

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

Ke-Xia Wang, Jiang-Long Peng, Xue-Feng Wang, Ye Tian, Jian Wang, Chao-Pin Li

**Ke-Xia Wang, Jiang-Long Peng, Xue-Feng Wang, Ye Tian, Jian Wang, Chao-Pin Li**, School of Medicine, Anhui University of Science and Technology, Huainan 232001, Anhui Province, China  
**Correspondence to:** Dr. Chao-Pin Li, Department of Etiology and Immunology, School of Medicine, Anhui University of Science and Technology, Huainan 232001, Anhui Province, China. cpli@aust.edu.cn  
**Telephone:** +86-554-6658770 **Fax:** +86-554-6662469  
**Received:** 2003-03-02 **Accepted:** 2003-06-02

## Abstract

**AIM:** To study the levels of T lymphocyte subsets and membrane interleukin-2 receptor (mIL-2R) on surface of peripheral blood mononuclear cells (PBMCs) of patients with hepatitis B and its role in the pathogenesis of hepatitis B.

**METHODS:** The levels of T lymphocyte subsets and mIL-2R in PBMC before and after being stimulated with PHA were detected by biotin-streptavidin (BSA) technique in 196 cases of hepatitis B.

**RESULTS:** In patients with hepatitis B, the levels of CD<sub>3</sub><sup>+</sup>, CD<sub>4</sub><sup>+</sup> cells, and the ratio of CD<sub>4</sub><sup>+</sup> cells/CD<sub>8</sub><sup>+</sup> cells were lower, but the level of CD<sub>8</sub><sup>+</sup> cells was higher than those in normal controls (42.20±6.01 vs 65.96±6.54, 38.17±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 CD<sub>3</sub><sup>+</sup>, CD<sub>4</sub><sup>+</sup>, CD<sub>8</sub><sup>+</sup> cells, the ratio of CD<sub>4</sub><sup>+</sup> cells/CD<sub>8</sub><sup>+</sup> cells and mIL-2R among different courses of hepatitis B were all significantly different ( $F=3\ 723.18$ ,  $P<0.01$ .  $F=130.43$ ,  $P<0.01$ .  $F=54.01$ ,  $P<0.01$ .  $F=2.99$ ,  $P<0.05$ .  $F=7.16$ ,  $P<0.01$ ).

**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.

Wang KX, Peng JL, Wang XF, Tian Y, Wang J, Li CP. Detection of T lymphocyte subsets and mIL-2R on surface of PBMC in patients with hepatitis B. *World J Gastroenterol* 2003; 9(9): 2017-2020  
<http://www.wjgnet.com/1007-9327/9/2017.asp>

## 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<sup>[1-18]</sup>. 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 patients with acute and chronic 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 10<sup>th</sup> 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 34.6 years), 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 posthepatic 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 Ca<sup>2+</sup> and Mg<sup>2+</sup>, PBMC were harvested from heparinized whole blood by centrifugation

**Table 1** Detection of T lymphocyte subsets and mIL-2R in PBMC of patients with hepatitis B ( $\bar{x}\pm s, \%$ )

Group	n	CD <sub>3</sub> <sup>+</sup>	CD <sub>4</sub> <sup>+</sup>	CD <sub>8</sub> <sup>+</sup>	CD <sub>4</sub> <sup>+</sup> /CD <sub>8</sub> <sup>+</sup>	mIL-2R	
						Silent	Induced
Control	30	65.96±6.54 <sup>a</sup>	41.73±6.40 <sup>b</sup>	30.02±4.54 <sup>c</sup>	1.44±0.31 <sup>d</sup>	4.52±1.49 <sup>e</sup>	37.95±3.00 <sup>f</sup>
Anti-HBs (+)	10	66.34±5.16	42.82±6.52	29.03±4.50	1.51±0.27	5.06±1.45	40.26±3.10
NBD	20	65.80±6.92	41.20±6.36	30.45±4.62	1.39±0.33	4.24±1.52	36.30±2.95
Hepatitis B	196	42.20±6.01 <sup>a</sup>	38.17±5.93 <sup>b</sup>	39.86±6.36 <sup>c</sup>	0.91±0.28 <sup>d</sup>	3.47±1.55 <sup>e</sup>	34.03±2.94 <sup>f</sup>
A HBsAg (+)	24	58.83±7.44 <sup>g</sup>	41.34±5.16 <sup>h</sup>	35.34±7.15 <sup>i</sup>	1.20±0.33 <sup>j</sup>	3.94±1.75 <sup>k</sup>	35.05±3.05 <sup>l</sup>
AH	22	57.38±7.73 <sup>g</sup>	40.21±6.12 <sup>h</sup>	39.47±6.25 <sup>i</sup>	1.01±0.30 <sup>j</sup>	3.67±1.68 <sup>k</sup>	34.22±2.25 <sup>l</sup>
SCH	46	38.54±7.56 <sup>g</sup>	39.56±6.44 <sup>h</sup>	41.10±7.64 <sup>i</sup>	0.98±0.31 <sup>j</sup>	3.44±1.40 <sup>k</sup>	31.96±3.80 <sup>l</sup>
MCH	37	40.14±5.85 <sup>g</sup>	37.22±5.38 <sup>h</sup>	41.45±6.23 <sup>i</sup>	0.88±0.25 <sup>j</sup>	3.25±1.50 <sup>k</sup>	32.81±2.76 <sup>l</sup>
SCH	20	40.01±6.23 <sup>g</sup>	35.51±6.33 <sup>h</sup>	42.86±5.58 <sup>i</sup>	0.81±0.22 <sup>j</sup>	3.06±1.56 <sup>k</sup>	33.83±3.52 <sup>l</sup>
SH	15	37.85±6.54 <sup>g</sup>	34.57±6.20 <sup>h</sup>	42.92±5.65 <sup>i</sup>	0.80±0.21 <sup>j</sup>	3.95±1.54 <sup>k</sup>	33.85±2.65 <sup>l</sup>
PC	18	38.72±6.22 <sup>g</sup>	36.11±4.23 <sup>h</sup>	41.89±8.98 <sup>i</sup>	0.90±0.19 <sup>j</sup>	3.31±1.60 <sup>k</sup>	31.55±2.34 <sup>l</sup>
HC	8	39.44±6.78 <sup>g</sup>	34.15±5.50 <sup>h</sup>	43.46±7.88 <sup>i</sup>	0.83±0.24 <sup>j</sup>	3.36±1.68 <sup>k</sup>	30.38±2.15 <sup>l</sup>

<sup>a</sup> $t=19.9295$ , <sup>a</sup> $P<0.001$ . <sup>b</sup> $t=3.0300$ , <sup>b</sup> $P<0.01$ . <sup>c</sup> $t=8.1551$ , <sup>c</sup> $P<0.001$ . <sup>d</sup> $t=9.5153$ , <sup>d</sup> $P<0.001$ . <sup>e</sup> $t=3.4722$ , <sup>e</sup> $P<0.001$ . <sup>f</sup> $t=6.7832$ , <sup>f</sup> $P<0.001$ . <sup>g</sup> $F=3723.18$ , <sup>g</sup> $P<0.01$ . <sup>h</sup> $F=130.43$ , <sup>h</sup> $P<0.01$ . <sup>i</sup> $F=54.01$ , <sup>i</sup> $P<0.01$ . <sup>j</sup> $F=2.99$ , <sup>j</sup> $P<0.05$ . <sup>k</sup> $F=7.16$ , <sup>k</sup> $P<0.01$ . <sup>l</sup> $F=1.60$ , <sup>l</sup> $P>0.05$ . NBD: normal blood donors, A HBsAg (+): asymptomatic HBsAg (+), AH: acute hepatitis, SCH: slight chronic hepatitis, MCH: moderate chronic hepatitis, SCH: severe chronic hepatitis, SH: severe hepatitis, PC: posthepatitic cirrhosis, HC: hepatocellular carcinoma.

**Table 2** Detection of T lymphocyte subsets and mIL-2R in PBMC with HBV-DNA (+) before and after induced with PHA ( $\bar{x}\pm s, \%$ )

Group	n	CD <sub>3</sub> <sup>+</sup>	CD <sub>4</sub> <sup>+</sup>	CD <sub>8</sub> <sup>+</sup>	CD <sub>4</sub> <sup>+</sup> /CD <sub>8</sub> <sup>+</sup>	mIL-2R	
						Silent	Induced
Control	30	65.96±6.54	41.73±6.40	30.02±4.54	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±1.45	40.26±3.10
NBD	20	65.80±6.92	41.20±6.36	30.45±4.62	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
HBV-DNA(+) of PBMC	118	39.57±7.11 <sup>a</sup>	34.36±7.16 <sup>b</sup>	37.82±6.54 <sup>c</sup>	0.88±0.33 <sup>d</sup>	2.82±1.62 <sup>e</sup>	31.56±3.00 <sup>f</sup>
HBV-DNA(-) of PBMC	78	44.36±5.43 <sup>a</sup>	40.75±5.87 <sup>b</sup>	41.72±6.21 <sup>c</sup>	0.99±0.27 <sup>d</sup>	3.85±1.47 <sup>e</sup>	35.84±2.83 <sup>f</sup>

<sup>a</sup> $t=5.3466$ , <sup>a</sup> $P<0.001$ . <sup>b</sup> $t=6.8525$ , <sup>b</sup> $P<0.01$ . <sup>c</sup> $t=4.2414$ , <sup>c</sup> $P<0.001$ . <sup>d</sup> $t=2.5641$ , <sup>d</sup> $P<0.05$ . <sup>e</sup> $t=4.6355$ , <sup>e</sup> $P<0.001$ . <sup>f</sup> $t=10.0352$ , <sup>f</sup> $P<0.001$ . NBD: normal blood donors.

on Ficoll-Hypaque sedimentation gradient and diluted to  $(1-3)\times 10^6 \cdot L^{-1}$  cells suspension with RPMI 1640 culture liquid. Ten  $\mu L$  suspension of PBMC was smeared on sheet glass pores so that the cells with CD<sub>3</sub><sup>+</sup>, CD<sub>4</sub><sup>+</sup>, CD<sub>8</sub><sup>+</sup> and the rest phrase of mIL-2R could be detected. Of the PBMC suspension, 0.5 mL was mixed with RPMI 1640 culture liquid, which had PHA 200  $\mu g \cdot L^{-1}$ . The cells were grown in continuous culture (37 °C, 50 mL  $\cdot L^{-1}$  CO<sub>2</sub> in atmosphere) for 72 h and its mIL-2R induced by PHA could be measured by the antibodies against membranes of T cells.

#### Immunocytochemical method of biotin- streptavidin system (BSA)

The different monoclonal antibodies (mAb) against CD<sub>3</sub>, CD<sub>4</sub>, CD<sub>8</sub> and Tac with biotin and SA-HRP were smeared on different sheet glasses. These smears were left dry naturally and fixed with acetone for 15-20 min. The cells were incubated in continuous culture (37 °C, 50 mL  $\cdot L^{-1}$  CO<sub>2</sub> in atmosphere) for 30 min. The immune sheet glass pores were measured after being stained with the color-developing agent and several washings with Tris buffer solution (TBS). The total number of 200 PBMCs was counted and its positive cells were statistically analyzed with the help of high power lens. The positive criterion was that the color of cell membrane was brown, if not being negative.

#### Detection of HBV-DNA

The levels of HBV-DNA in serum and PBMC were detected with negative, positive and vacuity controls being set up each

time. After routine process, the samples were placed at 94 °C for 300 s for denaturation at first, then at 94 °C for 30 s, at 55 °C for 30 s and at 72 °C for 30 s. After 35 cycles, the samples were extended at 72 °C for 300 s. After electrophoresis in 2 % sepharose with ethidium bromide (EB) for 20 min, products of amplification were observed with infrared-transmission meters. Samples with orange fluorescent bands as the positive controls were considered to be positive, while the others were negative.

#### Statistical analysis

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

## RESULTS

The results showed that the percentages of CD<sub>3</sub><sup>+</sup> and CD<sub>4</sub><sup>+</sup> cells, and the ratio of CD<sub>4</sub><sup>+</sup> cells /CD<sub>8</sub><sup>+</sup> cells were lower, the percentage of CD<sub>8</sub><sup>+</sup> cells was higher, and the levels of mIL-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 mIL-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

injury<sup>[15-30]</sup>. Immunologically competent cells and cytokines are the key points for body to eliminate hepatitis B virus<sup>[12]</sup>. Alterations of T lymphocyte subsets are an important reason for the disorder of immune function due to HBV infection. A lots of cytokines, especially IL-2, can facilitate proliferation of immunologically competent cells<sup>[14,31-37]</sup>, such as T lymphocytes, natural killer cells and lymphokine activated killer cells. While IL-2 is conjugated with mIL-2R on surface of the proper target cells, it will be efficient in immunoregulation. In order to further explore the pathogenesis of hepatitis B, we designed a series of correlation experiments to detect the levels of T lymphocyte subsets and mIL-2R on surface of PBMC in patients with hepatitis B.

CD<sub>3</sub><sup>+</sup>, CD<sub>4</sub><sup>+</sup> and CD<sub>8</sub><sup>+</sup> cells are major function 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<sup>[11,38-47]</sup>. In this study, the percentages of CD<sub>3</sub><sup>+</sup>, CD<sub>4</sub><sup>+</sup> cells and the ratio of CD<sub>4</sub><sup>+</sup> cells /CD<sub>8</sub><sup>+</sup> cells decreased, and CD<sub>8</sub><sup>+</sup> 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<sup>[48,49]</sup>. When entering into PBMCs, HBV can integrate with host cells and interfere with metabolism of cells, and can depress the expression of CD<sub>3</sub><sup>+</sup> and CD<sub>4</sub><sup>+</sup>. 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<sup>[50]</sup>. 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 CD<sub>3</sub><sup>+</sup>, CD<sub>4</sub><sup>+</sup>, CD<sub>8</sub><sup>+</sup> cells ( $P<0.01$ ), the ratio of CD<sub>4</sub><sup>+</sup> cells /CD<sub>8</sub><sup>+</sup> 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.

## REFERENCES

- Wei J, Wang YQ, Lu ZM, Li GD, Wang Y, Zhang ZC. Detection of anti-preS1 antibodies for recovery of hepatitis B patients by immunoassay. *World J Gastroenterol* 2002; **8**: 276-281
- Liu DX. A new hypothesis of pathogenetic mechanism of viral hepatitis B and C. *Med Hypotheses* 2001; **56**: 405-408
- Tennant BC, Gerin JL. The woodchuck model of hepatitis B virus infection. *ILAR J* 2001; **42**: 89-102
- Kao JH, Chen DS. Global control of hepatitis B virus infection. *Lancet Infect Dis* 2002; **2**: 395-403
- Bernardi M, Biselli M, Gramenzi A. Chronic hepatitis B. Recent advances in diagnosis and treatment. *Recenti Prog Med* 2002; **93**: 397-402
- Torbenson M, Thomas DL. Occult hepatitis B. *Lancet Infect Dis* 2002; **2**: 479-486
- Ohkubo K, Kato Y, Ichikawa T, Kajiji Y, Takeda Y, Higashi S, Hamasaki K, Nakao K, Nakata K, Eguchi K. Viral load is a significant prognostic factor for hepatitis B virus-associated hepatocellular carcinoma. *Cancer* 2002; **94**: 2663-2668
- Tai DI, Lo SK, Kuo CH, Du JM, Chen CJ, Hung CS, Chu CM. Replication of hepatitis B in HBsAg-positive siblings. *J Viral Hepat* 2002; **9**: 272-279
- Marusawa H, Osaki Y, Kimura T, Ito K, Yamashita Y, Eguchi T, Kudo M, Yamamoto Y, Kojima H, Seno H, Moriyasu F, Chiba T. High prevalence of anti-hepatitis B virus serological markers in patients with hepatitis C virus related chronic liver disease in Japan. *Gut* 1999; **45**: 284-288
- Izzo F, Cremona F, Ruffolo F, Palaia R, Parisi V, Curley SA. Outcome of 67 patients with hepatocellular cancer detected during screening of 1125 patients with chronic hepatitis. *Ann Surg* 1998; **227**: 513-518
- Sing G, Butterworth L, Chen X, Bryant A, Cooksley G. Composition of peripheral blood lymphocyte populations during different stages of chronic infection with hepatitis B virus. *J Viral Hepat* 1998; **5**: 83-93
- Bertoletti A, Ferrari C, Fiaccadori F. Role of the cell-mediated immune response in the pathogenesis of hepatitis B virus infection: possible immune-therapeutic strategies. *Acta Biomed Ateneo Parmense* 1996; **67**: 87-93
- Michalak TI, Hodgson PD, Churchill ND. Posttranscriptional inhibition of class I major histocompatibility complex presentation on hepatocytes and lymphoid cells in chronic woodchuck hepatitis virus infection. *J Virol* 2000; **74**: 4483-4494
- Tulek N, Saglam SK, Saglam M, Turkyilmaz R, Yildiz M. Soluble interleukin-2 receptor and interleukin-10 levels in patients with chronic hepatitis B infection. *Hepatogastroenterology* 2000; **47**: 828-831
- Helvacı M, Ozkaya B, Ozbal E, Ozinel S, Yaprak I. Efficacy of interferon therapy on serum fibronectin levels in children with chronic hepatitis B infection. *Pediatr Int* 1999; **41**: 270-273
- Park YN, Han KH, Kim KS, Chung JP, Kim S, Park C. Cytoplasmic expression of hepatitis B core antigen in chronic hepatitis B virus infection: role of precore stop mutants. *Liver* 1999; **19**: 199-205
- Ilan Y, Chowdhury JR. Induction of tolerance to hepatitis B virus: can we 'eat the disease' and live with the virus? *Med Hypotheses* 1999; **52**: 505-509
- Khettry U, Anand N, Gordon FD, Jenkins RL, Tahan SR, Loda M, Lewis WD. Recurrent hepatitis B, hepatitis C, and combined hepatitis B and C in liver allografts: a comparative pathological study. *Hum Pathol* 2000; **31**: 101-108
- Webster GJ, Reigat S, Maini MK, Whalley SA, Ogg GS, King A, Brown D, Amlot PL, Williams R, Vergani D, Dusheiko GM, Bertoletti A. Incubation phase of acute hepatitis B in man: dynamic of cellular immune mechanisms. *Hepatology* 2000; **32**: 1117-1124
- Chemin I, Ohgaki H, Chisari FV, Wild CP. Altered expression of hepatic carcinogen metabolizing enzymes with liver injury in HBV transgenic mouse lineages expressing various amounts of hepatitis B surface antigen. *Liver* 1999; **19**: 81-87
- Chomarat P, Rice JM, Slagle BL, Wild CP. Hepatitis B virus-induced liver injury and altered expression of carcinogen metabolising enzymes: the role of the HBx protein. *Toxicol Lett* 1998; **102-103**: 595-601
- Nakamoto Y, Guidotti LG, Kuhlen CV, Fowler P, Chisari FV. Immune pathogenesis of hepatocellular carcinoma. *J Exp Med* 1998; **188**: 341-350
- Hayashi N, Mita E. Fas system and apoptosis in viral hepatitis. *J Gastroenterol Hepatol* 1997; **12**: S223-226
- Sarin SK, Thakur V, Gupta RC, Saigal S, Malhotra V, Thyagarajan SP, Das BC. Profile of hepatocellular carcinoma in India: an insight into the possible etiologic associations. *J Gastroenterol Hepatol* 2001; **16**: 666-673

- 25 **Shoenfeld Y**, Aron-Maor A. Vaccination and autoimmunity- 'vaccinosis': a dangerous liaison? *J Autoimmun* 2000; **14**: 1-10
- 26 **Trobonjaca Z**, Kroger A, Stober D, Leithauser F, Moller P, Hauser H, Schirmbeck R, Reimann J. Activating immunity in the liver. II. IFN-beta attenuates NK cell-dependent liver injury triggered by liver NKT cell activation. *J Immunol* 2002; **168**: 3763-3770
- 27 **Rapicetta M**, Ferrari C, Levrero M. Viral determinants and host immune responses in the pathogenesis of HBV infection. *J Med Virol* 2002; **67**: 454-457
- 28 **Tanner MS**. Mechanisms of liver injury relevant to pediatric hepatology. *Crit Rev Clin Lab Sci* 2002; **39**: 1-61
- 29 **Rivero M**, Crespo J, Fabrega E, Casafont F, Mayorga M, Gomez-Fleitas M, Pons-Romero F. Apoptosis mediated by the Fas system in the fulminant hepatitis by hepatitis B virus. *J Viral Hepat* 2002; **9**: 107-113
- 30 **Okumura A**, Ishikawa T, Yoshioka K, Yuasa R, Fukuzawa Y, Kakumu S. Mutation at codon 130 in hepatitis B virus (HBV) core region increases markedly during acute exacerbation of hepatitis in chronic HBV carriers. *J Gastroenterol* 2001; **36**: 103-110
- 31 **Moe SM**, Zekonis M, Harezlak J, Ambrosius WT, Gassensmith CM, Murphy CL, Russell RR, Batiuk TD. A placebo-controlled trial to evaluate immunomodulatory effects of paricalcitol. *Am J Kidney Dis* 2001; **38**: 792-802
- 32 **Huang YH**, Wu JC, Tao MH, Syu WJ, Hsu SC, Chi WK, Chang FY, Lee SD. DNA-Based immunization produces Th1 immune responses to hepatitis delta virus in a mouse model. *Hepatology* 2000; **32**: 104-110
- 33 **Akbar SM**, Abe M, Masumoto T, Horiike N, Onji M. Mechanism of action of vaccine therapy in murine hepatitis B virus carriers: vaccine-induced activation of antigen presenting dendritic cells. *J Hepatol* 1999; **30**: 755-764
- 34 **Livingston BD**, Alexander J, Crimi C, Oseroff C, Celis E, Daly K, Guidotti LG, Chisari FV, Fikes J, Chesnut RW, Sette A. Altered helper T lymphocyte function associated with chronic hepatitis B virus infection and its role in response to therapeutic vaccination in humans. *J Immunol* 1999; **162**: 3088-3095
- 35 **Lau GK**, Nanji A, Hou J, Fong DY, Au WS, Yuen ST, Lin M, Kung HF, Lam SK. Thymosin-alpha1 and famciclovir combination therapy activates T-cell response in patients with chronic hepatitis B virus infection in immune-tolerant phase. *J Viral Hepat* 2002; **9**: 280-287
- 36 **Primiagi LS**, Tefanova VT, Tallo TG, Shmidt EV, Solomonova OV, Tuisk TP, Kikosh GV, Krupskaja LM, Lisitsyna SA. Th1-cytokines in chronic hepatitis B and C. *Vopr Virusol* 2002; **47**: 23-27
- 37 **Wang FS**, Xing LH, Liu MX, Zhu CL, Liu HG, Wang HF, Lei ZY. Dysfunction of peripheral blood dendritic cells from patients with chronic hepatitis B virus infection. *World J Gastroenterol* 2001; **7**: 537-541
- 38 **Chen M**, Sallberg M, Thung SN, Hughes J, Jones J, Milich DR. Nondeletional T-cell receptor transgenic mice: model for the CD4 (+) T-cell repertoire in chronic hepatitis B virus infection. *J Virol* 2000; **74**: 7587-7599
- 39 **Lin CM**, Wang FH. Selective modification of antigen-specific CD4 (+) T cells by retroviral-mediated gene transfer and *in vitro* sensitization with dendritic cells. *Clin Immunol* 2002; **104**: 58-66
- 40 **Lau GK**, Suri D, Liang R, Rigopoulou EI, Thomas MG, Mullerova I, Nanji A, Yuen ST, Williams R, Naoumov NV. Resolution of chronic hepatitis B and anti-HBs seroconversion in humans by adoptive transfer of immunity to hepatitis B core antigen. *Gastroenterology* 2002; **122**: 614-624
- 41 **Sing GK**, Li D, Chen X, Macnaughton T, Lichanska AM, Butterworth L, Ladhams A, Cooksley G. A molecular comparison of T lymphocyte populations infiltrating the liver and circulating in the blood of patients with chronic hepatitis B: evidence for antigen-driven selection of a public complementarity-determining region 3 (CDR3) motif. *Hepatology* 2001; **33**: 1288-1298
- 42 **Fei GZ**, Sylvan SP, Yao GB, Hellstrom UB. Quantitative monitoring of serum hepatitis B virus DNA and blood lymphocyte subsets during combined prednisolone and interferon-alpha therapy in patients with chronic hepatitis B. *J Viral Hepat* 1999; **6**: 219-227
- 43 **Polat Eyigun C**, Yasar Avci I, Sengul A, Hacibektasoglu A, Van Thiel DH. Immune status of individuals with differing clinical courses of HBV infection. *Hepatogastroenterology* 1999; **46**: 1890-1894
- 44 **Im EH**, Lee BS, Sung JK, Lee SO, Lee KT, Lee SM, Kim SH, Seo KS, Kim JH, Kim SG, Kim NJ, Lee HY. T cell subsets in chronic hepatitis B and the effect of prednisolone withdrawal and interferon alpha-2b. *Korean J Intern Med* 1999; **14**: 1-8
- 45 **Schirmbeck R**, Wild J, Reimann J. Similar as well as distinct MHC class I-binding peptides are generated by exogenous and endogenous processing of hepatitis B virus surface antigen. *Eur J Immunol* 1998; **28**: 4149-4161
- 46 **Bertoletti A**, D'Elia MM, Boni C, De Carli M, Zignego AL, Durazzo M, Missale G, Penna A, Fiaccadori F, Del Prete G, Ferrari C. Different cytokine profiles of intraphepatic T cells in chronic hepatitis B and hepatitis C virus infections. *Gastroenterology* 1997; **112**: 193-199
- 47 **Chen M**, Sallberg M, Thung SN, Hughes J, Jones J, Milich DR. Modeling the T-helper cell response in acute and chronic hepatitis B virus infection using T-cell receptor transgenic mice. *Antiviral Res* 2001; **52**: 99-111
- 48 **Jiang R**, Feng X, Guo Y, Lu Q, Hou J, Luo K, Fu N. T helper cells in patients with chronic hepatitis B virus infection. *Chin Med J* 2002; **115**: 422-424
- 49 **Sobao Y**, Tomiyama H, Sugi K, Tokunaga M, Ueno T, Saito S, Fujiyama S, Morimoto M, Tanaka K, Takiguchi M. The role of hepatitis B virus-specific memory CD8 T cells in the control of viral replication. *J Hepatol* 2002; **36**: 105-115
- 50 **Wang JP**, Li XH, Zhu Y, Wang AL, Lian JQ, Jia ZS, Xie YM. Detection of serum sIL-2R, IL-6, IL-8, TNF- $\alpha$  and lymphocytes subsets, mL-2R in patients with chronic hepatitis B. *Shijie Huaren Xiaohua Zazhi* 2000; **8**: 763-766

Edited by Zhang JZ and Wang XL