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Alteration of Interferon- α/β Receptors in Chronic Hepatitis B Patients

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Abstract

Background—The present study determined type I interferon (IFN) receptor (interferon- α/β receptor (IFNAR)) and its predictable role in interferon- α 2b treatment in chronic hepatitis B (CHB) patients.

Methods—Expression of IFN- α/β R-1 and IFN- α/β R-2 in peripheral blood mononuclear cells and in liver tissue was measured by flow cytometry, immunofluorescence, immunohistochemistry and RT-PCR.

Results—IFN- α/β R-1 and IFN- α/β R-2 in monocytes and lymphocytes increased in CHB patients. Expression of IFNAR-1 and IFNAR-2 in liver had positive correlation with HBV-DNA in liver tissue. Expression of IFN- α/β R in lymphocytes and monocytes increased in the first month, but then decreased during the subsequent interferon- α 2b treatment, patients who had higher levels of IFN- α/β R-2 in monocytes prior to therapy showed better viral response than those with lower levels.

Conclusions—Expression of IFN- α/β R-2 in monocytes can be used as a predictable parameter to evaluate the effect of IFN- α treatment in CHB patients.

Keywords

Interferon- α/β receptor; chronic hepatitis B; interferon-alpha; peripheral blood mononuclear cells (PBMCs)

Introduction

Patients with chronic hepatitis B (CHB) are at increased risk of developing hepatic decompensation, cirrhosis, and hepatocellular carcinoma [1, 2]. Interferon (IFN)- α , the first drug approved for use against CHB by the Food and Drug Administration (FDA) in the USA, has been shown to be effective in suppressing hepatitis B virus (HBV) replication [3] as well as in induction remission of liver disease. However, its efficacy is limited to a small percentage of highly selected patients.

It is now known that all type I IFNs bind to the same dimeric receptor, the interferon- α/β receptor (IFNAR). It is a heteromeric receptor composed of one chain with two subunits, referred to as interferon- α/β receptor 1 (IFNAR-1) and interferon- α/β receptor 2 (IFNAR-2) [4]. IFNAR-2 binds to type I IFNs with different affinities, mostly in the nanomolar range, and its low-affinity partner, IFNAR-1, which binds to IFNs with micromolar affinities, also differentially recognizes the type I IFNs [5, 6]. IFNs bind initially to IFNAR-2, and this complex then recruits IFNAR-1 [7, 8].

The expression of the type I IFN receptor in liver tissues may be directly involved in the pathogenesis of viral hepatitis and the response to IFN therapy. After a sequence of IFN- α receptor subunit genes was identified [9, 10], several investigators assessed the amounts of IFNAR-1 and IFNAR-2 expression in patients with hepatitis C by polymerase chain reaction (PCR) assay [11]. mRNA levels of IFNAR-1 and IFNAR-2 were significantly higher in

patients with sustained virological and biochemical response to interferon therapy compared with those with nonsustained response. Moreover, mRNA expression ratio of IFNAR-1 to IFNAR-2 was also significantly high in patients with sustained virological and biochemical response to IFN therapy. The expression level of IFNAR-2 in the liver was related to that in peripheral blood mononuclear cells (PBMCs). These results suggest that hepatitis C virus infection may up-regulate the expression of the type I interferon receptor and that the measurement of IFNAR-2 expression in PBMCs may be useful for monitoring its expression in liver.

The efficacy of IFN- α is limited to a small percentage of highly selected patients, only 20% to 40% have a complete, sustained inhibition of viral replication [12–14]. One possibility might be the difference in interferon- α/β receptor (IFNAR) expression in those patients, based on the observation that monocytes and B cells express the highest levels of IFNAR among peripheral hematopoietic cells [15]. These results prompted us to determine which parameter could be used for predicting response to IFN- α therapy; we detected IFNAR expression in CHB patients before and after application of interferon- α 2b.

To gain more insights into the alteration of IFNARs in CHB patients, we measured mRNA expression of IFNAR-1, IFNAR-2, interleukin (IL)-6, IL-8, tumor growth factor (TGF)- β 1 and tumor necrosis factor (TNF)- α both in PBMCs and liver tissues by real-time reverse-transcription polymerase chain reaction (RT-PCR). In addition, we evaluated the expression of IFN- α/β R-1 and IFN- α/β R-2 on peripheral lymphocytes and monocytes by flow cytometry, and performed immunofluorescence histochemistry to detect the expression of IFN- α/β R-1 and IFN- α/β R-2 in liver.

Patients and Methods

Patients

From March 2007 to June 2010, 84 patients (54 men and 30 women, age 21–51 years), who met the criteria for chronic hepatitis B according to the AASLD practice guideline and Chinese Society of Hepatology guideline [1, 16], and ten healthy people were enrolled in Qilu Hospital, Shandong University. All the patients were grouped into two groups: group A (56 patients): HBsAg+ (hepatitis B surface antigen), HBeAg+ (hepatitis B e antigen), Group B (28 patients): HBsAg+, HBeAg–. Of all the patients, 25 patients accepted liver biopsy. Twenty-five patients who met the criteria for IFN- α treatment [16] were treated with recombinant human interferon- α 2b (Yuan Ce Pharm, Beijing China) for at least 3 months (administered three million unit thrice a week), and blood samples were collected every 4 weeks to monitor the alteration of biochemical parameters. The effect of interferon- α 2b was verified by biochemical response (BR): decrease in serum ALT to within the normal range; virologic response (VR): decrease in serum HBV-DNA to undetectable levels by PCR assays, and loss of HBeAg in patients who were initially HBeAg positive; and early virologic response (EVR): decrease in serum HBV-DNA more than 2 log₁₀ copies/mL in 3 months.

Clinical characteristics were showed in Table I. All patients gave written informed consent for research testing under protocols approved by the local Research and Ethics Committee at

Qilu Hospital of Shandong University in accordance with the guidelines of the 1975 Declaration of Helsinki.

Cell Isolation

PBMCs were isolated from whole blood by means of Ficoll-Paque Plus (GE Healthcare Piscataway, NY, USA) density gradient centrifugation [17]; 10-mL heparinized whole blood diluted 1:1 with phosphate-buffered saline (PBS), were layered on top of 5-mL Ficoll and centrifuged without a brake at $500\times g$ (20°C) for 30 min and the PBMC ring was collected, and diluted with GIBCO RPMI 1640 (Invitrogen, Carlsbad, CA, USA) to a final volume of 50 mL, and centrifuged at $500\times g$ for 15 min at room temperature. The pellet was washed again with RPMI 1640 for the measurement. CD14^{+} monocytes and CD14^{-} lymphocytes were purified from PBMCs by positive or negative selection using microbeads MACS system (Miltenyi Biotech, Bergisch-Gladbach, Germany) according to the manufacturer's instructions.

Isolation of Human PBMCs and Quantitative RT-PCR

Total RNA was extracted and purified from PBMCs using RNeasy Mini Kit (Qiagen, Valencia, CA, USA). RNA was isolated from human liver tissue by homogenization and purification using TRIzol (Invitrogen) followed by RNeasy clean-up (Qiagen); 2 μg RNA was used as a template for cDNA synthesis according to the handbook of High Capacity cDNA Reverse-Transcription Kit (Applied Biosystem, Foster City, CA, USA). Quantitative RT-PCR was performed with the SYBR Green RT-PCR kit (Bio-Rad, Hercules, CA, USA) on the ABI PRISM 7000 (Applied Biosystems). The primers of IFNAR-1, IFNAR-2, IL-6, IL-8, TGF- β and TNF- α were designed using Primer Express software, version 2.0 (Applied Biosystems) (Table II). GAPDH was used to normalize the samples in each PCR reaction. The absence of nonspecific primer-dimer products was verified by melting-curve and gel-migration analyses. Quantification was performed by comparing the Ct values of each sample with a standard curve and normalization to GAPDH [18, 19].

Flow Cytometry

IFN- α/β R-1 and IFN- α/β R-2 expression was measured by flow-cytometric analysis [20]. Briefly, 2.0×10^5 cells were incubated with 10 μL (25 $\mu\text{g}/\text{mL}$) primary antibodies (goat monoclonal anti-human IFN- α/β R-1 antibody or rabbit polyclonal anti-human IFN- α/β R-2: R&D Systems, Minneapolis, MN, USA) for 1 h, followed by incubation with 1% fluorescein phycoerythrin (PE red)-conjugated donkey anti-goat IgG (Santa Cruz Biotech, Santa Cruz, CA, USA) or PerCP-cyanine dye 5.5 (Cy5.5 blue)-conjugated mouse anti-rabbit IgG (Santa Cruz Biotech) as the secondary antibodies. Background staining was determined using matched isotype controls, and all staining was done in the presence of 1% human serum to block nonspecific binding to Fc γ receptors. Mean fluorescence intensity (MFI) of the background stain was subtracted from IFN- α/β R-1 and IFN- α/β R-2 MFIs. The cells were analyzed using a FACSCalibur (Becton Dickinson, San Diego, CA, USA) to measure the forward and sideward light scatter and PE and Cy5.5 fluorescence [21].

Liver Histology and Immunofluorescence Histochemistry

All liver biopsies were obtained through percutaneous techniques, and histological examination was performed according to the Batts–Ludwig scoring system at a single pathology laboratory [22, 23]. Immunofluorescence double staining was carried out to detect IFN- α / β R-1 and IFN- α / β R-2 in liver biopsy tissue. After deparaffinization and dehydration, liver sections were microwaved to get antigen retrieval, blocked in the Dako protein block serum free (Dako, Carpinteria, CA, USA), and then incubated in a mixture of goat monoclonal anti-human anti-IFN- α / β R-1 (1:200) and rabbit polyclonal anti-human anti-IFN- α / β R-2 (1:200; R&D Systems.). One percent PE red-conjugated donkey anti-goat IgG (Santa Cruz Biotech, Santa Cruz, CA, USA) and Cy5.5 blue-conjugated mouse anti-rabbit IgG (Santa Cruz Biotech) as the secondary antibodies. After staining, the sections were mounted with Dako mounting medium (Dako.) and observed with a fluorescence microscope (Olympus 1X71, Japan). The positive area was measured in six high-power ($\times 200$) fields on each slide and quantified using NIH recommended software Image J.

Immunohistochemistry

Paraffin sections were deparaffinized by xylene and dehydrated in graded alcohol. Endogenous peroxide was inactivated using 3% cold hydrogen peroxide (Sigma-Aldrich St. Louis, MO) in methanol for 15 min. Slides were microwaved in citrate buffer for 20 min to get antigen retrieval, followed by blocking in the Dako protein block serum free (Dako) for 30 min, and an overnight incubation at 4°C in anti-IFN- α / β R-2 (1:200; R&D Systems.) in PBS. After washing, goat anti-rabbit IgG (horseradish peroxidase conjugated; Santa Cruz Biotech, Santa Cruz, CA, USA) was used as secondary antibody for 1 h at room temperature and then the signal was detected using an ABC Elite kit (Santa Cruz Biotech.). For negative control, sections were incubated with secondary antibodies only.

Statistical Analysis

Results were expressed as the mean $\pm$ standard deviation. Statistical significance between CHB patients and the control group were evaluated by the unpaired Student's *t* test. Differences among the patients groups were analyzed by Student's *t* test. The relationships between variations were analyzed by nonlinear (curve fit) and linear regression. A *p* value of <0.05 was considered statistically significant. All analysis was performed by Graph Pad Prism 5.0 (GraphPad Software, San Diego, CA, USA).

Results

Expression of IFN- α / β R in PBMCs Increased Significantly in CHB Patients

In concordance with a previous study [20], lymphocytes and monocytes from CHB patients showed up-regulated expression of IFN- α / β R-1 and IFN- α / β R-2 (Table III). IFN- α / β R-1 and IFN- α / β R-2 were predominantly expressed in the control group by lymphocytes, but not monocytes (IFN- α / β R-1, 26.65 ± 2.15 vs. 16.11 ± 1.96 ; IFN- α / β R-2, 25.25 ± 2.65 vs. 15.35 ± 3.98 ; Figure 1a). Both lymphocytes and monocytes from CHB patients expressed similar levels of IFN- α / β R-1 and IFN- α / β R-2 (IFN- α / β R-1, 39.27 ± 2.10 vs. 40.44 ± 1.49 ; IFN- α / β R-2, 38.40 ± 2.67 vs. 45.67 ± 3.59 . Figure 1b). The MFI of IFN- α / β R-1 and IFN- α /

β R-2 in peripheral lymphocytes was increased by 11.62 ± 2.34 and 14.15 ± 3.94 , respectively. Moreover, IFN- α/β R-1 and IFN- α/β R-2 expression in monocytes from CHB patients was increased much higher than in lymphocytes (25.33 ± 1.65 and 27.32 ± 2.56 , respectively). Monocytes showed much more IFN- α/β R-1 and IFN- α/β R-2 expression increase than lymphocytes in response to CHB virus ($p < 0.0001$).

Relationship Between IFNAR and IL-6, IL-8, TGF- β 1 and TNF- α in mRNA Levels

We found out that expression of IFNAR-1, IFNAR-2, IL-6, IL-8, TGF- β 1 and TNF- α in PBMCs and livers significantly increased in patients with CHB (Fig. 2a). While, mRNA levels of IFNAR-1 and IFNAR-2 in PBMCs had no correlation with the severity of viral infection in all the patients (data not shown). There was no difference in IFNAR-1 and IFNAR-2 as well as TGF- β 1, TNF- α , IL-6 and IL-8 mRNA levels between the two groups of patients (data not shown). Elevated hepatic mRNA expression of IFNAR-1 and IFNAR-2 was strongly correlated with HBV-DNA content in liver biopsy samples from CHB patients (Fig. 2b). The mRNA expression of IFNAR correlated with all the inflammatory cytokines such as IL-6, IL-8, TGF- β 1 and TNF- α detected in livers (Fig. 3).

Taken together, our data indicate that mRNA levels of hepatic IFNAR-1 and IFNAR-2 correlate with the HBV-DNA content in liver tissue of CHB patients.

Expression of IFN- α/β R-1 and IFN- α/β R-2 in Liver Tissues Increased Significantly in CHB Patient

Immunofluorescence histochemistry of liver tissues were double stained with IFN- α/β R-1 and IFN- α/β R-2 antibodies and showed increased expression compared with control liver tissues (Fig. 4a). Immunohistochemistry of liver tissues stained with IFN- α/β R-2 antibody had the same result (Fig. 4b). However, there was no relationship between IFN- α/β R-1 and IFN- α/β R-2 expression and inflammatory grade or fibrosis stage (data not shown).

Alterations of IFNAR Expression in Response to Interferon- α 2b Therapy

Among the 25 chronic hepatitis B patients treated with interferon- α 2b, 12 patients reached BR, only one patients achieved VR, while nine patients had EVR (Table IV).

During interferon- α 2b treatment, IFN- α/β R-1 and IFN- α/β R-2 in lymphocytes and monocytes in all the 25 patients treated with interferon- α 2b increased in the first month, but then decreased in the following 2 months (Fig. 5b). The mRNA levels of IFNAR-1 and IFNAR-2 in PBMCs showed similar patterns (data not shown). After we analyzed the difference between responders (patients who achieved EVR, BR or VR. Some patients could achieve BR, EVR and VR at the same time, so the total number of responders was 12.) and nonresponders (NR, $n=13$), we found out that before interferon- α 2b treatment, the expression of IFN- α/β R-1 and IFN- α/β R-2 in lymphocytes and monocytes magnified almost no difference between the two groups. But the expression of IFN- α/β R-2 in monocytes in responders was higher than that in nonresponders (Fig. 6d) before the application of interferon- α 2b. During the 3-month treatment, the expression of IFN- α/β R-1 and IFN- α/β R-2 in lymphocytes and monocytes was much higher in responders than non-responders (Fig. 6a – d).

Along with interferon- α 2b treatment, the mRNA levels of IL-6, IL-8, TGF- β and TNF- α , all decreased (Fig. 7a), implying that recombinant IFN- α can significantly suppress the inflammatory cytokines induced by hepatitis B virus. Expression of IFN- α / β R-2 in monocytes in the response patients was significantly higher than the NR patients (Fig. 7b).

Discussion

IFN- α is a critical mediator of immune response to HBV infection. IFN- α can stimulate the immune responses, which target infected hepatocytes leading to a decrease of hepatitis B virus. IFNs bind initially to IFN- α / β R-2, and this complex then recruits IFN- α / β R-1. The extracellular ligand-binding domain of IFN- α / β R-2, by virtue of its smaller size and higher affinity for IFNs than IFN- α / β R-1, is a more tractable target for mutagenesis and structural studies [24].

Sarah L. Pogue demonstrated that expression levels of IFNAR-1 and IFNAR-2 were highest on the monocyte population, followed by B cells. Neutrophils and NK cells expressed approximately one third the level of receptor as B cells, and the T cell subsets expressed low but detectable levels of the receptor [15]. Shiro Tochizawa's results indicated that all leukocyte subsets possessed IFNAR-2, and the expression was higher in monocytes and granulocytes than in lymphocytes [20]. We found that IFN- α / β R-1 and IFN- α / β R-2 were expressed ubiquitously in leukocytes, including lymphocytes, monocytes and neutrophils, while the expression of IFN- α / β R-1 and IFN- α / β R-2 in neutrophils shows no difference between CHB patients and healthy controls. To observe IFNAR's fluctuation during IFN- α treatment, we focused on IFN- α / β R-1 and IFN- α / β R-2 in lymphocytes and monocytes. Lau JY first reported the expression and regulation of IFNAR in chronic hepatitis B virus infection in 1991 [25]. Their data indicated that IFNARs are highly expressed and regulated normally in chronic hepatitis B virus infection by interferon- α therapy. Their results are limited by enrollment (only 20 HBV patients) and the fact that they did not measure the two subunits of IFNAR. Shiro Tochizawa also examined the effects of IFN- α on IFNAR-2 expression using heparin-treated blood. A decrease in IFNAR-2 expression in lymphocytes, monocytes, and granulocytes by 2-h treatment with IFN- α was observed in a concentration-dependent manner [20]. But he got the results only in vitro cultured leukocytes, there were no more data available about the effect of IFN- α on IFNAR expression in CHB patients.

From 2002 to 2007, three groups of researchers from Japan worked on the relationship between type I IFN receptors in response to interferon- α treatment in hepatitis C and gastrointestinal cancers. These results suggest that hepatitis C virus infection may up-regulate the expression of type I interferon receptor and that the measurement of IFN- α / β R-2 expression in PBMCs may be useful for monitoring its expression in liver [26–28]. Our data showed that in chronic hepatitis B patients, IFN- α / β R-1 and IFN- α / β R-2 in PBMCs and liver were elevated significantly after HBV infection. Moreover, IFNAR-1 and IFNAR-2 in livers have a positive relationship with the level of HBV-DNA in livers.

Our study revealed that the expression of IFN- α / β R-1 and IFN- α / β R-2 were higher in lymphocytes than in monocytes in the control group, while it is not the case in the CHB patients. During interferon- α 2b treatment, the levels of IFN- α / β R-1 and IFN- α / β R-2 on

lymphocytes and monocytes increased in the first month, but then decreased in the following 2 months. The pattern of alteration of IFNAR expression caused by interferon- α 2b in CHB patients is not the same as Shiro Tochizawa reported in cultured leukocytes. There are several possible explanations: (a) application of interferon- α 2b in the first month can induce the duplication and production of IFNAR in mRNA and protein levels in lymphocytes and monocytes, while continuous stimulation of interferon- α 2b can suppress the induction of IFNAR; (b) remaining IFNAR has a higher binding affinity, therefore partially compensating for the decrease in receptor number; (c) the intracellular effects are cumulative, so that with long-term treatment, less interferon- α 2b binding is required to sustain the same biological effects; and (d) the maximum biological response requires the occupation of only a small proportion of the accessible receptors. Our study only focused on the expression of IFNAR in PBMCs and in liver tissues, to gain better explanation to such alteration of IFNAR needs further studies.

The IFN molecule itself has no antiviral effect, the binding of IFN to its receptor is an essential first step required to exhibit antiviral activity. However, whether upregulation of the type I IFN receptor results in enhanced antiviral activity by IFN therapy is not confirmed. Patients who achieved EVR or BR had higher levels of IFN- α / β R-2 before interferon α 2b treatment in monocytes than the no-response patients. Perhaps the expression of IFN- α / β R-2 in monocytes can be used as a predictive parameter for the efficacy of interferon- α in the treatment of chronic hepatitis B. Perhaps this could explain the clinic manifestation that if a patient could not achieve early virus response in the first 3-month interferon- α 2b application, simply extending the course of IFN- α has no use in improving the rate of virus response. During the application of interferon- α 2b, the responders exhibited higher expression of IFN- α / β R-1 and IFN- α / β R-2 both in lymphocytes and monocytes than the nonresponders. These results presented a concept to us that higher IFN α / β R expression in PBMCs was a predictive factor associated with sustained virologic response to interferon- α 2b therapy in chronic hepatitis B patients.

In summary, we demonstrated that IFNAR expression in CHB patients is higher than in healthy controls, especially in peripheral monocytes. The expression of IFN- α / β R-2 on monocytes before interferon treatment may be used as a predictive parameter. Higher expression of IFN α / β R in lymphocytes and monocytes during interferon- α 2b treatment maybe predictive for response to IFN therapy.

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Abbreviations

HBV Hepatitis B virus

CHB	Chronic hepatitis B
HBsAg	Hepatitis B surface antigen
HBeAg	Hepatitis B e antigen
IFN-α	Interferon- α
IFNAR	Interferon- α/β receptor
RT-PCR	Real-time reverse-transcription polymerase chain reaction
GAPDH	Glyceraldehydes-3-phosphate dehydrogenase
BR	Biochemical response: decrease in serum ALT to within the normal range
VR	Virologic response: decrease in serum HBV-DNA to undetectable levels by PCR assays, and loss of HBeAg in patients who were initially HBeAg positive
EVR	Early virologic response: decrease in serum HBV-DNA more than 2 log ₁₀ copies/mL in 3 months

References

1. Liaw YF, Chu CM. Hepatitis B virus infection. *Lancet*. 2009; 373(9663):582–592. [PubMed: 19217993]
2. Lok AS, McMahon BJ. Chronic hepatitis B: update 2009. *Hepatology*. 2009; 50(3):661–662. [PubMed: 19714720]
3. Anonymous. Products of new biotechnologies. *FDA Drug Bull*. 1986; 16(2):19. [PubMed: 2950012]
4. Langer JA. Interferon at 50: new molecules, new potential, new (and old) questions. *Sci STKE*. 2007; 405:e53.
5. Cutrone EC, Langer JA. Contributions of cloned type I interferon receptor subunits to differential ligand binding. *FEBS Lett*. 1997; 404(2–3):197–202. [PubMed: 9119063]
6. Jaks E, Gavutis M, Uzé G, Martal J, Piehler J. Differential receptor subunit affinities of type I interferons govern differential signal activation. *J Mol Biol*. 2007; 366(2):525–539. [PubMed: 17174979]
7. Lamken P, Lata S, Gavutis M, Piehler J. Ligand-induced assembling of the type I interferon receptor on supported lipid bilayers. *J Mol Biol*. 2004; 341(1):303–318. [PubMed: 15312780]
8. Lamken P, Lata S, Gavutis M, Piehler J. Lateral ligand-receptor interactions on membranes probed by simultaneous fluorescence-interference detection. *Biophys J*. 2005; 88(6):4289–4302. [PubMed: 15778442]
9. Uzé G, Lutfalla G, Gresser I. Genetic transfer of a functional human interferon alpha receptor into mouse cells: cloning and expression of its cDNA. *Cell*. 1990; 60:225–234. [PubMed: 2153461]
10. Novick D, Cohen B, Rubinstein M. The human interferon alpha/ beta receptor: characterization and molecular cloning. *Cell*. 1994; 77(3):391–400. [PubMed: 8181059]
11. Taniguchi H, Iwasaki Y, Takahashi A, Shimomura H, Moriya A, Yu PC, et al. Intrahepatic mRNA levels of type I interferon receptor and interferon-stimulated genes in genotype 1b chronic hepatitis C. Association between IFNAR1 mRNA level and sustained response to interferon therapy. *Intervirology*. 2004; 50(1):32–39. [PubMed: 17164555]
12. Brook MG, Karayiannis P, Thomas HC. Which patients with chronic hepatitis B virus infection will respond to alpha-interferon therapy? A statistical analysis of predictive factors. *Hepatology*. 1989; 10(5):761–763. [PubMed: 2680866]
13. Perrillo RP, Schiff ER, Davis GL, Bodenheimer HC Jr, Lindsay K, Payne J, et al. A randomized, controlled trial of interferon alfa-2b alone and after prednisone withdrawal for the treatment of

- chronic hepatitis B. The hepatitis interventional therapy group. *New Engl J Med*. 1990; 323(5): 295–301. [PubMed: 2195346]
14. Perrillo R. Benefits and risks of interferon therapy for hepatitis B. *Hepatology*. 2009; 49(5 Suppl):S103–S111. [PubMed: 19399806]
 15. Pogue SL, Preston BT, Stalder J, Bebbington CR, Cardarelli PM. The receptor for type I IFNs is highly expressed on peripheral blood B cells and monocytes and mediates a distinct profile of differentiation and activation of these cells. *J Interferon Cytokine Res*. 2004; 24(2):131–139. [PubMed: 14980077]
 16. Chinese Society of Hepatology, Chinese Medical Association, Chinese Society of Infectious Diseases, Chinese Medical Association. Guideline on prevention and treatment of chronic hepatitis B in China (2005). *Chin Med J (Eng)*. 2007; 120(24):2159–2173.
 17. Lostia AM, Lionetto L, Ialongo C, Gentile G, Viterbo A, Malaguti P, et al. A liquid chromatography-tandem mass spectrometry method for the determination of 5-fluorouracil degradation rate by intact peripheral blood mononuclear cells. *Ther Drug Monit*. 2009; 31(4):482–488. [PubMed: 19571774]
 18. Higuchi R, Fockler C, Dollinger G, Watson R. Kinetic PCR analysis: real-time monitoring of DNA amplification reactions. *Biotechnology (N Y)*. 1993; 11(9):1026–1030. [PubMed: 7764001]
 19. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2^{−(Delta Delta C (T))} method. *Methods*. 2001; 25:402–408. [PubMed: 11846609]
 20. Tochizawa S, Akamatsu S, Sugiyama Y, Muraguchi M, Ohmoto Y, Ono Y, et al. A flow cytometric method for determination of the interferon receptor IFNAR2 subunit in peripheral blood leukocyte subsets. *J Pharmacol Toxicol Methods*. 2004; 50(1):59–66. [PubMed: 15233969]
 21. Shiba M, Nonomura N, Nakai Y, Nakayama M, Takayama H, Inoue H, et al. Type-I interferon receptor expression: its circadian rhythm and downregulation after interferon-alpha administration in peripheral blood cells from renal cancer patients. *Int J Urol*. 2009; 16(4):356–359. [PubMed: 19302507]
 22. Nguyen MH, Garcia RT, Trinh HN, Lam KD, Weiss G, Nguyen HA, et al. Histological disease in Asian-Americans with chronic hepatitis B, high hepatitis B virus DNA, and normal alanine aminotransferase levels. *Am J Gastroenterol*. 2009; 104(9):2206–2213. [PubMed: 19491836]
 23. Mani H, Kleiner DE. Liver biopsy findings in chronic hepatitis B. *Hepatology*. 2009; 49(5 Suppl):S61–S71. [PubMed: 19399798]
 24. Domanski P, Colamonici OR. The type-I interferon receptor, the long and short of it. *Cytokine Growth Factor Rev*. 1996; 7(2):143–151. [PubMed: 8899292]
 25. Lau JY, Sheron N, Morris AG, Bomford AB, Alexander GJ, Williams R. Interferon-alpha receptor expression and regulation in chronic hepatitis B virus infection. *Hepatology*. 1991; 13(2):332–338. [PubMed: 1825307]
 26. Fujiwara D, Hino K, Yamaguchi Y, Kubo Y, Yamashita S, Uchida K, et al. Type I interferon receptor and response to interferon therapy in chronic hepatitis C patients: a prospective study. *J Viral Hepat*. 2004; 11(2):136–140. [PubMed: 14996348]
 27. Yamaguchi Y, Hino K, Fujiwara D, Ren F, Katoh Y, Satoh Y, et al. Expression of type I interferon receptor in liver and peripheral blood mononuclear cells in chronic hepatitis C patients. *Dig Dis Sci*. 2002; 47(7):1611–1617. [PubMed: 12141824]
 28. Taniguchi H, Iwasaki Y, Takahashi A, Shimomura H, Moriya A, Yu PC, et al. Intrahepatic mRNA levels of type I interferon receptor and interferon-stimulated genes in genotype 1b chronic hepatitis C. Association between IFNAR1 mRNA level and sustained response to interferon therapy. *Intervirology*. 2007; 50(1):32–39. [PubMed: 17164555]

Fig. 1.

Expression of IFN- α / β R-1 and IFN- α / β R-2 in peripheral lymphocytes and monocytes increased in CHB patients. **a** IFN- α / β R-1 and IFN- α / β R-2 increased both in peripheral lymphocytes and monocytes in CHB patients. **b** In control group, IFN- α / β R-1 and IFN- α / β R-2 expression in lymphocytes was much higher than in monocytes, while in chronic HBV patients the levels of two kinds of receptor showed no difference between lymphocytes and monocytes. * $p < 0.05$; ** $p < 0.01$; # $p > 0.05$

Fig. 2. mRNA expression of IFNAR-1, IFNAR-2, IL-6, IL-8, TGF- β 1 and TNF- α in PBMCs and liver increased in CHB patients. **a** All cytokines (IL-6, IL-8, TGF- β 1 and TNF- α) and receptors (IFNAR-1, IFNAR-2) mRNA increased significantly in CHB patients, especially in the liver ($*p<0.01$). **b** IFNAR-1 and IFNAR-2 in liver displayed a positive correlation with HBV-DNA in liver tissues

Fig. 3.
Correlations between IFNAR and IL-6, IL-8, TGF-β1 and TNF-α in liver mRNA levels. **a, b**
Correlations between mRNA levels of IFNAR-1 and IL-6, IL-8, TGF-β1, TNF-α in liver

Fig. 4. Expression of IFN- α / β R-1 and IFN- α / β R-2 in liver went up in CHB patients. **a** Liver tissues double stained with IFN- α / β R-1 and IFN- α / β R-2 antibodies were more positive than the normal control. **b** Immunohistochemistry of liver tissues stained with IFN- α / β R-2 antibody exhibited the same result

Fig. 5. Alteration of IFN- α / β R in lymphocytes and monocytes in the 25 patients treated with interferon- α 2b. **a** IFN- α / β R-1 and IFN- α / β R-2 in lymphocytes and monocytes significant increased after interferon- α 2b treatment in the first month, while in the following 2 months, both IFN- α / β R-1 and IFN- α / β R-2 decreased dramatically. **b** In all the 25 patients treated with interferon- α 2b, IFN- α / β R-1 and IFN- α / β R-2 in lymphocytes and monocytes first increased in the first month, but then decrease in the following 2 months (* $p < 0.05$; ** $p < 0.01$)

Fig. 6.

Comparisons between responders ($n=12$) and nonresponders ($n=13$) at 3 months of treatment. **a, b** IFN- α / β R-1 and IFN- α / β R-2 in lymphocytes were almost at the same levels before interferon- α 2b treatment both in responders and nonresponders. Expression of IFN- α / β R-1 and IFN- α / β R-2 in lymphocytes in the two groups demonstrated the same trend of alteration (increased in the first month and then decreased dramatically in the following 2 months). Expression of IFN- α / β R-1 and IFN- α / β R-2 in lymphocytes in responders was much higher than nonresponders. **c, d** IFN- α / β R-1 and IFN- α / β R-2 in monocytes showed

almost the same results, but IFN- α / β R-2 expression in responders was much higher than nonresponders (** $p<0.01$)

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Fig. 7. Alteration of IL-6, IL-8, TGF-β1 and TNF-α in PBMCs during interferon-α2b treatment. **a** The mRNA levels of IL-6, IL-8, TGF-β1 and TNF-α in PBMCs continuously decreased according to interferon-α2b treatment in all the 25 treated patients. **b** Compared the expression of IFN-α/βR in PBMCs in responders (total, 12 of 25) and nonresponders (total, 13 of 25) before treatment, the expression of IFN-α/βR-2 in monocytes in responders was

significantly higher than the nonresponders (43.60 ± 5.69 vs. 29.53 ± 6.67). $*p < 0.05$;
 $**p < 0.01$; $\#p > 0.05$

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Table I

Clinic characteristics of chronic hepatitis B patients and control health group

Group	HBV-DNA 10 ⁵	HBV-DNA<10 ⁵	Control group
Case number (gender)	43 (M31/F12)	41 (M13/F18)	10 (M6/F4)
Age (year)	33±10	41 ±13	26±5
ALT (IU/L)	256.7±186.5	151.4± 167.2	25.8±15.2**
AST (IU/L)	221.3±145.2	121.5± 152.3	28.8±23.2**
ALP (IU/L)	102.5±56.8	121.4±87.8	102.3±76.4***
GGT (IU/L)	102.3± 119.3	123.5± 121.3	93.6±98.7***
TP (G/L)	73.9±9.3	71.3±8.6	72.7±8.7***
ALB (G/L)	38.6±8.9	39.2±9.1	38.8±7.8***
TBIL (umol/L)	18.8±20.2	17.6± 17.0	15.2± 18.3***
DBIL (umol/L)	10.5±12.3	11.5± 10.4	16.2±9.4*
Cho (mmol/L)	3.24±1.05	2.98± 1.32	3.12±2.05***
TG (mmol/L)	0.81 ±0.21	0.87±0.32	0.78±0.32***
GLU (mmol/L)	5.04±0.81	4.97±0.78	4.56±0.76***
BUN (mmol/L)	4.87±1.01	4.88± 1.03	4.84±0.89***
Ccr (mmol/L)	72.4±16.9	74.3± 15.7	68.2±17.3***
HBV-DNA in serum (copies/mL)	(12.1±16.5)×10 ⁵	(13.2±13.1)×10 ³	<10 ³ **
HBV-DNA in liver (copies/mL)	(10.1±6.5)×10 ⁵	(11.4±13.5)×10 ⁵	NC
	n=13	n=12	
Grade of inflammatory (number of patients)			
G0	0	0	
G1	3	4	
G2	5	5	
G3	4	3	
G4	1	0	
Stage of fibrosis (number of patients)			
S0	4	4	
S1	5	4	
S2	3	4	
S3	1	0	
S4	0	0	

All the data are presented as means±standard error

ALT alanine aminotransferase, AST aspartate transaminase, ALP alkaline phosphatase, GGT r-glutamyltransferase, TP total protein, ALB albumin, TBIL total bilirubin, DBIL direct bilirubin, Cho total cholesterol, TG triglycerides, GLU glucose, BUN blood urea nitrogen, Ccr endogenous creatinine clearance

*
p<0.05;

**
p<0.01;

 $p>0.05$

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Table II

RT-PCR primers

Gene	RefSeq ID	Forward	Reverse
h IFNAR-1	NM_000629	5'-TGACCAGAAATGAACTGTGTCA-3'	5'-TTTAAATAGTTAAGAGCTTGCCCG-3'
h IFNAR-2	NM_000874	5'-GAAGGTGGTTAAGAACTGTGC-3'	5'-CCCGCTGAATCCTTCTAGGACGG-3'
h IL-6	NM_000600	5'-GTCAGGGGTGGTTATTGCAT-3'	5'-AGTGAGGAACAAGCCAGAGC-3'
h IL-8	NM_000584	5'-AAATTTGGGGTGGAAGGTT-3'	5'-TCCTGATTCTGCAGCTCTGT-3'
h TGF- β 1	NM_000660	5'-CTTCAGCCGAGGTCCTT-3'	5'-CCCTGGACACCAACTATTGC-3'
h TNF- α	NM_000594	5'-AGATGATCTGACTGCCTGGG-3'	5'-CTGCTGCACTTTGGAGTGAT-3'
h GAPDH	NM_002046	5'-AATGAAGGGGTCATTGATGG-3'	5'-AAGGTGAAGGTCGGAGTCAA-3'

Table IIIExpression of IFN- α / β R in control group and CHB patients (By FACS)

	Control group	CHB patients	Increase
IFN- α / β R-1 in monocytes (MFI)	16.11 $\pm$ 1.96	40.44 $\pm$ 1.49*	25.33 $\pm$ 1.65
IFN- α / β R-2 in monocytes (MFI)	15.35 $\pm$ 3.98	45.67 $\pm$ 3.59*	27.32 $\pm$ 2.56
IFN- α / β R-1 in lymphocytes (MFI)	26.65 $\pm$ 2.15	39.27 $\pm$ 2.10*	11.62 $\pm$ 2.34
IFN- α / β R-2 in lymphocytes (MFI)	25.25 $\pm$ 2.65	38.40 $\pm$ 2.67*	14.15 $\pm$ 3.94

All the data are presented as means $\pm$ standard error*MFI* mean fluorescence intensity*
 $p < 0.01$

Table IVClinic parameters of interferon- α 2b-treated patients

Group	Before treatment	1 month	2 months	3 months
Case number (gender)	25 (M16/F9)	25 (M16/F9)	25 (M16/F9)	25(M16/F9)
Age (year)	32 $\pm$ 12	32 $\pm$ 12	32 $\pm$ 12	32 $\pm$ 12
ALT (IU/L)	267.7 $\pm$ 176.5	165.8 $\pm$ 147.6	151.4 $\pm$ 137.2	87.2 $\pm$ 52.3**
AST (IU/L)	196.7 $\pm$ 185.4	213.3 $\pm$ 167.2	132.5 $\pm$ 107.3	97.3 $\pm$ 67.6**
AKP (IU/L)	96.5 $\pm$ 48.6	101.3 $\pm$ 87.6	123.4 $\pm$ 78.9	101.4 $\pm$ 75.2***
GGT (IU/L)	94.3 $\pm$ 109.2	100.4 $\pm$ 79.2	103.5 $\pm$ 97.3	110.8 $\pm$ 78.4***
TP (G/L)	74.1 $\pm$ 8.9	73.1 $\pm$ 7.5	73.2 $\pm$ 6.9	71.4 $\pm$ 8.9***
ALB (G/L)	37.6 $\pm$ 7.9	37.2 $\pm$ 6.7	38.2 $\pm$ 8.4	37.8 $\pm$ 7.6***
TBIL (umol/L)	35.8 $\pm$ 10.2	26.9 $\pm$ 9.1	25.6 $\pm$ 10.1	24.8 $\pm$ 9.4***
DBIL (umol/L)	15.5 $\pm$ 12.3	16.4 $\pm$ 10.4	15.5 $\pm$ 8.4	13.4 $\pm$ 8.1***
HBsAg	3,387.6 $\pm$ 1,434.3	3,476.8 $\pm$ 1,132.4	2,987.6 $\pm$ 1,034.5	3,076.5 $\pm$ 1,213.4***
HBeAg	1,209.2 $\pm$ 978.6	1,023.6 $\pm$ 765.4	874.5 $\pm$ 654.7	543.6 $\pm$ 467.9**
HBV-DNA in serum (copies/mL)	(12.1 $\pm$ 16.5) $\times$ 10 ⁵	(11.2 $\pm$ 13.6) $\times$ 10 ⁵	(14.2 $\pm$ 6.1) $\times$ 10 ⁴	(10.2 $\pm$ 5.8) $\times$ 10 ⁴ *
Biochemical respond (BR)		1/25	8/25	12/25
Virologic responds (VR)		0/25	0/25	1/25
Early virologic responds (EVR)		2/25	4/25	9/25

All the data are presented as means $\pm$ standard error*
 $p < 0.05$;**
 $p < 0.01$;***
 $p > 0.05$