Hepatitis B virus DNA is more powerful than HBeAg in predicting peripheral T-lymphocyte subpopulations in chronic HBV-infected individuals with normal liver function tests

Jing You, Hutcha Sriplung, Alan Geater, Virasakdi Chongsuvivatwong, Lin Zhuang, Hong-Ying Chen, Jun-Hua Huang, Bao-Zhang Tang

Abstract

AIM: To investigate the peripheral T-lymphocyte subpopulation profile, and its correlations with hepatitis B virus (HBV) replication level in chronic HBV-infected (CHI) individuals with normal liver function tests (LFTs).

METHODS: Frequencies of T-lymphocyte subpopulations in peripheral blood were measured by flow cytometry in 216 CHI individuals. HBV markers were detected with ELISA. Serum HBV DNA load was assessed with quantitative real-time PCR. Information of age at HBV infection, and maternal HBV infection status was collected. ANOVA linear trend test and linear regression were used in statistical analysis.

RESULTS: CHI individuals had significantly decreased relative frequencies of CD3⁺, CD4⁺ subpopulations and CD4⁺/CD8⁺ ratio, and increased CD8⁺ subset percentage compared with uninfected individuals (all P < 0.001). There was a significant linear relationship between the load of HBV DNA and the parameters of T-lymphocyte subpopulations (ANOVA linear trend test P < 0.01). The parameters were also significantly worse among individuals whose mothers were known to be HBV carriers, and those having gained infection before the age of 8 years. In multiple regressions, after adjustment for age at HBV infection and status of maternal HBV infection, log copies of HBV DNA maintained its highly significant predictive coefficient on T-lymphocyte subpopulations, whereas the effect of HBeAg was not significant.

CONCLUSION: HBV DNA correlates with modification in the relative T-lymphocyte subpopulation frequencies. High viral load is more powerful than HBeAg in predicting the impaired balance of T-cell subsets.

Key words: Hepatitis B virus; Chronic hepatitis B virus infection; Hepatitis B virus DNA; T-lymphocyte subpopulation; Immune function

INTRODUCTION

Hepatitis B virus (HBV) infection is a global public...
health problem. It is estimated that approximately 2 billion people have serological evidence of past or present HBV infection and more than 350 million individuals worldwide are chronically infected with HBV. In infected adolescents or adults, 5%-10% will develop into a chronic carrier state, whereas in infected neonates up to 90% develop chronicity. HBV infection is especially prevalent in African and Asian countries such as Korea, Japan, Taiwan and mainland China because most patients with chronic HBV infection have acquired the infection perinatally from carrier mothers. China has the highest prevalence of HBV, with over one-third of the world's total estimated HBV carriers. Out of the chronic HBV-infected patients, 70%-80% could have persistent normal liver function for many years or a lifetime. Further persistent viral infection can, however, lead to subclinical hepatitis and chronic active hepatitis, even liver cirrhosis and the development of hepatocellular carcinoma.

The pathogenesis of persistent viral infection and hepatitis B is complex. Generally, it is not HBV itself that damages hepatocytes directly, but the result of function disorder of cell-mediated immunity. The cellular immune response to HBV is thought to be responsible for viral clearance, and disease pathogenesis during infection. The T-cell response to HBV is vigorous, polyclonal, and multispecific in acutely infected patients who successfully clear the virus, and it is relatively weak and narrowly focused in chronically infected patients. The outcome of HBV infection would depend upon the balance between development of immunity (leading to virus elimination) and tolerance (leading to chronic viral persistence). HBeAg may play an important role in the interaction of the virus with the immune system. Secreted HBeAg has been proposed to have an immunoregulatory function in uterus by establishing T-cell tolerance to HBeAg and HBeAg that may predispose neonates born to HBV-infected mothers to develop persistent HBV infection. Recent studies have further demonstrated an immunomodulatory role of HBeAg in antigen presentation and recognition by CD4+ cells.

It is essential to study the HBV replication status and its effects on cellular immune function in normal LFTs chronic HBV-infected (CHI) individuals. Firstly, they are the majority of chronic HBV-infected individuals; secondly, the understanding of the immune response upon HBV infection is useful to develop appropriate therapeutic strategies for controlling viral hepatitis and disease progression, as well as to improve current knowledge regarding persistent HBV infection prognosis. However, the correlations between HBV-specific T-cell response, and HBV viral load and HBeAg expression in CHI individuals are complicated. So are the effects of age at first infection and maternal HBV infection status. The aim of the work reported herein was to evaluate the peripheral blood T-lymphocyte subpopulation profile, and its correlations with HBV replication level, and to determine further which active marker of HBV active replication, HBV DNA or HBeAg is more powerful in predicting peripheral T-lymphocyte subpopulation in CHI individuals.

MATERIALS AND METHODS

Enrollment of study subjects

Two hundred and sixteen consecutive CHI individuals with normal LFTs were recruited from the Department of Infectious Diseases and of Hepatology of the First Affiliated Hospital of Kunming Medical University, the Third Municipal People's Hospital of Kunming and the Yunnan General Hospital of The Chinese People's Armed Police Forces, between January 2004 and May 2007.

The following criteria were fulfilled by all individuals: (1) steady positivity for HBsAg in their serum for at least 12 mo and persistently normal liver function tests; and (2) exclusion of other concomitant causes of liver disease (hepatitis C, D and HIV infection and alcohol consumption of more than 60 g/day) and relatively rare liver disease (autoimmune hepatitis and metabolic liver disease) and treated with immunosuppressive therapy or antiviral therapy for HBV-infection within the recent 12 mo before entry. None of the patients was a drug user, or exposed to hepatotoxin. Informed consent was obtained from each study subject. The study protocol conformed to the guidelines of Declaration of Helsinki and was approved by ethics committees of the Faculty of Medicine of Prince of Songkla University and the First Affiliated Hospital of Kunming Medical University.

One hundred individuals who were free of HBsAg were identified from individuals coming to the outpatient service for a health check-up; 61 of the participants were male, 39 were female; mean age, 33.24 (SD, 10.28) years. These served as the control group for comparison of T-lymphocyte subpopulation with those who had HBV infection.

Serological liver function tests and hepatitis B virus markers evaluation

Serum alanine amino-transferase (ALT), aspartate transaminase (AST) and total bilirubin (TBil) were tested with routine automated techniques (upper limit of normal: 40 U/L, 40 U/L and 17.1 μmol/mL, respectively) (AU2700, Japan). HBV markers (HBsAg, HBsAb, HBeAg, HBeAb, HBcAb, and anti-HBeAb IgM) were measured at a virological laboratory with enzyme-linked immunosorbent assay (ELISA) (Anthos 2010, Austria). The experimental methods followed those specified within the reagent kit (Sino-American Biotech Co., Ltd) package insert.

Quantitative measurement HBV DNA (viraemia)

Serum HBV DNA load in individuals was assessed by the real-time fluorescent quantitative polymerase chain reaction method (Real-Time-PCR) using a Lightcycler PCR system (FQD-33A, Bioer) with a lower limit of detection of approximately 1000 viral genome copies/mL. The handling procedures were performed in strict accordance with the reagent kit (Shenzhen PG Biotech Co., Ltd) package insert. The primer was provided in the kit, the reaction volume was 40 μL, and the reaction condition was 37℃ for 5 min, 94℃ for 1 min then 40 cycles as 95℃ for 5 s and 60℃ for 30 s.

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Peripheral blood T lymphocyte subsets measurement

The key components of cellular immunity are T-lymphocyte and its subpopulations. CD3+, CD4+ and CD8+ cells are major functional subgroups of T cells, and play an important role in response to HBV infection, which can reflect the situations of cellular immune function and immunoregulation, and are usually regarded as a valuable index to forecast the changes of patients’ immunity[1-3]. These indices were included in our study for evaluating cellular immune function status of normal LFTs CHI individuals.

Blood samples were collected in heparinized vacutainer tubes. Whole blood samples were analyzed with a Multi-Q-Prep processor (Coulter, USA) and thereafter Epics-XL flow cytometry (FCM) (Coulter, USA). Lymphocytes were analyzed using a gate set on forward scatter versus side scatter, and a three color flow cytometry to combination reagent of CD3, CD4 and CD8. Anti-human monoclonal antibodies CD3-PE-CY5/CD4-FITC/CD8-PE were purchased from Immunotech, Ltd, USA. The detection was analyzed with the CELLQuest software (Coulter, USA) for each sample. The results were expressed as the percentages of CD3+, CD3+/CD4+ (short for CD4+ below) and CD3+/CD8+ (short for CD8+ below) cells found to be positive for the marker antigen in the total T-cell population. The handling procedures were performed in strict accordance with the instructions within the reagent kit package insert.

Maternal HBV infection status (MH)

All mothers of the subjects were reviewed in medical records for previous HBV infection and most of those who were infected could be identified. In addition, all of them were invited to undergo HBV-marker tests. For those with a positive result, a second set of tests was conducted 3 mo after the first test to confirm chronic HBV carrier status. If the mother had died, the cause of death was investigated based on medical records and history taking whether it was from HBV-related liver diseases such as chronic hepatitis B, HBV-related liver cirrhosis or hepatocellular carcinoma. If so, the MH was classified as positive.

Age at HBV infection

In the recent three decades in China, all children have been obligated to be tested for HBV markers when they first enter kindergarten and elementary schools. Subsequent obligatory tests are made when they apply for university or for a job. The results of these tests were obtained from medical records and interview. Based on this setting, we classified the age of first positive test as before 8 years, between 8-20 years and after 20 years.

Statistical analysis

Initial calculation came up with a sample size of 50 subjects with HBV DNA positive and the same number of HBV DNA negative group. This could provide the study with a statistical power of 80% at the 0.025 level of significance to detect a difference in T-cell variation values of 33 versus 38. However, to cover the problem of being potentially confounded by other variables and to have enough subjects for stratifying levels of HBV DNA load to examine dose-response relationship, we ultimately recruited 216 CHI individuals and 100 controls.

Descriptive statistics were used to examine the age, gender, serum HBV viral load, HBeAg status, age at HBV-infection and maternal HBV infection status. The levels of T-lymphocyte subpopulation in normal individuals (HBsAg-negative) were summarized as means and standard deviation to serve as a control reference. Effects of various independent demographic, clinical and serological variables on T-cell profile were analyzed only among HBsAg-positive individuals. In univariate analysis, breakdown of these profiles by individual independent variables was carried out. Independent t test was done for 2-level independent variables and one-way ANOVA for more than 2-level variables. The relationship of HBV replication level and peripheral T-lymphocyte subpopulation was analyzed by correlation analysis and ANOVA linear trend test. Finally, multiple linear regression models were employed in multivariate analysis to assess the independent effects of variables on peripheral blood T lymphocytes. Variables yielding a P value ≤ 0.2 in the univariate analysis were included in the multivariate analysis, and the models were refined by backward elimination guided by the change in log likelihood of successive models. A final P value of less than 0.05 was considered statistically significant. Computations were carried out with the aid of R software version 2.5.1[10].

RESULTS

Demographic characteristics and clinical features of CHI individuals

Demographic, serological, and clinical characteristics of the CHI individuals are summarized in Table 1. They were predominated by male (57.9%). One hundred and twenty four (57.4%) were less than 30 years old.

Of the CHI individuals, 37% got the infection before the age of 8 years. Almost three quarters had detectable serum levels of HBV DNA. Among these, the majority (68.4%, 93/136) had over 10^3 copies per milliliter. Just over half of them were HBeAg positive (56.5%).

Around 60% of the individuals’ mothers were HBV positive. Among these individuals, nearly half had young age of infection and five-sixths had detectable serum levels of HBV DNA, of whom the majority (79.2%) had high viral load. Over 75% were HBeAg positive, whereas non-MH individuals were characterized by high age of infection, low viral load and low positivity of HBeAg.

Of those who had young age at infection, 80% (64/80) were HBeAg positive, and the majority (69/80) had detectable serum levels of HBV DNA, of whom nearly 74% (51/69) had high viral load.
Peripheral T lymphocyte subpopulation composition in CHI individuals with normal LFTs

CHI individuals had significantly decreased relative frequencies of CD3+ and CD4+ subpopulations and CD4+/CD8+ ratio, and increased CD8+ subset percentage compared with the control group. Univariate analyses showed that the impaired balance of T-cell subpopulations and viral load is also shown in Figure 1 (r = -0.67, -0.54, 0.61, -0.67, respectively, for CD3+, CD4+, CD8+ and CD4+/CD8+ ratio; all P < 0.0001) and Figure 2.

Linear regression predicting peripheral blood T-lymphocyte subpopulation from relevant parameters

In Table 3, linear regression models are separately summarized for CD3+, CD4+ and CD8+ cells and CD4+/CD8+ ratio, which are the dependent variables. After adjustment for all independent variables listed in the table, serum level of HBV viral load was the only significant predictor for each outcome variable, whereas the effects of HBeAg and other variables were not significant.

Figure 3 shows the relationship between T-lymphocyte subpopulations and viral load for all children. The correlation coefficient for each measure and each T-cell parameter was statistically significant at the 0.01 level. The relationship between T-lymphocyte subpopulations and viral load is also shown in Figure 1 (r = -0.67, -0.54, 0.61, -0.67, respectively, for CD3+, CD4+, CD8+ and CD4+/CD8+ ratio; all P < 0.0001) and Figure 2.

Table 1  Characteristics of chronic HBV-infected individuals with normal liver function tests

| Characteristics | All individuals (n = 216) | Maternal HBV-infection status (MH) |
|-----------------|---------------------------|-----------------------------------|
|                 | Individuals with MH (n = 129) | Individuals without MH (n = 87) | P |
| Sex (male/female) | 125/91 | 75/54 | 50/37 | 0.922 |
| Mean age (yr) | 31.53 ± 11.23 | 29.11 ± 11.44 | 35.13 ± 9.94 | < 0.001 |
| Age of HBV-infection (yr) (%) | | | |
| < 8 | 80 (37.0) | 62 (48.1) | 18 (20.7) | | |
| 8-20 | 56 (25.9) | 36 (27.9) | 20 (23.0) | | |
| > 20 | 58 (26.9) | 21 (16.3) | 37 (42.5) | | |
| Unknown | 22 (10.2) | 10 (7.8) | 12 (13.8) | | |
| HBV DNA positive (%) | 136 (63.0) | 106 (82.2) | 30 (34.5) | < 0.001 |
| Serum HBV DNA (copies/mL) (%) | | | |
| < 1.0 × 10^5 | 80 (37.0) | 23 (17.8) | 57 (65.5) | | |
| 1.0 × 10^3-1.0 × 10^5 | 14 (6.5) | 5 (3.9) | 9 (10.3) | | |
| 1.0 × 10^-2-1.0 × 10^2 | 29 (13.4) | 17 (13.2) | 12 (13.8) | | |
| > 1.0 × 10^5 | 93 (43.1) | 84 (65.1) | 9 (10.3) | | |
| HBV DNA load (log, copies/mL) | 5.90 ± 2.61 | 7.13 ± 2.36 | 4.07 ± 1.74 | < 0.001 |
| HBeAg positive (%) | 122 (56.5) | 97 (73.2) | 35.75 ± 6.08 | < 0.001 |

1Chi-square test P value; 2Student t test P value; HBV: Hepatitis B virus; MH: Maternal HBV-infection status.

Table 2  Peripheral T-cell subsets in normal control and CHI individuals broken down by various factors (mean ± SD)

| Groups | n | CD3+ (%) | CD4+ (%) | CD8+ (%) | CD4+/CD8+ ratio |
|--------|---|----------|----------|----------|-----------------|
| HBV status | | | | | |
| Negative | 100 | 71.07 ± 4.76 | 38.94 ± 3.39 | 24.02 ± 4.35 | 1.67 ± 0.33 |
| Positive | 216 | 57.35 ± 13.81 | 32.97 ± 7.00 | 33.08 ± 7.99 | 1.07 ± 0.41 |
| Maternal HBV-infection status | | | | | |
| Negative | 87 | 64.67 ± 10.74 | 35.75 ± 6.08 | 28.71 ± 5.56 | 1.29 ± 0.35 |
| Positive | 129 | 52.42 ± 13.49 | 31.10 ± 6.98 | 36.03 ± 8.04 | 0.93 ± 0.38 |
| Age at HBV-infection (yr) | | | | | |
| < 8 | 80 | 66.35 ± 8.19 | 30.78 ± 7.03 | 35.36 ± 7.12 | 0.91 ± 0.30 |
| 8-20 | 56 | 66.46 ± 9.33 | 31.65 ± 5.06 | 35.80 ± 7.79 | 0.93 ± 0.28 |
| > 20 | 58 | 69.35 ± 9.85 | 36.37 ± 7.31 | 28.22 ± 7.11 | 1.37 ± 0.46 |
| Unknown | 22 | 69.35 ± 9.85 | 35.35 ± 6.62 | 30.69 ± 7.48 | 1.24 ± 0.46 |
| HBV DNA load (copies/mL) | | | | | |
| ≤ 1.0 × 10^5 | 80 | 65.84 ± 9.39 | 37.11 ± 6.29 | 28.12 ± 5.65 | 1.38 ± 0.40 |
| 1.0 × 10^3-1.0 × 10^5 | 14 | 65.36 ± 5.15 | 34.70 ± 2.79 | 28.66 ± 6.21 | 1.28 ± 0.38 |
| > 1.0 × 10^5 | 29 | 66.20 ± 9.99 | 33.66 ± 6.59 | 32.40 ± 6.54 | 1.06 ± 0.24 |
| HBeAg status | | | | | |
| Negative | 94 | 64.45 ± 10.44 | 35.81 ± 6.69 | 29.05 ± 6.43 | 1.30 ± 0.42 |
| Positive | 122 | 51.89 ± 13.63 | 30.78 ± 6.46 | 36.19 ± 7.69 | 0.89 ± 0.31 |

1P < 0.001 for all comparisons of +ve vs -ve for each measure and each T-cell parameter; 2P < 0.01 for ANOVA linear trend test; 3P < 0.05, 4P < 0.01, 5P < 0.001 vs < 8 yr group; 6P < 0.01, 7P < 0.001 vs 8-20 yr group.
Phocyte subpopulations and viral load stratified by age at HBV infection. There was no significant difference of T-cell subsets among groups of age at HBV infection after adjustment for serum level of HBV viral load. A similar pattern is also seen in the figures that show the relationship between T-lymphocyte subpopulations and viral load stratified by maternal HBV carrier status and by HBeAg status in CHI individuals with normal LFTs respectively.

**DISCUSSION**

This study demonstrated an impaired balance of the T-cell subsets related to an increased proportion of CD8⁺ T-lymphocytes and decreased proportion of CD4⁺ T-lymphocytes and CD4⁺/CD8⁺ ratio in CHI individuals who had normal liver function tests. The level of the T-cell impairment had a linear dose-response relationship with the load of HBV DNA. The study also

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**Figure 1** Correlation between peripheral T-cell subsets and serum HBV viral load. The numbers in the boxes refer to correlation coefficients. There is a negative correlation between the CD3⁺ and CD4⁺ cells and CD4⁺/CD8⁺ ratio and serum viral load in CHI individuals with normal LFTs (r = -0.67, -0.54, -0.67; P < 0.0001), and a positive correlation between the levels of CD8⁺ cells and viral load (r = 0.61, P < 0.0001).

**Table 3** Multiple linear regression predicting peripheral blood T lymphocyte subpopulation (n = 216)

|                    | CD3⁺ T lymphocyte | CD4⁺ T lymphocyte | CD8⁺ T lymphocyte | CD4⁺/CD8⁺ ratio |
|--------------------|-------------------|-------------------|-------------------|-----------------|
| Intercept          | β: 79.54          | SE: 3.03          | P: -               |                 |
| Serum HBV load     | -3.65             | 0.43              | < 0.0001          |                 |
| (log, copies/mL)²  | -1.38             | 0.25              | < 0.0001          |                 |
| HBeAg negative     | 0.61              | 1.16              | 0.6               | 0.69            |
| Age at HBV-infection(yr)³ | 0.06 | 0.06 | 0.06 | 0.74 |
| 8-20               | -1.53             | 1.8               | 0.27              | 1.05            | 0.03 | 0.05 |
| > 20               | -4.77             | 2.08              | 1.2               | 1.22            | -1.82 | 0.15 | 0.06 |
| Unknown            | 1.28              | 2.55              | 1.64              | 1.49            | -0.77 | 1.58 | 0.13 | 0.08 |
| Maternal HBV-infection status | 2.17 | 1.74 | 0.21 | 0.31 | 1.02 | 0.77 | -2.45 | 1.08 | 0.02 |

β: Coefficients from the model; SE: Standard error. ¹Continuous variable; ²control group, < 8 yr of age at HBV infection.
illustrated the strong independent effects of HBV DNA, which eliminate the effects of maternal carrier status, younger age of infection and HBeAg positivity.

Our findings indicate that normal LFTs chronic HBV-infected individuals have an impaired balance of T-cell profile. The same finding also has been proved by previous researches in patients with chronic hepatitis B (CHB) that the chronicity of HBV infection is caused by a deficiency in cellular immune function, and hepatocytic damage is mainly caused by immunological injury\textsuperscript{[12-21]}. However, the mechanism has not been defined\textsuperscript{[5]}. Recently, the results have been reported by Tian et al\textsuperscript{[22]} that CD4\textsuperscript{+} and CD8\textsuperscript{+} T cells decreased in both 33 CHB patients and 21 asymptomatic HBV carriers. Thus, most evidence has come from research in experimental animals\textsuperscript{[23-25]} and in CHB patients\textsuperscript{[26-30]}.

Our results reveal that T-cell impairment was significantly associated with viral replication level. The substantial linear dose-response relationship and strong independent predictive ability of HBV DNA, but not of other variables, on T-cell subpopulations suggests a close proximity between them in the causal pathway. However, cross-sectional study nature of our data does not allow us to identify the temporal direction of the causal relationship between these two variables. Mizukoshi et al\textsuperscript{[31]} suggested that antiviral therapy of persistently infected patients appeared to increase the frequency of HBV-specific CD4\textsuperscript{+} T cell responses during the first year of treatment. Boni et al\textsuperscript{[32-35]} reported that antiviral treatment can overcome CD8\textsuperscript{+} T cell hypo-responsiveness in subjects with chronic HBV infection, suggesting that the T cells are present, but suppressed. It was reported by Pham et al\textsuperscript{[36]} in 21 CHB patients that the ratio of CD4\textsuperscript{+}/CD8\textsuperscript{+} liver-derived lymphocytes, and not of peripheral blood lymphocytes appeared to be related to the level of HBV replication, and it revealed a positive correlation with viral load. The evidence that efficient antiviral T cell response can be restored by mono-antiviral treatment in CHB patients concurrently with reduction of viremia, indicates the importance of viral load in the pathogenesis of T cell hypo-responsiveness in these patients.

The stronger independent effect of viral load on the T-cell impairment and viral factor (viral variants) might explain the disappearance of the effect of other variables in multivariate analysis. Among our research subjects, the majority were characterized by young age of first HBV infection, maternal carrier status and high viral load in serum, and also high HBeAg expression. As a matter of fact, in addition to HBV DNA, HBeAg is also a seromarker for high viral replication which plays...
a crucial role in chronicity of HBV infection and high viral load by inducing immunological tolerance to HBV in the fetus. The tolerating effect of HBeAg has been well characterized in mice and likely contributes to the low level of core-specific T-cell responses present in HBeAg+ chronic patients. Clinical evidence supports the tolerogenic effect of HBeAg. Also, viral mutations that abrogate or antagonize antigen recognition by virus-specific T cells have been reported in patients with chronic HBV infection, although the results from univariate analysis in our study showed that dysfunction of T-cell was significantly related with HBeAg, which later disappeared in multivariate analysis. One possible reason is that some of the subjects were infected with pre-C stop codon mutation virus (pre-C/C mutant), which resulted in a loss of HBeAg. In these patients, therefore, viral replication may persist despite elimination of HBeAg and seroconversion to anti-HBe. The loss of HBeAg appears irrelevant for the biology of the virus, it may play an important role in the interaction of the virus with the immune system. This may weaken the independent association between HBeAg and the T-cell impairment so that the sample size in our study cannot detect this magnitude of association. Those who had maternal carrier history usually got infection at a younger age (Table 1) and a higher HBV viral load was detected in the majority of those who had infection at a younger age. This phenomenon suggests that infection from the mother and/or at younger age predisposes to tolerance to HBV infection and, thus, higher viral load.

The strength of this study lies in the large sample size of CHI individuals with normal LFTs and the measurements of T-lymphocyte subpopulations using modern advanced flow cytometric technology and viral load by the quantitative real-time PCR method. A limitation of this study is the unknown age at HBV-infection of 22 individuals, the specificity of T-lymphocyte subpopulations, and liver-derived T-lymphocyte were not explored concurrently. Although the strong relationship of T-lymphocyte subpopulations with viral load is illustrated, further studies are needed to confirm the causal relationship between them.

Our results, which suggest that high viral load contributes to the impaired balance of the T-cell subsets in normal LFTs CHI individuals, have practical implications for understanding of pathogenesis and controlling of persistent viral infection, disease progression and prognosis because these individuals are at risk of persistent viral infection leading to sub-clinical hepatitis, and chronic active hepatitis, even
liver cirrhosis and the development of hepatocellular carcinoma\[3\]. Perhaps, we should take this contribution into account in designing Interventional strategies such as anti-viral therapeutic and/or immunotherapeutic strategies to prevent the progression and long-term consequences, which have been proved effective in CHB patients. Further clinical studies are needed to explore this possibility not only in CHB patients but also in normal LFTs chronic HBV-infected individuals.

In conclusion, we found that a strong independent predictive effect of HBV DNA load on T-lymphocyte subpopulations suggests a close proximity in the causal pathway between HBV viral load and the T-cell impairment. This information is of great interest because, first, it will be possible to predict the variation of T-lymphocyte subpopulations in peripheral blood in the future by measuring serum viral load level in chronic HBV-infected individuals with normal LFTs and second, this parameter can be monitored in blood easily and cheaply. Therefore, the measurement of viral load in serum of individuals suffering from chronic HBV infection could represent a simple parameter for the evaluation of cellular immune function status.

**COMMENTS**

**Background**

Hepatitis B virus (HBV) infection is a serious public health problem worldwide and a major cause of chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma. HBV infection is especially prevalent in African and Asian countries because most patients with chronic HBV infection have acquired the infection perinatally from carrier mothers. The pathogenesis of persistent viral infection is very complex and has not been clarified until now. Generally, it is not HBV itself that damages hepatocytes directly, but the results of disorder of cell-mediated immunity. The outcome of HBV infection would depend on the balance between development of immunity (leading to virus elimination) and tolerance (leading to chronic viral persistence).

**Research frontiers**

Outcome of HBV infection, and the pathogenesis of liver disease are determined by immune-mediated host-virus interaction, which have been difficult to fully elucidate because the host range of HBV is limited to man and chimpanzees. The pathogenesis of liver disease and interaction between virus and host remain the research hotspots in this field.

**Innovations and breakthroughs**

The pathogenesis and correlation of cellular immune disorder and HBV viral replication level remain unknown. In the present study, peripheral T-lymphocyte subpopulations of chronic HBV-infected (CHI) individuals who had normal liver function tests in big sample size were measured using advanced flow cytometry technology and HBV viral load with sensitive quantitative real-time-PCR method. The results suggest that the impaired balance of T-cell subpopulations was significantly associated with viral replication level. The substantial linear dose-response relationship and strong independent predictive effect of viral load on T-lymphocyte subpopulations suggests a close proximity of the causal pathway between them, and indicates the importance of viral load in the pathogenesis of T-cell impairment in these patients.

**Applications**

The results, which suggest that high viral load contributes to the impaired balance of the T-cell subsets in normal liver function tests (LFTs) CHI individuals, have practical implications for understanding of pathogenesis and controlling of persistent viral infection and disease progression and prognosis because these individuals are also at risk of persistent viral infection leading to subclinical hepatitis and chronic active hepatitis, even liver cirrhosis and the development of hepatocellular carcinoma. In addition, it is possible to predict the variation of T-lymphocyte subpopulations in peripheral blood in the future by measuring serum viral load level in chronic HBV-infected patients.

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