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Dynamic analysis of lymphocyte subsets of peripheral blood in patients with acute self-limited hepatitis B

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ABSTRACT

Purpose: To investigate dynamic changes and significance of lymphocyte subsets (T lymphocytes, B lymphocytes, NK cells and T cell subsets) of peripheral blood in patients with acute self-limited hepatitis B (AHB). Methods: Immune cells of peripheral blood were compared among 17 cases of self-limited acute hepatitis B patients, 36 patients with chronic hepatitis B (CHB) and 32 healthy controls by flow cytometry (FCM). $CD4^+/CD8^+$ was monitored dynamically, meanwhile relations between T lymphocyte subsets and ALT and clearance of HBV DNA were explored. Results: Dynamic changes of lymphocyte subsets were found in AHB, the level of $CD3^+$T cells was significantly higher compared to CHB group and healthy control group. Frequencies of $CD3^+CD4^+$ T cells in the third and fourth week and $CD4^+/CD8^+$ in the second week were higher compared to other groups. Frequency of NK cells was low and was significantly lower compared to other groups in the third week specially. It was showed that $CD4^+/CD8^+$ was low followed by high abnormal ALT during early stage by dynamic monitoring of $CD4^+/CD8^+$, and $CD4^+/CD8^+$ was increasing accompanied by normal ALT set by set, but $CD4^+/CD8^+$ had no significant relation to ALT and HBV DNA. Conclusion: Immune status of AHB, compared to CHB and healthy controls, was significantly different and dynamic changes of lymphocyte subsets may be related to progress of disease.

Keywords: Acute Hbv; Self-Limited; Lymphocyte Subsets; Facs

1. INTRODUCTION

Hepatitis B virus (HBV) is one of the most prevalent viral pathogens in humans, with almost a third of the world population having evidence of infection, and about 350 million chronically infected patients. Approximately 1 million people die annually from HBV-related disease, such as liver failure, cirrhosis and hepatocellular carcinoma [1]. After body was infected by Hepatitis B virus (HBV), complex immune responses could be caused, and cell-mediated immunity is an important factor to determine results of HBV infection [2]. Different subsets of lymphocyte have different responses to viral antigens and effects on clinical course and prognosis of development. Many precious researches focused on lymphocyte subsets in peripheral blood of chronic hepatitis B [3,4], which indicated that there was an imbalance in peripheral blood T-lymphocyte subsets and turbulence in cellular immunity. Researches about stable detection of lymphocyte subsets for adults with acute self-limited hepatitis B (referred to as "acute hepatitis B") [5] showed that acute-phase CD4 responses were efficient and CD8 responses were multispecific irrespective of the outcome of infection. In this paper, by comparing differences of lymphocyte subsets among acute hepatitis B, chronic hepatitis B and normal controls, dynamic characteristics of lymphocyte subsets in acute hepatitis B were observed and the relationship between body's immune response to HBV infection and disease prognosis was explored.

2. MATERIALS AND MEHTODS

2.1. Characteristics of Patients

Patients with acute hepatitis B, chronic hepatitis B were out-patient clinics and hospitalized patients of Department of Infectious Diseases, the First Affiliated Hospital with Nanjing Medical University from May 2007 to May 2008, diagnosis were in line with the revised diagnostic criteria of the Tenth National Conference on viral hepatitis and Liver Disease for viral hepatitis in 2000 [6] and the "prevention and treatment of chronic hepatitis B

guide" [7], and exclusion of other hepatitis viruses and CMV, EBV, HIV co-infection. The information of patients was shown in **Table 1**.

2.2. Instruments and Reagents

IgG1-PE/IgG2a-FITC,CD3/CD4/CD8/CD45,CD3/CD4/CD28, CD3/CD8/CD28, CD3/CD19/CD56/CD45 monoclonal antibodies (fluorescent-labeled), as well as hemolytic agents were purchased from BD company(USA). Flow cytometry (BD FACS Calibur, USA). Automatic biochemical analyzer and its reagents were Beckman Company's products (USA). HBV DNA quantitative detection equipment were production of Roche's Light Cycler 1.0(USA).

2.3. Detection of Lymphocyte Subsets

Methods were referred Ya Pinghan [8] etc. and modified it slightly. 100 μl blood samples were collected into heparinized vacuum tubes and blended with mouse anti-human monoclonal antibody CD3/CD8/CD45/CD4, CD3/CD16 + 56/CD45/CD19 (fluorescent-labeled) 20 ul respectively, reacted at room temperature protected from light for 30 min, and was detected by FACS in 2 h. A "gate" of the leukocyte common antigen (CD45)-positive cells was set up according to forward and side scatter two-parameter point diagram and lymphocyte surface markers were analyzed by two-parameter. Statistical results were analyzed by use of CellQuest software.

2.4. Dynamic Detection of Lymphocyte Subsets

The frequencies of lymphocyte subsets were measured and dynamically analyzed for acute hepatitis B when admitted to hospital for the first, second, third and fourth week, compared to normal controls and chronic hepatitis B. Dynamical $CD4^+/CD8^+$ ratios were detected and dynamical relationships between $CD4^+/CD8^+$ ratios and alanine aminotransferase (ALT) were analyzed. In order to explore relationship between $CD4^+/CD8^+$ ratios and clearance of HBV DNA, $CD4^+/CD8^+$ ratios of acute hepatitis B with HBV DNA positive and negative patients were analyzed and compared.

2.5. Statistical Analysis

Measurement data were expressed using $\bar{X} \pm s$. By application of SPSS 11.0 statistical software, means of two groups were compared using *t* test or paired *t* test and multiple sets of means were compared by single-factor ANOVA analysis of variance. The correlation among ratios of lymphocyte subsets, alanine aminotransferase and HBV DNA level were analyzed by use of correlation analysis. $P < 0.05$ was referred as statistically significant.

Table 1. Some characteristics of different groups in patients.

Group	No	Male	Female	Average age
AHB	17	11	6	32.4 ± 4.8
CHB	36	20	16	37.5 ± 6.2
Normal	32	18	14	35.5 ± 3.9

A total of 17 cases of acute hepatitis B group, 11 males and 6 females, aged from 18 to 57 years old, and average (32.4 ± 4.8) years of age. Chronic hepatitis B group, 36 cases were recruited, 20 males and 16 females, aged from 23 to 75 years old, and mean (37.5 ± 6.2) years of age. Healthy controls, 32 cases without past and current HBV infection, as a normal control group, 18 male cases and14 female, aged from 22 to 53 years old, and mean (35.5 ± 3.9 years).

3. RESULTS

3.1. Changes of Lymphocyte Subsets for Acute Hepatitis B

For acute hepatitis B at admission and the dynamic changes trend of lymphocyte subsets for the first, second, third and fourth week (**Figure 1(a)**), and lymphocyte subsets of normal controls and chronic hepatitis B (**Figure 1(b)**).Peripheral blood $CD3^+$ T cells within 4 weeks of acute hepatitis B hospitalization were significantly higher, and the difference was statistically significant (**Figure 2**) compared to normal controls and CHB group. Frequencies of $CD4^+$ T cells from admission to 4th week showed a rising trend which were higher than normal controls and CHB group, and the differences of the third week and fourth week were statistically significant (**Figure 3**).For acute hepatitis B,$CD8^+$ T cells showed a upward trend first and then showed a downward trend, and the difference was statistically significant for the results of the second week (**Figure 4**).$CD56^+$ cells in 4 weeks were lower than both normal controls and CHB group, however, the third week compared with other two groups and the fourth week compared with CHB group respectively, the difference were statistically significant (**Figure 5**).

3.2. Level of ALT in Acute Hepatitis B and Changes in the Ratio of Lymphocyte Subsets

For acute hepatitis B, the trends of $CD4^+/CD8^+$ ratio means and ALT dynamic changes for hospitalized in the first week, second week, third week and fourth week were showed in **Figure 6**. For third week and fourth week, difference of $CD4^+/CD8^+$ ratio was statistically significant ($P < 0.05$) compared with chronic hepatitis B

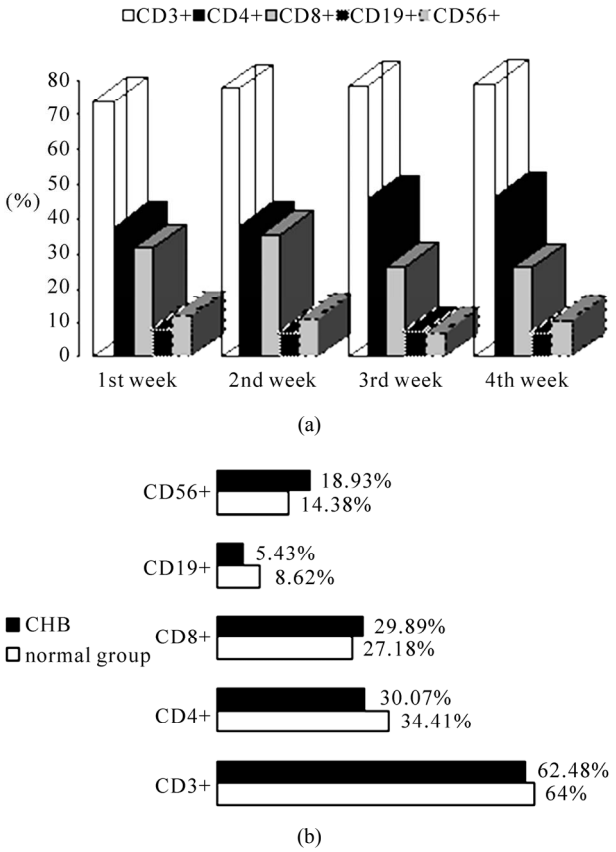


Figure 1. (a) Dynamic changes of acute hepatitis B lymphocyte subsets. Patients admitted to hospital and dynamic changes of lymphocyte subsets $CD3^+$,$CD4^+$,$CD8^+$,$CD19^+$ and $CD56^+$ for the first, second, third and fourth week. (b) Frequencies of lymphocyte subsets of normal controls and chronic hepatitis B.

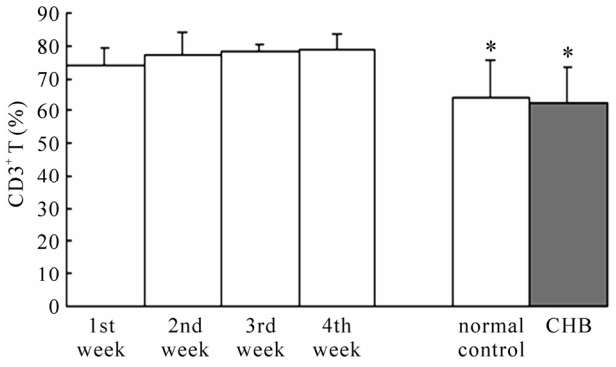
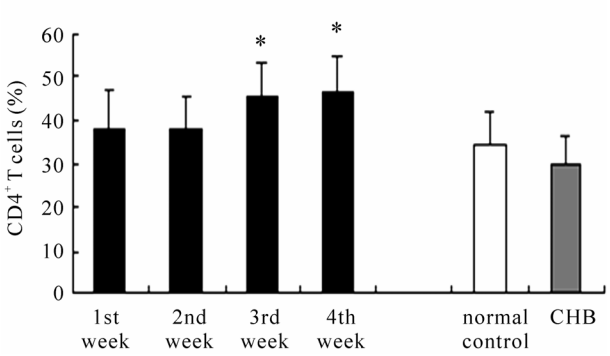


Figure 2. The results of dynamic changes of $CD3^+$ T cells in patients with AHB compared to normal controls and CHB. The mean ratios of different weeks for AHB were 73.97 ± 5.21%, 77.67 ± 6.64%, 78.54 ± 1.78% and 78.81 ± 5.01% respectively. And the ratios of normal controls and CHB were 64 ± 11.54% and 62.48 ± 11.33% respectively. Compared to the other groups, $CD3^+$T cells of AHB were higher and the differences were statistically significant. ($^*P < 0.05$).



Finger 3. The results of dynamic changes of $CD4^+$ T cells in patients with AHB, compared to normal controls and CHB. For AHB, the mean ratios of $CD4^+$ T cells were 37.86 ± 9.15%, 37.93 ± 7.34%, 45.71 ± 7.42% and 46.37 ± 8.09% respectively. The mean ratios of $CD4^+$ T cells in normal control and CHB were 34.41 ± 7.53% and 30.07 ± 6.67% respectively. Compared to the other groups, $CD4^+$ T cells of AHB in third and fourth week were higher and the differences were statistically significant. ($^*P < 0.05$).

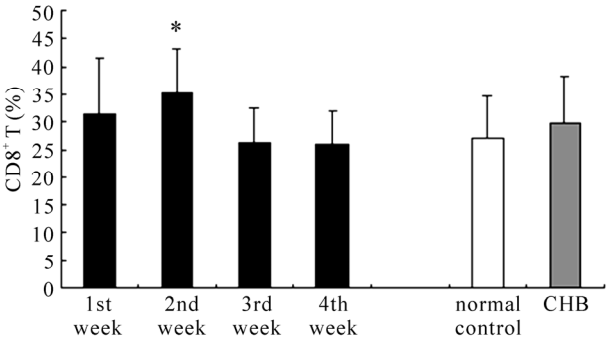


Figure 4. The results of $CD8^+$ T cells in patients with AHB compared to normal controls and CHB. The mean ratios of $CD8^+$ T cells in patients with AHB in four weeks were 31.58 ± 10.05%, 35.24 ± 7.79%, 26.45 ± 6.01% and 26.1 ± 5.95%, respectively.And the mean ratios of normal control and CHB were 27.18 ± 7.59% and 29.89 ± 8.36% respectively. The differences between 2nd week of AHB and the other groups were statistically significant. ($^*P < 0.05$).

(1.12% ± 0.28%).For chronic hepatitis B, difference was statistically significant (P < 0.05) compared with normal control (1.41% ± 0.60%). $CD4^+/CD8^+$ ratio and ALT changes were observed dynamically, and at early stage high levels of abnormal ALT followed lower $CD4^+/CD8^+$ ratio. As ALT normalization, the $CD4^+/CD8^+$ ratio showed a gradual increasing trend. The correlation between dynamic $CD4^+/CD8^+$ ratio and ALT was analyzed for different weeks, and r values were 0.700, –0.286, –0.179, 0.286, P values were 0.188, 0.535, 0.702, 0.535 (all > 0.05), respectively ,which suggested no significant correlation.

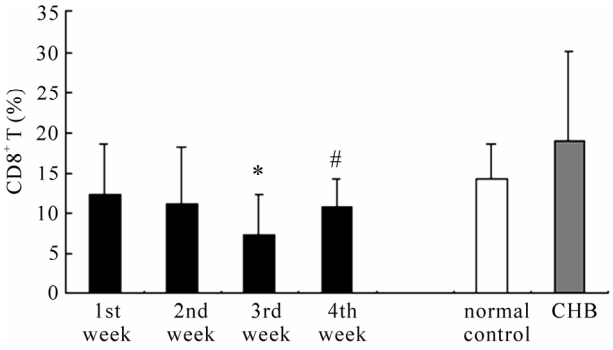


Figure 5. The results of $CD56^+$ cells in patients with AHB compared to normal controls and CHB. The mean ratios of $CD56^+$ cells in patients with AHB were 12.28 ± 6.32, 11.15 ± 7.12, 7.26 ± 5.04% and 10.89 ± 3.33 within four weeks, respectively. The mean ratios of normal control and CHB were 14.38 ± 4.23% and 18.93 ± 11.22% respectively. The difference between the 3rd of AHB and the other groups was statistically significant ($^*P < 0.05$) and the difference between the 4th of AHB and the CHB was statically significant. ($^\#P < 0.05$).

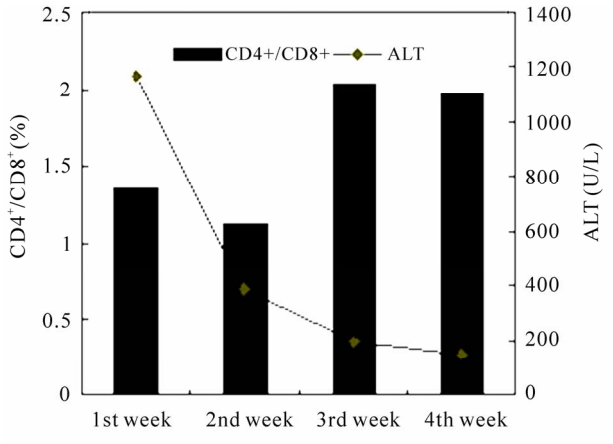


Figure 6. Trends of $CD4^+/CD8^+$ ratio means and ALT dynamic changes. It showed that ALT became gradual normalization followed $CD4^+/CD8^+$ gradually increased over time.

3.3. The Ratio of Lymphocyte Subsets and HBV DNA

HBV DNA of acute hepatitis B on admission were detected, of which 5 cases were negative (HBV DNA < 10^3 copies/ml) and 12 cases were positive (for the 10^3 copies/ml < HBV DNA < 10^7 copies/ml), and positive patients happened to be HBV DNA negative conversion within two weeks. The $CD4^+/CD8^+$ ratio of 12 patients with positive HBV DNA (mean 1.18% ± 0.93%) was lower than that of negative patients (mean 1.59% ± 0.47%), but no statistically significant difference was found between them (t = –0.701, P = 0.501).Twelve cases were positive in patients with $CD4^+/CD8^+$ ratio and the presence or absence of HBV DNA and no significant correlation (r = 0.433, P = 0.466). The $CD4^+/CD8^+$ of and HBV DNA of 12 cases of patients with positive HBV DNA were no significant correlation (r = 0.433, P = 0.466).

4. DISCUSSION

When adults are infected with HBV, the vast majority of people can clear virus and only a little infected persons continued to be chronic infection. Most studies have confirmed that the prognosis of HBV infection is closely related to functional status of the immune system which is an important factor to determine the prognosis. Acute hepatitis B triggers the body's immune response, particularly cell-mediated immunity. In our study, dynamic changes of peripheral blood lymphocyte subsets in acute hepatitis B and characteristics of peripheral blood lymphocyte subsets with normal controls and chronic hepatitis B were observed and analyzed, in order to initially explore the relationship between lymphocytes change and disease progression and prognosis.

$CD4^+$ T cells play a important role in viral clearance [9], and play a central role in controlling immune response. Subsets of $CD8^+$ T cells include cytotoxic T cells (CTL) and suppressive T cells (Ts), however, inactivated CTL are differentiated to special killing effective T cells dependent on identifying processing antigen and by IL-2 secreted by $CD4^+$ T cells. Past studies about lymphocyte subsets of peripheral blood such as Urbani S, D. Vassilopoulos and others [10,11] used more static detection, but we detected lymphocyte subsets dynamically. It showed that lymphocyte subsets showed a certain degree of dynamic change for acute hepatitis B: $CD3^+$ T cells increased significantly within four weeks of hospitalization and $CD4^+$ T cells showed increasing trend compared to normal controls and chronic hepatitis B. However, for chronic hepatitis B, $CD3^+$ T cells and $CD4^+$ T cells ratios were lower than normal controls. These figures showed that the weaker $CD4^+$ T cells immune response may be related to chronic infection. Differences of $CD3^+$ T and $CD4^+$ T cells ratios between acute and chronic hepatitis B suggested that when adults happened to be acute infection, T lymphocytes of peripheral blood, especially a higher proportion of $CD4^+$ T cells, may be related to a strong Th1 cell response. As disease progression, more obvious advantage of $CD4^+$ T cell ratio was essential to be ridding of virus. $CD8^+$ T cells ratio of acute hepatitis B had a peak, and was higher than other two groups, but the ratio has fallen and were lower than other two groups in third and fourth week. As for chronic hepatitis B, $CD8^+$ T cells slightly increased compared with normal controls. As we know, $CD8^+$ T cells have different sub-

sets and most studies confirmed that for acute hepatitis B there was a strong polyclonal, multi-specific CTL response. In our study $CD8^+$ T cells had advantage of ratio during early stage, however, specific CTL proportion might explain the phenomenon, which injured body's own cells while clearing the virus. But for chronic hepatitis B, CTL response was not likely to dominate and Ts cell ratio mainly increased which inhibited cellular immune function, leading to sustained and persistent infection.

In part $CD4^+/CD8^+$ reflects state of immune system within a certain range [12]. The up-regulation of ratio indicates that immune response is strong and the reduction of ratio or even less than one indicates that low immune function. As for chronic hepatitis B, Yin Ying [13], You Jing [14] and so on confirmed existence of $CD4^+/CD8^+$ down. Our study showed that $CD4^+/CD8^+$ of acute hepatitis B in the first, third and fourth week were higher than that of normal controls and chronic hepatitis B which because activation and reproduction of specific CTL depending on $CD4^+$ T cells activated, and a higher proportion of $CD4^+$ T cells was behalf of inducing a strong anti-viral response. As $CD4^+$ T cells of acute hepatitis B increased significantly, compared to chronic hepatitis B, it indicated that arise of $CD4^+/CD8^+$ in acute hepatitis B showed advantage of positive regulation and a good prognosis disease.

For acute hepatitis B, ALT became gradual normalization followed $CD4^+/CD8^+$ gradually increased over time. Although a significant correlation between them was not found, a moderate cellular immune response happened during acute infection, which was conducive to completely remove virus, meanwhile which played a role in immune regulation function to restore tissue damage. HBV DNA was likely to have been largely cleared before liver damage for majority of acute hepatitis B, and for twelve cases of positive patients, HBV DNA continued to decline rapidly and serology turned to be negative. Virus was cleared mainly through hepatic cell disruption or non-dissolving mechanism [15]. For acute hepatitis B with positive and negative HBV DNA, $CD4^+/CD8^+$ of them had no statistical difference and HBV DNA level and $CD4^+/CD8^+$ also had no significant correlation. However as for chronic hepatitis B, You Jing [9] suggested that there was significant negative correlation between $CD4^+/CD8^+$ and HBV DNA level, which suggested that immune status of chronic hepatitis B was at a low level and viral replication was relatively stable, and immune response could not completely inhibit virus replication, leading to persistent infection. Nevertheless, for acute hepatitis B, whether positive or negative HBV DNA, immune response was at a relatively positive adjustment advantage, and a strong particularly cellular immunity, which could rapidly clear HBV DNA and play a strong and appropriate regulation function in later reaction, ultimately got to be clinical recovery.

NK cells are component of innate immune. They express molecular CD56 and play the role of first line of defense by leading to infectious cell disrupted non-specifically. Moretta L. *et al*. [16] confirmed that in early stage of acute hepatitis B, NK cells could play a role compared to chronic hepatitis B and normal controls. Our study showed that NK cells of acute hepatitis B had a high proportion in early stage, then dropped to a trough in the third week. NK cells of chronic hepatitis B was higher than normal controls. Chronic hepatitis B had a protracted course and persistent inflammation, leading to NK cells always maintained a relatively high proportion. For acute infection NK cells of peripheral blood showed dynamic changes, and it may be related to balance and accumulation of NK cells between blood circulation and liver tissue, so that it was more conducive for NK cells to produce IFN, and thus inhibited viral replication and cleared infected cells.

In summary, by dynamically analyzing lymphocyte subsets of peripheral blood in acute self-limited hepatitis B in a certain period of time, the relationship between clearing virus and immune system changes was explored. It showed that when acute hepatitis B occurred, there was a more comprehensive and effective immune response, which can be completely rid of virus and access to clinical recovery. However, for different individuals infected with HBV, how body activates a strong immune response in order to completely remove virus, as well as limitations of this study to a certain extent, additional studies are required further study in the future.

REFERENCES

[1] World Health Organization: Department of Communicable diseases surveillance and response. (2004) Hepatitis B, WHO Fact Sheets, http://www.who.int

[2] Robert, T., Stefan, W. and Carola, S. (2003) $CD8^+$T cell mediate viral clearance and disease pathogenesis during acute hepatitis B virus infection. *Journal of Virology*, **77(1)**, 68-86.

[3] You, J., Zhuang, L. and Zhang, Y.F. (2009) Peripheral T-lymphocyte subpopulations in different clinical stages of chronic HBV infection correlate with HBV load. *World Journal of Biological Chemistry*, **15(27)**, 3382-3393.

[4] Tian, Y., Qiu, Z.F. and Li, T.S. (2005) Difference and significance of peripheral blood T-lymphocyte subsets in patients with chronic hepatitis B and asymptomatic HBV carriers. *National Medical Journal of China*, **85(47)**, 3354-3358.

[5] Urbani, S., Boni, C. and Amadei, B. (2005) Acute phase HBV-specific T cell responses associated with HBV per-

sistence after HBV/HCV co-infection. *Hepatology*, **41(4)**, 826-831.

[6] Chinese Society of Infectious Disease and Parasitology and Chinese Society of Hepatology of Chinese Medical Association. (2001)The program of prevention and cure for viral hepatitis. *Chinese Journal of Infectious Disease*, **19(1)**, 56-62.

[7] Chinese Society of Hepatology and Infectious Disease. The guidelines of prevention and cure for chronic hepatitis B. (2005) *Chinese Journal of Hepatology*, **13(12)**, 881-891.

[8] Han, Y.P., Liu, Y. and Li, J. (2005) Study on the difference between the lymphocyte subsets of liver tissue and peripheral blood in liver disease patients. *Labotary Medicine*, **20(5)**, 448-451.

[9] Bertoletti, A. and Gehring, A.J. (2006) The immune response during hepatitis B virus infection. *Journal of General Virology*, **87(6)**, 1439-1449.

[10] Urbani, S., Boni, C. and Amadei, B. (2005) Acute phase HBV-specific T cell responses associated with HBV persistence after HBV/HCV co-infection. *Hepatology*, **41(4)**, 826-831.

[11] Vassilopoulos, D., Rapti, I. and Nilolaou, M. (2008) Cellular immune responses in hepatitis B virus e antigen negative chronic hepatitis B. *Journal of Viral Hepatitis*, **15(11)**, 817-826.

[12] Brian, D.L. and Jeff, A. (1999) Altered helper t lymphocyte function associated with chronic hepatitis b virus infection and its role in response to therapeutic vaccination in humans. *Immunol*, **162(5)**, 3088-3095.

[13] Ying, Y., Zhang, Y.H. and Zhang, L.C. (2002) Significance of the changes of the levels of TNF-α and sIl-2R and CD4/CD8 in patients with hepatitis B. *Immunological Journal*, **18(1)**, 74.

[14] You, J., Zhuang, L. and Chen, H.Y. (2007) Relationship between variarations in peripheral T lymphocyte subsets and viral replication levels in Chinese chronic HBV carriers with normal liver function tests. *World Chinese Journal of Digestology*, **15(35)**, 3722-3727.

[15] Rehermann and Nascimbeni, M, (2005) Immunology of hepatitis B virus and hepatitis C virus infection. *Nature Reviews Immunology*, **5(3)**, 215-229.

[16] Moretta, L., Bottino, C., Pende, D., Vitale, M., Mingari, M.C. and Moretta, A. (2005) Human natural killer cells: Molecular mechanisms controlling NK cell activation and tumor cell lysis. *Immunology Letters*, **100(1)**, 7-13.

