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• *BRIEF REPORTS* •

Influence of HLA-DRB1 alleles and HBV genotypes on interferon-α therapy for chronic hepatitis B

Rui-Hai Chu, Li-Xian Ma, Gang Wang, Li-Hua Shao

Rui-Hai Chu, Li-Xian Ma, Gang Wang, Li-Hua Shao, Department of Infectious Diseases, Qilu Hospital of Shandong University, Jinan 250012, Shandong Province, China
Correspondence to: Dr. Rui-Hai Chu, Department of Infectious Diseases, Qilu Hospital of Shandong University, Jinan 250012, Shandong Province, China. rhchu@163.com
Telephone: +86-531-8380313
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Abstract

AIM: To investigate the influence of HLA-DRB$_1$ alleles and HBV genotypes on interferon-α therapy for chronic hepatitis B.

METHODS: HLA-DRB1*03, *07, *09, *12, *15 alleles were determined using polymerase chain reaction/sequence specific primer (PCR/SSP) technique in 126 patients with chronic hepatitis B and 76 normal control subjects in Shandong Province, and HBV genotypes were determined by nested-PCR analysis using type-specific primers in 126 patients.

RESULTS: The positivity of HLA-DRB1*07 allele in chronic hepatitis B group was significantly higher than that in normal control group ($\chi^2 = 6.33$, $P<0.025$, $RR = 2.37$). Among the 126 patients, genotype B was found in 38 (30.2%), genotype C in 69 (54.8%), and mixed genotype (B+C) in 19 (15.0%), genotypes D-F were not found. Among the 46 DRB1*07(+) patients, 7 were responders and 39 were non-responders among them ($\chi^2 = 6.71$, $P<0.05$). The positivity of HLA-DRB1*07 and prevalence of HBV genotype C were significantly higher in non-responders than in responders.

CONCLUSION: High positivities of HLA-DRB1 *07 allele and HBV genotype C are closely associated with the lower response to interferon-α therapy for chronic hepatitis B.

Key words: HLA-DRB1 alleles; HBV genotypes; Interferon-α therapy; Chronic hepatitis B

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INTRODUCTION

Hepatitis B virus (HBV) is a well-known agent of acute and chronic hepatitis. Seventy-five percent of the estimated 400 million patients with chronic HBV infection in the world are Asians[1]. Transient infections may cause fatal, fulminant hepatitis and chronic infections may lead to liver cirrhosis and even hepatocellular carcinoma.

Interferon-α (IFN-α) has anti-viral, immuno-modulatory, anti-proliferative, and gene induction properties. Treatment with interferon-α results in the clearance of HBeAg, and is associated with improved clinical outcomes[2]. It is well-known that several factors influence the response, including viral factors (particularly serum HBV-DNA loads), host factors, which may be genetic (sex, cytokine polymorphism), other factors such as age, serum aminotransferase activities, grade of inflammatory activity in liver, presence of cirrhosis, and race. The aim of this study was to identify the influence of HLA-DRB1 alleles and HBV genotypes on the response to interferon-α therapy.

MATERIALS AND METHODS

Subjects

A total of 126 patients (94 males, 32 females, mean age: 33.2±10.6 years) with chronic hepatitis B and 76 healthy controls (54 males, 22 females, mean age: 33.8±9.8 years) were enrolled in this study. These patients received treatment in the Department of Infectious Diseases, Qilu Hospital of Shandong University, China. The diagnoses of these patients were made according to the criteria established in the National Viral Hepatitis Conference of China (2000). All the patients and controls were Chinese Han people from Shandong Province. HCV infection, auto-immune hepatitis and metabolic disorders were excluded by appropriate clinical and laboratory evaluations. They had no histories of malignancy or depression, immunodeficiency virus infection, liver cirrhosis, decompensated liver diseases, use of IFN and thyroid abnormality, chemotherapy, or other agents that could influence treatment outcomes. HLA-DRB1*03, *07, *09, *12, *15 alleles were determined in all patients and healthy controls. HBV genotypes were detected only in patients.

The 126 patients received IFN-α treatment subcutaneously at a dose of 3 MU thrice weekly for 24 wk. The response to IFN-α therapy was defined as loss of HBeAg, sero-conversion to anti-HBe, normalization of serum ALT levels and HBV-DNA (-). The patients with a response were described as 'responders' and those without response as 'non-responders' in this report.

Serological tests

Sera were tested for ALT, HBeAg, anti-HBeAg, and HBV-

DNA. The remaining sera were stored at -70 ℃.

Genotyping of HLA alleles

Genomic DNA was extracted from frozen blood clots by proteinase K digestion followed by phenol/chloroform extraction and ethanol precipitation. DNA was typed for HLA class II alleles DRB1*03, *07, *09, *12, *15 by polymerase chain reaction (PCR), using published sequence-specific primers[2]. A total amount of 50 μL PCR reaction solution contained 1 μL of each sequence specific primer (10 μmol/L), 2 μL of genomic DNA (60-80 ng/μL), 5 μL of 10× buffer, 5 μL of $MgCl_2$ (15 mmol/L), 5 μL of dNTP (2 mmol/L), 0.4 μL of Taq polymerase (5 U/μL) and 30.6 μL of deionized H_2O. The PCR cycling parameters of HLA-DRB1 alleles were as follows: pre-denaturation at 94 ℃ for 5 min, denaturation at 94 ℃ for 30 s, annealing at 55 ℃ for 1 min, extension at 72 ℃ for 50 s, repetition for 30 cycles and final extension at 72 ℃ for 5 min.

Genotyping of HBV

Nucleic acid of HBV was extracted from 200 μL serum samples by heating at 100 ℃ for 10 min and the HBV genome was amplified by nested PCR using universal primers for the outer primers, followed by two different mixtures containing type-specific inner primers[3]. The first round of PCR was carried out in a tube containing 40 μL of a reaction buffer containing 4 μL of HBV-DNA, 4 μL of 10× buffer, 4 μL of $MgCl_2$ (15 mmol/L), 4 μL of dNTPs (10 mmol/L), 0.2 μL of Taq polymerase (5 U/μL), 4 μL of each outer primer (12.5 μg/mL), and 15.8 μL of deionized H_2O. The thermocycler was programmed to incubate the samples for 2 min at 94 ℃, followed by 35 cycles, each at 94 ℃ for 15 s, at 58 ℃ for 30 s, at 72 ℃ for 30 s, and final extension at 72 ℃ for 7 min. Two second rounds of PCR were performed for each sample, with the common universal anti-sense primers for mixA and mixB. Two microliters of the first PCR product was added to two tubes containing the second sets of each of the inner primer pairs, each of the deoxynucleotides, Taq polymerase and PCR buffer, as in the first reaction, then amplified with the following parameters: preheating at 94 ℃ for 2 min, then followed by 35 cycles, each at 94 ℃ for 15 s, at 60 ℃ for 30 s, at 72 ℃ for 30 s, and final extension at 72 ℃ for 7 min.

HLA-DRB1*03, *07, *09, *12, *15 alleles and genotypes of HBV for each sample were determined by identifying the genotype-specific DNA bands. All the primers were synthesized by Shanghai Branch, Canadian Sangon Company. Taq DNA polyenzyme and dNTPs were also purchased from Shanghai Branch, Canadian Sangon Company. pUC19 DNA/*Msp*I (*Hpa*II) marker 23 was purchased from MBI Fermentas. Amplifications of all samples were performed in a GeneAmp PCR system (GeneAmp PCR system 9600; Perkin Elmer).

Detection of PCR products

PCR products were electrophoresed on 2% agarose gel, stained with ethidium bromide, and evaluated under ultraviolet light. The sizes of PCR products were estimated according to the pUC19 DNA/*Msp*I (*Hpa*II) marker 23.

Statistical analysis

The positivity of HLA-DRB1 alleles was calculated by direct count. The positive number (PN) of the study group was compared with that of the control group using χ^2-test. Relative risk frequencies (RR) were calculated according to Wolf formula. $P<0.05$ was considered statistically significant.

RESULTS

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

The distribution of HLA-DRB1*03, *07, *09, *12, *15 alleles in patients with chronic hepatitis B and healthy controls is shown in Table 1. The positivity of HLA-DRB1*07 allele in patients with chronic hepatitis B group was markedly higher than that in normal controls, there was a significant difference between them. The positivity of HLA-DRB1*03, *09, *12, *15 alleles in patients with chronic hepatitis B was not different from that in normal controls. The electrophoresis of HLA-DRB1*03, *07, *09, *12, *15 alleles is shown in Figure 1.

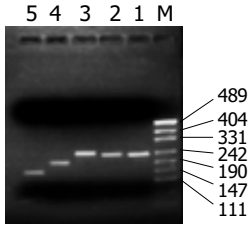

Figure 1 Electrophoresis of HLA-DRB1*03,*07,*09,*12,*15 alleles. M: pUC19 DNA/*Msp*I (*Hpa*II) marker 23; 1: DRB1*0701/0702, 232 bp; 2: DRB1*0901, 236 bp; 3: DRB1*1201/1202, 248 bp; 4: DRB1*1501/1502, 197 bp; 5: DRB1*0301/0302, 151 bp.

Table 1 Distribution of HLA-DRB1*03,*07,*09,*12,*15 alleles in patients with chronic hepatitis B and healthy controls

DRB1* Allele	Chronic hepatitis (n = 126)		Normal control (n = 76)		*RR*	χ^2	*P*
	PN	PR (%)	PN	PR (%)			
0301 / 0302	10	7.9	5	6.6	1.24	0.13	>0.75
0701 / 0702	46	36.5	15	19.7	2.37	6.33	<0.025
0901	31	24.6	22	28.9	0.80	0.46	>0.50
1201 / 1202	17	13.5	12	15.8	0.85	0.20	>0.75
1501 / 1502	16	12.7	9	11.8	1.08	0.03	>0.90
Blank	19	15.0	8	10.5	1.51	0.85	>0.50

PN: positive number, PR: positive rate, RR: relative risk.

Prevalence of HBV genotypes in patients with chronic hepatitis B

The prevalence of different HBV genotypes in patients with chronic hepatitis B is shown in Table 2. Genotype B was found in 38 patients (30.2%), genotype C in 69 (54.8%), mixed genotype (B+C) in 19 (15.0%), genotypes D- F were not found. Genotypes C and B were the major genotypes in this area. The electrophoresis of HBV genotypes is shown in Figure 2.

Table 2 Prevalence of different HBV genotypes in patients with chronic hepatitis B

Genotype	Number	%
B	38	30.2
C	69	54.8
B+C	19	15.0
D	0	0
E	0	0
F	0	0

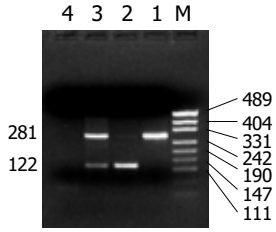

Figure 2 Electrophoresis of HBV genotypes. M: pUC19 DNA/*Msp*I (*Hpa*II) marker 23; lane 1: genotype B (281 bp); lane 2: genotype C (122 bp); lane 3: genotype B+C; lane 4: negative control.

*No response of HLA-DRB1*07 to IFN-α therapy*

Among the 46 DRB1*07(+) patients, 7 were responders and 39 were non-responders. Compared with the DRB1*07 (-) patients, the positivity of DRB1*07 in responder group was significantly lower than that in non-responder group ($\chi^2 = 4.51$, $P<0.05$), indicating that DRB1*07 had no response to IFN-α therapy (Table 3).

Table 3 Correlation between DRB1*07 and IFN-α therapy, *n* (%)

	Responder (*n* = 33)	Non-responder (*n* = 93)	Total (126)	χ^2	*P*
HLA-DRB1*07(+)	7 (15.2)	39 (84.8)	46	4.51	<0.05
HLA-DRB1*07(-)	26 (32.5)	54 (67.5)	80		

Response of HBV genotypes C and B to interferon-α therapy

Patients with HBV genotype C had a significantly lower response to IFN-α, only 13% *vs* 50% among patients with genotype B and 26.3% with genotype B+C ($\chi^2 = 17.3$, $P<0.005$, Table 4).

*Response to interferon-α therapy in patients with HLA-DRB1*07 (+) and genotype C (+)*

The patients with both HLA-DRB1*07(+) and genotype C (+) were compared with those with HLA-DRB1*07(+) or genotype C(+), significant difference was found among them ($\chi^2 = 6.71$, $P<0.05$, Table 5).

Table 4 Response to interferon-α therapy in patients with different genotypes, *n* (%)

	Responder	Non-responder	Total	χ^2	*P*
Genotype				17.3	<0.005
B	19 (50)	19 (50)	38		
C	9 (13)	60 (87)	69		
B+C	5 (26.3)	14 (73.7)	19		

Table 5 Response to interferon-α therapy in patients with HLA-DRB1*07 and genotype C, *n* (%)

		Responder	Non-responder	Total	χ^2	*P*
HLA-DRB1*07+						
genotype C					6.36	<0.05
+	+	2 (7.7)	24 (92.3)	26		
+	-	7 (35)	13 (65)	20		
-	+	14 (32.6)	29 (67.4)	43		

DISCUSSION

Currently, two therapeutic agents, interferon and lamivudine, are used in treatment of chronic hepatitis B in most countries, and the only agent known to have a lasting beneficial effect on chronic hepatitis B is interferon-α. It was reported that IFN treatment can result in sustained clearance of HBeAg in approximately 25-40% of patients, and a loss of HBsAg in 10% of Western patients[4]. The response of chronic hepatitis B to IFN-α is generally determined by a combination of virological factors, host immunological factors and genetic factors[5-10].

The present study aimed to investigate the role of HBV genotypes and HLA-DRB1 alleles in response to IFN-α in Chinese Han hepatitis B patients. Until now, HBV can be classified into eight genotypes (A-H)[11-14]. HBV genotypes have a characteristic geographic distribution[13-18]. Genotype A is more common in North America and northwestern Europe, genotypes B and C are the two most common genotypes prevailing in Asia. Genotype D is predominant in the Mediterranean area, the Middle East, and in India. The distribution of genotype H is less studied. Studies suggested that genotype A is associated with a higher rate of IFN-α-induced HBeAg sero-conversion than genotype D[19], and genotype B is associated with a higher rate of IFN-α-induced HBeAg clearance than genotype C[20,21]. Our study showed that HBV genotype C was associated with a lower rate of anti-viral response to IFN-α treatment in Chinese Han patients with HBeAg-positive chronic hepatitis B than genotype B and genotype B+C (50% *vs* 13% *vs* 26.3%, $P<0.005$). Further studies are needed to determine if HBV genotypes also play a role in response to IFN-α treatment of chronic hepatitis B patients from other geographic regions where other HBV genotypes prevail.

Great inconsistencies exist in studies about the association between HLA-DRB1 alleles and chronic hepatitis B. Jiang *et al*., suggested that the allele frequencies of HLA-DRB1*0301

in chronic hepatitis B group (17.31%) are markedly higher than those in normal control group (5.67%), and that HLA-DRB1*0301 is closely associated with susceptibility to chronic hepatitis B. They also found that the allele frequencies of HLA-DRB1*1101/1104 in chronic hepatitis B group were markedly lower than those in acute hepatitis B group, and that HLA-DRB1*1101/1104 is closely associated with resistance to chronic hepatitis B. Shen *et al*.[9] suggested that susceptibility to chronic hepatitis B is strongly associated with HLA-DRB1*10 allele in northern Chinese patients. Cotrina *et al*.[22], reported that HLA-DRB1*1301 and -DRB1*1302 alleles are associated with the clearance of HBV infection and protect people against chronic hepatitis B. Diepolder *et al*.[10], found that a strong virus-specific $CD4^+$ and $CD8^+$ T lymphocyte response to HBV is associated with viral clearance, patients with acute hepatitis B carrying HLA-DR13 have 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. In the present study, we did not examine HLA-DRB1*11 and HLA-DRB1*13, but there was no significant difference in HLA-DRB1*03 between the control group and the chronic hepatitis B group, which is inconsistent with Jiang's report. On the contrary, we found that HLA-DRB1*07 was significantly higher in chronic hepatitis B group than in control group, the response to IFN-α therapy was significantly lower in HLA-DRB1*07(+) group than in HLA-DRB1*07(-) group ($P<0.05$), suggesting that susceptibility to persistent infection and lower response to interferon-α are significantly associated with the presence of HLA-DRB1*07[23-25].

It is more important that the response to IFN-α therapy in patients with both HLA-DRB1*07(+) and genotype C (+) was even lower than that in patients with HLA-DRB1*07 (+) or genotype C (+, $P<0.05$), suggesting that HBV genotype C and HLA-DRB1*07 are closely associated with lower response of chronic hepatitis B to IFN-α therapy. Therefore, early exclusion of their existence before anti-viral therapy is very important for selection of patients and judgment of their prognosis.

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