Article

The Expression of CD2 in Chronic HBV Infection

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It was previously reported that several kinds of intercellular adhesion molecules are closely related to chronic HBV infection. The complex of CD2 and CD58 plays an important role in enhancing the adhesion of T lymphocytes to target cells, and promoting hyperplasia and activation of T lymphocytes. In this study, we detected the level of CD2 expressed on the surface of PBMC, the expression level of CD2 mRNA in PBMC and the percentage of CD2 positive cells in PBMC of patients with chronic HBV infection and compared them with the expression level of normal controls. We also determined the level of serum HBV DNA from patients with chronic HBV infection and from normal controls. The clinical characteristics of hepatic function were tested as well. The results showed that the expression of CD2 significantly increased with the severity of chronic HBV infection, which suggested that CD2 might contribute to the hepatocyte damage in chronic HBV infection. *Cellular & Molecular Immunology*. 2008;5(1): 69-73.

Key Words: chronic HBV infection, CD2, RT-PCR

Introduction

Chronic hepatitis B is caused by hepatitis B virus (HBV), which is epidemic throughout the world. However, it is not well known about the pathogenesis of chronic HBV infection and the role of the cellular immune response in the pathogenesis. Recently, some reports suggested that HBV does not directly cause any cytopathic effect (1-4). It has been proved that the cellular immune response is attributed to the hepatocellular damage after HBV infection during the clearance of HBV. The infected hepatocytes are identified and eliminated by the cytotoxic T lymphocytes (CTL), and the balance between Th1 cells and Th2 cells may play an important role in the hepatocyte damage (5-7).

Double stimulating signals are necessary in the activation of T lymphocytes; one comes from the specific binding of TCR to the complex of MHC molecules and antigen peptide, the other is provided by the binding of antigen-presenting cells to the adhesion molecules on the surface of T cells. Those adhesion molecules are also named co-stimulating molecules, which include CD58/CD2, LFA-1/ICAM-1 and CD28/B7, etc. Only part of the T lymphocytes could be activated in the insufficient condition of co-stimulating signals, while most of them were in a situation of clonal non-response (8, 9). The recent studies have shown that LFA-1/ICAM-1 was highly related with the HBV infection and the degree of the liver damage. The complex of CD2 and CD58 plays important roles in enhancing the adhesion of T lymphocytes to target cells, and promoting the hyperplasia and activation of T lymphocytes (10-12).

In this study, we detected the expression levels of CD2 mRNA in PBMC and the percentage of CD2 positive cells in PBMC of patients with chronic HBV infection and compared them with the levels of normal controls to evaluate the role of CD2 in the pathogenesis of hepatitis B.

Materials and Methods

Patients

Thirty patients were selected from outpatients and inpatients of the First Hospital and the Second Hospital of Xi'an Jiaotong University for the study of the expression of CD2 in PBMC. The patients were divided into 3 groups, namely mild chronic HBV infection group (n = 10), moderate chronic HBV infection group (n = 10) and severe chronic HBV infection group (n = 10). Among the 30 patients, 19 were male, 11 were female, and the average age was 40.8 years old. Fifteen healthy persons were taken as normal control group. Patients with other viral hepatitis (hepatitis A, C, D, etc.) were excluded by the serological test and anamnesis. None of the patients received any antiviral or immunomodulatory treatment in the preceding 6 months of the study. The diagnosis code for chronic HBV infection edited by the 12th

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Chinese National Academic Conference of Viral Hepatitis and Liver Disease in 2005 was used as the classification criteria. Informed consent was obtained from each patient and the study was approved by the hospital.

Isolation of PBMC

To isolate PBMC, 4 ml of fresh blood was collected from the vein into tubes containing sodium heparin aseptically. PBMC were isolated according to standard methods, then were washed twice in phosphate buffered saline, and the cell concentration was adjusted to 1×10^6/ml.

Real-time PCR

Total cellular RNA was isolated using TRIzol (Invitrogen, USA) and reverse-transcribed to cDNA using the cDNA synthesis Kit (Fermentas Co., USA) according to the manufacturer's protocols. The obtained cDNAs were stored at -20°C. Real-time PCR was performed with the ABI Prism 7900HT Fast Real-time PCR System Instrument (Applied Biosystems) using Qpcr SYVR Green Core Kit (Eurogentec) according to the manufacturer's instructions. The reaction mixture (50 μl) included PCR buffer 5 μl, dNTP 1 μl (10 mmol/L), primer sequence-1 5 μl (5 μmol/L), primer sequence-2 5 μl (5 μmol/L), Taq DNA polymerase 0.4 μl (5 U/μl), Mg^{2+} 3 μl (25 mmol/L), MGB probe 5 μl (5 μmol/L), and template 10 μl. Distilled water was added to final reaction volume of 50 μl. The amplification program included an initial denaturation step at 94°C for 5 min, followed by denaturation at 94°C for 30 s, and annealing at 55°C and extension at 72°C for 30 s with a final extension at 72°C for 10 min for 40 cycles. The type 7700 Fluorescence Ratio Instrument (ABI Co., USA) was used for amplification and data collection. Primer and probe were designed by using Primer Express 2.0 software. Forward prime sequence: 5'-CAA GTG CAC AGC AGG GAA CA-3'; reverse primer sequence: 5'-CCA CAT ATG CCA ATG ATG AGA TAG A-3'; product size: 77 bp; MGB-TaqMan Probe sequence: 5'-ACC TTT CTC TGG ACA G-3'. Lux gene was linked to 5' end, and MGB molecule and quenched gene (NFQ) were linked to 3' end, and house keeping gene β-actin (GeneCore Biotech, China) was applied as an internal control. The quantities of CD2 mRNA and β-actin were calculated based on standard curve (standard curve comes from amplification of standard sample). The levels of CD2 mRNA expression were presented as the ratio of CD2 mRNA to β-actin. Every sample was run in three parallel reactions.

Detection of the percentage of CD2 positive cells in PBMC

For flow cytometric analysis of surface markers, PBMC were collected as described above. PBMC were washed twice with PBS and stained for 20 min with mouse anti-human CD2 antibody conjugated by FITC (Biolegend). PBMC were washed once with PBS and fixed in PBS containing 2% paraformaldehyde (pH 7.4) for 15 min. Following fixation, the cells were washed once with PBS and resuspended at a concentration of 1×10^6 cells/ml. Fluorescence profiles were acquired on a FACSCalibur (BD).

Detection of the levels of HBV DNA and the parameters of hepatic function in serum

Serum samples was isolated from 2 ml of fresh peripheral blood, which was collected aseptically from vein, following the standard methods, and stored at -20°C. The levels of HBV DNA were determined by the HBV FQ-PCR Kit (Daangene Company, China) and the standard of negative we used was $< 10^3$ copies/ml. The levels of ALT, AST, TBIL, DBIL and IBIL were tested by the CHEMIX-180 Automated Biochemistry Analyzer (Japanese SYSMEX Corporation).

Statistical analysis

Statistical analysis were performed using the commercially available software SPSS. The significance among different groups was examined by one-way analysis of variance followed by two-sample Student's *t* test. Differences between groups were considered significant when p values < 0.05 were obtained. The data were presented by mean ± SD. The correlations between the levels of CD2 and ALT and AST, which reflect the liver functions, were analyzed by the one tailed rectilinear correlation method. Correlations between groups were considered significant if provability values of $p < 0.05$ were obtained. The data were presented by correlation coefficient (r).

Results

Clinical characteristics of patients with different types of chronic HBV infection

The clinical characteristics of patients were illustrated in Table 1. The results showed that the levels of the clinical characteristics of patients with different types of chronic

Table 1. Clinical characteristics of patients with different types of chronic HBV infection and normal controls

Groups	n	TBIL (μmol/L)	DBIL (μmol/L)	IBIL (μmol/L)	ALT (IU/L)	AST (IU/L)
Normal	15	11.25 ± 2.14	3.00 ± 1.54	6.41 ± 1.85	25.19 ± 2.58	19.57 ± 3.06
Chronic HBV infection (mild)	10	15.14 ± 3.26*	5.92 ± 2.05*	10.39 ± 2.63*	73.33 ± 3.68*	55.67 ± 9.81*
Chronic HBV infection (moderate)	10	43.20 ± 8.73*	34.18 ± 7.11*	33.74 ± 3.8*	98.20 ± 18.90*	104.4 ± 12.80*
Chronic HBV infection (severe)	10	165.3 ± 17.67*	59.88 ± 8.62*	50.80 ± 16.05*	221.6 ± 18.19*	157 ± 22.54*

Data were shown as mean ± SD. * $p < 0.05$ compared with normal control group.

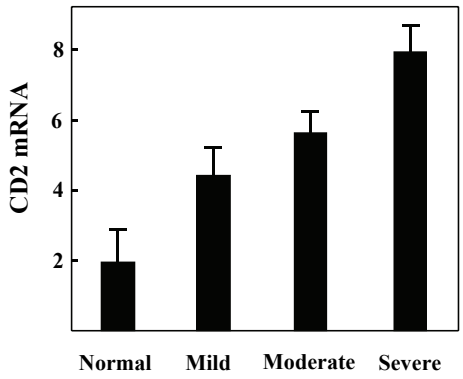

Figure 1. The expression of the levels of CD2 mRNA in PBMC from patients with different types of chronic HBV infection. Total RNA was isolated from PBMC of the patients with chronic HBV infection. The levels of CD2 mRNA expression were analysed by real-time PCR. Data were shown as mean ± SD.

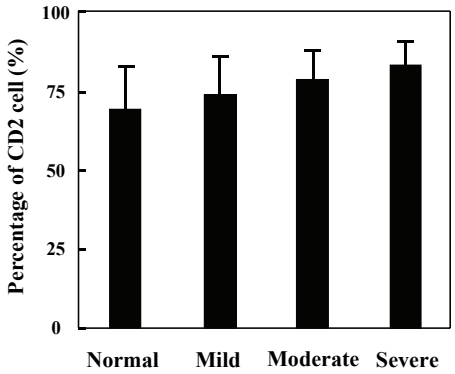

Figure 2. The percentage of CD2 positive cells in PBMC from patients with different types of chronic HBV infection. PBMC were collected from the patients with chronic HBV infection. The percentages of CD2 positive cells were analysed by flow cytometry. Data were shown as mean ± SD.

HBV infection were significantly higher than those in normal controls ($p < 0.05$). The levels of the clinical characteristics increased in an order from mild chronic, moderate chronic, to severe chronic hepatitis groups and the differences among the groups were significant ($p < 0.05$).

Expression of CD2 mRNA and protein in PBMC

The levels of CD2 mRNA in PBMC were significantly higher than those in normal controls ($p < 0.05$). The levels of CD2 mRNA in PBMC increased in an order from mild chronic, moderate chronic, to severe chronic hepatitis groups and the differences among the groups were significant ($p < 0.05$) (Figure 1).

The percentage of CD2 positive cells in PBMC in patients with different types of chronic HBV infection was detected by FACS. Results showed that the percentages of CD2 positive cells in PBMC in patients with different types of chronic HBV infection were higher than those in normal controls ($p < 0.05$). The percentages increased in an order from mild chronic, moderate chronic, to severe chronic and the differences among the groups were significant ($p < 0.05$) (Figure 2). The levels of serum HBV DNA in patients with different types of chronic HBV infection were examined by real-time PCR. The results showed that the logarithm averages of the levels of HBV DNA in positive serum samples of patients with chronic HBV infection had not significant difference among the groups ($p > 0.05$) (Table 2).

We next attempted to assess the relationship between the levels of AST or ALT and the expression of CD2 in PBMC. Significant correlation was found between AST or ALT and the level of CD2 in PBMC (Figures 3, 4).

Discussion

The complex of CD2 and CD58 plays an important role in enhancing the adhesion of T lymphocytes to target cells, and promoting the hyperplasia and activation of T lymphocytes (10-14). Recently, some studies demonstrated that several kinds of intercellular adhesion molecules were closely related to the infection of HBV (15-19). CD2/CD58 was considered associated with the liver inflammation and the hepatolysis, and the expression of CD58 on the surface of hepatocyte was increased significantly in chronic HBV infected patients and positively related with severity of disease. *In vitro* studies showed that cytokines could up-regulate the expression of CD58 on the liver cell surface as well as increase the level of soluble CD58 in serum and the levels of sCD58 might reflect the degree of the liver inflammation (20-23).

Cell-mediated immune reaction is thought to be the predominant mechanism of virus elimination and hepato-cellular damage, through CTL, natural killer cells. It was

Table 2. Comparison of the percentages of HBV DNA in patients with different types of chronic HBV infection

Chronic HBV infection (n)	Positive number of HBV-DNA	Positive percentage of HBV-DNA	Average logarithm quantity of HBV-DNA (ml^{-1})
Mild (10)	6	60%	$7.89 \pm 1.04^{*}$
Moderate (10)	7	70%	$8.05 \pm 0.56^{*}$
Severe (10)	6	60%	$8.17 \pm 0.63^{*}$

Data were shown as mean ± SD. $^{*}p > 0.05$ compared among the three patient groups.

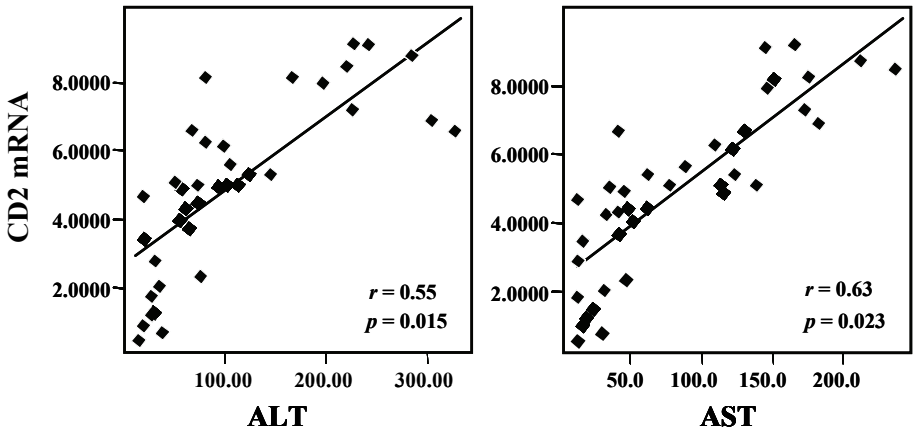

Figure 3. The relationship between the expression level of CD2 mRNA and the serum level of ALT and AST.

accepted that infected hepatocytes were identified and eliminated by the CTL (5-7). In the present study, we found that the levels of CD2 mRNA and the percentages of CD2 positive cells in PBMC were all higher than those in the normal controls, and the higher levels of CD2 mRNA and the percentages of CD2 positive cells, the more severity of disease. The increased expression of CD2 in PBMC of patients with chronic HBV infection could promote the adhesion between HBV-infected hepatocytes and T cells, increase the activity of T and NK cells, and stimulate the differentiation of $CD4^+$ T cells towards Th1 cells which could accelerate cell-mediated immunity (13, 14). T cells activated by interaction with complex of CD2 and CD58 would increase the transcription and translation of IL-2 and IFN-γ mRNA, and then differentiate into Th1 which would enhance the cellular immune response. Binding with CD2 molecules on the surface of NK cells, CD58 would activate NK cells and increase the cytotoxic effect (25). This might contribute to the elimination of HBV. The cytotoxic effect of the immune cells might be implemented by releasing PF or inducing apoptosis of CD95 positive cells (29, 30).

In addition, the levels of HBV DNA, which were considered the molecular biological parameter of the HBV infection and reflected the level of the virus replication *in vivo*, was determined by the real-time FQ-PCR (24-28), and the result showed that the logarithm averages of the levels of HBV DNA in positive serum samples had not significant difference among the three chronic HBV infection groups ($p > 0.05$). This result suggested that HBV may not be a direct cause of the liver damage, but the immune response of host to HBV might damage the infected liver cells as well as eliminate HBV. To evaluate the relationship between CD2 and the hepatocellular damage of the chronic HBV infection, we also detected the liver function indexes of ALT, AST, TBIL, DBIL, and IBIL, which reflect the degree of the hepatocellular damage. The findings showed that the levels of CD2 mRNA and the percentages of CD2 positive cells in PBMC of patients with chronic HBV infection were positively correlated with liver function indexes ($p < 0.05$).

In summary, this study showed that the expression of CD2 was correlated with the degree of the hepatocellular damage of HBV infection. CD2 probably increased cell-

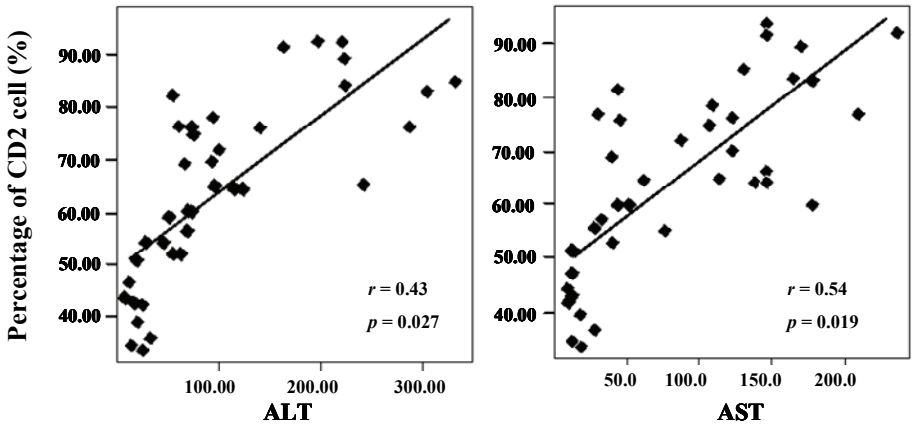

Figure 4. The relationship between the percentages of CD2 positive cells and the serum levels of ALT and AST.

mediated immune response to eliminate HBV and lead to damage of liver tissue, through accelerating activation of T cells and NK cells.

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