Original Article

Association of Human Leukocyte Antigen Polymorphism with Hepatitis B Virus Infection and Genotypes

Shu-Yun Zhang1,2, Hong-Xi Gu1*, Di Li1, Shu-Fen Yang2, Zhao-Hua Zhong1, Xing-Ku Li1 and Xi Jin2
1Department of Microbiology and 2Research Center of the Second Affiliated Hospital, Harbin Medical University, Harbin, China

(Received May 16, 2006. Accepted August 1, 2006)

SUMMARY: Associations were studied between the polymorphism of northern Han Chinese leukocyte antigen (HLA) alleles and the outcomes of hepatitis B virus (HBV) infection and HBV genotypes. HLA-A, B, and DRB1 alleles in peripheral blood mononuclear cells (PBMCs) were detected by polymerase chain reaction (PCR) with sequence-specific primers. The PBMCs were collected from 61 persons who tested positive for hepatitis B surface antigen (HBsAg) for more than 6 months (Persistent group), 32 persons who tested negative for both HBsAg and HBV DNA but positive for both anti-HBc and anti-HBs (Recovered group), and 40 persons who tested negative for all serologic markers of HBV infection (Uninfected group). HBV genotypes in serum specimens from 56 of 61 patients with persistent HBV infection were determined by nested PCR with 6 pairs of HBV genotype-specific primers (A to F). The frequency of HLA-DRB1*12 was significantly higher in the Persistent group than in the Recovered group (P = 0.004). HLA-A*02 was significantly higher in the Recovered group than in the Persistent group (P = 0.044). HLA-DRB1*15 was significantly higher in the HBV genotype B group than in the C group (P = 0.013). These findings suggested that there were associations not only between HLA polymorphisms and outcomes of HBV infection but also between HBV polymorphisms and the infected HBV genotypes.

INTRODUCTION

The hepatitis B virus (HBV) is an important cause of chronic HBV infection (CHB), which affects about 350 million persons and is the leading cause of cirrhosis and hepatocellular carcinoma (HCC) worldwide. HBV infection in adulthood results in viral persistence and the development of chronic hepatitis in approximately 15% of cases, but the factors that determine HBV persistence or clearance are not well understood (1-3). A variety of viral (viral load, genotype, mutation), host (age at infection, gender, immune status), and external (concurrent viral infections, alcohol consumption, chemotherapy) factors influence the risk of persistent HBV infection (4-6). Eight genotypes (A - H) of HBV have been designated, based on genome sequence divergence. Each genotype has a distinct geographical and ethnic distribution. Genotypes A and D infections occur frequently in Africa, Europe, and India, while genotypes B and C are prevalent in Asia. Genotype E is localized in West Africa, and genotype F is found only in Central and South America. The distributions of genotypes G and H are less clear. Remarkable clinical and pathogenic differences exist among HBV genotypes (1). In addition, the host immune response plays a key role in determining the outcomes of HBV infection. The immune response is coordinated by the human leukocyte antigen (HLA) class I and class II molecules, which present viral antigens to CD8+ cytotoxic T cells and CD4+ helper T cells, respectively. The genes encoding these molecules are of the most polymorphism in the human genome and are ideal candidates for investigating associations with outcomes of HBV infection (5,6). Using a Han Chinese cohort with well-defined viral persistence or clearance, we molecularly typed HLA-A, B, and DRB1 alleles as well as HBV genotypes to determine the associations of HLA polymorphisms with the outcomes of HBV infection and infected HBV genotypes.

MATERIALS AND METHODS

Study population: The study included 93 persons who visited the Infectious Disease Department and Health Check Center of the Second Affiliated Hospital of Harbin Medical University, Harbin, China, between July 2003 and October 2005. Of the 93 persons, 61 were hepatitis B surface antigen (HBsAg)-positive for more than 6 months (Persistent group) and 32 were negative for both HBsAg and HBV DNA but positive for both anti-HBc and anti-HBs (Recovered group); 60 persons were males and 33 were females, with a mean age of 38.4 ± 11.9 years. They were diagnosed according to the Virus Hepatitis Diagnosis Standard of China (2000). In addition, 40 persons who were negative for HBsAg, anti-HBs, HBeAg, anti-HBe, anti-HBc, and HBV DNA were enrolled in this study as controls (Uninfected group). Of these, 30 were males and 10 were females, with a mean age of 33.6 ± 7.5 years. None of the subjects were positive for the HCV, HDV, and HIV antibody. None of the participants had a familial relationship with each other. Informed consent was obtained from all participants. This study was approved by the Harbin Medical University Committee on Clinical Investigation.

Laboratory findings: HBV serology was studied with enzyme-linked immunosorbent assay (ELISA) using the HBV Diagnostic Kit (Kehua Co., Shanghai, China). Serum HBV DNA was quantitated by real-time quantitative polymerase chain reaction (PCR) performed in a LightCycler (Roche, Basel, Switzerland) using a commercial kit (PG Biotechnology, Shenzhen, China).
HLa allele typing: Genomic DNA was extracted from peripheral blood mononuclear cells using the guanidine sulfocyanate method with reagents supplied by the Shanghai Blood Center. HLA alleles were determined by a low-resolution DNA typing method, a PCR sequence-specific primer (PCR-SSP) technique developed by the 13th International Histocompatibility Workshop, according to the manufacturer’s instructions (HLA-ABDR SSP kit; Biotest, Dreieich, Germany). The typing results were analyzed with the software supplied by Biotest. This kit can be used to detect HLA A*01~A*08, B*07~B*83, and DRB1*01~DRB1*16.

HBV genotyping: HBV DNA was extracted from sera according to the manufacturer’s instructions (PG Biotechnology). HBV genotypes were determined by nested PCR (Bioer XP Cycler; Bioer, Hangzhou, China) with 6 pairs of HBV genotype-specific primers (A to F) (7,8).

Statistical analysis: The frequencies of HLA alleles were calculated by direct counting. Data were analyzed by the χ²-test and the two-tailed Fisher’s exact test with the SPSS 10.0 data analysis software package (SPSS, Chicago, Ill., USA).

RESULTS

Frequencies of HLA-A, B, and DRB1 alleles in different study groups: Thirteen HLA-A alleles, 17 HLA-B alleles, and 12 HLA-DRB1 alleles were detectable in the three study groups (Table 1). The most frequent HLA-A allele was A*02 (frequency of alleles [AF] = 0.252), while A*11 and A*24 were also frequent (AF = 0.117 and AF = 0.124). The frequencies of the HLA-B and DRB1 alleles were the same. B*13 and B*40 were the most frequent HLA-B alleles (AF = 0.128 and AF = 0.139), DRB1*07, DRB1*09, DRB1*12, and DRB1*15 were the same HLA-DRB1 alleles (AF = 0.120, AF = 0.139, AF = 0.143, and AF = 0.113, respectively).

Comparison of frequency of HLA-A, B, and DRB1 alleles between the Persistent group and the Recovered group: The frequency distributions of HLA-A, B, and DRB1 alleles were compared in the Persistent group and the Recovered group, and the detectable alleles with total AF ≥ 0.20 are listed in Table 2. A*02 was the most frequent HLA-A allele in both groups. Moreover, A*02 was more frequent in the Recovered group than in the Persistent group, with a statistically significant difference (0.360 versus 0.221, P = 0.044) by the χ²-test. It was also borderline significant (P = 0.055) by the Fisher’s exact test. This suggested A*02 might be associated with HBV clearance, with an OR of 0.507 (95% CI = 0.260-0.986). In addition, the frequency of HLA-DRB1*12 was significantly higher in the Persistent group than in the Recovered group (0.230 versus 0.063, P = 0.004). It was still significant (P = 0.004) by the Fisher’s exact test. This suggested DRB1*12 might be associated with persistent HBV infection, resulting in an OR of 4.468 (95% CI = 1.492 - 13.377). Though the frequencies of HLA-A*31, B*13, and DRB1*07 were higher and those of HLA-B*46, B*51, and DRB1*14 were lower in the Persistent group than in the Recovered group, no statistically significant difference was observed (P > 0.05).

Analysis of HBV genotypes: Among the 61 serum specimens in the Persistent group, 56 specimens were HBV DNA ≥ 1.0 × 10⁵ copies/mL and genotyped. The most common genotype among the genotyped specimens was C, which accounted for 89.3% (50/56), while genotype B accounted for 10.7% (6/56). Other genotypes were not detected (genotypes A, D, E, and F). No statistically significant differences were observed in age, sex, HBeAg positive ratio, and serum DNA levels between genotypes B and C (Table 3).

Comparison of frequencies of HLA-A, B, and DRB1 alleles between HBV genotypes B and C in the Persistent group: The PBMC samples from the 56 HBV-genotyped patients in the Persistent group were detected for HLA-A, B, and DRB1 alleles. The frequency distributions of HLA-A, B, and DRB1 in HBV genotypes B and C are listed in Table 4. Comparing HLA alleles between HBV genotypes B and C, four HLA alleles were significantly higher in HBV genotype B: HLA-A*31 (0.167 versus 0.030, P = 0.030), A*33 (0.250 versus 0.070, P = 0.039), B*52 (0.167 versus 0.020, P = 0.032), and DRB1*12 (0.120 versus 0.057, P = 0.032). Moreover, A*11 was more frequent in genotype B and significantly lower in genotype C (AF = 0.117 versus 0.213, P = 0.004), and DRB1*07 was higher and those of HLA-B*46, B*51, and DRB1*14 were lower in the Persistent group than in the Recovered group, no statistically significant difference was observed (P > 0.05).
0.010), and DRB1*15 (0.333 versus 0.090, \( P = 0.013 \)). By the two-tailed Fisher's exact test, DRB1*15 was significantly more frequent only in HBV genotype B (0.333 versus 0.090, \( P = 0.033 \)). HLA-A*02 and DRB1*12 were more frequent in HBV genotype C, but there was no statistically significant difference with HBV genotype B (0.240 versus 0.083, \( P = 0.218 \)). These results suggested that DRB1*15 might be associated with HBV persistence in persons infected with HBV genotype B (OR = 5.056, 95% CI = 1.269 - 20.133).

**DISCUSSION**

Host and viral factors undoubtedly influence the outcomes of HBV infections. HLA polymorphisms and viral genotypes, representing the host and viral factors, respectively, have shown their importance in the progress of HBV infections (1,6). In this study on the correlations between HLA polymorphisms and the outcomes of HBV infection and viral genotypes, the correlation between HLA-DRB1*12 and the HBV genotype distribution and characteristics of the patients in Persistent group

### Table 2. Comparison of frequencies of HLA-A, B, DRB1 alleles between Persistent group and Recovered group

<table>
<thead>
<tr>
<th>HLA alleles</th>
<th>Persistent group</th>
<th>Recovered group</th>
<th>( P )</th>
<th>OR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>A0</td>
<td>4 (0.033)</td>
<td>2 (0.031)</td>
<td></td>
<td>1.051</td>
<td>0.187 - 5.898</td>
</tr>
<tr>
<td>B0</td>
<td>27 (0.221)</td>
<td>23 (0.360)</td>
<td>0.044</td>
<td>0.507</td>
<td>0.260 - 0.986</td>
</tr>
<tr>
<td>C0</td>
<td>8 (0.066)</td>
<td>4 (0.063)</td>
<td></td>
<td>1.053</td>
<td>0.305 - 3.638</td>
</tr>
<tr>
<td>11</td>
<td>15 (0.123)</td>
<td>7 (0.109)</td>
<td></td>
<td>1.142</td>
<td>0.440 - 2.960</td>
</tr>
<tr>
<td>24</td>
<td>18 (0.148)</td>
<td>8 (0.125)</td>
<td></td>
<td>1.212</td>
<td>0.496 - 2.962</td>
</tr>
<tr>
<td>26</td>
<td>5 (0.041)</td>
<td>1 (0.016)</td>
<td></td>
<td>2.692</td>
<td>0.303 - 23.551</td>
</tr>
<tr>
<td>30</td>
<td>9 (0.074)</td>
<td>3 (0.047)</td>
<td></td>
<td>1.619</td>
<td>0.423 - 6.205</td>
</tr>
<tr>
<td>31</td>
<td>8 (0.066)</td>
<td>1 (0.016)</td>
<td></td>
<td>4.421</td>
<td>0.541 - 36.157</td>
</tr>
<tr>
<td>32</td>
<td>3 (0.025)</td>
<td>1 (0.016)</td>
<td></td>
<td>1.588</td>
<td>0.162 - 15.585</td>
</tr>
<tr>
<td>33</td>
<td>10 (0.082)</td>
<td>4 (0.063)</td>
<td></td>
<td>1.339</td>
<td>0.403 - 4.452</td>
</tr>
<tr>
<td>35</td>
<td>6 (0.049)</td>
<td>2 (0.031)</td>
<td></td>
<td>1.051</td>
<td>0.279 - 3.791</td>
</tr>
<tr>
<td>44</td>
<td>9 (0.074)</td>
<td>4 (0.060)</td>
<td></td>
<td>0.170</td>
<td>0.054 - 0.585</td>
</tr>
<tr>
<td>46</td>
<td>4 (0.033)</td>
<td>2 (0.031)</td>
<td></td>
<td>1.051</td>
<td>0.353 - 2.986</td>
</tr>
<tr>
<td>48</td>
<td>3 (0.025)</td>
<td>1 (0.016)</td>
<td></td>
<td>1.051</td>
<td>0.162 - 15.585</td>
</tr>
<tr>
<td>51</td>
<td>8 (0.066)</td>
<td>3 (0.047)</td>
<td></td>
<td>1.195</td>
<td>0.403 - 3.791</td>
</tr>
<tr>
<td>52</td>
<td>6 (0.049)</td>
<td>2 (0.031)</td>
<td></td>
<td>1.051</td>
<td>0.279 - 3.791</td>
</tr>
<tr>
<td>53</td>
<td>5 (0.042)</td>
<td>2 (0.031)</td>
<td></td>
<td>1.051</td>
<td>0.279 - 3.791</td>
</tr>
<tr>
<td>55</td>
<td>2 (0.016)</td>
<td>1 (0.016)</td>
<td></td>
<td>1.051</td>
<td>0.279 - 3.791</td>
</tr>
<tr>
<td>57</td>
<td>8 (0.066)</td>
<td>3 (0.047)</td>
<td></td>
<td>1.051</td>
<td>0.279 - 3.791</td>
</tr>
<tr>
<td>58</td>
<td>4 (0.033)</td>
<td>2 (0.031)</td>
<td></td>
<td>1.051</td>
<td>0.279 - 3.791</td>
</tr>
<tr>
<td>60</td>
<td>3 (0.025)</td>
<td>1 (0.016)</td>
<td></td>
<td>1.051</td>
<td>0.279 - 3.791</td>
</tr>
<tr>
<td>61</td>
<td>2 (0.016)</td>
<td>1 (0.016)</td>
<td></td>
<td>1.051</td>
<td>0.279 - 3.791</td>
</tr>
<tr>
<td>62</td>
<td>1 (0.008)</td>
<td>3 (0.047)</td>
<td></td>
<td>1.051</td>
<td>0.279 - 3.791</td>
</tr>
</tbody>
</table>

**Table 3.** HBV genotype distribution and characteristics of the patients in Persistent group.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Men/women</th>
<th>Age (mean ± SD)</th>
<th>HBsAg* (%)</th>
<th>HBV DNA (×10^6 copies/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>B</td>
<td>6 (10.7)</td>
<td>5/1</td>
<td>38.1 ± 10.2</td>
<td>4 (66.7)</td>
</tr>
<tr>
<td>C</td>
<td>50 (89.3)</td>
<td>41/9</td>
<td>39.4 ± 11.1</td>
<td>37 (74.0)</td>
</tr>
</tbody>
</table>

0.010), and DRB1*15 (0.333 versus 0.090, \( P = 0.013 \)). By the two-tailed Fisher's exact test, DRB1*15 was significantly more frequent only in HBV genotype B (0.333 versus 0.090, \( P = 0.033 \)). HLA-A*02 and DRB1*12 were more frequent in HBV genotype C, but there was no statistically significant difference with HBV genotype B (0.240 versus 0.083, \( P = 0.218 \)). These results suggested that DRB1*15 might be associated with HBV persistence in persons infected with HBV genotype B (OR = 5.056, 95% CI = 1.269 - 20.133).

**DISCUSSION**

Host and viral factors undoubtedly influence the outcomes of HBV infections. HLA polymorphisms and viral genotypes, representing the host and viral factors, respectively, have shown their importance in the progress of HBV infections (1,6). In this study on the correlations between HLA polymorphisms and the outcomes of HBV infection and viral genotypes, the correlation between HLA-DRB1*12 and the outcome of HBV infection was more than fourfold higher in the Persistent group than in the Recovered group (OR = 4.468, \( P = 0.004 \)). That of HLA-A*02 was nearly twofold higher in the Persistent group than in the Recovered group (OR = 4.468, \( P = 0.004 \)). By the two-tailed Fisher's exact test, DRB1*15 was more than fivefold higher in HBV genotype B than in genotype C in the Persistent group (OR = 5.056, \( P = 0.013 \)). These findings suggested that there were relationships not only between the specific HLA alleles and outcomes of HBV infections, but also between the specific HLA alleles and the genotype of infected HBV in Northern Han Chinese with persistent HBV infection in China.

Although previous reports found that HLA-B*08, B*35,
alleles between HBV genotypes B and C in the Persistent genotype B and those with genotype C (21). Comparing HLA ence in characteristics was observed between patients with was the majority (89.3%). No statistically significant differ-
genotypes in the Persistent group. In this study, genotype C
relationships between HLA polymorphisms and infected HBV
infection, we examined the Northern China.
showed that HLA-DRB1*12 might be correlated with viral
persistence in Han Chinese in Northern China.
HLA-DRB1*1301/1302 was reported to be associated with
acute self-limited hepatitis B or with spontaneous viral clear-
ance after HBV infection in Gambians (18), Europeans (19),
and Koreans (20). In this study, however, the frequency of
HLA-DRB1*13 was low in both the Persistent group (0.016)
and the Recovered group (0.016) without a statistical differ-
ence. In addition, A*02 was the most frequent HLA-A allele
in either group. Moreover, A*02 was significantly higher in
the Recovered group than in the Persistent group (0.360
versus 0.221, \( P = 0.044 \)). Even by Fisher’s exact test, the
difference was borderline significant (\( P = 0.055 \)). These data
suggested that HLA-A*02 might be somehow related to
viral clearance following HBV infection in Han Chinese in
Northern China.

Besides analysis of the association between HLA polymor-
phisms and outcomes of HBV infection, we examined the
relationships between HLA polymorphisms and infected HBV
genotypes in the Persistent group. In this study, genotype C
was the majority (89.3%). No statistically significant differ-
ence in characteristics was observed between patients with
genotype B and those with genotype C (21). Comparing HLA
a alleles between HBV genotypes B and C in the Persistent
group, DRB1*15 was significantly more frequent in HBV
genotype B (0.333 versus 0.090, \( P = 0.033 \)). This suggested that
HLA-DRB1*15 might be correlated to viral persistence in
patients infected with HBV genotype B. DRB1*15 was
relatively more frequent in the Persistent group (0.107) and
the Recovered group (0.125), but did not differ between the
two groups (\( P > 0.05 \)) (Table 2). Studies of DRB1*15 sub-
types are needed to elucidate whether or not the different
DRB1*15 subtypes may contribute to the difference in
DRB1*15 between HBV genotypes B and C in the Persistent
group. The clinical significance of these results needs to be
confirmed by further research, such as follow-up studies on
newly HBV-infected persons and their relatives.

Our results suggested that HLA-DRB1*12 might be corre-
lated with viral persistence, HLA-A*02 might be somehow
related with viral clearance after HBV infection, and HLA-
DRB1*15 might be correlated with the viral persistence in
patients infected with HBV genotype B in Han Chinese
in Northern China. These findings imply that various HLA
molecules could present different HBV epitopes, which
may partly come from different HBV genotypes, to induce
effective immune responses or to cause immune-tolerance
responses.

ACKNOWLEDGMENTS

We thank Dr. Shu-Chen Li, Shu-Lan Lu, Xiao-Yan Wang,
et al. for providing clinical samples. We are grateful to Dr.
Ren Rui for data analysis.

This work was supported by Specialized Research Fund for the Doctoral Program of Higher Education (No. 20050226002), the Natural Science Foundation of Heilongjiang Province (No. D0307), and Foundation of Health Department of Heilongjiang Province (2005 - 311).

REFERENCES

1. Pan, C.Q. and Zhang, J.X. (2005): Natural history and
clinical consequences of hepatitis B virus infection. Int.
2. Tran, T.T. and Martin, P. (2004): Hepatitis B: epidemi-
epidemiology and immunology of hepatitis B virus
on human genetic alleles associated with hepatitis B virus
infection. World J. Gastroenterol., 9, 641-644.
responses and therapy in chronic hepatitis B. J. Hepatol.,
39, 631-634.
Influence of genetic factors on the susceptibility to HBV
infection, its clinical pictures, and responsiveness to HBV
specific genotyping system for hepatitis B virus corres-
sponding to six major genotypes by PCR using type-
Epidemiology of HBV genotypes by nested PCR with
multi-paired primers. World Chin. J. Digestol., 12, 1073-
9. Thio, C.L., Thomas, D.L., Karacki, P., Gao, X., Marti,
D., Kaslow, R.A., Goedert, J.J., Hilgarter, M., Stratthdee,
S.A., Duggal, P., O’Brien, S.J., Astemborski, J. and
I and class II HLA antigens and chronic hepatitis B virus
infection. J. Virol., 77, 12083-12087.
Influence of HLA-DRB1 alleles and HBV genotypes on
interferon-a therapy for chronic hepatitis B. World J.
Gastroenterol., 11, 4753-4757.
Association between HLA class II gene and suscepti-
bility or resistance to chronic hepatitis B. World J.
Gastroenterol., 9, 2221-2225.
polymorphism of human leucocyte antigen-DRB1,
-DQA1 and -DQB1 alleles in patients with hepatitis B.
II genes with the susceptibility to hepatitis B virus
infection and the response to interferon in HBV-infected
patients. World J. Gastroenterol., 11, 5721-5724 (in
Chinese).
14. Han, Y.N., Yang, J.L., Zheng, S.G., Tang, Q. and Zhu,
II genes with the susceptibility to hepatitis B virus
infection. World Chin. J. Digestol., 12, 255-266.
15. Chen, H.G., Jiang, G.F., Meng, X.Q., Ma, Y.L. and Liu,


