

Relationship of human leukocyte antigen class II genes with the susceptibility to hepatitis B virus infection and the response to interferon in HBV-infected patients

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Abstract

AIM: To study the relationship of human leukocyte antigen (HLA)-DRB1 and -DQB1 alleles with the genetic susceptibility to HBV infection and the response to interferon (IFN) in HBV-infected patients.

METHODS: Low-resolution DNA typing kit was used to determine HLA-DR-1 and -DQB1 genes in 72 patients with chronic hepatitis B (CHB) and HLA-DRB1 in 200 healthy people ready to donate their bone marrow in Shanghai. Among CHB patients, 35 were treated with IFN α -1b for 24 wk.

RESULTS: The frequencies of HLA-DRB1*06, DRB1*08 and DRB1*16 alleles in 72 patients were higher than in 200 healthy people (2.08% vs 0%, OR = 3.837, P = 0.018; 11.11% vs 5.50%, OR = 2.148, P = 0.034; and 6.94% vs 3.00%, OR = 0.625, P = 0.049, respectively); whereas that of DRB1*07 allele was lower (2.78% vs 7.75%, OR = 0.340, P = 0.046). The frequency of HLA-DRB1*14 allele was higher in 11 responders to IFN compared with 24 non-responders (18.18% vs 2.08%, OR = 10.444, P = 0.031), whereas that of DQB1*07 allele was inverse (9.09% vs 37.50%, OR = 0.167, P = 0.021).

CONCLUSION: The polymorphism of HLA class II may influence the susceptibility to HBV infection and the response to IFN in studied CHB patients. Compared with other HLA-DRB1 alleles, HLA-DRB1*06, DRB1*08, and DRB1*16 may be associated with chronicity of HBV infection, HLA-DRB1*07 with protection against HBV infection, and HLA-DRB1*14 allele may be associated with a high rate of the response of CHB patients to IFN treatment. Compared with other HLA-DQB1 alleles, HLA-DQB1*07 may be associated with low response rate to IFN.

INTRODUCTION

It is generally accepted that viral clearance or chronic viremia following HBV infection is determined by the host immune response against HBV, in which human leukocyte antigens (HLA) play a central role. The progressions of antigen-presenting cells presenting viral antigens to B and T cells, B and T cells recognizing antigens, and B and T cells being reactivated are all restricted by HLA. The genes of HLA are polymorphous and difficult types of HLA are combined with difficult motifs of the antigens. It is, therefore, presumed that the HLA polymorphism possibly determines the pathogenesis and outcome of HBV infection. It has been reported that the HLA polymorphism correlates with the outcome of HBV infection, but this relationship is not universal on the basis of the investigated population. In Caucasia^[1,2] and Korea^[3], for example, HLA-DRB1*1301-02 has been found to be associated with acute self-limited hepatitis B. In Taiwanese, HLA-DRB1*0406 is associated with recovery from HBV infection in Han Chinese, and so is HLA-B*4001 in Aborigines^[4]. Han Chinese with HLA-DR12 (especially one of its alleles of DRB1*1201) or HLA-DRB1*1101/1104, respectively, have been shown to be able to resist HBV infection, and those with HLA-DR9, DQ9, HLA-DRB1*0301, HLA-DQB1*0301 (one of DQ7 isoforms)^[5,6] and DRB1*10, respectively, have been revealed to have a relationship with susceptibility to chronic HBV infection^[7]. DRB1*15 and its link-inequilibrium haploid-DRB1*15-DQB1*05 have been found to be associated with sustained response to interferon (IFN) treatment in Taiwanese patients with chronic hepatitis C^[8]. It has not been known, however, whether HLA genes correlate with the responsiveness to IFN treatment in patients with chronic hepatitis B (CHB). The aim of this study was therefore to assess whether HLA class II alleles play a critical role in the susceptibility to HBV infection and the response rate to IFN treatment in CHB patients.

MATERIALS AND METHODS

Subjects

A total of 72 patients with chronic HBV infection in the present study were positive for HBV surface antigen (HBsAg), e antigen (HBeAg) or anti-HBe and anti-HBc paralleled with serum ALT elevation for at least 6 mo. None of the patients were positive for hepatitis C virus antibody, hepatitis D virus antibody and HIV antibody; 62 of them were males and 10 were females with mean age of 32 ± 7 years; 200 healthy people ready to donate their bone marrow in Shanghai were selected as control group; 112 of them were males and 88 were females with mean age of 35 ± 7 years. All participants had no relative relationship.

Thirty-five of seventy-two patients without immunosuppressive or antiviral therapy within 1 year before entry, and without alcoholism, intravenous drug abuse, fatty liver, cirrhosis, pregnancy, or other serious diseases that may interfere with IFN- α therapy or that were forbidden for IFN therapy were treated with IFN- α -1b (Sinogen, Kexing Company, Shenzhen) at a dose of 3 MU per 2 d for 24 wk. Thirty-one of thirty-five patients were males and four were females with mean age of 31 ± 7 years. Response to IFN- α treatment was defined as normalization of serum elevated ALT, undetectable HBV DNA by PCR method, and HBeAg seroconversion to anti-HBe until 6 mo after the treatment had ended; and non-response as detectable HBV DNA by PCR, and/or presence of HBeAg regardless of serum ALT level.

Methods

HLA class II typing Genomic DNA was extracted from peripheral blood using guanidine sulfocyanate method and the reagents were supplied by Shanghai blood center. HLA class II alleles were determined by low resolution DNA typing method, one of polymerase chain reaction-sequence specific primers techniques (Pel-Freez, USA) according to the manufacturer's instruction. Gene typing was performed with the use of software supplied by the company.

Serological testing

HBsAg, HBeAg, and antibodies to HBsAg, HBeAg and

HBeAg were tested by an ELISA (Roche, Switzerland).

Quantification of HBV DNA

Serum HBV DNA was quantitated by fluorescence PCR performed in LightCycle amplifier (Roche, Switzerland) using domestic commercial kit (Piji Company, Shenzhen).

Statistical analysis

The frequency of HLA class II alleles was calculated by direct counting. The statistical significance of the associations of HLA class II alleles with the clinical outcome or the response to IFN- α was determined by the two-tailed Fisher's exact test using the SPSS 10.0 data analysis software package (Chicago, USA); *P* values <0.05 were considered statistically significant.

RESULTS

Relationship between HLA-DRB1 and the susceptibility to HBV infection

Frequency distribution of HLA-DRB1 in CHB patients and wealthy controls is illustrated in Table 1. The frequency of DRB1*06 allele in CHB patients was higher than in wealthy controls (2.08% *vs* 0%, *P* = 0.018, odds ratio [OR] = 3.837, 95% confidence interval [95%CI] 3.329-4.422), so are those of DRB1*08 allele (11.11% *vs* 5.50%, *P* = 0.034, OR = 2.148, 95%CI 1.094-4.216) and DRB1*16 allele (6.94% *vs* 3.00%, *P* = 0.049, OR = 0.625, 95%CI 0.252-1.552); the frequency of DRB1*07 allele was lower in CHB patients than in wealthy controls (2.78% *vs* 7.75%, *P* = 0.046, OR = 0.340, 95%CI 0.118-0.981). No significant differences of other HLA-DRB1 alleles' distribution were found between the two groups.

Relationship of HLA-DRB1 and HLA-DQB1 with the response to IFN therapy in patients with CHB

Frequency distribution of HLA-DRB1 and -DQB1 in responders and non-responders to IFN treatment is shown in Table 2. The frequency of DRB1*14 allele in 11 responders was higher than in 24 non-responders (18.18% *vs* 2.08%, *P* = 0.031, OR = 10.444, 95%CI 1.092-99.855); that of DQB1*07 allele was inverse (9.09% *vs* 37.50%,

Table 1 Distribution of HLA-DRB1 alleles in CHB patients and wealthy people

| HLA-DRB1 | Patients with CHB | | Wealthy people | | OR (95%CI) | <i>P</i> |
|----------|-------------------|------------------------------------|----------------|------------------------------------|----------------------|----------|
| | <i>N1</i> | Allele frequency <i>n</i> = 144 | <i>N2</i> | Allele frequency <i>n</i> = 400 | | |
| DRB1*01 | 2 | 1.39 | 3 | 0.75 | 1.864 (0.308-11.269) | 0.612 |
| DRB1*04 | 14 | 9.72 | 53 | 13.25 | 0.705 (0.378-1.314) | 0.303 |
| DRB1*06 | 3 | 2.08 | 0 | | 3.837 (3.329-4.422) | 0.018 |
| DRB1*07 | 4 | 2.78 | 31 | 7.75 | 0.340 (0.118-0.981) | 0.046 |
| DRB1*08 | 16 | 11.11 | 22 | 5.50 | 2.148 (1.094-4.216) | 0.034 |
| DRB1*09 | 34 | 23.61 | 75 | 18.75 | 1.339 (0.846-2.120) | 0.225 |
| DRB1*10 | 4 | 2.78 | 4 | 1.00 | 2.829 (0.698-11.462) | 0.217 |
| DRB1*11 | 11 | 7.64 | 29 | 7.25 | 1.058 (0.514-2.178) | 0.854 |
| DRB1*12 | 20 | 13.89 | 66 | 16.50 | 0.816 (0.475-1.402) | 0.507 |
| DRB1*13 | 2 | 1.39 | 15 | 3.75 | 0.362 (0.082-1.601) | 0.262 |
| DRB1*14 | 9 | 6.25 | 25 | 6.25 | 1.000 (0.455-2.197) | 1.000 |
| DRB1*15 | 9 | 6.25 | 39 | 9.75 | 0.617 (0.291-1.308) | 0.234 |
| DRB1*16 | 10 | 6.94 | 12 | 3.00 | 2.413 (1.019-5.713) | 0.049 |
| DRB1*17 | 6 | 4.17 | 26 | 6.50 | 0.625 (0.252-1.552) | 0.409 |

$P = 0.021$, OR = 0.167, 95%CI 0.035-0.796). No significant differences of other HLA-DRB1 and -DQB1 alleles' distribution were found between the responders and non-responders.

DISCUSSION

It has been well known that human HLA-II antigens are expressed in the member surface of antigen presentation cells and immune response cells, taking part in the regulation of host immune response against foreign antigens, which is determined by the HLA genes' polymorphism and their expression levels in the above-mentioned cell member surface. It is generally acknowledged that some HLA polymorphisms are associated with auto-immune diseases. A close attention has been recently paid to the relationship between HLA polymorphisms and viral hepatitis.

It has been reported that in western Caucasians the frequencies of HLA-DRB1*1301/1302 in German patients with acute self-limited hepatitis B and USA patients with spontaneous viral clearance after HBV infection are both higher than in respective patients with CHB^[1,2], whereas in the USA, Afro-American persistent HBV infection is associated with HLA-II gene homozygotes, and DQA1*0501 and DQB1*0301, and their haploids DQA1*0501-DQB1*0301 and DQA1*0501-DQB1*0301-DRB1*1102^[9]. It has also revealed that in Korea DR6 and DR13 (especially DR13) have a close relationship with spontaneous viral clearance and DR9 is associated with disease chronicity following HBV infection^[2]. These findings suggest that there are different relationships between HLA gene polymorphisms and HBV infection in different racial population, implying that various HLA molecules could present different HBV epitopes to induce effective immune responses^[4].

It has been found that in China, the frequencies of

HLA-DR10, -DRB1*0301 and -DQB1*0301 all increase in patients with CHB compared with wealthy people, whereas that of HLA-DRB1*1101/1104 is inverse^[6,7]; the frequency of HLA-DR12 (especially one of its alleles DRB1*1201) declines compared with those with spontaneous cure following HBV infection, whereas frequencies HLA-DR9, -DQ9 are inverse^[5]. In the present study the frequencies of HLA-DRB1*06, -DRB1*08 and -DRB1*16 were all higher in CHB patients than in wealthy people, and that of HLA-DRB1*07 lower in CHB patients than in wealthy people, suggesting that individuals with HLA-DRB1*06, -DRB1*08 or -DRB1*16 alleles were susceptible to HBV infection, whereas those with HLA-DRB1*07 allele was resistant to HBV infection in Shanghai people. Four HLA genes mentioned above, however, are all infrequent among the Shanghai population, the proportion of which accounts for 16.25% of the whole population, suggesting that HLA polymorphism has a less of a role in pathogenesis of CHB.

A lot of domestic and foreign studies including this one demonstrate that some of HLA alleles were susceptible genes to HBV infection and some were resistant genes to HBV infection, but the different genes associated with the outcome of HBV infection were found in different studies. This divergence may be elucidated by gene background of different nations of subjects, research designs and the fact that disease chronicity following HBV infection is probably not influenced by HLA gene polymorphisms.

Whether hepatitis B onsets and disease chronicity occurs after HBV infection is dependent on immune response condition of host who is infected. If there are no primary and secondary immune defects, host's age can reflect his/her mature level of immune functions. Hyams *et al.*, reviewed the literatures on chronic HBV infection and found that chronicity rate is about 80-90%, when infection occurs in infants and young persons less than 6 years; that there are usually no self-conscious symptoms accompanying HBV

Table 2 Distribution of HLA class II alleles in IFN responders and non-responders

| HLA | Responder | | Non-responder | | OR (95%CI) | P |
|---------|-----------|----------------------------|---------------|----------------------------|-----------------------|-------|
| | N1 | Allele frequency n = 22 | N2 | Allele frequency n = 48 | | |
| DRB1*01 | 0 | 0.00 | 1 | 2.08 | 1.468 (1.249-1.725) | 1.000 |
| DRB1*04 | 2 | 9.09 | 7 | 14.58 | 0.586 (0.111-3.080) | 0.709 |
| DRB1*06 | 0 | 0.00 | 1 | 2.08 | 1.468 (1.249-1.725) | 1.000 |
| DRB1*07 | 1 | 4.55 | 2 | 4.17 | 1.095 (0.094-12.760) | 1.000 |
| DRB1*08 | 2 | 9.09 | 3 | 6.25 | 1.500 (0.232-9.685) | 0.646 |
| DRB1*09 | 3 | 13.64 | 11 | 22.92 | 0.531 (0.132-2.135) | 0.524 |
| DRB1*10 | 1 | 4.55 | 2 | 4.17 | 1.095 (0.094-12.760) | 1.000 |
| DRB1*11 | 1 | 4.55 | 5 | 10.42 | 0.410 (0.045-3.731) | 0.657 |
| DRB1*12 | 2 | 9.09 | 9 | 18.75 | 0.433 (0.085-2.199) | 0.483 |
| DRB1*14 | 4 | 18.18 | 1 | 2.08 | 10.444 (1.092-99.855) | 0.031 |
| DRB1*15 | 2 | 9.09 | 1 | 2.08 | 4.700 (0.403-54.835) | 0.231 |
| DRB1*16 | 3 | 13.64 | 2 | 4.17 | 3.632 (0.561-23.500) | 0.316 |
| DRB1*17 | 1 | 4.55 | 3 | 6.25 | 0.714 (0.070-7.281) | 1.000 |
| DQB1*02 | 2 | 9.09 | 3 | 6.25 | 1.500 (0.232-9.685) | 0.646 |
| DQB1*04 | 1 | 4.55 | 1 | 2.08 | 2.238 (0.134-37.516) | 0.533 |
| DQB1*05 | 7 | 31.82 | 8 | 16.67 | 2.333 (0.720-7.557) | 0.210 |
| DQB1*06 | 4 | 18.18 | 2 | 4.17 | 5.111 (0.860-30.310) | 0.073 |
| DQB1*07 | 2 | 9.09 | 18 | 37.50 | 0.167 (0.035-0.796) | 0.021 |
| DQB1*08 | 1 | 4.55 | 5 | 10.42 | 0.410 (0.045-3.731) | 0.657 |
| DQB1*09 | 5 | 22.73 | 11 | 22.92 | 0.989 (0.297-3.295) | 1.000 |

that clear on their own and immunity being acquired, and only a minority with HBV infection show clinical manifestations of acute hepatitis B, chronicity rate less than 5% when infection occurs in adults^[10]. The result of a study has shown that only 0.3% of patients become chronic HBV carriers in which a total of about 300 000 patients with HBV infection due to inoculations of yellow fever vaccine populated by HBV were followed up for 40 years^[11]. In China 30-50% of population are detected with one antibody or more antibodies to HBV in their whole life, suggesting that they have been infected with HBV and virus has been cleared spontaneously and immunity against HBV has been acquired, which demonstrates that acute transient infection is a principal pattern of HBV infection^[12]. These phenomena are not obviously explained by individual discrepancy of human gene background, including HLA polymorphisms.

The effects of HLA genes on IFN treatment to CHB patients have not yet been reported, although domestic and foreign studies have shown that gene polymorphisms of HLA-I and -II antigens have an influence on host immune response to HBsAg vaccine^[13-15]. Our study has revealed that HLA-DRB1*14 may be associated with a high rate of the response of CHB patients to IFN treatment, compared with other HLA-DRB1 alleles and HLA-DQB1*07 may be associated with low response rate to IFN, compared with other HLA-DQB1 alleles.

DRB1*14 is also an infrequent HLA gene in Shanghai, its frequencies in 200 wealthy people and 72 CHB patients being 6.25% and 7.14%, respectively. DRB1*14 was found in five patients treated with IFN, four of them were responders. Among these five patients, four of them were infected with type B HBV and one was infected with type C HBV, and serum HBV DNA level was low (data not shown). It has been generally accepted that low level of serum HBV DNA has benefit to IFN treatment and the response rate to IFN treatment is higher in patients with type B HBV infection than in those with type C HBV infection^[16]. Therefore, it is not excluded that HLA-DRB1*14 had an occasional relationship with IFN therapy in our study.

Further studies are required for elucidating whether HLA-DQB1*07 allele can serve as one of the indices for selecting patients to be treated with IFN because of a small sample in our study, though it is a frequent HLA gene in the Chinese and has been found to be associated with low response rate to IFN treatment in the present study.

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