Clinical Immunology

Downregulation of HLA Class II Molecules by G1896A Pre-Core Mutation in Chronic Hepatitis B Virus Infection

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Abstract

Over the past decade, increasing attention has been focused on the contribution of naturally occurring mutations in the hepatitis B virus (HBV) genome to the clinical course of the chronic infection. The aim of this study was to investigate the effect of the HBV pre-core mutation G1896A on the expression of HLA class II molecules and the core protein of hepatitis B in liver biopsies of chronic hepatitis B (CHB) infection. In 30 HBeAg-negative CHB patients the pre-core region of the HBV genome was amplified and sequenced to determine the presence of the mutation G1896A. Liver biopsies were scored based on the Histology Activity Index (HAI) system and immunohistochemistry (IHC) was performed to study the expression of HLA class II molecules on the antigen-presenting cells and the core protein in hepatocytes. We found that 19 of the 30 patients (63%) harbored the G1896A mutation. Compared to the patients without this mutation, those with G1896A had lower HAI scores (5.0 ± 2.8 versus 7.9 ± 4.2, p = 0.03). The study of the expression of HLA-II molecules in our patients revealed that subjects with the G1896A mutation had lower expression of HLA-II compared to wild-type infected subjects (1.87 ± 0.6 versus 3.27 ± 1.5, p < 0.01). Core protein expression was present in four patients (13.3%) who had higher HBV DNA levels than patients without core protein expression (3.81 ± 0.78 versus 2.02 ± 0.16, p < 0.001). These results suggest that the G1896A pre-core mutation may directly interfere with antigen presentation, most likely by decreasing the availability of HLA class II molecules, and this may result in less aggressive liver disease in HBeAg-negative CHB infection.

Introduction

There are over 350 million people who are persistently infected with hepatitis B virus (HBV) and they are at increased risk of developing complications such as cirrhosis and hepatocellular carcinoma (10). It is apparent that liver-related adverse outcomes in chronic hepatitis B (CHB) depend on the interaction between the host immunological system, viral replication, and genetic heterogeneity of the virus, including genotypes and mutations.

In the pathogenesis of HBV infection, the virus-specific immune response can elicit a protective effect or act as a mediator for liver tissue damage (7,16). The major histocompatibility complex (MHC) II molecules are an important regulatory factor in the adaptive immune response, and they play a role in presentation of processed viral antigens to T-helper lymphocytes (1,17,18). Although the host immune response plays a major role in determining if an individual undergoes either viral clearance or disease progression, a variety of viral adaptive mechanisms may also influence the outcome. Frequently, mutations in critical regions of the viral genome are the result of these adaptive mechanisms (11,21). The substitution of A for G at nucleotide 1896 (G1896A) of the HBV pre-core region is an example of an adaptive variant that leads to premature interruption of the translation of the HBe antigen (5,6). The prevalence of HBV harboring the G1896A mutation in different geographic areas is related to the distribution of HBV genotypes with a high frequency in patients infected with genotype D (20,23), the predominant HBV genotype in Iran (26).

The nucleotide at position 1896 is located within the RNA encapsidation signal epsilon, a conserved stem-loop structure important for replication. The G1896A mutation helps to stabilize the base-pairing of the stem structure for the common HBV genotypes B, C, and D, but not for genotype A (23,25).

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Several independent studies suggest that in addition to blocking HBeAg synthesis (28), the pre-core mutation has other effects on HBV gene expression and replication (14,19). However, other studies revealed little or no effect of this mutation on viral replication and RNA encapsidation (30).

Since presentation of multiple epitopes of HBcAg/HBeAg through HLA class II molecules to CD4+ T lymphocytes represents the trigger event for the adaptive immune response and for the activation of chronic inflammatory processes, we were interested in examining the clinical relevance of the G1896A pre-core mutation on the host's antiviral immune response. Thus in this study we examined the effect of G1896A pre-core stop mutation on the expression of HLA class II molecules and HBV core antigen (HBcAg) in liver biopsies of HBeAg-negative CHB patients.

Materials and Methods

Patients

Thirty HBeAg-negative perinatally acquired CHB patients with detectable HBV DNA by a non-commercial hemi-nested PCR were recruited for this study from Iranian patients with CHB. All perinatally acquired chronic infection, as they had a clear history of familial HBV infection with the presence of HBsAg. Demographic data and serum samples were collected at the initial assessment before liver biopsy and stored at −70°C prior to analysis. None of patients received antiviral treatment prior to liver biopsy. The study protocol was approved by the ethics committee of Shariati Hospital.

Clinical evaluation and HBV-DNA quantification

Data concerning age and sex were obtained and serologic markers including HBsAg, HBeAg, and anti-HBe were detected using commercially available enzyme-linked immunosorbent assay kits from Radim Diagnostics (Pomezia, Italy). HBV DNA was extracted from 200 μL of serum using the semi-automated Roche Magna Pure system (Version 2.1; Roche Diagnostics, Branchberg, NJ) according to the manufacturer's instructions. Extracted DNA was eluted in a final volume of 50 μL of elution buffer. PCR products were purified using PCR purification columns from MO Bio Laboratories Inc. (Carlsbad, CA) according to the manufacturer's instructions, and eluted in 100 μL of elution buffer. PCR products were sequenced using the Big Dye Terminator Cycle sequencing Ready Reaction Kit Version 3.1 (Applied Biosystems, Foster City, CA).

Statistical analysis

To compare the characteristics of HBV harboring the G1896A mutation with wild-type HBV genome from patients, we used the chi-square test for categorical variables, and the t-test for continuous variables. Normality of variables was checked using the Kolmogorov-Smirnov test. Data for stage variable were not normally distributed, so the Mann-Whitney U test was used. The Pearson or Spearman correlation was used to evaluate any association between variables. A p-value <0.05 was considered significant. Analysis was carried out using STATA ver. 8. (StataCorp, LP, US)

Results

Demographic characteristics and the frequency of the G1896A pre-core mutation, along with the clinical and biochemical profiles of the 30 study subjects are summarized in Table 1. The mean age was 43 ± 11 y, and 18/30 (60%) were male. Amplification of the HBV pre-core region by hemi-nested PCR was successful in samples from all 30 patients, and sequence analysis showed the presence of the pre-core mutation G1896A in 19/30 (63%) of the samples.

Histological and immunohistochemical findings

The mean necroinflammatory grade according the modified HAI system was 4.8 ± 2.3, suggesting mild inflammation in most patients. Immunohistochemistry for the detection of HBcAg showed positive staining in the liver biopsies of four subjects (13.3%), with nuclear or cytoplasmic staining or sometimes both present (Fig. 1). There was no significant relationship between HBcAg expression and the presence of the G1896A mutation (p = 0.8). However, patients with HBcAg expression had significantly higher viral loads than those who were HBcAg-negative (3.37 ± 0.16 versus 1.82 ± 0.16, p < 0.001). Patients who had HBcAg in their hepatocytes had lower expression of HLA class II (p = 0.05, r = −0.37).

Numbers of positive Kupffer cells per microscopic field (400× magnification) were counted in 10 fields per section. The mean numbers of positive Kupffer cells were calculated, and the intensity of staining for HLA class II molecules was scored from 1–5 for the amount of positive reactions in the Kupffer cells, finding a mean of 2.3. Fig. 2 shows the expression pattern of HLA class II molecules in Kupffer cells, and in sinusoidal and central vein endothelium in the liver biopsies of patients with a difference in intensity of staining. The histological and immunohistochemical findings in liver biopsies of the CHB patients are shown in Table 2.

Pre-core G1896G mutation detection and direct sequencing

The pre-core region was amplified using hemi-nested PCR as described previously. The amplified products were purified using PCR purification columns from MO Bio Laboratories Inc. (Carlsbad, CA) according to the manufacturer's instructions, and eluted in 100 μL of elution buffer. PCR products were sequenced using the Big Dye Terminator Cycle sequencing Ready Reaction Kit Version 3.1 (Applied Biosystems, Foster City, CA).
The G1896A mutation in relation to grade and stage of liver disease. As illustrated in Table 2, subjects with the G1896A pre-core mutation had lower stage and HAI scores compared to wild-type patients. There was no correlation of the median level of serum alanine aminotransferase (ALT) and viral load with the expression of HLA class II.

We next sought to examine the significance of the G1896A pre-core mutation in relation to grade and stage of liver disease. As illustrated in Table 2, subjects with the G1896A mutation had lower stage and HAI scores compared to subjects infected with wild-type virus (2.0 ± 0.7 versus 2.0 ± 1.6, p = 0.02 and 5.0 ± 2.8 versus 7.9 ± 4, p = 0.03). However, the mean level of serum ALT and viral load for patients with virus having the G1896A mutation was not significantly different from wild-type patients. Table 2 shows the univariate analysis of the relationship between the G1896A mutation with the expression of HLA class II molecules, stage, and HAI score of liver disease.

**Discussion**

Our data demonstrate that the G1896A mutation occurs frequently in HBeAg-negative patients (19/30 or 63%), consistent with previous observations (26). In this study, we showed a close association between the frequency of G1896A pre-core stop codon mutation and reduced expression of HLA class II molecules; this may affect the clinical course of HBV infection in hepatitis B infection has not been well studied. Viruses infecting antigen-presenting cells have evolved their own strategies that impair surface expression of HLA class II and co-stimulatory molecules (9,24). Central vein endothelium and Kupffer cells in the liver normally contain HLA class II, and the intensity of staining for HLA class-II molecules is increased in liver-tropic viruses such as HBV (3). Viral clearance and disease pathogenesis in HBV infection depends on the activation of HLA class-II-restricted T-helper lymphocytes (7,17,18).

In the present study we were able to show that patients having the G1896A mutation variant had significantly lower expression of HLA class II molecules on antigen-presenting cells. Indeed, the expression of HLA class II molecules is an important part of the mechanism leading to the activation of the inflammatory response involved in the progression and severity of HBeAg-negative CHB infection. Data obtained from gene expression analysis in HBV-transgenic mice showed an overexpression of HLA class II molecules that induces hepatocarcinogenesis (4). We found that HLA class-II staining was more intense in patients with a higher necroinflammatory grade, consistent with the effects of HLA class II molecules on cellular damage following the immune response.

Recent studies have demonstrated that the interaction between DNA and HLA class II molecules inhibits the
To address the relationship between the presence of the pre-core mutation and a significant decrease in the expression of HLA class II molecules, we postulate that HBV with the pre-core stop mutation at nt 1896 can enhance viral base pairing within the stem-loop region of the encapsidation signal, epsilon. This stability with higher frequency in genotype D may interfere with the presentation of HLA class II molecules, or involve in the activation of proteasome-mediated degradation. Further studies with gene expression analysis are required to eluci-

FIG. 2. Immunohistochemical detection of HLA class-II molecules expression. Original magnification ×400. Arrows indicate the Kupffer cells. (A) Patient with wild type variant of HBV in pre-core region. Immunostaining of the HLA class-II molecules showing a staining score of 4 (when the mean number of positive Kupffer cells was 40–50 cells with strong positive reactivity for HLA class-II). (B) A patient with G1896A pre-core mutant. Immunostaining of the HLA class-II molecules showing a staining score of 2 (the mean number of positive Kupffer cells was 20–30 cells, with moderately positive reactivity for HLA class-II).
Table 2. Comparison of Clinical Findings for the G1896A Mutation Versus Wild-Type HBcAg-Negative Chronic Hepatitis B

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mutation (n = 19)</th>
<th>Wild-type (n = 11)</th>
<th>p Value</th>
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<tr>
<td>Age (mean ± SD)</td>
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<td>Alanine (U/L)</td>
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<td>Viral load (log copies/mL)</td>
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<td>Grade of inflammation</td>
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<td>Stage of fibrosis</td>
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<tr>
<td>HLA class II presentation</td>
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*Mean ± SD.

date the inhibitory mechanisms of HLA class II presentation by the G1896A mutation.

Conclusion

In summary, in CHB patients with virus containing the G1896A mutation, the expression of HLA class II molecules is downregulated, and was accompanied by weaker antiviral immune response to persistent infection with less aggressive liver disease.

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Author Disclosure Statement

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References


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