Detection of T lymphocyte subsets and mIL-2R on surface of PBMC in patients with hepatitis B

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INTRODUCTION
Hepatitis B virus (HBV) parasitizing in hepatocytes is a pathogen of viral hepatitis B, which easily develops into hepatic fibrosis and cirrhosis, even hepatocellular carcinoma. But the pathogenesis of hepatitis B is very complex and has not been clarified until now. Generally, it is not HBV itself that damages hepatocytes directly, but the results of function disorder of cell-mediated immunity[1-10]. Peripheral blood mononuclear cells (PBMCs), which are aggregation of abundant immunologically competent cells, such as T lymphocytes, natural killer cells and lymphokine activated killer, likely play an important role in anti-HBV infection. Interleukin-2 (IL-2) has a crucial role in several immunologic functions. Its effect is dependent on the conjugation with membrane interleukin-2 receptor (mIL-2R) expressed on surface of activated T lymphocytes and other immunocompetent cells and can release from them. Biotin-streptavidin (BSA) has a high specificity and sensitivity. In order to study possible changes of T lymphocyte subsets and mIL-2R on surface of peripheral blood mononuclear cells (PBMC) of patients with hepatitis B and its role in the pathogenesis of hepatitis B, 196 cases of hepatitis B were detected by the BSA methods in this study. The results suggest that there is a state of depression rather than of activation of T lymphocyte subsets and mIL-2R system in viral hepatitis B, and that the pathogenesis of viral hepatitis B is related to the cellular and humoral immune function of patients.

MATERIALS AND METHODS
Subjects
According to the diagnostic criteria passed by the 10th National Conference on Viral Hepatitis and Hepatopathy 2000 (Xi’an), 196 patients with hepatitis B (male 113 and female 83), aged 19-52 years (average 34.52±4.54), were chosen from our affiliated teaching hospitals. Among them, 24 patients were HBsAg-positive without symptoms, 22 with acute hepatitis B, 46 with slight chronic hepatitis, 37 with moderate chronic hepatitis, 26 with severe chronic hepatitis, 15 with severe hepatitis, 18 with posthepatitic cirrhosis and 8 with hepatocellular carcinoma. In addition, the controls were selected from HBsAb-positive volunteers (n=10) and normal blood donors from the local central blood bank (n=20), aged 10-45 years (average 32.6 years).

Reagents and instruments
Antibodies against T lymphocyte subsets were provided by Shanghai Jing’an Medical Institute, Ficoll-Hypaque sedimentation gradients were offered by Shanghai Second Reagent Factory, and HBV-DNA reagents were made in Shanghai Middle Asia Gene Institute. Carbon dioxide incubator (MDF-135) was made in Japan.

Samples
Five mL peripheral vein blood 5 mL from each patient with hepatitis B and the controls was collected at 8:00 a.m., and 2.5 mL was distributed in a sterile test tube and 2.5 mL into an anticoagulant test tube with heparin.

Separation of PBMC and detection of T cell subsets, mIL-2R
After the heparinized anticoagulant blood was mixed with equal volume of Hanks’ liquid without Ca2+ and Mg2+, PBMC were harvested from heparinized whole blood by centrifugation.

RESULTS: In patients with hepatitis B, the levels of CD4+, CD8+ cells, and the ratio of CD4+ cells/CD8+ cells were lower, but the level of CD19+ cells was higher than those in normal controls (42.20±6.01 vs 65.96±6.54, 36.17±5.93 vs 41.73±6.40, 0.91±0.28 vs 1.44±0.31, 39.86±6.36 vs 30.02±4.54, P<0.01). The total expression level of mIL-2R in PBMC before and after being stimulated with PHA was also lower than those in normal controls (3.47±1.55 vs 4.52±1.49, 34.03±2.94 vs 37.95±3.00, P<0.01). In all the patients with hepatitis B, the levels of T lymphocyte subsets and mIL-2R in PBMC with HBV-DNA (+) were lower than those with HBV-DNA (-), which were significantly different (39.57±7.11 vs 44.36±5.43, 34.36±7.16 vs 40.75±5.87, 37.82±6.54 vs 41.72±6.21, 0.88±0.33 vs 0.99±0.27, 2.82±1.62 vs 3.85±1.47, 31.56±3.00 vs 35.84±2.83, P<0.01). In addition, the levels of CD4+ cells/CD8+ cells, CD4+ cells, the ratio of CD4+ cells/CD8+ cells and mIL-2R among different courses of hepatitis B were all significantly different (F=3.723, P<0.01, F=130.43, P<0.01, F=54.01, P<0.01, F=2.99, P<0.05, F=7.16, P<0.01).

CONCLUSION: Both cellular and humoral immune functions are obviously in disorder in patients with hepatitis B, which might be closely associated with the chronicity in patients.

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Detection of T lymphocyte subsets and mL-2R in PBMC of patients with hepatitis B (x ±%,)

| Group       | n  | CD3⁺ | CD4⁺ | CD8⁺ | CD4⁻/CD8⁻ | mL-2R       |
|-------------|----|------|------|------|-----------|------------|
| Control     | 30 | 65.96±6.54 | 41.73±6.40 | 30.02±6.54 | 1.44±0.31 | 4.52±1.49 | 37.95±3.00 |
| Anti-HBs (+) | 10 | 66.34±5.16 | 42.82±6.52 | 29.03±4.50 | 1.51±0.27 | 5.06±0.45 | 40.26±3.10 |
| NBD         | 20 | 65.80±6.92 | 41.20±6.36 | 30.45±6.62 | 1.39±0.33 | 4.24±1.52 | 36.30±2.95 |
| Hepatitis B | 196| 42.20±6.01 | 38.17±5.93 | 39.86±6.36 | 0.91±0.28 | 3.47±1.55 | 34.03±2.94 |
| A HBsAg (+) | 24 | 58.83±7.44 | 41.34±5.16 | 35.34±7.15 | 1.20±0.33 | 3.94±1.75 | 35.05±3.05 |
| AH          | 22 | 57.38±7.39 | 40.21±6.12 | 39.47±6.25 | 1.01±0.30 | 3.67±1.68 | 34.22±2.25 |
| SCH         | 48 | 38.54±5.56 | 39.56±6.44 | 41.10±7.64 | 0.98±0.31 | 3.44±1.40 | 31.96±8.00 |
| MCH         | 37 | 40.14±6.58 | 37.22±5.38 | 41.45±6.29 | 0.88±0.29 | 3.25±1.50 | 32.81±7.76 |
| SH          | 20 | 40.01±6.23 | 35.51±4.33 | 42.86±5.58 | 0.81±0.22 | 3.06±1.56 | 33.82±5.32 |
| PC          | 18 | 38.72±6.22 | 36.11±4.23 | 41.89±8.98 | 0.90±0.19 | 3.31±1.60 | 31.55±2.34 |
| HC          | 8  | 39.44±6.78 | 34.15±5.50 | 43.46±7.88 | 0.83±0.24 | 3.36±1.68 | 30.38±2.15 |

Statistical analysis was made by $t$ and $F$ tests.

**RESULTS**

The results showed that the percentages of CD3⁺ and CD4⁺ cells, and the ratio of CD4⁺/CD8⁻ cells were lower, the percentage of CD8⁻ cells was higher, and the levels of mL-2R before and after stimulation with PHA were lower in patients with hepatitis B than those in normal controls ($P<0.01$). Among different courses of hepatitis B, T lymphocyte subsets and mL-2R were all significantly different from each other. The detailed results are shown in Tables 1 and 2.

**DISCUSSION**

Recently, studies have shown that patients with hepatitis B are usually accompanied by disorder of immune function, and hepatocytic damage is mainly caused by immunological...
infecting PBMC, HBV can interfere with the normal metabolism eliminating HBV and contribute to chronicity of hepatitis B. The body’s immune function and the course of illness. While humoral immune functions, and a close relationship between according to the above findings, it is concluded that in patients which suggested that there is a close correlation between levels CD4+ cells were lower in patients with hepatitis B. The levels of CD4+ cells /CD8+ cells decreased, and CD8+ cells increased, suggesting that disorders of cellular immune function and pathologic damages occurred in the 196 patients with hepatitis B detected by the method of BSA. PBMCs are easily infected by HBV[48,49]. When entering into PBMCs, HBV can integrate with host cells and interfere with metabolism of cells, and can depress the expression of CD4+ and CD8+. As seen in this study, the expression levels of T lymphocyte subsets between positive and negative HBV-DNA and HBV-DNA in PBMC were significantly different (P<0.01).

mIL-2R plays a key role in biologic effect of IL-2 and its expression levels can reflect the course of T cell activity and the immune situation of body[50]. From this study, we can see the expression levels of mIL-2R in PBMCs in silence and induction were lower in hepatitis B patients than in normal controls (P<0.01), and the expression levels of mIL-2R in PBMCs were lower in HBV-DNA positive patients than in HBV-DNA negative patients (P<0.01). After stimulation with PHA, the levels of mIL-2R obviously increased, which showed that mIL-2R could be induced by PHA, but its expression levels were still significantly lower than those in normal controls (P<0.001). In addition, due to deterioration and chronicity of hepatitis B, the expression levels of mIL-2R had a tendency of descent in the patients. These also showed that T cell activity was interfered and humoral immune function was decreased in patients with hepatitis B.

The levels of CD4+, CD8+, CD4+ /CD8+ cells (P<0.01), the ratio of CD4+ cells /CD8+ cells (P<0.01) and mIL-2R (P<0.05) among different courses of hepatitis B were all significantly different, which suggested that there is a close correlation between levels of T lymphocyte subsets and different courses of hepatitis B. According to the above findings, it is concluded that in patients with hepatitis B, there is an obvious disorder of cellular and humoral immune functions, and a close relationship between the body’s immune function and the course of illness. While infecting PBMC, HBV can interfere with the normal metabolism of these cells, and prevent lymphocytic membranes from accepting signals from antigen presenting cells (APC), and depress the expression of mIL-2R. All of these do no good to eliminating HBV and contribute to chronicity of hepatitis B.

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