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• BRIEF REPORTS •

Curative effects of interferon-α and HLA-DRB1 -DQA1 and -DQB1 alleles in chronic viral hepatitis B

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Abstract

AIM: To investigate the association between curative effects of interferon-α and partial human leucocyte antigen (HLA) II alleles in chronic viral hepatitis B.

METHODS: Sixty patients with chronic viral hepatitis B in Shanghai were treated with a standard course of treatment with interferon-α for 6 mo. HLA-DRB1, -DQA1, and -DQB1 alleles were detected by polymerase chain reaction-sequence specific primer (PCR-SSP) method.

RESULTS: Frequencies of HLA-DRB1*04 ($P<0.025$) and HLA-DQA1*0303 ($P<0.01$) in non-responders were significantly higher than those in partial and complete responders. Frequencies of HLA-DQA1*0505 ($P<0.025$) and HLA-DQB1*0301 ($P<0.005$) in partial and complete responders were significantly higher than those in non-responders.

CONCLUSION: Non-response to interferon-α therapy is positively correlated with HLA-DRB1*04 and HLA-DQA1*0303, and negatively correlated with HLA-DQA1*0505 and -DQB1*0301 in patient with chronic viral hepatitis B. HLA II genes of the identification alleles provide a method for evaluating outcome of interferon-α treatment.

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INTRODUCTION

Chronic viral hepatitis B is a contagious disease with the higher infection and incidence rate in China, and approximately 0.3 million peoples died of chronic viral hepatitis B per year[1]. Currently, it is mainly treated with interferon-α, lamivudine, *etc*. However, the effect of treatment is varying in different patients. Normally, complete response rate is about 30-40%, complete curability is less than 10%. What is the determinant of interferon-α curative effect on different individuals? Reports from domestic and overseas showed that individuals had different endings after being infected by HBV and HCV[2-3] and different response after being treated with interferon-α[4]. Researches indicated that these phenomenons were correlated with HLA alleles[5]. HLA gene contributes to the host response against HBV. Individuals with different HLA alleles may differ in susceptibility or resistance to HBV[6-8]. Our study tried to analyze HLA-DRB1, -DQB1, -DQA1 alleles in chronic viral hepatitis B to be treated with interferon-α for 6 mo, and study the association between curative effects of interferon-α and partial HLA alleles, which will help to direct the treating process of anti-virus in clinic.

MATERIALS AND METHODS

Research subjects and prescription

Sixty patients with chronic viral hepatitis B were enrolled in this study. The diagnosis of all the cases was made according to the criteria established on the Viral Hepatitis Conference held in 2000[9]. All patients had abnormal serum transaminase levels. HBsAg, HBeAg, HBcAb in serum were detected by ELISA and HBV-DNA was detected by immunofluorescent semi-quantitative polymerase chain reaction. All patients' HAV, HCV, HDV and HEV in serum were negative, and did not have a history of using adrenal cortical hormone before. There were 41 male and 19 female patients with average age 35±8 years. They were all treated with 5.0 million units interferon-α daily for 2 wk and then every other day for an additional 22 wk. Liver function was detected every 2 wk. Hepatitis B viral marks were detected by Abbott Laboratories and HBV-DNA was determined by PCR at every 3-mo therapy.

Sampling and action

Five milliliter blood from each research subject was taken, and treated with EDTA for anti-coagulation. After mixed with 1 mL cell membrane cracking solution, the samples were centrifuged for 30 s, and then the supernatant was removed. Another 1 mL of cell membrane cracking solution was added after drying the test tube by bibulous paper. Centrifuged for 20 s and the top clear water was removed again. The cell mass at the bottom of the tube was vibrated and dissolved thoroughly. When mixed equally with 0.4 mL karyen cracking solution, separated out floccule DNA by adding 1 mL absolute ethanol. Supernatant was abandoned, and washed with 70% ethanol. After drying by blot paper, put it under room temperature to let ethanol volatilize. Then 0.1 double distilled water was added, and kept at -40 ℃ for testing.

Study method

HLA-DRB1, HLA-DQA1, and HLA-DQB1 alleles were detected by applying the PCR-SSP technique[10]. PCR buffer solution was vibrated and mixed. Taq enzyme was put on the icebox. Distilled water 67 μL and 1.8 μL of Taq enzyme were added to the PCR buffer solution and vibrated. Then the solution was aspirated and added to the monitor hole. And 19 μL of DNA samples were added to the spare mixing solution. Except negative contrast hole, the solution was added

to every hole. Color changed from yellow to pink. The reagent was sealed up, and sent to PCR apparatus for amplification. Sample solution 6 μL was electrophoresed on 20 g/L agarose gel for 12 min under 150 V, and observed the under ultraviolet light.

Statistical analysis

HLA-DRB1, -DQA1, and -DQB1 alleles frequencies for the partial and complete responders were compared with those of the non-responders using the χ^2 test. $P<0.05$ was considered statistically significant.

RESULTS

Based on the results of HBV markers and HBV-DNA after 6-mo therapy with interferon-α, patients were divided into 3 groups: (1) Complete response group: HBeAg and HBV-DNA were negative, while HBeAb was positive, and ALT was normal; (2) Partial response group: HBeAg and HBV-DNA level decreased, while ALT was normal; (3) Non-response group: HBeAg and HBV-DNA were stable. After inspection, it was found that the frequencies of HLA-DRB1*04($P<0.025$) and HLA-DQA1*0303($P<0.01$) in non-responders were significantly higher than those in partial and complete responders, and the frequencies of HLA-DQA1*0505($P<0.025$) and HLA-DQB1*0301($P<0.005$) in partial and complete responders were significantly higher than those in non-responders (Tables 1, 2 and 3).

Table 1 Comparison of frequency of HLA-DRB1 allele among non-responders and partial and complete responders

Allele	Partial and complete responders (n=34)	Non-responders (n=26)	χ^2
DRB1*10(+)	1	0	0.778
DRB1*11(+)	9	3	2.053
DRB1*4(+)[a]	1	6	2.053
DRB1*12(+)	9	7	0.002
DRB1*8(+)	4	5	0.644
DRB1*9(+)	15	11	0.020
DRB1*14(+)	4	4	0.167
DRB1*15(+)	8	10	1.564
DRB1*17(+)	1	2	0.700
DRB1*16(+)	3	0	2.415
DRB1*7(+)	4	0	3.277

[a]$P<0.025$.

Table 2 Comparison of HLA-DQA1 allele frequencies among non-responders and partial and complete responders

Allele	Partial and complete responders (n=32)	Non-responders (n=28)	χ^2
DQA1*0105(+)	1	0	0.890
DQA1*0505(+)[a]	12	3	5.714
DQA1*0303(+)[b]	0	6	7.619
DQA1*0601(+)	10	7	0.287
DQA1*0103(+)	9	7	0.075
DQA1*0302(+)	15	12	0.097
DQA1*0104(+)	4	4	0.041
DQA1*0102(+)	4	6	0.857
DQA1*0301(+)	1	4	2.435

[a]$P<0.025$, [b]$P<0.01$.

Table 3 comparison of frequencies of HLA-DQB1 allele among non-responders and partial and complete responders

Allele	Partial and complete responders (n=32)	Non-responders (n=28)	χ^2
DQB1*0502(+)	6	1	3.338
DQB1*0301(+)[a]	20	7	8.485
DQB1*0401(+)	1	3	1.382
DQB1*0303(+)	15	12	0.097
DQB1*0503(+)	3	4	0.431
DQB1*0601(+)	6	9	1.429

[a]$P<0.005$.

DISCUSSION

Different individuals infected with HBV show different complicated symptoms. This is not only due to virus itself, but immunity itself [11]. A great deal of evidences suggested that both cellular and humoral immunities were required for viral clearance. The latter is mainly subjected to major histocompatibility complex (MHC). HLA, the genetic offspring of MHC, is the first inherited system discovered to be associated with diseases definitely. Genes for HLA are located on the short arm of chromosome 6 with high polymorphism, and it is closely associated with immunoreactions of anti-HBV[12]. Some special HLA genes may have influence on the rate of HBV infection and strength of immunoreactions[13]. 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[14]. Jiang *et al*.[15] found that HLA-DRB1*0301, -DQA1*0501 and -DQB1*0301 might be the susceptible genes, and HLA-DRB1*1101/1104 and -DQA1*0301 might be the resistant genes to chronic hepatitis B, and that host HLA class II gene was an important factor for determining the outcome of HBV infection. HLA spread on the cell surface through membrane protein with function of integrating with inner and outer antigen peptide and taking immune response when detected by $CD4^+$ (cluster of differentiation) or $CD8^+$ T cell on the surface of antigen presenting cells and target cells. Class II molecule, on the surface of antigen presenting cells, submits outer antigen including virus molecule group to the $CD4^+$ T cell, which stimulates the releasing of the cell gene to take the effect of adjusting $CD8^+$ cytotoxic T lymphocyte response and determine the antibody produce. Diepolder *et al*.[16] found that people carrying HLA-DR13 had stronger $CD4^+$ T cell response. That might be depended on the more accurate submission function of DR13, or associated with multiple peptide property of immunity adjusting gene chain near DR13. Thursz *et al*.[17] discovered that DRB1*1302 possessed high frequency of clearance of hepatitis B virus in the Gambia people. Cotrina *et al*.[5] also reported that predominance of the DRB1*1302 allele was observed in acute viral hepatitis B versus chronic viral hepatitis B in adult American. And the HLA-DRB1*0401 antigen was lower in the cases of chronic viral hepatitis B and C than that in the controls. Hohler *et al*.[18] reported that the MHC class II allele DRB1*1301-02 was associated with protection from chronic viral hepatitis B in African Americans. Furthermore Bhimma *et al*.[19] demonstrated that there was a high frequency of DQB1*0603 in subjects compared to controls in black children with hepatitis B virus-associated membranous nephropathy. Jiang *et al*.[20] recently found that the possibility of fulminant hepatitis was increased in chronic hepatitis B with HLA-DRB1*1001. Tibbs *et al*.[21] showed that the HLA-DQB1*0302 and HLA-DQA1*03 alleles confered protection from chronic HCV-infection in Northern European

Caucasoid. These studies showed that HLA-II molecules were associated with clearance and prognosis of chronic viral hepatitis.

Currently, factors for forecasting interferon treating effect are as follows: ALT level before treatment; level of HBV-DNA; gene types of hepatitis B virus; sex of patients; and the duration of virus infection, *etc*. Interferon can induce the expression of IL-12 (interleukin-12) β_2 subpopulation, which induce Th0 (help T cell) cell to differentiate into Th1 cell. Previous studies showed that Th1 type response was beneficial for the clearance of chronically infected viruses[7]. The balance of HBV differential antigen may influence the persistent HBV infection. Superiority of Th1 tends to occur acute hepatitis, while superiority of Th2 tends to occur persistent infection[22]. There were fewer reports about association between curative effects of interferon-α with partial HLA allele. Qian *et al*.[23] reported that the frequency of HLA-DRB1*07 in non-responders was higher than that in partial and complete responders in Guangdong Province of China, and the level of IL-4 and IFN-γ of each patient was higher than that of pre-treatment. It indicated that after treatment of chronic viral hepatitis B with IFN-γ, TH1 expression was relevant to the HLA-DRB1*07. Dincer *et al*.[24] reported that in the HCV patient treated with interferon-α for 6 mo, the frequency of HLA-DRB1*13 was significantly higher in the non-responder group compared to the responder group. Our study showed that the frequency of HLA-DRB1*04 and HLA-DQA1*0303 in non-responders were obviously higher than those in partial and complete responders, and the frequency of HLA-DQA1*0505 and HLA-DQB1*0301 in partial and complete responders were markedly higher than those in non-responders. HLA-II molecules might be used for the treatment prognosis of interferon -α in patients with chronic hepatitis B.

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