Association between HLA class II gene and susceptibility or resistance to chronic hepatitis B

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Abstract

AIM: To investigate the association between the polymorphism of HLA-DRB1, -DQA1 and -DQB1 alleles and viral hepatitis B.

METHODS: HLA-DRB1, -DQA1 and -DQB1 alleles in 54 patients with chronic hepatitis B, 30 patients with acute hepatitis B and 106 normal control subjects were analyzed by using the polymerase chain reaction/sequence specific primer (PCR/SSP) technique.

RESULTS: The allele frequency of HLA-DRB1*0301 in the chronic hepatitis B group was markedly higher than that in the normal control group (17.31 % vs 5.67 %), there was a significant correlation between them ($\chi^2=12.3068, Pc=0.0074, RR=4.15$). The allele frequency of HLA-DQA1*0501 in the chronic hepatitis B group was significantly higher than that in the normal control group (25.96 % vs 13.68 %), there was a significant correlation between them ($\chi^2=9.0022, Pc=0.0157, RR=2.87$). The allele frequency of HLA-DQB1*0301 in the chronic hepatitis B group was notably higher than that in the normal control group (35.58 % vs 18.87 %), there was a significant correlation between them ($\chi^2=15.5938, Pc=0.0075, RR=4.07$). The allele frequency of HLA-DRB1*1101/1104 in the chronic hepatitis B group was obviously lower than that in the normal control group (0.96 % vs 13.33 %), there was a significant correlation between them ($\chi^2=11.9206, Pc=0.0145, RR=18.55$). The allele frequency of HLA-DQA1*0301 in the chronic hepatitis B group was remarkably lower than that in the normal control group (14.42 % vs 30 %), there was a significant correlation between them ($\chi^2=8.7396, Pc=0.0167, RR=0.35$).

CONCLUSION: HLA-DRB1*0301, HLA-DQA1*0501 and HLA-DQB1*0301 are closely related with susceptibility to chronic hepatitis B, and HLA-DRB1*1101/1104 and HLA-DQA1*0301 are closely related with resistance to chronic hepatitis B. These findings suggest that host HLA class II gene is an important factor determining the outcome of HBV infection.

INTRODUCTION

The progression of hepatitis B virus (HBV) infection may be influenced by a number of factors including the viral genotype and the level of viremia, but these factors alone do not account for the variability in outcome. There is an increasing awareness that host factors are involved. A great deal of evidences suggest that both cellular and humoral immune responses are required for viral clearance$^{[1-3]}$. Polymorphisms of human leukocyte antigen (HLA) influence immune responses. Variability in immune response is often associated with HLA polymorphism. HLA genotype of an individual may influence the progression of HBV infection. Patients who have successfully recovered from acute hepatitis B develop strong HLA classes I and II restricted T cell response, whereas these responses are weak or absent in patients with chronic hepatitis B$^{[4, 5]}$. In the present study, we have analyzed the polymorphism of HLA-DRB1, -DQA1 and -DQB1 alleles in patients with chronic and acute hepatitis B and healthy controls using the polymerase chain reaction with sequence specific primers (PCR/SSP). This study aimed at investigating whether these alleles might be associated with susceptibility or resistance to chronic hepatitis B.

MATERIALS AND METHODS

Subjects

Fifty-two patients (43 males, 9 females, mean age: 33.46 years) with chronic hepatitis B and 30 patients (24 males, 6 females, mean age: 33.25 years) with acute hepatitis B, and 106 healthy blood donors (88 males, 18 females, mean age: 31.27 years) were included in this study. All the patients were from the Institute of Infectious Diseases, Southwest Hospital of Third Military Medical University. The diagnosis of all the cases was made according to the criteria established on the Viral Hepatitis Conference held in 2000. All the patients and controls were Chinese Han people without relatives from Chongqing. The subjects were divided into chronic hepatitis B group, acute hepatitis B group and healthy control group.

Primer synthesis and reagents

The polymorphisms of HLA-DRB1, -DQA1 and -DQB1 alleles were assessed by PCR/SSP technique. HLA-DRB1, -DQA1 and -DQB1 loci of specific PCR primers were designed by Olerup et al$^{[6, 7]}$, and synthesized by Shanghai Branch, Canadian Sangon Company. The primers amplifying human growth hormone gene (5’-primer: 5’-GGG TCC CCA ACC ATT CCC TTA-3’, 3’-primer: 5’-TCA CGG ATT TCT GTT GTG TTT-3’) were synthesized by Shanghai Branch, Canadian Sangon Company. Taq DNA polymerase and dNTP were purchased from Shanghai Branch, Canadian Sangon Company, pBR322/Hand III marker and the ReadyPCR™ whole blood genomic DNA purification system were provided by Sino-American Biotechnology Company.

Methods

DNA extraction Genomic DNA was extracted from peripheral blood by using the Ready PCR™ whole blood genomic DNA purification system.
**PCR amplification**

A total amount of 25 µl PCR reaction solution contained 8 pmol each of sequence specific primer (3.2 µl), 0.8 pmol of each internal control primer (0.32 µl), 50-100 ng of genomic DNA (2 µl), 2.5 µl of 10×buffer, 25 mmol/L of MgCl₂ (2.5 µl), 10 mmol/L of dNTP (1 µl), 5 unit/µl of Taq polymerase (0.5 µl) and 13 µl of deminized H₂O. The PCR cycling parameters of HLA-DRB1 alleles were as follows: pre-denaturation at 94 °C for 5 min, denaturation at 94 °C for 50 s, annealing at 65 °C for 1 min, extension at 72 °C for 1 min, and final extension at 72 °C for 5 min. The PCR cycling parameters of HLA-DQA1 and -DQB1 alleles were as follows: pre-denaturation at 94 °C for 4 min, denaturation at 94 °C for 1 min, annealing at 65 °C for 1 min, extension at 72 °C for 1 min, and final extension at 72 °C for 2 min. In each PCR reaction a primer pair was included to amplify the human growth hormone gene, which functioned as an internal positive amplification control and gave rise to a 429 base pair fragment.

**Detection of PCR products** PCR products were loaded in 2 % agarose gel containing 0.5 µg/ml of ethidium bromide, electrophoresed for 20 min at 15 V/cm, examined under ultraviolet light. The individual alleles were assigned for the specific pattern of appropriately sized bands.

**Statistical analysis**

Allele frequencies of HLA-DRB1, -DQA1 and -DQB1 were calculated by direct count. AF for the study group was compared with that for the control group using Chi-square (χ²) test. The Fisher’s exact test was used when χ² value exceeded 3.84, the P values were corrected for the number of alleles (corrected P=PC). Relative risk frequencies (RR) were calculated according to Wolf formula.

**RESULTS**

**HLA-DRB1 alleles in patients with chronic and acute hepatitis B and healthy controls**

The distribution of HLA-DRB1 alleles is shown in Table 1. The allele frequencies of HLA-DRB1*0301 in the chronic hepatitis B group (17.31 %) were markedly higher than those in the normal control group (5.67 %), there was a significant correlation between them (χ²=12.3068, P=0.0074, RR=4.15). The allele frequencies of HLA-DRB1*0401/0411 in the chronic hepatitis B group (0.96 %) were significantly lower than those in the acute hepatitis B group (13.33 %), with significant correlation between them (χ²=11.9206, P=0.0145, RR=18.55). The data of electrophoresis of HLA-DRB1 alleles amplification are shown in Figure 1.

**Table 1 Allele frequency of HLA-DRB1 in patients with chronic and acute hepatitis B and normal healthy individuals**

| HLA-DRB1 allele | Normal control (n=100) | Chronic hepatitis B (n=52) | Acute hepatitis B (n=30) |
|-----------------|------------------------|----------------------------|-------------------------|
| PN AF           | PN AF                  | PN AF                      |
| 0101/0103       | 1 0.47                 | 1 0.96                     | 1 1.67                  |
| 0102            | 12 5.66                | 18 17.31                   | 6 10.00                 |
| 0401/0411       | 24 11.32               | 13 12.50                   | 7 11.67                 |
| 0701/0702       | 11 5.19                | 8 7.69                     | 4 6.67                  |
| 0801/0804       | 9 4.25                 | 6 5.77                     | 3 5.00                  |
| 0901            | 32 15.09               | 16 15.39                   | 8 13.33                 |
| 1001            | 2 0.94                 | 2 1.92                     | 1 1.67                  |
| 1101/1104**     | 13 6.13                | 1 0.96                     | 8 13.33                 |
| 1201/1202       | 34 16.04               | 15 14.42                   | 8 13.33                 |
| 1301/1302       | 4 1.89                 | 1 0.96                     | 1 1.67                  |
| 1303/1304       | 1 0.47                 | 1 0.96                     | 1 1.67                  |
| 1401/1404       | 14 6.60                | 6 5.77                     | 4 6.67                  |
| 1402/1403       | 0 0.00                 | 0 0.00                     | 0 0.00                  |
| 1501/1502       | 34 16.04               | 11 10.58                   | 5 8.33                  |
| 1601/1602       | 13 6.13                | 2 1.92                     | 1 1.67                  |
| Blank           | 8 3.77                 | 3 2.89                     | 2 3.33                  |

**HLA-DQA1 alleles in patients with chronic and acute hepatitis B and healthy controls**

The distribution of HLA-DQA1 alleles is shown in Table 2. The allele frequencies of HLA-DQA1*0501 in the chronic hepatitis B group (25.96 %) were markedly higher than those in the normal control group (13.68 %), there was a significant correlation between them (χ²=9.2002, P=0.0157, RR=2.87). The allele frequencies of HLA-DQA1*0301 in the chronic hepatitis B group (14.42 %) was significantly lower than those in the acute hepatitis B group (30 %), there was a significant correlation between them (χ²=7.6781, P=0.0388, RR=3.70). The data of electrophoresis of HLA-DQA1 alleles amplification are shown in Figure 2.

**Table 2 Allele frequency of HLA-DQA1 in patients with chronic and acute hepatitis B and normal healthy individuals**

| HLA-DQA1 allele | Normal control (n=106) | Chronic hepatitis B (n=52) | Acute hepatitis B (n=30) |
|-----------------|------------------------|----------------------------|-------------------------|
| PN AF           | PN AF                  | PN AF                      |
| 0103            | 17 8.02                | 9 8.65                     | 4 6.67                  |
| 0102            | 45 21.23               | 22 21.15                   | 12 20.00                |
| 0103            | 9 4.25                 | 5 4.81                     | 2 3.33                  |
| 0104            | 3 1.42                 | 1 0.96                     | 1 1.67                  |
| 0201            | 7 3.30                 | 3 2.88                     | 1 1.67                  |
| 0301*           | 57 26.89               | 15 14.42                   | 18 30.00                |
| 0302            | 1 0.47                 | 0 0.00                     | 0 0.00                  |
| 0401            | 2 0.49                 | 1 0.96                     | 1 1.67                  |
| 0501**          | 29 13.68               | 27 25.96                   | 10 16.67                |
| 0601            | 23 10.85               | 12 11.54                   | 6 10.00                 |
| Blank           | 19 8.96                | 9 8.65                     | 5 8.33                  |

**Figure 1 Electrophoresis of HLA-DRB1 alleles amplification by PCR/SSP. M: pBR322DNA/MSP I marker, 1: negative control, 2: 0101/0103, 3: 0301, 4: 0401/0411, 5: 0701/0702, 6: 0801/0804, 7: 0901, 8: 1001, 9: 1101/1104, 10: 1201/1202, 11: 1301/1302, 12: 1303/1304, 13: 1401/1404, 14: 1402/1403, 15: 1501/1502, 16: 1601/1602.**
Electrophoresis of HLA-DQA1 alleles amplification by PCR/SSP. M: pBR322DNA/MSP I marker, 1: negative control, 2: 0101/0104, 3: 0101/0102/0104, 4: 0102/0103, 5: 0103, 6: 0201, 7: 0301, 8: 0302, 9: 0401, 10: 0501, 11: 0601, 12: A (when the amplification product was -DQA1*0104, “A” was negative. When the amplification product was non-DQA1*0104, “A” was positive).

**Figure 2** Electrophoresis of HLA-DQA1 alleles amplification by PCR/SSP. M: pBR322DNA/MSP I marker, 1: negative control, 2: 0101/0104, 3: 0101/0102/0104, 4: 0102/0103, 5: 0103, 6: 0201, 7: 0301, 8: 0302, 9: 0401, 10: 0501, 11: 0601, 12: A (when the amplification product was -DQA1*0104, “A” was negative. When the amplification product was non-DQA1*0104, “A” was positive).

**Table 3** Allele frequency of HLA-DQB1 in patients with chronic and acute hepatitis B and normal healthy individuals

| HLA-DQB1 allele | Normal control (n=106) | Chronic hepatitis B (n=52) | Acute hepatitis B (n=30) |
|-----------------|-------------------------|-----------------------------|--------------------------|
|                 | PN | AF | PN | AF | PN | AF |
| 0201            | 23 | 10.85 | 10 | 9.62 | 6 | 10.00 |
| 0301*           | 40 | 18.87 | 37 | 35.58 | 16 | 26.67 |
| 0302            | 14 | 6.61 | 6 | 5.77 | 3 | 5.00 |
| 0303            | 35 | 16.51 | 15 | 14.42 | 10 | 16.67 |
| 0401            | 11 | 5.19 | 5 | 4.81 | 3 | 5.00 |
| 0402            | 2 | 0.94 | 1 | 0.96 | 1 | 1.67 |
| 0501            | 9 | 4.25 | 3 | 2.88 | 2 | 3.33 |
| 0502            | 20 | 9.43 | 7 | 6.73 | 3 | 5.00 |
| 0503            | 6 | 2.83 | 2 | 1.92 | 1 | 1.67 |
| 0601            | 20 | 9.43 | 7 | 6.73 | 7 | 11.67 |
| 0602            | 12 | 5.66 | 4 | 3.85 | 3 | 5.00 |
| 0603            | 5 | 2.36 | 2 | 1.92 | 1 | 1.67 |
| 0604            | 7 | 3.30 | 2 | 1.92 | 2 | 3.33 |
| Blank           | 8 | 3.77 | 3 | 2.89 | 2 | 3.33 |

PN: positive number, AF: allele frequency. \( \chi^2=45.5938, P=0.0075, RR=4.07. \)

**Table 3** Allele frequency of HLA-DQB1 in patients with chronic and acute hepatitis B and normal healthy individuals

**DISCUSSION**

Host and viral factors undoubtedly influence the clinical expression and behavior of chronic hepatitis B. Attempts to explain the clinical expression and the behavior of chronic hepatitis B by viral factors have shown the importance of viral genotypes and viraemia level for the clinical presentation. However, there remain large inconsistencies, and it is very likely that immune response to hepatitis B virus (HBV) of the host can modify disease outcomes. HLA is a critical genetic factor that determines individual variations of immune response. The ternary structure of HLA molecules and their roles in the control of immune response have been clearly elucidated. There are many reports about statistical associations between HLA and diseases. HLA gene contributes to the host response against HBV. Individuals with different HLA types may differ in susceptibility or resistance to disease, and associations between HLA polymorphism and susceptibility or resistance to diseases have been identified.

Researches on the correlation between HLA and hepatitis B have been performed for many years. Traditional serological method was used in some investigations, but it has become obsolete and inaccurate. To have a better understanding of the disease, correlation between hepatitis B and HLA should be further studied using nucleotide-typing techniques. Therefore, in the present study, we examined the HLA-DRB1, -DQA1 and -DQB1 alleles by PCR/SSP technique in patients with hepatitis B in an attempt to investigate the association between the polymerase of HLA class II gene and hepatitis B. Fourteen HLA-DRB1 alleles, ten HLA-DQA1 alleles and thirteen HLA-DQB1 alleles were detected. The allele frequencies of HLA-DRB1, -DQA1 and -DQB1 in healthy individuals tallied with genetic characteristics of the Han people in southern region of China.

A previous study showed that the allele frequencies of HLA-B8, DR3, A30, DQA1*0501 in patients with chronic hepatitis B were markedly increased, suggesting that these alleles are associated with chronic hepatitis B. Thio et al. [8] found that HBV persistence was significantly associated with class II alleles, DQA1*0501 (OR=2.6) and DQB1*0301 (OR=3.9), the two-locus haplotype consisted of these same two alleles (OR=3) and the three-locus haplotype consisted of DQA1*0501, DQB1*0301 and DRB1*1102 (OR=10.7). The study by Shen et al. suggested that the susceptibility to chronic hepatitis B was strongly associated with HLA-DRB1*10 allele in northern Chinese patients [9]. In the present study, we found that the allele frequencies of HLA-DRB1*0301, -DQA1*0501 and -DQB1*0301 in the chronic hepatitis B group were markedly higher than those in the normal control group, there was a significant correlation between them (Tables 1, 2 and 3). These findings suggest that HLA-DRB1*0301, -DQA1*0501 and -DQB1*0301 are closely associated with the susceptibility to chronic hepatitis B, and may be the susceptible gene.

Cotrina et al. [9] analyzed the HLA-DRB1 genotype in a series of patients with chronic hepatitis B and acute hepatitis B, which further confirmed that HLA-DRB1*1301 and -DRB1*1302 alleles were associated with the clearance of HBV infection and protected people against chronic hepatitis B. Diepolder et al. [10] found that a strong virus-specific CD4+ and CD8+ T lymphocyte response to hepatitis B virus was associated with viral clearance, patients with acute hepatitis B carrying HLA-DR13 had a more vigorous CD4+ T cell response to HBV core than patients not carrying HLA-DR13, suggesting that HLA-DR13 is associated with a self-limited course of HBV infection, and the beneficial effect of HLA-DR13 alleles on the outcome of HBV infection could be explained by a more vigorous HBV core-specific CD4+ T cell response, which might be either due to a more proficient antigen presentation by HLA-
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