

T cell subsets in chronic hepatitis B and the effect of prednisolone withdrawal and interferon alpha-2b

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Objectives : *The evaluations of the pathogenetic roles of cell mediated immunity and of the preventive effect for disease progression with interferon(IFN) treatment in patients with chronic active hepatitis-B(CAH-B) are the objectives of this study.*

Methods : *Thirty-two patients with CAH-B were treated with interferon -2b(IFN -2b) with prednisolone withdrawal and 30 control patients were treated with conventional hepatotonics for 6 months. Peripheral total T cell fractions and T cell subsets of the patients with CAH-B, treated with IFN -2b with prednisolone withdrawal, were examined 1 month before administration of prednisolone, and compared with 12 normal controls for assessing the potential role of cellular immunity in the development of CAH-B. To estimate the effectiveness of IFN therapy for the patients with CAH-B, levels of various liver function tests, HBsAg, anti-HBs, HBeAg, anti-HBe, HBV DNA, anti-HCV and others were assessed for the treatment group and compared with control patients at pre- and post-treatment period each.*

Results : *The value of CD4 was significantly lower in patients with CAH-B than normal controls ($36.3 \pm 7.7\%$ vs $42.1 \pm 5.7\%$, $p < 0.05$) and the value of CD8 was significantly higher in patients with CAH-B than normal controls ($30.6 \pm 10.3\%$ vs $24.3 \pm 5.2\%$, $p < 0.05$) before prednisolone administration. The patients in responder group ($n=26$) had significantly lower CD4 cells compared with normal controls, but non-responders ($n=6$) did not have. The levels of liver function test(LFT) in the patients with IFN -2b treatment with prednisolone withdrawal were not different from the control patient group at pretreatment, but significantly lower than control patient group's after treatment, regardless of response to IFN -2b treatment with prednisolone withdrawal.*

Conclusions : *The cellular immunity of the host may have a potential role in the pathogenesis of chronicity of hepatitis B infection. IFN -2b treatment with prednisolone withdrawal may be regarded as one of the effective treatment modalities for the inhibition of disease progression in patients with CAH-B.*

Key Words : *chronic active hepatitis, interferon, T cell subsets*

INTRODUCTION

Although the development mechanism of chronic viral hepatitis B(CVH-B), which is the most important etiologic

factor in liver cirrhosis(LC) and hepatocellular carcinoma (HCC) in Korea, has not been fully understood until now, immune mechanism of the host(especially cellular immunity) seems to play a major role in this area^{1).}

Hepatitis B virus(HBV) by itself does not appear to induce hepatic lesion directly, and lysis of infected hepatocytes depends on the immune response of the host^{2).} In these circumstance, IFN induces the recognition of cytotoxic T cells to infected hepatocytes

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and lysis of the infected hepatocyte¹⁻⁵).

Immune system abnormality of the host accompanies incomplete removal of the infected hepatocytes and persistent replication of HBV introduces the development of CVH-B^{6,7}. Several studies⁸⁻¹⁰ reported that decrease of the IFN concentration, CD4/CD8 ratio and value of CD4, and increase of the value of CD8 were discovered in patients with CVH-B. These results suggested that the defective immune system of the host participated in the pathogenesis of CVH-B¹¹⁻¹⁵.

According to the above results, one hypothesis^{18, 19} was that therapeutic agents (which improve the host immunity and prevent the proliferation of HBV) prevent the progression of CVH-B to LC or HCC and, therefore, many antiviral and immunosuppressive agents were tried and variable therapeutic effects were observed¹⁸⁻²². Among these, IFN may be the most valuable and effective treatment modality¹⁵⁻²².

Therefore, the authors have undertaken a controlled study to determine the effectiveness of combination treatment with prednisolone withdrawal and IFN -2b to find out the abnormalities of cellular immunity on patients with CAH-B, and compared the effectiveness of combination treatment with IFN -2b with prednisolone withdrawal to IFN alone.

MATERIALS AND METHODS

1. Patients

Sixty two patients with CAH-B and 12 normal controls were examined. All patients had HBsAg, HBeAg and positive results for HBV DNA. Among these patients with CAH-B, 32 patients were treated with prednisolone withdrawal followed by IFN -2b for a total of 6 months, and 30 patients were treated with conventional hepatotoxicity for the same duration as the control patient group.

All cases of the normal control group had negative results for serum HBsAg and anti-HCV, and the results of LFT were within normal limits.

2. Treatment protocol

Patients had been taking 45 mg prednisolone per day for the initial 2 weeks and then 30 mg per day and 15 mg per day for the subsequent 2 weeks and, thereafter, they had not been taking any drug for 2 weeks.

IFN -2b (Intron A[®], Schering-Plough, Kenilworth,

New Jersey, USA) was injected in doses of 3×10^6 units subcutaneously. The same dose was given every day during the first week and, thereafter, three times a week for the subsequent 15 weeks.

3. Serological assays, T cell subsets determinations and LFTs

HBsAg, anti-HBs, HBeAg and anti-HBe were tested with radioimmunoassay using CIS[®] Kit (Abbott Laboratories, North Chicago, USA) and HBV-DNAs were quantitated by the spot hybridization²⁶ (EXPIDITE[®] Persepiv Biosystem INC, USA) 1 month before administration of prednisolone and 1 month after termination of IFN -2b treatment in patient with CAH-B, and 1 month before the study in normal controls.

Anti-HCV was checked by second generation of enzyme immunoassay (IMX[®], Abbott GmbH Diagnostica, Max-Planck-Ring 2, Wiesbaden-Delekenheim, Germany) 1 month before the study in all patient and normal controls.

The subsets of lymphocytes were examined by flow cytometry (COULTER EPICS XL[®], Coulter Corporation, Miami, Florida, USA) with mouse monoclonal antibody (OKT3, OKT4 and OKT8)²³ in all participants in this study at 1 month before administration of prednisolone. Thereby, peripheral total T cell fraction and T cell subsets were assessed in IFN -2b treated patients with CAH-B at 1 month before administration of prednisolone and after IFN -2b treatment.

Serum levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (AP), total bilirubin (TB), total protein (TP), albumin (Alb), blood urea nitrogen (BUN) and creatinine (Cre) were examined by 17 Hi-cell autoanalyzer before prednisolone treatment and 1, 3 and 6 months after the beginning of treatment with IFN -2b.

Blood cell counts were examined every 2 weeks during this study for the evaluation of leukopenia and thrombocytopenia.

The patients who were treated with prednisolone withdrawal and IFN -2b were divided into two groups, responder and non-responder, according to the response to treatment. The response was defined as one of these was achieved: normalization, disappearance of HBV DNA and seroconversion of HBeAg. Assessment was carried out at 1 month after IFN -2b treatment and based on seroconversion of HBeAg and HBV DNA at 1 month

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after IFN -2b treatment, and LFTs after 1, 3, and 6 months.

4. Statistical Analysis

All data were analysed by using a paired t-test and given as mean±SEM.

RESULTS

1. Clinical characteristics of the patients

Numbers, mean age(years old) and sex ratios of IFN -2b treated patients with CAH-B, control patient group treated with conventional hepatotonics and normal control group were 32, 35.7, 28:4, 30, 31.6, 21:9 and (12, 33.1, 9:3).

Among these groups, age and sex ratios were not significantly different from each other(Table 1). All the clinical profiles and laboratory findings were not significantly different in both groups of patients at the beginning of this study(p>0.05).

2. Changes of the levels of liver function test and adverse effects according to IFN -2b treatment in patients with CAH-B

Both serum levels of AST and ALT were significantly decreased after IFN -2b treatment(p<0.001) in patients with CAH-B.

In 26(81.3%) of 32 patients with IFN -2b treatment, levels of AST and ALT significantly decreased to normal or one and a half of normal level. On the other hand, levels of these enzymes in the control patient group decreased to normal range or one and a half of normal level in only 8 (26.7%) of 30 patients.(p<0.05).

The levels of AP, TP, BUN, creatinine(Cre) and hemoglobin(Hb) had no significant difference statistically between pre- and post-treatment periods(p>0.05, Table 2).

Flu-like syndromes were observed in all patients, but the syndromes disappeared with sulindac preparation in all cases within 3 to 14 days.

Thrombocytopenia and leukopenia were noted in 7(21.9%) and 6 cases(18.8%) of the 32 patients respectively, and mostly prominent in the second week,

Table 1. Characteristics of patients and controls (before treatment with interferon)

	Interferon group	Patients control	Normal control
Numbers	32	30	12
Sex(M:F)	28:4	21:9	9:3
Age(years)	35.7 ± 11.1	31.6 ± 7.3	33.1 ± 18.4
Hb(g/dl)	15.3 ± 1.5	14.8 ± 2.2	14.5 ± 0.6
AST(IU/L)	155.0 ± 115.0	147.8 ± 89.4	24.9 ± 3.9*
ALT(IU/L)	227.0 ± 126.0	202.1 ± 112.3	26.4 ± 5.1*
ALP(IU/L)	182.9 ± 38.9	180.7 ± 28.8	127.9 ± 14.8*
TB(mg/dl)	1.23 ± 1.00	1.2 ± 0.9	0.6 ± 0.1*

* : statistically significant data(p<0.05)

Table 2. Changes in serum levels of liver function test according to treatment for the patients with chronic active hepatitis B

	Responder group (n=26)		Non-responder group(n=6)	
	Pre-treatment	Post-treatment	Pre-treatment	Post-treatment
AST(IU/L)	178.0 ± 63.1	26.4 ± 9.6*	142.0 ± 138.0	46.1 ± 15.9*
ALT(IU/L)	289.0 ± 150.0	27.0 ± 4.9*	192.0 ± 104.0	70.9 ± 36.5*
ALP(IU/L)	186.3 ± 54.2	197.7 ± 36.9	180.8 ± 34.2	170.9 ± 29.7
TB(mg/dl)	1.0 ± 0.3	1.0 ± 0.1	1.4 ± 1.3	0.8 ± 0.3

* : statistically significant data(p<0.05)

Table 3. Changes of HBsAg and HBeAg in the patients with chronic active hepatitis B

	Pre-treatment		Post-treatment	
	IFN	-2b group control	IFN	-2b group control
HBsAg/Anti- HBs (+/-)	32/0	30/0	32/0 (0%)	30/0(0%)
HBeAg/Anti- HBe (+/-)	32/0	30/0	20/12(37.5%)	23/7(23.3%)
HBV DNA(+/-)	32/0	30/0	1/21(65.6%)	23/7(23.3%)

(%): seroconversion rate

but almost all of the patients had improved during IFN -2b treatment.

3. Changes of HBeAg, Anti HBe and HBV DNA in patient group with IFN -2b treatment and control patient group

A marked reduction of serum HBV DNA was noted in the IFN -2b group as compared with the patient control group. In the IFN -2b group, loss of HBV DNA occurred in 21 of 32 cases(65.6%) and was significantly more frequent than in control patient group(7 of 30 cases, 23.3%, $p<0.05$) (Table 3).

A seroconversion rate of HBeAg was significantly higher in the IFN -2b treatment group(12 of 32 cases, 37.5%) than in patient control group(7 of 30 cases, 23.3%, $p<0.05$). None of both the IFN -2b treatment group and patient control group had resulted in loss of HBsAg(Table 3).

Table 4. T cell subsets of the patients and normal controls

	Patients(n=62)	Normal controls(n=12)
CD3(%)	65.9 ± 8.1	65.4 ± 10.1
CD4(%)	36.3 ± 7.7*	42.1 ± 5.7
CD8(%)	30.6 ± 10.3*	24.3 ± 5.2
CD4/CD8	1.4 ± 0.8	1.7 ± 0.3
Lymphocytes (/mm ³)	2,557 ± 971	2,396 ± 648

* : statistically significant data($p<0.05$)

4. Comparison of the peripheral total lymphocytes and T cell subsets between IFN -2b treatment group and normal control group

Before administration of prednisolone, the numbers of CD3 cells of the IFN -2b group were similar to those

of the control group($p>0.05$), CD4 cells were significantly reduced in the IFN -2b group than in the control patient group($36.3 \pm 7.7\%$ vs $42.1 \pm 5.7\%$, $p<0.05$) and CD8 cells also were significantly increased in the IFN -2b group than in that of the control group($30.6 \pm 10.3\%$ vs $24.3 \pm 5.2\%$, $p<0.05$). The ratio of CD4 to CD8 cells was more decreased in the IFN -2b group than in the control group, but was statistically insignificant ($p>0.05$) (Table 4).

5. Comparison of T cell subsets between the responder group and non-responder group for IFN -2b treatment with prednisolone withdrawal

Before administration of prednisolone, CD3 cells in the responder group were slightly higher than those of the non-responder group, but insignificant($p>0.05$). CD4 cells in the responder group were significantly lower than in the normal control group($35.2 \pm 6.8\%$ vs $42.1 \pm 5.7\%$, $p<0.05$). CD8 cells in both responder and non-responder group were higher than in the normal control, but insignificant($31.6 \pm 9.6\%$, $31.0 \pm 8.9\%$ vs $24.3 \pm 5.2\%$, $p>0.05$)(Table 5).

6. Comparison of changes of T cell subsets according to IFN -2b treatment between responder and non-responder group

In the responder group, numbers of CD4 cells were increased after IFN -2b treatment, but statistically insignificant($35.2 \pm 6.8\%$ vs $40.0 \pm 9.3\%$, $p>0.05$) and numbers of CD8 cells were decreased but insignificant also(Table 6). In contrast to the responder group, numbers of CD4 cells were decreased from $36.9 \pm 8.3\%$ to $35.4 \pm 4.0\%$ and numbers of CD8 cells were increased from $31.0 \pm 8.9\%$ to $33.9 \pm 9.7\%$ after IFN -2b treatment in the non-responder group, but these results were not statistically significant, too($p>0.05$, Table 6).

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Table 5. Peripheral T lymphocytes and T cell subsets in the patients with CVH-B and normal controls (before treatment with interferon -2b)

	CAH-B		Normal Controls (n=12)
	Responder (n=26)	Non-responder (n=6)	
CD3(%)	65.8 ± 7.1	66.1 ± 8.8	65.4 ± 10.1
CD4(%)	35.2 ± 6.8*	37.0 ± 8.3	42.1 ± 5.7
CD8(%)	31.6 ± 9.6*	31.0 ± 8.9*	24.3 ± 5.2
CD4/CD8	1.3 ± 0.7	1.4 ± 0.8	1.7 ± 0.3
Lymphocytes	2,258 ± 848	2,740 ± 1,020	2,396 ± 648

* : statistically significant difference to normal controls (p<0.05)

Table 6. Changes of T cell subsets according to treatment

	Responder group(n=26)		Non-responder group(n=6)	
	Pre-treatment	Post-treatment	Pre-treatment	Post-treatment
CD3(%)	65.8 ± 7.1	68.8 ± 5.9	66.1 ± 8.8	61.6 ± 8.2
CD4(%)	35.2 ± 6.8#	40.0 ± 9.3*	37.0 ± 8.3	35.4 ± 4.1
CD8(%)	31.6 ± 9.6#	30.2 ± 8.8*	31.0 ± 8.9#	33.9 ± 9.7
CD4/CD8	1.3 ± 0.7	1.5 ± 0.7	1.4 ± 0.8	1.1 ± 0.4*
Lymphocytes	2,258 ± 848	2,500 ± 1,140	2,740 ± 1,020	2,500 ± 574

* : statistically significant difference between response and group after prednisolone and IFN -2b treatment.

: statistically significant difference to normal controls

DISCUSSION

CAH-B is one of the most important causes of the development of LC and HCC. Its clear pathogenesis was not proven until now, but defective cellular immunity of the host has been suggested as playing an important role in the development of CVH-B¹⁻⁵.

With HBV infection to the hepatocytes, HBeAg and HBcAg were expressed on the surface of the hepatocyte²⁴, and major histocompatibility antigen complex-I(MHC-I) was expressed concomitantly⁴. These MHC-I and HBeAg or HBcAg interact synergically and induce cytolysis of the infected liver cells by the cytotoxic T cell⁶.

IFN is released by activated lymphocytes and activates the intracellular enzyme(2,5-oligoadenylate synthetase) and this enzyme induces the release of ribonuclease which destroys viral mRNA, and eventually prevents viral translation and propagation to the adjacent tissue⁵. IFN increases the expression of MHC-I on the hepatocytes also, and induces the cytolysis of the infected

hepatocytes by cytotoxic T cell⁶.

Several studies reported that reduced serum IFN level and qualitative or quantitative abnormalities of cellular immunity of the host were discovered in patients with CVH-B⁸⁻¹⁰.

Many reports for T cell subsets in CVH-B showed variable results, but usually many authors^{10, 11, 24, 25} agree that decreased ratio of CD4 to CD8 cells is largely dependent on significantly decreased CD4 cells in patients with CVH-B. Barnaba et al⁶ had demonstrated that the ratio of CD4 to CD8 cells was decreased during acute viral hepatitis B and that it resulted from increased CD8 cells or decreased CD4 cells or both. Thomas et al¹² also reported similar results in chronic liver disease and that it resulted from decreased CD4 cells mostly.

In this report, increase of CD8 cells appeared but was statistically insignificant. In our study, decreased CD4 cells and increased CD8 cells were also significantly noted(Table 4).

Similarly as in previous reports^{11, 12, 24-26}, the ratio of CD4 to CD8 cells was decreased in patients with CAH-B as compared with normal control in our study too, but

was statistically insignificant ($p > 0.05$, Table 4). These results may be due to both an decrease of CD4 cells and an increase of CD8 cells. In our study, CD4 cells of patients were lower significantly in the responder group compared with that of the normal control group, but insignificant in the non-responder group (Table 5). It is suggested that decrease of CD4 cells may be used as a potential prognostic factor to predict the course of CVH-B, but further studies can be assessed to confirm this suggestion.

In our study, a larger decrease of CD4 cells and a larger increase of CD8 cells also appeared in the responder group than in the non-responder group at the pre-treatment period. It is interesting that CD4 cells have increased significantly from $35.2 \pm 6.8\%$ to $40.0 \pm 9.3\%$ ($P < 0.05$) and that CD8 cells have decreased from $31.6 \pm 9.6\%$ to $30.2 \pm 8.8\%$ ($P > 0.05$) in the responder group, but were insignificant in the non-responder group in our study (Table 6).

Many authors agree that persistent proliferation of HBV may play an essential role in the progression of CVH to LC or HCC^{6, 7}. In our study, all of the patients with CVH-B had HBeAg and HBV DNA which were serologic markers for proliferation of HBV (Table 3). Loss of these markers was accompanied by improvement of symptoms and laboratory data²⁷⁻²⁹. These findings propose that various antiviral therapies will prevent the progression of CVH-B to LC or HCC.

In various reports, immunosuppressants (prednisolone, azathioprine etc) and several antiviral or immunomodulating agents (adenosine arabinoside, adenosine arabinoside monophosphate, IFN, and interleukin-2 etc) had been studied and many authors agree that IFN is the most effective agent among these^{15-22, 30}.

Yasushi et al¹⁰ reported that loss of HBV DNA occurred in 42% with treatment of IFN for 12 to 24 months, and 54% in Lok et al¹⁷ and 52% in Thomas et al¹⁸. In Korea, Choi et al¹⁵ and Choi et al¹⁶ reported that seroconversion rates of HBeAg were 25% and 17.6% apart. These results were superior to the spontaneous conversion rate of HBeAg in Korea reported by Yoon et al¹¹, but inferior to those in western countries¹⁶⁻¹⁸.

However, the reason why these results were worse than western countries is suspected as follow; first, transmaternal transmission of HBV was more common in Korea and patients with CVH-B occurred in this manner were more resistant to IFN treatment and, second, we

commonly used a lower dose than western countries¹⁶⁻¹⁸. Therefore, if the dose of IFN is increased, a better response will be acquired.

Recently, several studies^{32, 33} reported that IFN therapy with prednisolone withdrawal may be more effective for the seroconversion of HBeAg than in IFN therapy alone. In Korea, Kim et al¹⁴ reported that combined therapy with IFN and short-term prednisolone withdrawal was more effective (50% rate of seroconversion) than single IFN therapy. It has been demonstrated that seroconversion of HBeAg occurred in 12 cases (37.5%) of 32 patients with interferon treatment, and it is significantly higher than in the control patient group (7 of 30 cases, 23.3%, $p < 0.05$) in our study (Table 3) and this result was inferior to Kim et al¹⁴, but superior to Choi et al¹⁵ (17.6%) and Choi et al¹⁶ (25.0%). Therefore it may be suggested that combination therapy of IFN with prednisolone withdrawal is more effective than IFN alone.

Disappearance of HBV DNA occurred in 21 cases (65.6%) of 32 patients and these results were similar to those of other previous reports. The above results were significantly better than the control patients. Loss of HBV DNA occurred faster than seroconversion of HBeAg and similar results were reported by other authors³⁷⁻³⁹.

The good responder group for the IFN treatment includes the patients with recently acquired infection, higher levels of serum aminotransferases, lower serum level of HBV DNA and active hepatitis on liver biopsy. Heterosexuals and women respond better than homosexuals and men³⁵. However, in our study, the levels of aminotransferase and sex difference did not influence the prognosis of IFN treatment ($p > 0.05$).

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