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Review Article

Naturally Occurring Mutations of Hepatitis B virus and Hepatitis C Virus in Korean Chronic Patients by Distinct CD4 T Cell Responses

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Hepatitis B virus (HBV) and hepatitis C virus (HCV) infections are among the most common causes of chronic liver disease worldwide. The host immune pressure against hepatitis viruses during the chronic infection has led to mutations in their coding genes, which could play a pivotal role in the clinical outcomes of chronic patients. Our recent molecular epidemiologic studies regarding the HBV precore/core (preC/C) regions and HCV nonstructural 5B (NS5B) protein suggest the presence of distinct CD4 T cell immune pressure against HBV and HCV in Korean chronic patients. However, induced HBV and HCV mutations seem to exert an opposite effect on Korean chronic hepatitis B (CHB) and chronic hepatitis C (CHC) patients, respectively. On the basis of two of our recent papers, we focused in this review on the relationships between the mutation patterns of HBV preC/C and HCV NS5B, which were presumed to be caused by distinct CD4 T cell pressure in the Korean population and their effect on the clinical outcomes and liver disease progression of CHB and CHC patients.

Key Words: Hepatitis B virus (HBV), Hepatitis C virus (HCV), Mutations, Precore/core (preC/C), Nonstructural 5B (NS5B)

Introduction

Hepatitis B virus (HBV) and Hepatitis C virus (HCV) infections are the major causes of chronic liver disease, cirrhosis, and hepatocellular carcinoma (HCC). Despite the availability of an effective vaccine, more than 350 million people worldwide are chronically infected with HBV. Approximately, 130 million people suffer from chronic HCV and a vaccine for HCV is not currently available (1).

The Republic of Korea has been recognized as an endemic area for HBV infection. For instance, according to the Korean National Health and Nutrition Survey of 2010, the prevalence of HBsAg was 2.7% in men and 3.1% in women (2). Although HBV is by far the more important risk factor for the development of HCC in Korea, HCV infection is more closely associated with HCC in elderly patients. An overall estimate of the prevalence of anti-HCV among Koreans older than 40 years was 1.29% during the years 1995-2000 (3).

There is increasing evidence that HBV and HCV genotypes play a significant role in causing different disease profiles and antiviral responses in chronic hepatitis B (CHB) and chronic hepatitis C (CHC) infections (4, 5). An

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extraordinary prevalence of HBV genotype C2, which is known to be more virulent than genotype B, has been reported in Korea (6). Combinatorial treatment with pegylated interferon (IFN)-α and ribavirin provides good clinical efficacy in patients infected with HCV genotypes (GTs) 2 and 3, but is less efficacious in the most prevalent GT 1-infected patients (7, 8). The most prevalent HCV genotype in Korea is GT 1b followed by 2a (9).

Mutations in HCV and HBV have been reported to play a very pivotal role in their viral life and the liver disease progression of chronic patients. Recently, we have reported that the pronounced high frequency of mutation and distinct mutation patterns were found within hepatitis B virus (HBV) antigens (preS, surface, core, and X) from Korean chronic patients. Compared to the other areas, these patterns may have contributed to the progression of liver diseases in Korean chronic patients (6, 10~25). Particularity, in the preC/C region, distinct mutation types related to clinical severance were found to be mainly located at the MHC class II restricted regions (26). Furthermore, our recent paper showed that a vigorous and distinct immune pressure at the T cell level in Korean patients may also lead to the high frequency of mutation in HCV nonstructural proteins, NS5B, which contributes to the high sustained virological response (SVR) rates of genotype 1b infected patients via the loss of functional activity of RNA-dependent RNA polymerase (RdRp) (27). This raises the possibility that there may be a vigorous and distinct intrahepatic immune pressure leading to epitope escape mutations in Korean populations.

In this review, on the basis of two of our recent papers (11, 28) we focused on the relationships between the mutation patterns of HBV preC/C and HCV NS5B, which were presumed to be caused by distinct CD4 T cell pressure in the Korean population, and their effect on the clinical outcomes and liver disease progression of hepatitis B and hepatitis C patients.

The HBV preC/C mutations from Korean patients infected with genotype C

The HBV C protein (HBcAg), which is the protein capsid of the virus core, is 183 residues long, of which the N-terminal 149 residues are the assembly domain (29, 30). The HBcAg is the principal target for the host immune response, particularly the cytotoxic T lymphocyte (CTL) attack, in which nonsynomyous mutations that change immune epitopes could lead to the production of immune escape variants, resulting in the persistence of HBV (31~33). Moreover, since the mutation in the C region can lead to simultaneous mutations in Hepatitis B envelop Antigen (HBeAg), a key HBV immunoregulatory protein, the mutation may also profoundly affect the natural course of chronic hepatitis B (CHB) (34).

In our recent paper (11), the mutation patterns within the entire preC/C region from the DNAs of a total of 70 chronic hepatitis B patients were investigated using a nested PCR protocol. In general, non-random distribution was shown in the mutations in the preC/C region of Korean chronic patients infected with genotype C. The mean values of all the mutation rates in the entire preC/C region were 2% (4.2 mutations in the 212 amino acids). The mutation rates in the MHC class I restricted region (designated M1RR) (61 mutations/ 2870 aa, 2.2%) or class II restricted region (designated M2RR) (170 mutations/ 7350 aa, 2.3%) were significantly higher than in the non-restricted region (designated NRR) without T cell epitopes (0.8%) ($p < 0.001$). In particular, this was more pronounced considering the mutation rates (72 mutations/ 1750 aa, 4.1%) in "hot spots", aa residue 81-105 region in MHC class II restricted regions (26). This suggests that the host immune pressure against the T cell, particularly CD4+ T cell response, may be the major driving force of preC/C mutations in Korean chronic patients. Furthermore, our data showed that M2RR (109 mutations/ 3675 aa, 3.0%), which is the target of the CD4 T cell, and particularly the aa residue 81-105 region (44 mutations/ 875 aa, 5.0%) was subjected more to the mutations induced by HBeAg seroconversion than M1RR

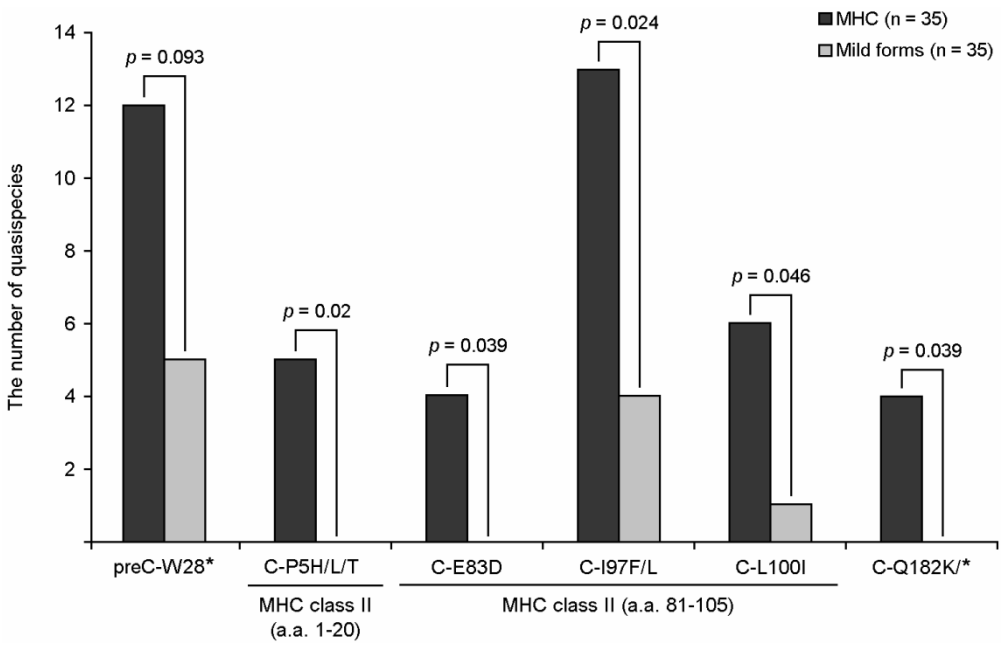

Figure 1. Six types of HBV preC/C mutations related to the severity of liver diseases in Korean chronic patients. The types of mutations within the region of MHC II-restricted T-cell epitopes are marked. For comparison between patients with HCC and other disease types, preC/C mutations of 35 HCC patients and 35 patients with other types of liver diseases (27 of chronic hepatitis and 8 patients of liver cirrhosis) were analyzed. To analyze the mutation patterns and their frequencies of deletions and insertions in the entire preC/C region, a nested PCR protocol was used. First-round PCR was performed using the sense primer CoreF1 (59-AAC GAC CGA CCT TGA GGC ATA CTT-39) and the antisense primer CoreR1 (59-ATT TGG TAA GGT TAG GAT AGA A-39) to yield a 1,017 bp amplicon between 1,682 nt to 2,698 nt of the HBV genome. Second-round PCR was performed using the sense primer CoreF2 (59-GAG TTG GGG GAG GAG ATT AGG TTA-39) and the antisense primer [11].

(37 mutations/ 1435 aa, 2.6%), the target of the CD8 cytotoxic T cell. This information strongly supports the above hypothesis.

Six mutations (preC-W28*, C-P5H/L/T, C-E83D, C-I97F /L, C-L100I and C-Q182K/*) in the preC/C region were found to be related to HCC patients compared to patients in other stages of the disease (LC and CH). Of interest, the following 4 of 6 HCC related preC/C mutations (C-P5H/ L/T, C-E83D, C-I97F/L, and C-L100I) were located in the MHC class II restricted T cell epitopes (one in a.a. 1-20 and three in a.a. 81-105, Fig. 1). This suggests that a distinct CD4 T cell response against HBcAg in Korean chronic patients, which may lead to immune escape mutations within MHC class II restricted regions, may contribute to hepato-carcinogenesis. Therefore, inhibition of the CTL function by deregulating the CD4 T cell, rather than direct evasion of the CTL function, at least in Korean patients, may be the principal strategy for HBV immune evasion.

In summary, our recent paper regarding HBV preC/C mutations in Korean patients indicated that together with epidemiologic traits, such as an extraordinary high prevalence of genotype C infections prone to HBV mutations, as well as perinatal infection rather than horizontal infection, the presence of a distinct CD4+ T cell immune response may lead to the generation of a higher level of HBcAg variants. As a result, this contributes to the HCC progress via immune evasion in Korean patients with CHB.

The HCV NS5B mutation from Korean chronic patients with genotype 1b

The HCV RNA-dependent RNA polymerase (RdRp) is an essential enzyme of an RNA dependent RNA polymerase that lacks proofreading activity, leading to viral quasispecies

within an infected individual (35). Monitoring of HCV quasispecies diversity is important to predict liver disease progression as well as HCV treatment outcome (36~39). Recently, variations in NS5B specific codons were reported to be positively related to the sustained virological response (SVR) and early virological response (EVR) of genotype 1b infected patients.

It was also reported that the SVR rate in patients with HCV genotype-1 treated with pegylated-IFN plus ribavirin are higher in Asian patients, particularly in Korean patients ranging from 56% to 62%, as compared to Caucasian patients. Recently, two IL28B gene polymorphisms (rs-12979860 and rs8099917) showing the strongest association with treatment response have been reported to be found with high frequency in Korean patients with hepatitis C virus (HCV) genotype-1 compared to other ethnic groups. Although prior investigations can partially explain the high SVR rates in Korean patients, other mechanisms still remain to be offered. To address this, in our recent paper, the mutations in the partial NS5B gene (492 bp) from 166 quasispecies of 15 genotype 1b treatment naive Korean chronic patients were determined. Mutation patterns and frequencies that mainly focused on the T cell epitope regions were also evaluated (28). Our previous data showed that the mutation frequencies inside CD8+ epitope regions (2.9%) are significantly higher than those outside epitope regions (2.3%, $p = 0.001$). The mutation frequencies inside predicted CD4+ epitopes (4.8%) are significantly higher than those outside CD4+ epitope (1.4%), as well as even inside known CD8+ epitopes ($p < 0.001$). This contrast was more pronounced in the mutational hotspot region of the NS5B (aa 333-355) of CD4+ epitopes, where an extraordinary high mutation frequency (6.7%) was observed (28). It is noteworthy that the region was predicted to have high binding affinity for the various MHC class II HLA types prevalent in Korean patients, raising the possibility that there may be a distinct MHC class II restricted immune pressure against HCV genotype 1b in Korean chronic patients. This suggests that there may be distinct intra-hepatic MHC class II restricted immune pressures, at least against HCV NS5B, among the Korean population. A broadly directed virus specific immune pressure at the CD4 T cell level was recently reported to play a very pivotal role in spontaneous resolution at the very early phase of HCV acute infection. Furthermore, the presence of multi-specific CD4+ T cell response against HCV not only can aid the induction of a vigorous antiviral CD8+ T cell response, but can also provide antibody production for the inhibition of viral spread (39). In particular, since 3 codons (A333, V338, and Q355) of 4 were reported to have been related to the high SVR located in a mutational hotspot region of the NS5B (aa 333-355) of CD4+ epitopes (28) (Fig. 2), the acquisition of mutations induced by Korean distinct immune pressure at the CD4 T cell level may have contributed to the high SVR found in genotype 1b infected Korean patients.

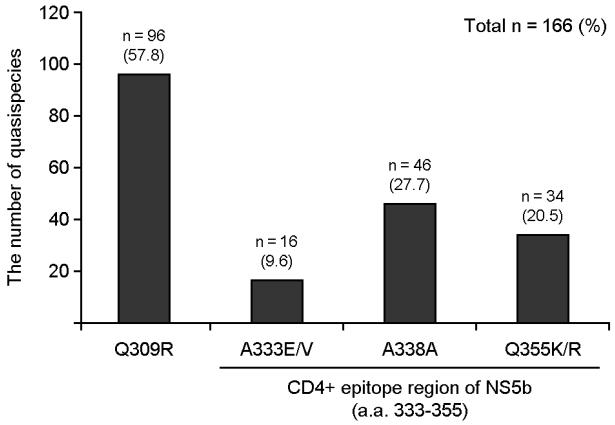

Figure 2. Mutation frequency at the 309, 333, 338 and 355 codons related to SVR and ETR in the NS5B region. The 166 quasispecies clones of HCV NS5B from 15 genotype 1b infected Korean patients were analyzed. For quasispecies analysis, the nested PCR protocol was performed. The first round of amplification was carried out using the sense primer A1b (O/S) (accession no. M62321, positions 8113-8135 59 - CTGACRACTAGCTGYGG-TAAYAC - 39) and the antisense primer F1b (O/A) (positions 8678-8699, 59 - CCTGGAGAGTAACTRTGGAGTG - 39). The second round of amplification was carried out using the sense primer B1b (I/S) (positions 8181-8205, 59 - GCTCCRGGAC-TGCACSATGCTCGTG - 39) and the antisense primer E1b (I/A) (positions 8654-8675, 59 - AATGCGCTRAGRCCATGGAGTC - 39) to amplify 495 bp of the GT-1b NS5B region [28].

Conclusion

Our recent molecular epidemiologic studies have showed

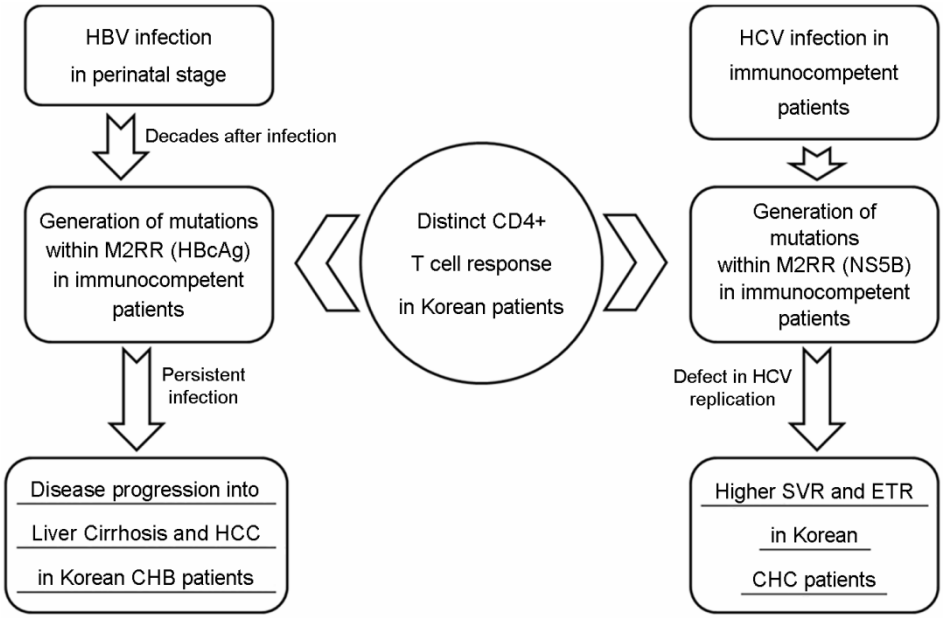

Figure 3. The presence of distinct CD4 T cell response in Korean patients may lead to opposite clinical outcomes in CHB and CHC Korean patients. The difference in clinical outcomes by viral mutations via distinct immune pressure between patients with CHB and CHC may be mainly due to the difference between HBV and HCV in the infections of Korean chronic patients, such as the vertical infection from mother to child versus horizontal infection.

that there may be a distinct MHC class II restricted immune pressure against HCV NS5B and HBV HBcAg in Korean patients. This immune pressure could produce immune evasion variants within M2RR, leading to the different clinical outcomes in chronic hepatitis B (CHB) and chronic hepatitis C (CHC) patients. Generally, immune pressure induced viral mutations are likely to contribute to the progression of severe forms of liver diseases, such as HCC and liver cirrhosis in CHB patients, while in contrast, the mutations contribute to the SVR and EVR in CHC patients.

The difference in clinical outcomes induced by viral mutations via distinct immune pressure between patients with CHB and CHC may be mainly due to the difference between HBV and HCV in the infections of Korean chronic patients, such as the vertical infection from mother to child versus horizontal infection. Most of the HBV infections in Korea occurred in the perinatal stage where a complete immune response does not work. Therefore, the immune response induced HBV variants may have been generated decades after the wild type of HBV infections appeared in South Korea, which may have contributed to the disease's progression via increases in persistent infection. But, in HCV infection, most of the cases occurred in immune competent adults by horizontal transmission. Therefore, the presence of distinctly competent MHC class II restricted immune response in Korean patients may have produced mutations in HCV NS5B, resulting in the high rates of SVR in Korean patients. In conclusion, our data has provided a likely explanation why there may be opposite clinical outcomes between CHB and CHC Korean patients, including the higher prevalence of HCC and the higher relapse after antiviral therapy in CHB versus the higher SVR rate in CHC patients compared to other areas (Fig. 3).

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