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Original Article

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

Shu-Yun Zhang[1,2], Hong-Xi Gu[1]*, Di Li[1], Shu-Fen Yang[2], Zhao-Hua Zhong[1], Xing-Ku Li[2] and Xi Jin[2]

[1]Department of Microbiology and [2]Research Center of the Second Affiliated Hospital, Harbin Medical University, Harbin, China

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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 HLA 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.

*Corresponding author: Mailing address: Department of Microbiology, Harbin Medical University, Harbin 150086, Heilongjiang Province, China. Tel & Fax: +86-451-86685122, E-mail: hxgu2432@163.com

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 χ^2-test and the two-tailed Fisher's exact test with the SPSS 10.0 data analysis software package (SPSS, Chicago, Ill., USA). $P < 0.05$ was considered statistically significant. The odds ratio (OR), which reflects the likelihood of a subject carrying a specific allele, and the 95% confidence interval (95% CI) were calculated.

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 most frequent 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 ≧ 0.020 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 χ^2 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 ≧ 1.0×10^3 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, $P =$

Table 1. Frequencies of HLA-A, B, DRB1 alleles in study groups

HLA alleles		Total		Persistent group		Recovered group		Uninfected group	
		$n = 133$	AF[1)]	$n = 61$	AF	$n = 32$	AF	$n = 40$	AF
A	01	13	0.049	4	0.033	2	0.031	7	0.088
	02	67	**0.252**	27	0.221	23	0.360	17	0.213
	03	21	0.079	8	0.066	4	0.063	9	0.113
	11	31	**0.117**	15	0.123	7	0.109	9	0.113
	24	33	**0.124**	18	0.148	8	0.125	7	0.088
	26	8	0.030	5	0.041	1	0.016	2	0.025
	29	3	0.011	0	0.000	1	0.016	2	0.025
	30	17	0.064	9	0.074	3	0.047	5	0.063
	31	13	0.049	8	0.066	1	0.016	4	0.050
	32	5	0.019	3	0.025	1	0.016	1	0.013
	33	19	0.071	10	0.082	4	0.063	5	0.063
	68	6	0.023	3	0.025	1	0.016	2	0.025
	69	3	0.011	0	0.000	0	0.000	3	0.037
B	07	8	0.030	4	0.033	2	0.031	2	0.025
	13	34	**0.128**	21	0.172	5	0.078	8	0.100
	15	21	0.079	11	0.090	6	0.094	4	0.050
	27	5	0.019	1	0.008	0	0.000	4	0.050
	35	26	0.098	8	0.066	5	0.078	13	0.163
	37	3	0.011	1	0.008	0	0.000	2	0.025
	38	5	0.019	2	0.016	1	0.016	2	0.025
	40	37	**0.139**	21	0.172	8	0.125	8	0.100
	44	16	0.060	9	0.074	4	0.063	3	0.037
	46	20	0.075	6	0.049	7	0.109	7	0.088
	48	9	0.034	3	0.025	3	0.047	3	0.037
	51	18	0.068	8	0.066	8	0.125	2	0.025
	52	8	0.030	4	0.033	1	0.016	3	0.037
	55	7	0.026	3	0.025	2	0.031	2	0.025
	56	4	0.015	0	0.000	1	0.016	3	0.037
	57	4	0.015	3	0.025	0	0.000	1	0.013
	58	10	0.036	4	0.033	2	0.031	4	0.050
DRB1	01	10	0.036	4	0.033	2	0.031	4	0.050
	03	7	0.026	3	0.025	3	0.047	1	0.013
	04	21	0.079	10	0.082	7	0.109	4	0.050
	07	32	**0.120**	17	0.139	4	0.063	11	0.138
	08	18	0.068	8	0.066	5	0.078	5	0.063
	09	37	**0.139**	18	0.148	10	0.156	9	0.113
	11	17	0.064	7	0.057	4	0.063	6	0.075
	12	38	**0.143**	28	0.230	4	0.063	6	0.075
	13	10	0.036	2	0.016	1	0.016	7	0.088
	14	15	0.056	4	0.033	6	0.094	5	0.063
	15	30	**0.113**	13	0.107	8	0.125	9	0.113
	16	5	0.019	1	0.008	3	0.047	1	0.013

[1)]: AF, frequency of alleles. AF ≧ 0.10 are shown in bold.

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

HLA alleles		Persistent group		Recovered group		$P^{1)}$	OR	95% CI
		$n = 61$	AF	$n = 32$	AF			
A	01	4	0.033	2	0.031	–	1.051	0.187 - 5.898
	02	27	0.221	23	0.360	**0.044**	0.507	0.260 - 0.986
	03	8	0.066	4	0.063	–	1.053	0.305 - 3.638
	11	15	0.123	7	0.109	–	1.142	0.440 - 2.960
	24	18	0.148	8	0.125	–	1.212	0.496 - 2.962
	26	5	0.041	1	0.016	–	2.692	0.308 - 23.551
	30	9	0.074	3	0.047	–	1.619	0.423 - 6.205
	31	8	0.066	1	0.016	0.132	4.421	0.541 - 36.157
	32	3	0.025	1	0.016	–	1.588	0.162 - 15.585
	33	10	0.082	4	0.063	–	1.339	0.403 - 4.452
	68	3	0.025	1	0.016	–	1.588	0.162 - 15.585
B	07	4	0.033	2	0.031	–	1.051	0.187 - 5.898
	13	21	0.172	5	0.078	0.079	2.453	0.879 - 6.851
	15	11	0.090	6	0.094	–	0.958	0.337 - 2.722
	35	8	0.066	5	0.078	–	0.828	0.259 - 2.643
	40	21	0.172	8	0.125	–	1.455	0.605 - 3.500
	44	9	0.074	4	0.063	–	1.195	0.353 - 4.041
	46	6	0.049	7	0.109	0.126	0.421	0.135 - 1.311
	48	3	0.025	3	0.047	–	0.513	0.100 - 2.616
	51	8	0.066	8	0.125	0.170	0.491	0.175 - 1.377
	52	4	0.033	1	0.016	–	2.136	0.234 - 19.517
	55	3	0.025	2	0.031	–	0.782	0.127 - 4.801
	57	3	0.025	0	0.000	0.206	–	–
	58	4	0.033	2	0.031	–	1.051	0.187 - 5.898
DRB1	01	4	0.033	2	0.031	–	1.051	0.187 - 5.898
	03	3	0.025	3	0.047	–	0.513	0.100 - 2.616
	04	10	0.082	7	0.109	–	0.727	0.263 - 2.011
	07	17	0.139	4	0.063	0.116	2.429	0.781 - 7.551
	08	8	0.066	5	0.078	–	0.828	0.259 - 2.643
	09	18	0.148	10	0.156	–	0.935	0.403 - 2.165
	11	7	0.057	4	0.063	–	0.913	0.257 - 3.243
	12	28	0.230	4	0.063	**0.004**	4.468	1.492 - 13.377
	13	2	0.016	1	0.016	–	1.050	0.093 - 11.805
	14	4	0.033	6	0.094	0.080	0.328	0.089 - 1.207
	15	13	0.107	8	0.125	–	0.835	0.327 - 2.133
	16	1	0.008	3	0.047	0.084	0.168	0.017 - 1.650

[1)]: Based on χ^2 test. $P < 0.05$ are shown in bold; those ≧ 0.30 have been omitted (–).
AF, frequency of alleles; OR, odds ratio; 95% CI, 95% confidence interval.

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

Genotype	*n* (%)	Men/ women	Age (mean ± SD)	HBeAg$^+$ (%)	HBV DNA ($\times 10^6$ copies/mL)
B	6 (10.7)	5/1	38.1 ± 10.2	4 (66.7)	12.9 ± 21.0
C	50 (89.3)	41/9	39.4 ± 11.1	37 (74.0)	11.3 ± 22.3

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 HBV genotype B (OR = 5.056, 95% CI = 1.269 - 20.133).

Table 4. Comparison of frequencies of HLA-A, B, DRB1 alleles between HBV genotypes B and C in Persistent group

HLA alleles		B		C		$P^{1)}$	OR	95% CI
		$n = 6$	AF	$n = 50$	AF			
A	01	0	0.000	4	0.040	–	0.000	–
	02	1	0.083	24	0.240	0.218	0.288	0.035 - 2.346
	03	2	0.167	5	0.050	0.115	3.800	0.651 - 22.188
	11	2	0.167	13	0.130	–	1.338	0.263 - 6.805
	24	1	0.083	13	0.130	–	0.608	0.072 - 5.112
	26	0	0.000	5	0.050	–	0.000	–
	30	0	0.000	9	0.090	0.279	0.000	–
	31	2	0.167	3	0.030	**0.030**	6.467	0.963 - 43.406
	32	0	0.000	3	0.030	–	0.000	–
	33	3	0.250	7	0.070	**0.039**	4.429	0.973 - 20.162
	68	0	0.000	3	0.030	–	0.000	–
B	07	0	0.000	4	0.040	–	0.000	–
	13	1	0.083	18	0.180	–	0.388	0.047 - 3.188
	15	2	0.167	6	0.060	0.175	3.133	0.557 - 17.639
	35	2	0.167	5	0.050	0.115	3.800	0.651 - 22.188
	38	0	0.000	2	0.020	–	0.000	–
	40	1	0.083	18	0.180	–	0.388	0.047 - 3.188
	44	2	0.167	7	0.070	0.244	2.657	0.485 - 14.566
	46	0	0.000	5	0.050	–	0.000	–
	48	0	0.000	3	0.030	–	0.000	–
	51	0	0.000	7	0.070	–	0.000	–
	52	2	0.167	2	0.020	**0.010**	9.800	1.243 - 77.287
	55	0	0.000	3	0.030	–	0.000	–
	57	0	0.000	3	0.030	–	0.000	–
	58	1	0.083	3	0.030	–	2.939	0.281 - 30.744
DRB1	01	0	0.000	4	0.040	–	0.000	–
	03	0	0.000	2	0.020	–	0.000	–
	04	1	0.083	9	0.090	–	0.919	0.106 - 7.960
	07	2	0.167	15	0.150	–	1.133	0.226 - 5.695
	08	0	0.000	6	0.060	–	0.000	–
	09	3	0.250	12	0.120	0.212	2.444	0.580 - 10.308
	11	1	0.083	5	0.050	–	1.727	0.185 - 16.159
	12	1	0.083	24	0.240	0.218	0.288	0.035 - 2.346
	13	0	0.000	2	0.020	–	0.000	–
	14	0	0.000	4	0.040	–	0.000	–
	15	4	0.333	9	0.090	**0.013**	5.056	1.269 - 20.133

[1)]: See footnote [1)] of Table 2.
Abbreviations are in Table 2.

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 Recovered group than that in the Persistent group (OR = 1/0.507, $P = 0.044$). HLA-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,

DRB1*03, DRB1*07, and DRB1*09 were significantly more frequent in hepatitis B carriers and patients of chronic active hepatitis (CAH) (6,9-15), this was not the case in the present study. HLA-B*08 was not included in the analysis of this study due to its total AF < 0.020. Furthermore, we found that HLA-DRB1*12 was significantly more frequent in the Persistent group than in the Recovered group (0.230 versus 0.063, $P = 0.004$). This was consistent with the studies on Han Chinese in Taiwan and Hubei, China (16,17). This result 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 clearance 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 difference. 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 polymorphisms 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 difference in characteristics was observed between patients with genotype B and those with genotype C (21). Comparing HLA 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 subtypes 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 correlated with viral persistence, HLA-A*02 might be somehow correlated 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.

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