Turk J Gastroenterol 2010; 21 (4): 401-410

Determination of intracellular Th1/Th2 type cytokines in lymphocytes of chronic hepatitis B patients treated with interferon-alpha

Interferon alfa tedavisi alan kronik hepatit B hastalarında intrasellüler Th1/Th2 sitokin düzeyleri

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Background/aims: *Host-related immune factors in childhood chronic hepatitis B and change in the initial profile with interferon (IFN)-α treatment need to be clarified.* ***Methods:*** *Sixteen patients were included in the study, and 10 million units of IFN-α treatment 3 times per week for 6 months was initiated. Pre- and post-treatment percentages of interleukin (IL)-2 and IFN-γ in CD4+ T cells were assessed to determine intracellular T helper cell 1 (Th1) type cytokine expression. Similarly, percentages of intracellular IL-2 and IFN-γ were detected to verify cytotoxic T cell 1 (Tc1) type cytokine expression in CD8+ T cells. Percentages of Th2 and Tc2 type cytokine expression (IL-4 and IL-13) were determined in CD4+ and CD8+ T cells, respectively.* ***Resuts:*** *Six (50%) of these were evaluated as having no response and the other half with partial/complete response. All patients had higher percentages of Th2 cells with respect to healthy controls pre-treatment. Tc percentages, both Tc1 and Tc2, were significantly different between these groups, being higher in the patient group. When values of the nonresponder group were compared with healthy controls, IL-4 expression was higher and the percentages of Th1 type cells were significantly low. IL-13 expression in Th and Tc cells decreased after 6 months of treatment in the unresponsive group. The decrease we observed in Th1 percentages with treatment, in the responsive group, may be due to Th1 deposition shifting from the periphery to liver tissue, as reported before. Intracellular cytokine profiles of treatment responders and normal controls were not different.* ***Results:*** *This is the first study in children comparing baseline and post-treatment intracellular cytokine profiles with values in healthy controls.*

Key words: Chronic hepatitis B, interferon alpha, intracellular cytokines, cytotoxic T cell, helper T cell

Amaç: *Çocukluk çağı kronik hepatit B hastalarında konakla ilişkili immün faktörler ve IFN-α tedavisi ile gözlenen sitokin profil değişikliğinin tanımlanması gereklidir.* ***Yöntem:*** *Onaltı hasta çalışmaya alınmış ve 6 ay süre ile haftada üç gün 10 milyon ünite IFN-α tedavisi başlanmıştır. Tedavi öncesi ve sonrası T yardımcı 1(Th1) hücre sitokin ekspresyonunu değerlendirmek için CD4+T hücrelerde IL-2 ve IFN-γ yüzdeleri ölçülmüştür. T sitotoksik hücre(Tc1) sitokin ekspresyonu için CD8+T hücrelerde IL-2 and IFN-γ, Th2 ve Tc2 sitokin ekspresyon oranları için IL-4 ve IL-13 sırasıyla CD4+ ve CD8+ T hücrelerde bakılmıştır.* ***Bulgular:*** *Olguların yarısında hiç yanıt alınmazken, yarısında parsiyel/tam yanıt alınmıştır. Olguların tümünde tedavi öncesi Th2 ve Tc hücre oranı sağlıklı kontrollere göre yüksek saptanmıştır. Altı aylık tedavi sonrası tedaviye yanıtsız grup ile sağlıklı kontroller karşılaştırıldığında, IL-4 ekspresyonu daha yüksek ve Th1 hücre oranları daha düşük saptanmıştır. Th ve Tc hücrelerde IL-13 ekspresyonu tedaviye yanıtsız grupta, tedavi sonrası azaldığı görülmüştür. Tedaviye yanıtı olan grupta gözlenen Th1 hücre oranındaki düşme periferden karaciğere doğru Th1 depozisyonu ile ilişkilendirilebilir. Tedaviye yanıtlı grup ve normal kontroller arasında intrasellüler sitokin profilleri açısından fark saptanmamıştır.* ***Sonuç:*** *Bu çalışma, çocukluk çağı kronik hepatit B hastalarında tedavi öncesi ve tedavi sonrası intrasellüler sitokin düzeylerini karşılaştıran ilk çalışmadır.*

Anahtar kelimeler: Kronik hepatit B, interferon alfa, intrasellüler sitokinler, sitotoksik T hücre, yardımcı T hücre

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Manuscript received: 20.01.2010 **Accepted:** 01.12.2010

doi: 10.4318/tjg.2010.0127

INTRODUCTION

Hepatitis B virus (HBV) produces significant worldwide morbidity causing acute, fulminant and chronic hepatic disease (1, 2). More than a billion of the world's population has evidence of HBV exposure, and chronic HBV infection is estimated in 300-400 million cases (3). Once the virus is acquired, down-regulation of HBV transcription/translation and viral clearance are shown to occur by the noncytolytic mechanism of primed cytotoxic T (Tc) cells (4). Age, ethnicity, and geographic and genetic features are reported determinants that affect the nature of the disease. Depending on the balance between viral factors and immune response of the host, HBV is cleared by strong multispecific T cell-mediated response, whereas specific unresponsiveness of Tc cells results in chronic infection (5). It is well documented that T helper 2 (Th2) type cells are dominant in chronic disease, and the T helper 1 (Th1) phenotype is assumed to be responsible for the cure of chronic disease; however, there is limited data concerning the functional behavior of these cell subtypes during the chronic as well as the treatment stages of the disease (4, 5).

Available treatment modalities of chronic HBV infection have focused on ameliorating the host response whether by interfering with viral replication or by modulating the host's immune response. Therapy with conventional interferon alpha (IFN-α) has produced encouraging results, with a HBeAg loss rate of 20-50% (5,6). Fischler and coworkers (7) documented that HBcAg-specific T cell responses increased during IFN-α treatment, especially in those patients who lost HBeAg, and related studies are reported concerning the influence of IFN on progression of T cell functions.

The objective of this study was to analyze (i) the type of T cell dominance, (ii) intracellular cytokine response in the chronic and immune tolerant phase of HBV infection, and (iii) changes in T cell subtypes and cytokine release before and after IFN-α treatment.

MATERIALS AND METHODS

This study was performed in Ege University Faculty of Medicine, Department of Pediatrics and Pediatric Immunology and Pediatric Gastroenterology units with a one-year follow-up. The patients of the study were recently diagnosed and had not received any prior treatment against chronic hepatitis B. Sixteen patients (Group I) with the following criteria were included and longitudinally followed during the study: Patients were HBsAg / HBeAg positive and anti-HBe negative for at least six months. HBV DNA was positive (hybridization method). Serum alanine aminotransferase (ALT) level was at least 1.5 times the upper limit of normal. Patients with coexisting hepatic infection other than HBV or chronic liver disease were excluded from the study. Informed parental consent was obtained from each patient. Patients received 10 million units IFN-α (Intron 2a, Schering Plough, Sweden) 3 times per week for 6 months. All of the patients were evaluated at the end of the treatment as having no response (Group IIA) or as partial or complete response (Group IIB). "Complete response criteria" were: negative HBV DNA, negative HBeAg, normal serum ALT level, positive anti-HBe. "Partial response criteria" were: negative HBV DNA, normal serum ALT level, negative anti-HBe and positive HBeAg. Presence of positive HBV DNA was evaluated as "no response" to treatment. Twelve patients out of Group I (n=16) were able to complete the study. Six (50%) of these were evaluated as having no response (Group IIA) and the other half as having partial/complete response (Group IIB).

Since genotype is an important factor influencing the outcome of the therapy, genetic analysis for HBV was also performed.

In this study, pre- and post-treatment data of the patients were compared. The control group (Group III) included 12 healthy children without any humoral or cellular immune deficiency or liver disease. The annual infection rate of these children was $\leq$2, and none had experienced any infectious episode within the last 3 months. Pre- and post-treatment values of the patients were also compared with healthy controls.

Liver function tests, prothrombin time and white blood cell counts, and serological markers (HBs Ag, HBe Ag, anti-HBe, anti-HBc IgM, anti-HBc IgG, anti-HBs, HBV DNA, anti-HCV) for all of the patients were documented before and after the treatment. Liver biopsy was performed before the treatment and scored according to HAI (Hepatic Activity Index) (data not shown) (8).

Percentages of surface anti-human CD3, CD8, CD4, CD45, CD14 (Becton-Dickinson, Belgium) (data not shown) in addition to intracellular anti-human interleukin (IL)-2, anti-human IL-13, anti-

human IL-4, anti-human IFN-γ were analyzed with monoclonal antibodies labeled with fluorescein isothiocyanate (FITC), phycoerythrin (PE) or peridininchlorophyll-protein (PerCp) (BDIS Fastimmune Cytokine System, Becton Dickinson, Belgium). Lymphocytes were activated by phorbol 12-myristate 13 acetate (PMA) (Sigma-Aldrich, USA) + ionomycine (I) (Sigma-Aldrich, USA) permeabilizing solution (Becton Dickinson, Belgium), and brefeldin-A (BFA) and CD69 monoclonal antibody were used in accordance with manufacturer's recommended protocol for the detection of intracellular cytokines in activated lymphocytes. Percentages of the IL-2 and IFN-γ in CD4+ T cells were assessed to determine intracellular Th1 type cytokine expression. Similarly, percentages of intracellular IL-2 and IFN-γ were detected to verify Tc1 type cytokine expression in CD8 +T cells. Percentage of Th2 and Tc2 type cytokine expression (IL-4 and IL-13) were determined in CD4+ and CD8+ T cells, respectively. Samples were analyzed by flow cytometer (FACSCAN, Becton-Dickinson, Belgium) using Cell-Quest software (Becton Dickinson, Belgium).

At the end of 24 weeks of treatment with IFN-α, all patients were reevaluated with regard to serological, biochemical and immunological parameters.

Statistical analysis of data was performed using Mann-Whitney U, Kruskal-Wallis and chi-square tests in an SPSS 12 environment. P values less than 0.05 were accepted as statistically significant.

RESULTS

The mean ages of the patients in the pre-treatment group (Group I, n=16) and in healthy controls (Group III, n=12) were 12.3±4.4 and 7.2±1.2 years, respectively.

Fifty percent of the patients responded completely or partially (Group IIB, n=6) to treatment, while 50% were unresponsive (Group IIA, n=6). Nine of the 12 patients in Group II (75%) and all patients (100%) in the unresponsive group (Group IIA) had genotype D.

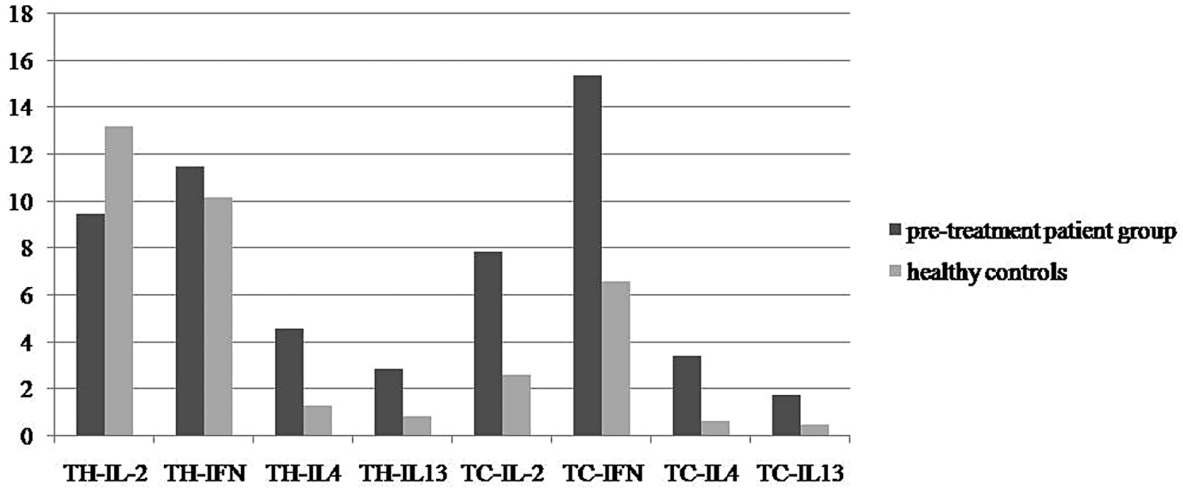
Percentages of Th1 (IL-2, -IFN-γ), Th2 (IL-4, IL-13), Tc1 (IL-2, IFN-γ), and Tc2 (IL-4, IL-13) phenotypes between the pre-treatment patient group (Group I) and healthy controls (Group III) were compared (Figure 1). It was noted that the Th1 cytokine profile did not differ between these two groups. Th2 type cytokine expression was accentuated in chronic hepatitis patients before treatment with respect to healthy controls, but this was not statistically significant (For IL-4, 4.57±4.39% versus 1.27±1.24%; for IL-13, 2.83±2.81% versus 0.83±0.81% in Group I and Group III, respectively; p=0.06 and p=0.07). Tc cell percentages, both Tc1 and Tc2, were significantly different between these groups, being higher in the patient group. P values for Tc1 were 0.001 and 0.005 for IL-2 and IFN-γ, respectively; p values for Tc2 were 0.022 and 0.002 for IL-4 and IL-13, respectively (Figure 1).

Percentage of IL-4-bearing Th2 cells was higher in the unresponsive group when compared to the healthy controls (Figure 2). In contrast, the unresponsive group had lower Th1 type cytokines, which was statistically significant (for IL-2, p=0.000). When the two groups were compared according to Tc lymphocyte percentages, Tc1 phenotypes were significantly lower in the unresponsive group (p=0.001 and p=0.013 for IL-2 and IFN-γ percentages, respectively).

No statistically significant difference was observed in terms of intracellular cytokine profiles of treatment responders and normal controls (Figure 3).

Pre- and post-treatment intracellular cytokine profiles of Group IIA patients were compared (Figure 4). This group was unresponsive to IFN-α treatment and no difference in IL-4 levels was observed (p=0.52). On the other hand, percentage of IL-13-bearing Th cells was higher in the pre-treatment period, which was statistically significant (p=0.016). Tc1 type cytokines were significantly higher at the pre-treatment stage (p=0.004 and p=0.006), whereas there was no significant difference in Tc2 profiles (p>0.05).

When pre- and post-treatment intracellular cytokine levels of the IFN-α responsive group (Group IIB) (n=6) were compared, percentage of IFN-γ-bearing CD4 cells was significantly higher in the pre-treatment period (p=0.020) (Figure 5). There was no statistically significant difference in Th2 type cytokine expression. There was a statistically significant difference between the two groups with respect to Tc1 type cytokine expression (for IL-2, p=0.045 and for IFN-γ, p=0.020), leading to lower percentages after treatment. There was no significant difference in the percentages of Tc2 cells.



	Th1		Th2		Tc1		Tc2	
	IL-2	IFN-γ	IL-4	IL-13	IL-2	IFN-γ	IL-4	IL-13
Group I	9,44 ± 6,36	11,47±4,95	4,57±4,39	2,83±2,81	7,83±4,51	15,37±9,29	3,43±4,06	1,72±1,78
Group III	13,19±8,67	10,17±7,24	1,27±1,24	0,83±0,81	2,62±1,48	6,58±4,37	0,65±0,77	0,46±0,86
P	0,35	0,27	0,06	0,07	0,001	0,005	0,022	0,002

Figure 1. Comparison of percentages of Th1 (IL-2, -IFN-γ), Th2 (IL-4, IL-13), Tc1 (IL-2, IFN-γ), and Tc2 (IL-4, IL-13) phenotypes between pre-treatment patient group (Group I) and healthy controls (Group III).

DISCUSSION

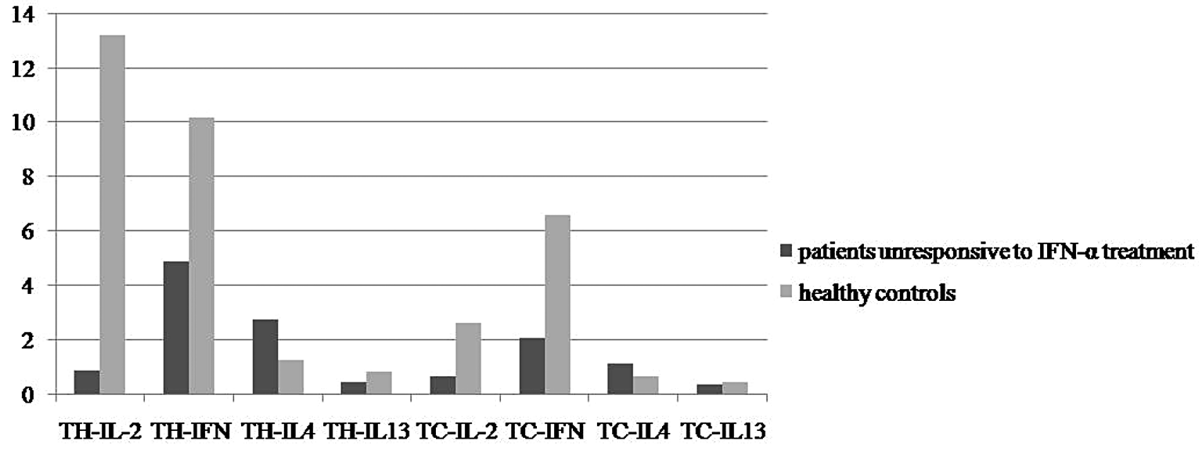
IFN-α is a very important treatment option for chronic hepatitis B. However, duration for optimum treatment is not yet certain. In a meta-analysis in 1993, at least three months of treatment with IFN-α was shown to be effective (12), and a duration of six months was shown to be more effective. HBeAg negativity was 33%, HBV DNA negativity was shown to be 37% and HBsAg negativity was 7.8% when compared to 12%, 12% and 1.8% values, respectively, in the placebo group of the same study. In our study, after 24 weeks of IFN-α treatment, 50% of our patients were negative for HBeAg and HBV DNA.

The success of treatment also varies with ethnicity. Generally, Asian patients are positive for HBeAg and HBV DNA with normal serum ALT levels (13). These patients are reported to have acquired the virus perinatally. In that study, the probability of successful treatment was only 10% among Asian patients. In our study, all patients were Caucasians from the Mediterranean region and were positive for HBeAg and HBV DNA.

In different studies, different rates of eradication of HBV with IFN-α treatment have been reported. The largest number of patients and the longest duration belongs to the study of van Zonneveld et al. (14). The author had followed 165 patients with chronic hepatitis B from 1978 until 2002. Median time for follow-up was 8.8 years. At the end of the study, 33% of 54 patients were responsive to treatment. In our study, the response rate was 50%.

Hepatitis B virus has 8 genotypes from A to H. Response rates also vary with genotypes. Genotype D is the most common type in India and Middle Asia (15). Erhardt et al. (16) followed 64 German chronic hepatitis B patients; seroconversion rate for genotype A was 37%, whereas it was 6% with genotype D. In our study, nine patients (75%) were genotype D and six of these (Group IIB) were responsive to therapy.

In studies revealing the pathogenesis, acute hepatitis B infection seems to be controlled by cellular immunity, while humoral immunity is dominant in chronic illness. Im et al. (17) found the CD8+ T cell ratios to be lower in chronic active hepatitis B



	Th1		Th2		Tc1		Tc2	
	IL-2	IFN-γ	IL-4	IL-13	IL-2	IFN-γ	IL-4	IL-13
Group IIA	0,88±0,6	4,90±3,53	2,77±1,88	0,46±0,58	0,67±0,37	2,05±2,00	1,12±0,65	0,35±0,25
Group III	13,19±8,67	10,17±7,24	1,27±1,24	0,83±0,81	2,62±1,48	6,58±4,37	0,65±0,77	0,46±0,86
p	0,000	0,053	0,151	0,494	0,001	0,013	0,151	0,125

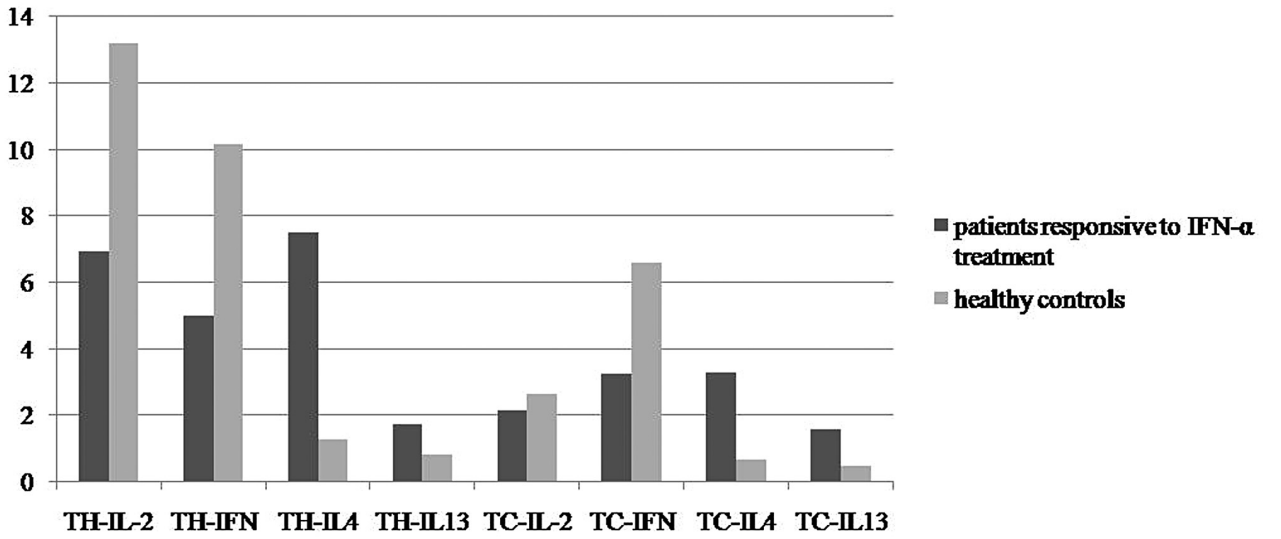
Figure 2. Comparison of percentages of Th1 (IL-2, -IFN-γ), Th2 (IL-4, IL-13), Tc1 (IL-2, IFN-γ), and Tc2 (IL-4, IL-13) phenotypes between patients unresponsive to IFN-α treatment (Group IIA) and healthy controls (Group III).

patients than healthy controls. The responders had lower CD4+ T cells when compared to the healthy control values. Pham et al. (18) (1994) found out that CD4/CD8 ratios in liver lymphocytes of HBV DNA positive non-cirrhotic patients were high.

Kakumu et al. (19) (1990) analyzed liver biopsy samples of chronic hepatitis B patients and found that the percentages of hepatocyte CD8+ T cells and HLA-DR positive cells were higher than those of peripheral blood while hepatocyte CD4+ T cells were lower when compared to peripheral blood. As a result, it was suggested that CD8+ T cells had increased in order to destroy the hepatic cells for the establishment of viral clearance. Unal et al. (20) showed that CD3+ T cells were higher in chronic hepatitis B patients more obviously than CD8+ T cells. In a study by Genel et al. (21), combined therapy of IFN-α and lamivudine was given to chronic hepatitis B patients, and the only significant factor predicting immune response was found to be the pretreatment CD4+/CD8+ T cell ratios. The responsive patients had low pre-treatment ratios of CD4+/CD8+. This was because of high CD8+ T cells, revealing the importance of CD8+ T cell amount for the clearance of HBV (21). In our study, CD4+ T cell percentages were significantly lower than in controls before treatment with relatively increased CD8+ T cells (data not shown). In addition, the CD8+ T cell percentages in non-responders were found to be very low significantly. These results were concordant with the findings of Genel et al.

Gilles et al. (22) showed that IFN-γ and IL-2 produced by Th1 cells stop the viral replication. Maruyama et al. (23) reported that chronic hepatitis B patients had dominance of Th2 cells at the pretreatment stage. Similar with these findings, pretreatment chronic hepatitis B patients also had higher percentages of Th2 cells with respect to healthy controls in our study. We believe this difference will gain statistical significance with a larger group of patients.

In a study by Xing et al. (24), the balance of Th1/Th2 was emphasized, and treatment responsiveness was evaluated as “no, partial and comple-



	Th1		Th2		Tc1		Tc2	
	IL-2	IFN-γ	IL-4	IL-13	IL-2	IFN-γ	IL-4	IL-13
Group IIB	6,94±6,86	4,99±2,60	7,50±7,58	1,74±2,48	2,14±2,48	3,24±2,69	3,30±3,08	1,56±1,78
Group III	13,19±8,67	10,17±7,24	1,27±1,24	0,83±0,81	2,62±1,48	6,58±4,37	0,65±0,77	0,46±0,86
P	0,151	0,083	0,125	0,553	0,335	0,125	0,102	0,102

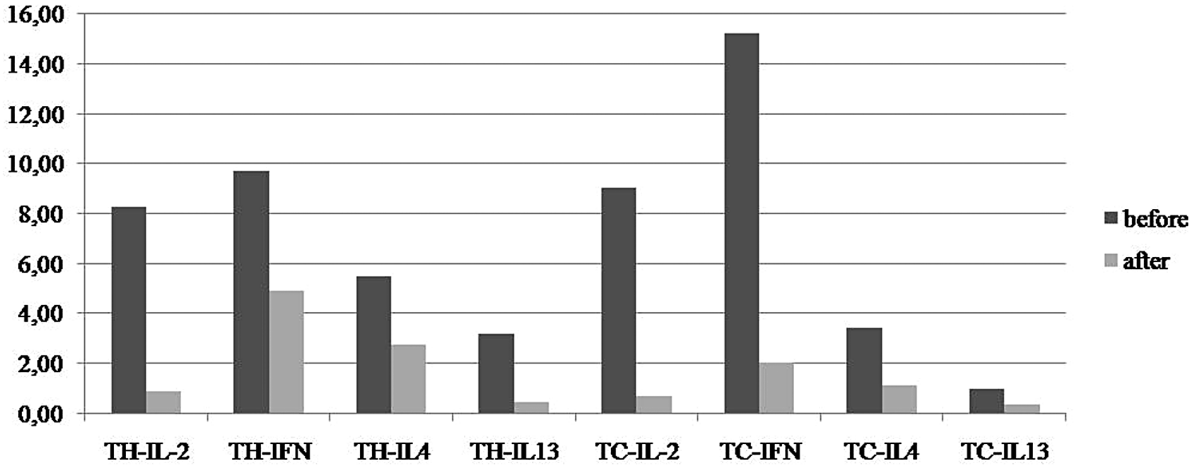
Figure 3. Comparison of percentages of Th1 (IL-2, IFN-γ), Th2 (IL-4, IL-13), Tc1 (IL-2, IFN-γ), and Tc2 (IL-4, IL-13) phenotypes between patients responsive to IFN-α treatment (Group IIB) and healthy controls (Group III).

te". Our study differs from their study, as they stimulated peripheral blood mononuclear cells with mitogens and determined cytokine levels in cell culture supernatants without discriminating T cells as Th or Tc. In addition to focusing on T cells, we investigated the intracellular cytokine profiles of T cells in response to treatment for chronic HBV infection in children. IFN-γ and IL-2 represented Th1 type cytokines, while IL-4 and IL-13 represented Th2 type cytokines in our study. This is the first study to compare baseline intracellular cytokine profiles with those of healthy controls. Differences in Th1/Th2 as well as Tc1/Tc2 balance in chronic hepatitis B patients with respect to IFN-α treatment were also studied.

There were nine responsive patients, eight partially responsive patients and five unresponsive patients out of 22 patients in the study of Xing et al. (24). All of the patients were evaluated for Th1/Th2 balance with their pre- and post-treatment cytokine profiles. Initially, IFN-γ levels were lower and IL-4 levels were higher in chronic hepatitis B patients when compared to healthy controls. These patients received IFN-α therapy for 24 weeks. At the 4th, 12th and 24th week evaluations, a gradual increase in IFN-γ expression percentages and a decrease in IL-4 were observed among the responsive patients. At the end of the IFN therapy, IFN-γ expression was significantly higher in responsive patients than their pre-treatment values. In contrast to this finding, IFN-γ levels did not change significantly in unresponsive patients in comparison to initial values (24).

In another study, by Jiang et al. (25), CD4+ T cells of chronic hepatitis B patients were found to be Th0 type, and the Th1 type cells were increased in accordance with the degree of hepatic inflammation. In addition, Th2 cells were found to be at higher levels in chronic hepatitis B patients when compared to healthy controls. In the acute phase of disease, IFN-γ producing Th1 type cytokines were found to be dominant (25).

Lee et al. (26) studied the cytokine profiles of peripheral blood mononuclear cells by reverse trans-



	Th1		Th2		Tc1		Tc2	
	IL-2	IFN-γ	IL-4	IL-13	IL-2	IFN-γ	IL-4	IL-13
Group I* (n=6)	8,25±4,72	9,72±3,50	5,47±5,31	3,19±3,43	9,05±2,21	15,23±10,02	3,42±3,94	0,99±1,02
Group IIA (n=6)	0,88±0,60	4,90±3,53	2,77±1,88	0,46±0,58	0,67±0,37	2,05±2,00	1,12±0,65	0,35±0,25
p	0,004	0,025	0,522	0,016	0,004	0,006	0,522	0,055

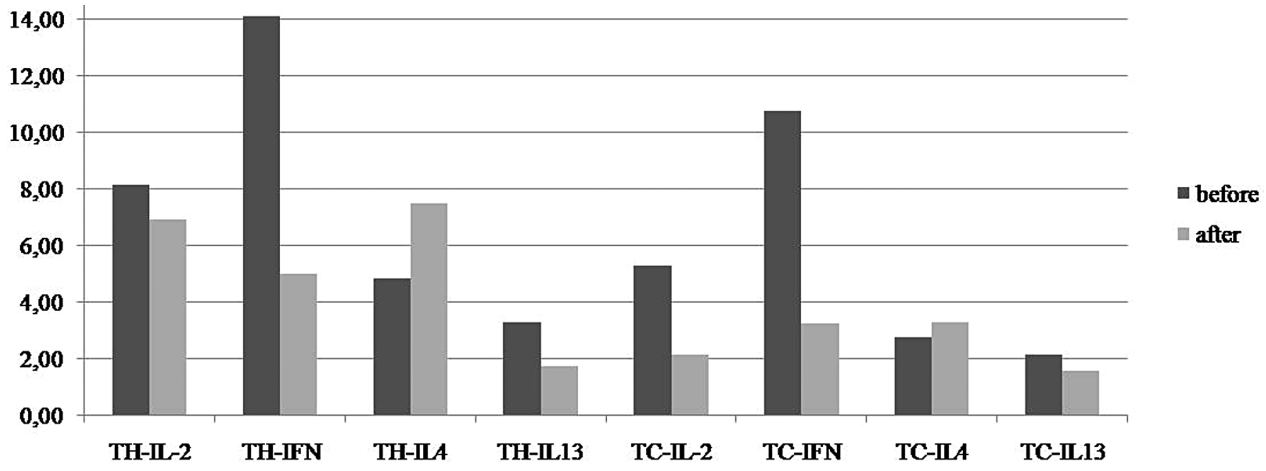
* Pretreatment values of non-responders

Figure 4. Comparison of percentages of Th1 (IL-2, IFN-γ), Th2 (IL-4, IL-3), Tc1 (IL-2, IFN-γ), and Tc2 (IL-4, IL-3) phenotypes between pre-treatment and post-treatment values of patients unresponsive to IFN-α treatment.

cription-polymerase chain reaction (RT-PCR) method. After stimulation with HBsAg, there was an increase in IFN-γ, IL-2, IL-4, and IL-10 as 41%, 8%, 41%, and 50%, respectively. In this study, levels of ALT and aspartate aminotransferase (AST) were correlated with percentage of IFN-γ expression in T cells. As a result, we can assume that Th1 type cytokines were related with hepatocyte damage, whereas Th2 type cytokines did not have any counter-effect. In our study, when pre-treatment Th2 type cytokine (IL-4, IL-13) profiles were compared with values of healthy controls, it was seen that they were higher (for IL-4 in Group I=4.57%, Group III=1.27%; for IL-13 in Group I=2.83 %, Group III= 0.83%) in the chronic hepatitis B group. When values of the no responder group were compared with healthy controls, IL-4 expression was higher and the percentages of Th1 type cells were significantly low. This may suggest that Th1 deficiency may be responsible for the following unresponsiveness. When healthy controls were compared with responders with respect to Th1 and Th2 profiles, no significant difference was seen. Thus, Th1/Th2 balance in responders was well established as in healthy controls. These profiles were also compared in pre- and post-treatment period of responders. After six months of therapy, Th2-Tc2 cytokine expression differed in responders with a tendency of IL-13 to decrease and IL-4 to increase in the post-treatment phase compared with pre-treatment values, but neither was statistically significant.

Although the concept of our study differs from that of the previous ones, the obtained results almost support similar hypotheses as far as pathogenesis and response to treatment are concerned. This is more striking in our findings reflecting low percentages of Th1 and high percentages of Th2 in the non-responders.

As a cytokine, IFN-α supports cellular immune response by promoting a strong Tc cell response. However, as we see from studies, there are some other unknown factors contributing to unresponsiveness, such as the virus genotype and immunological awareness of the host. Recently, Tc cells ha-



	Th1		Th2		Tc1		Tc2	
	IL-2	IFN-γ	IL-4	IL-13	IL-2	IFN-γ	IL-4	IL-13
Group I* (n=6)	8,14±4,98	14,12±6,26	4,83±4,06	3,28±2,53	5,30±3,17	10,74±5,66	2,76±2,72	2,15±2,11
Group IIB (n=6)	6,94±6,86	4,99±2,60	7,50±7,58	1,74±2,48	2,14±2,48	3,24±2,69	3,30±3,08	1,56±1,78
p	0,575	0,020	0,810	0,259	0,045	0,020	0,936	0,259

* Pretreatment values of responders

Figure 5. Comparison of percentages of Th1 (IL-2, IFN-γ), Th2 (IL-4, IL-3), Tc1 (IL-2, IFN-γ), and Tc2 (IL-4, IL-3) phenotypes between pre-treatment and post-treatment values of patients responsive to IFN-α treatment.

ve been further divided in to two groups as Tc1 and Tc2.

The main cytokines of Tc1 are IL-2, IFN–γ and TNF-α and -β. The main cytokines of Tc2 are IL-4 and IL-13. There are few clinical studies about Tc1 and Tc2 subsets, and thus far, no study concerning Tc1 and Tc2 balance in chronic hepatitis B has been performed in adults or children. Our study is unique, as Tc1/Tc2 in addition to Th1/Th2 profiles were both investigated for the first time in childhood chronic hepatitis B patients.

Huang et al.'s (27) study on a mouse model showed that when HBsAg expressing recombinant adenovirus was injected in to bone marrow dendritic cells, this adenovirus infection caused neither a Th1/Th2 response nor IFN-γ production. However, the infusion of HBsAg + adenovirus caused an induction of Tc1 cells. Comparison of pre-treatment Tc1 (IL-2, IFN-γ) and Tc2 (IL-4, IL-13) cytokines of patients (Group I) and healthy controls (Group II-I) in our study yielded significantly increased Tc1 and Tc2 percentages in the pre-treatment group. The post-treatment unresponsive group had decreased levels of Tc1 type cytokines with statistical significance. Genel et al. (21) suggested that unresponsiveness was correlated with low Tc percentages. However, in our study, no statistical difference between the cytokine profiles of responders and the healthy group was observed.

The major factor in chronic hepatitis B is the disturbed Th1/Th2 balance in favor of Th2 cells. Activity of HBeAg-specific Th1 cells avoids chronicity. Thus, disease resolution occurs when HBV antigen-specific Th1 cells become dominant and vice versa. IFN-α treatment decreases Th2 cells and increases the levels of Th1 type cytokines. We observed increased Th2 and Tc (both, Tc1 and Tc2) expression before treatment. It may be speculated that the decrease we have observed in Th1 percentages with treatment, in the responsive group, may be due to Th1 deposition shifting from the periphery to liver tissue, as also suggested by previous studies. The fact that IL-13 expression in Th

and Tc cells decreased after treatment in the unresponsive group raises the question of whether this cytokine may have a relevant impact on treatment failure. The most striking finding in our study is that cytokine profiles in treatment-responsive patients closely resembled those of healthy controls.

A predominant shift of the immune response towards a Th1 profile has been suggested to be crucial for the eradication of HBV, while a prevalent Th2 response seems to favor viral persistence. With the speculation that the use of an immunomodulatory agent capable of reinforcing the Th1 response could be advantageous to control HBV infection, Loggi et al. (28) investigated effects of thymosin alpha-1 and IFN-α on cytokine production by peripheral blood mononuclear cells (PBMC) in 12 patients with eAg-negative chronic hepatitis B. Thymosin alpha-1 alone and IFN-α alone were found to reduce and increase, respectively, only the synthesis of IL-10 by PBMCs of patients with HBeAg-negative chronic HBV infection. The association of thymosin alpha-1 plus IFN-α induced a significant increase in IL-2 and blocked the IL-10 increase induced by IFN-α.

As it is very expensive and difficult to study intracellular cytokine profiles, we only have intracellular cytokine levels after six months of therapy. According to biochemical findings and the clinical well-being of the patients, we can assume these patients have sustained response for two years. With our findings, we can conclude that treatment efficacy of IFN-α in childhood chronic hepatitis B infection is promising. Still, more extensive studies with larger number of patients are required to confirm our results.

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