5. Mutations of Hepatitis B Virus Correlate with Liver Disease

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Abstract

Hepatitis B virus infection leads to a wide spectrum of liver injury, including self-limited acute hepatitis, fulminant hepatitis and chronic hepatitis with progression to cirrhosis or acute exacerbation to liver failure, as well as an asymptomatic chronic carrier state. The hepatitis B core antigen could be an immunological target of cytotoxic T lymphocytes. To investigate the reason why the severe immunological attack occurs, the entire precore and core region was sequenced. No significant change in deduced amino acid residue was noted in all the asymptomatic healthy carriers and all the self-limited acute hepatitis patients. In contrast, clustering changes in small segments of amino acids were found in all the patients with severe chronic liver disease and in all the fatal hepatitis cases. These data suggest that these regions with mutations may play an important role in the pathogenesis of hepatitis B viral disease, and such mutations are related to severe liver damage.

Introduction

Hepatitis B virus (HBV) infection leads to a wide spectrum of liver injury. The hepatic injury due to HBV is thought to be immune mediated (1). The hepatitis B core antigen could be an immunological target of cytotoxic T lymphocytes (CTL) (2). Since studies on endogenously processed viral peptides demonstrated that a peptide as small as 8 to 9 amino acids could be recognized by CTL (3, 4), it should be supposed that if there is "pressure" from CTL, there may be substitutions in the amino acid residues in a restricted segment of the core gene. Although recent studies revealed that a precore defective mutant is correlated with fulminant hepatitis and severe exacerbation of chronic hepatitis (5, 6), the mechanism by which the precore defective mutant induces severe hepatocyte injury is not known yet (5, 6). In this study, we analyzed the nucleotide sequence of the entire precore (29 codon) and core region (183–185 codon) of HBV DNA from patients with various types of diseases using the polymerase chain reaction (PCR) and direct sequencing methods.

Comparison with reported amino acid residues of the core region

Fourteen previously reported amino acid residues of the

precore and core region of HBV are aligned in Fig. 1 according to their subtypes. These sequences were divided into two groups. The first group had almost the same core amino acid sequence and was defined as type 1 core sequence. All 4 reported sequences of subtype adr showed well conserved amino acid residues of core peptides (Fig. 1). The core amino acid sequence of all our patients infected with subtype adr belonged to type 1. The second group was different from type 1 core sequence at almost the same positions and was defined as type 2. Type 2 includes the majority of subtype adw. The core amino acid sequences of all our patients infected with subtype adw belonged to type 2 core sequence. We used two of our own prototypes, HBVPT1 as type 1 prototype and HBVPT2 as type 2 prototype, for the analysis of amino acid substitutions.

Patients

1. Patients infected with subtype adr (type 1 core sequence)
 - 10 HBe antigen-positive asymptomatic healthy carriers
 - 20 chronic hepatitis patients
 - 5 fatal severe exacerbation of chronic hepatitis patients
 - 2 fatal fulminant hepatitis patients
 - 6 self-limited acute hepatitis patients
2. Patients infected with subtype adw (type 2 core sequence)
 - 5 fatal severe exacerbation of chronic hepatitis patients
 - 3 fatal fulminant hepatitis patients
 - 3 self-limited acute hepatitis patients

Amplification and sequencing of precore and core region of HBV DNA

To amplify the precore and core gene, we prepared several synthetic oligonucleotide primers according to the reported sequence by Kobayashi and Koike (7) (Fig. 1): sense primers, F1 (nt 1618–1635), F2 (nt 1518–1537); antisense primers, F3 (nt 2328–2347), F4 (nt 2363–2382). With these primers, a segment of the entire precore and core region, was amplified by PCR. Amplification of HBV DNA was performed basically by the method described previously (8). To sequence the amplified segment, we prepared several sequencing primers: sense primers, S1 (nt 1644–1662), S2 (nt 1739–1758), S3 (nt 1828–1847), S4 (nt 1934–1953), S5 (nt 2103–2121), S6 (nt 2228–2247); antisense primers, S7 (nt 1828–1847), S8 (nt 1934–1953), S9 (nt 2103–2121), S10 (nt 2228–2247) (Fig. 1). The direct sequencing of the PCR products was performed as previously

Fig. 1. Schematic presentation of precore and core peptide with hydrophobicity profile of HBV. Locations of amplification and sequencing primers used for PCR and direct sequencing are shown by arrows. Deduced amino acid residues of precore and core peptide, different from prototype adr HBVPT1, are plotted against the corresponding position of peptides. Amino acid residues were numbered from start of precore or core gene. The dashed line indicates the same amino acid with prototype HBVPT1. Amino acid residues are indicated by a single letter. * indicates termination codon.

described (9).

Results

Deduced amino acid residue of precore and core gene

1. Patients infected with subtype adr (type 1 core sequence)

No significant changes in deduced amino acid residue from type 1 prototype (HBVPT1) were noted in the 10 healthy carriers (Fig. 2). In contrast, the 39 "missense" nucleotide changes from HBVPT1 were found in the core region in chronic liver disease patients. The 39 deduced amino acid substitutions from HBVPT1 in the core region are given in Fig. 2. Of these changes, 21 (54%) were clustered in a small segment (from core codon 84 to 101, 9.8% of the core peptide) (Fig. 2). At least one amino acid substitution in this segment was found in 15 of the 20 chronic liver disease patients. Of interest was that 15 patients with amino acid substitutions in this region had advanced liver disease (chronic active hepatitis and cirrhosis), whereas all 5 patients without such substitutions had only mild liver disease (chronic persistent hepatitis). The precore defective mutation

was found in only 4 out of 16 chronic liver disease patients with core substitutions (Fig. 2).

In 5 fatal severe exacerbation of chronic hepatitis, 2 fatal fulminant hepatitis, and 6 self-limited acute hepatitis patients, the 29 "missense" changes from HBVPT1 in the core region were only found in fulminant and severe acute exacerbation patients (Fig. 2). The locations of 29 amino acid substitutions in the core region are shown in Fig. 2. Of these 29 amino acid changes, 12 (41.4%) were clustered in a small segment (from core codon 84 to 99, 8.7% of the core peptide) (Fig. 2). At least one amino acid substitution in this segment was found in all 7 fatal cases (Fig. 2). Most of the substitutions were not identified in any of the 14 previously reported sequences. A precore defective mutation was found in 4 out of 5 severe exacerbation patients and 1 out of 2 fulminant hepatitis patients. All of the 6 self-limited acute hepatitis patients had no precore defective mutation (Fig. 2).

2. Patients infected with subtype adw (type 2 core sequence)

In 5 fatal severe exacerbation, 3 fatal fulminant hepatitis and 3 self-limited acute hepatitis patients, the 34 "missense" changes

Fig. 2. The deduced amino acid substitutions in patients infected with subtype adr, including 10 HBe antigen-positive asymptomatic healthy carriers, 20 chronic hepatitis patients, 5 fatal severe exacerbation of chronic hepatitis patients, 2 fatal fulminant hepatitis patients and 6 self-limited acute hepatitis patients. Only the deduced amino acid residues which are different from the prototype (HBVPT1), are indicated by vertical solid lines. The mutation clustering region from core codon 84 to 101 is shaded.

Fig. 3. The deduced amino acid substitutions in patients infected with subtype adw, including 5 fatal severe exacerbation of chronic hepatitis patients, 3 fatal fulminant hepatitis patients and 3 self-limited acute hepatitis patients. Only the deduced amino acid residues which are different from the prototype (HBVPT2), are indicated by vertical solid lines. The mutation clustering region from core codon 48 to 60 is shaded.

from type 2 prototype (HBVPT2) in the core region were only found in severe acute exacerbation and fulminant hepatitis patients (Fig. 3). The location of 34 amino acid substitutions from HBVPT2 in the core region are shown in Fig. 3. Of these 34 amino acid changes, 9 (26.5%) were clustered in a small segment (from core codon 48 to 60, 7.1% of the core peptide) (Fig. 3). At least one amino acid substitution in this segment was found in all of the 3 fulminant and 4 of 5 severe exacerbation patients (Fig. 3). Seven out of eight fatal patients had an amino acid substitution at core codon 77. The precore defective mutation was found in all fatal 8 cases (Fig. 3). All 3 self-limited acute hepatitis patients had no precore defective mutation (Fig. 3).

Discussion

It is not known why there are differences in the severity of liver disease among HBV infected individuals. In this study, precore and core genes were investigated in patients with chronic subtype adr HBV infection at the beginning. Despite active virus replication, no significant hepatocellular injury has been observed in 10 HBeAg-positive asymptomatic healthy carriers and the deduced amino acid sequences of the core peptide were identical to the prototype HBVPT1 (Fig. 2). In contrast, a mutation clustering region (core codon from 84 to 101) was found in 15 patients with chronic active liver disease infected with subtype adr. This indicates the mutation in the core region is related to active liver disease (10).

Secondly, it was shown that all the fatal hepatitis cases infected with subtype adr had amino acid substitutions in this mutation clustering region (Fig. 2). All the precore and core sequences from self-limited acute hepatitis patients were identical to prototypes of each subtype (HBVPT1 and HBVPT2) (Figs. 2, 3). Whereas almost all fatal patients infected with subtype adw had another mutation clustering region (core codon from 48 to 60) (Fig. 3). These results indicated that "mutation clustering regions" differed between type 1 and type 2 core sequences. If the immunological pressure induces amino acid substitutions, the immunological targets might differ between type 1 and type 2 sequences, because there are already amino acid differences at core codon 12, 27, 67, 74, 83, 87 and 91 between type 1 (HBVPT1) and type 2 (HBVPT2) prototype. This might be the reason why "mutation clustering regions" differed between type 1 and type 2 core sequences (Figs. 2, 3) and suggest the importance of minute alteration of the prototype sequence (11).

Recent studies revealed that the endogenously processed viral peptides bound to the class I major histocompatibility complex (MHC) are recognized by CTL and the size of the

processed viral peptide could be as small as 8 to 9 amino acids (3, 4). Furthermore, a recent study on amino acid sequences eluted from MHC molecules revealed that class I MHC has allele-specific binding motifs (12). The core mutations tend to cluster but are located in relatively diverse regions. This diversity might be due to the different of HLA type of each individual patient.

The natural course of hepatitis B viral liver diseases is quite variable. In this study, the absence of substitutions in the core region was related to an uneventful course, and by contrast the presence of such changes was associated with severe liver disease. Thus, the most important clinical implication of the present findings is that the presence of an amino acid substitution in the "mutation clustering regions" might be used as an indicator of worsening of the disease.

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