the production of HBeAg by introducing a premature stop codon into the open reading frame (ORF) of the precore region. Several studies have associated the mutation A1896 with an exacerbation of the clinical symptoms of liver disease caused by HBV. In other studies, however, such an association has not been observed. The occurrence of the A1896 mutation depends upon the nucleotide (C or T) at position 1858, that forms a base pair with nt 1896 in the pregenomic RNA loop. The presence of a C at position 1858 precludes the G to A mutation at nt 1896 as this would destabilize the stem-loop structure of the RNA encapsidation signal. The presence of the A1896 mutation is thus restricted to genotypes that have a T at nt 1858, as is the case for genotypes B, C, D, and E. Genotype A usually shows a C at this position while genotype F may present a T or a C. HBV genotypes are not uniformly distributed around the world, and the A1896 mutation has been found to be more prevalent in geographic regions where genotypes B, C and D are predominant, such as Asia and the Mediterranean area, and less prevalent in North America and Europe where genotype A is commonly found. In South America, where genotypes A, D, and F have been found, the frequency of the mutation is unknown. Besides the A1896 mutation, a number of point mutations leading to initiation failure or premature termination, as well as deletions and insertions of nucleotides inducing frameshifts, have been detected in the precore region. Regulation of transcription and expression of the precore and core genes has been extensively studied. Mutations in the core promoter, notably the double mutation at positions 1762 and 1764 changing AGG to TGA, have been suggested to mediate down-regulation of HBeAg production. Such mutations, able to prevent or reduce the transcription of precore mRNA and HBe synthesis, have been found in anti-HBe positive chronic carriers. In this study, we identify the mutations present in the precore and core promoter regions of HBV isolates (genotypes A, D, and F) derived from anti-HBe positive Brazilian patients.

CONCLUSION

Conclusions The pattern of core promoter and precore mutations of HBVs derived from anti-HBe Brazilian carriers appeared to be unique among

those already described. First, a low frequency (20%) of TGA 1762-1764 was noted among the genotype A isolates. Second, a strong association between A1896 and TGA 1762-1764 mutations was observed among isolates from genotype D ($p < 0.05$): all isolates analyzed showed either none or both mutations (Table II). Third, the common point mutations (positions 1896 and 1762-1764) were often accompanied by other mutations, notably at positions 1727, 1740, 1773, and in the hypervariable region (nt 1751-1755). Finally, only two genotype A isolates that were wild type at both positions 1896 and 1762-1764 showed other mutations which have already been associated with the anti-HBe phenotype. On the other hand, follow up of anti-HBe patients for measurement of viral load should be important to better associate the replication competence of HBV strains with anti-HBe phenotype It has been assumed that the outcome of hepatitis B infection depends on HBV genotypes. Recent studies have reported recombination events between different genotypes of HBV, which could contribute to geographical differences in the natural history of hepatitis B. Central and South America are the unique regions of the world where genotypes A, D and F co-circulate at a large scale. The presence of these three genotypes may account for specific variations of HBV isolates affecting the pattern of core promoter and precore mutations. To date, only a few HBV genomes from South America have been completely sequenced. Nucleotide sequencing of the whole genome of a large number of strains should help to explain the single behavior of South American HBV isolates in relation to precore-core mutations.