

# The role of serum Th1 and Th2 cytokines in patients with chronic hepatitis B and hepatitis C virus infection

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**Background.** The imbalance of pro-inflammatory Th1 and anti-inflammatory Th2 cytokine production may play an important role in the immunopathogenesis of hepatitis B and C virus infection.

The aim of this study was to analyze serum levels of Th1 and Th2 cytokines in patients with chronic hepatitis virus infection of different etiology.

**Materials and methods.** The serum levels of Th1 cytokines: interferon-gamma (IFN- $\gamma$ ), interleukin-2 (IL-2), soluble IL-2 receptors (sIL-2R), and a Th2 cytokine, interleukin-10 (IL-10) were measured by EIA assay in 20 healthy controls and in 62 patients (47 males and 15 females, mean age 26.0 years) with chronic hepatitis virus infection according to the etiology: 15 patients with hepatitis B, 21 patients with hepatitis C and 26 patients with mixed hepatitis (B + C). The levels of the cytokines were compared with the traditional biochemical (alanine transaminase, ALT) and viral (HBV DNA or HCV RNA) indicators of infection.

**Results.** Apart from etiology, the serum levels of IFN- $\gamma$ , sIL-2R and IL-10 cytokines were higher in patients with chronic hepatitis B and/or C than those in the healthy controls ( $P < 0.05$ ). The sIL-2R and IL-10 levels were significantly correlated with ALT activity, although there was no correlation between the mentioned cytokines and the presence of HBV DNA or HCV RNA. However, the levels of IL-2 were significantly lower only in cases of chronic HCV monoinfection, while they were close to normal in chronic hepatitis B and in mixed hepatitis. The mean levels of sIL-2R tended to be increased in the cases of chronic hepatitis B, hepatitis C and mixed hepatitis, but the differences were not statistically significant.

**Conclusion.** Amongst the Th1 and Th2 cytokines studied, sIL-2R, IFN- $\gamma$  and IL-10 co-participate in the pathogenesis of chronic hepatitis B or C and can be used for evaluating the immune state of the organism. Our data suggest that sIL-2R is closely related with the severity of the pathological process of liver lesions.

**Key words:** Th1 and Th2 cytokines, chronic viral hepatitis B and C

## BACKGROUND

T-lymphocyte immunoregulatory cytokines play an important role in the host response to hepatitis B virus (HBV) and hepatitis C virus (HCV) infection. In fact, the immune response which is associated with a T-helper (Th)1 cytokine profile, suggests that cell-

mediated immunity is associated with recovery (1), while Th2 cytokine response takes place in development of persistent infection (2).

The imbalance of pro-inflammatory Th1 and anti-inflammatory Th2 cytokine production may play an important role in the immunopathogenesis of hepatitis B and C virus infection. It can be used for the estimation of chronic liver disease development, progression and outcome (3, 4).

The objective of the present study was to analyze the serum level of Th1 and Th2 cytokines in patients with chronic hepatitis B and hepatitis C virus infection. The levels of the cytokines were also compared with the traditional biochemical (alanine transaminase, ALT) and viral (HBV DNA or HCV RNA) indicators of infection activity.

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The study was reported under the same name as a poster at the Second European Congress of Virology "Eurovirology 2004" in Madrid, Spain on September 7, 2004 and as an oral presentation at the VIII All-Russian Scientific Forum "Days of Immunology in St. Petersburg" on September 29, 2004.

## MATERIALS AND METHODS

**Patients.** Sixty-two randomly selected patients with chronic viral hepatitis were studied, of them 29 (24 males and 5 females aged 16–67, mean  $29.0 \pm 1.1$  years) comprised Group 1 and 33 (23 males and 10 females, age 9–61, mean  $23.1 \pm 1.9$  years) Group 2 from Department for Infectious Diseases of Ida-Viru Central Hospital. Serum samples were collected and tested during 2000–2001 (Group 1 of patients) and in 2003 (group 2).

Based on the profile of specific serological markers of HBV and HCV infection, results of detection of HBV DNA or HCV RNA, clinical laboratory and anamnestic data, all patients were divided into three subgroups according to the etiological diagnoses: chronic hepatitis B (cHBV,  $n = 15$ ), chronic hepatitis C (cHCV,  $n = 21$ ) and chronic mixt hepatitis (cHBV + HCV,  $n = 26$ ). The duration of the disease was from 1.5 to 5 years, mean 2.5 years.

Twenty healthy subjects (17 males, 3 females, age 34–59) with normal liver function tests and without markers of viral hepatitis and HIV-infection were used as normal controls.

**Viral hepatitis markers** were tested by the ELISA method using third generation commercial test-kits according to manufacturer's instructions.

**HBV DNA** was detected by polymerase chain reaction (PCR) for the S-region of HBV genome in 20/41 (48.8%) of HBsAg-positive sera, and **HCV RNA** was detected by reverse transcription PCR (RT-PCR) for the NS5B region of HCV genome in 20/47 (42.5%) of anti-HCV-positive sera.

**The serum levels of Th1 cytokines:** interferon-gamma (IFN- $\gamma$ ), interleukin-2 (IL-2), soluble IL-2 receptors (sIL-2Rxc), and a **Th2 cytokine,** interleukin-10 (IL-10) were measured by EIA assay with commercial kits from Bender MedSystems Diagnostics, Austria and Roche and Boehringer Mannheim, Germany; Diacorne Research, France. The concentration of cytokines was detected based on the titration of standards. The lowest limit of sensitivity of test systems for IFN- $\gamma$  was  $<1.5$  pg/ml, for IL-2  $<10$  pg/ml, for IL-10  $<5$  pg/ml and for sIL-2R  $<8$  pg/ml or 15 U/ml.

### Statistical analysis

The concentrations of IFN- $\gamma$ , IL-2, sIL-2R and IL-10 in blood sera were calculated using optical density

derived by regression analysis. The differences between the groups of patients were evaluated using dispersion analysis.

To define the validity of cytokine concentration and its relation to the diagnosis, correlation and dispersion analysis as well as criterion of Student's and  $\chi^2$  were used. Calculations were performed with the Excel 2000 program.

**Ethics.** The study was approved by the Tallinn Medical Research Ethics Committee.

## RESULTS

Apart from etiology the serum IFN- $\gamma$  and sIL-2R levels in patients from both study groups as well as the levels of IL-10 from Group 2 were significantly increased as compared with those of healthy controls. The data are shown in Tables 1 and 2.

However, the mean levels of IFN- $\gamma$  and IL-2 in patients of Group 1 did not differ significantly from control.

Amongst the cytokines measured, only the serum IL-2 levels were significantly lower in individuals with cHCV.

Table 1. Serum levels of IFN- $\gamma$ , IL-2 and sIL-2R in patients with chronic hepatitis, 2000–2001

Groups	Cytokines		
	IFN- $\gamma$ , pg/ml	IL-2, pg/ml	sIL-2r, pg/ml
Group 1, n = 29	$7.85 \pm 1.2$	$20.8 \pm 1.7$	$6306.0 \pm 478.0^*$
P*	$>0.05$	$>0.05$	$<0.05$
Of them			
cHBV, n = 8	$9.9 \pm 3.4^*$	$23.6 \pm 3.3$	$4692.2 \pm 683.6^*$
cHCV, n = 7	$4.8 \pm 0.35^*$	$14.2 \pm 1.9^*$	$5795.6 \pm 1037.9^*$
cHBV + HCV, n = 14	$7.95 \pm 1.4^*$	$21.9 \pm 2.4$	$7443.6 \pm 548.6^*$
Control, n=10	$3.90 \pm 0.25$	$23.8 \pm 1.8$	$1373 \pm 143.2$
P*	$<0.05$	$<0.05$	$<0.05$

P\* – compared with control.

Table 2. Levels of IFN- $\gamma$ , IL-10 and sIL-2R in blood sera of patients with chronic hepatitis, 2003

Groups	Cytokines		
	IFN- $\gamma$ , pg/ml	IL-10, pg/ml	sIL-2r, U/ml
Group 2, n = 33	$0.613 \pm 0.100^*$	$41.2 \pm 6.3^*$	$127.5 \pm 15.9^*$
P*	$<0.01$	$<0.05$	$<0.05$
Of them			
cHBV, n = 7	$0.646 \pm 0.14^*$	$50.6 \pm 19.5^*$	$99.6 \pm 32.3^*$
cHCV, n = 14	$0.68 \pm 0.18^*$	$32.0 \pm 9.9^*$	$112.5 \pm 20.9^*$
cHBV + HCV, n = 12	$0.50 \pm 0.12^*$	$48.1 \pm 8.4^*$	$166.5 \pm 31.3^*$
Control, n = 10	$0.188 \pm 0.075$	$10.8 \pm 7.8$	$57.1 \pm 5.6$
P*	$<0.001$	$<0.05$	$<0.05$

P\* – compared with control.

The serum levels of sIL-2R in both groups of patients tended to be increased in cases of chronic hepatitis B to hepatitis C and to mixed hepatitis, but the differences were not statistically significant (Tables 1 and 2).

In the present study, the ALT levels were 2–200 times higher than normal and were in the range 13–3590 U/L (Table 3). The highest elevation of ALT was observed in patients with chronic mixed hepatis

Table 3. ALT indices (normal 10–60 U/L) in patients with chronic viral hepatitis

Study groups	ALT
Chronic viral hepatitis, n = 62 (total)	532.7 ± 253.9(13–3590)
cHBV, n = 15	624.4 ± 351.8
cHCV, n = 21	138.8 ± 42.3
cHBV + HCV, n = 26	834.8 ± 367.5

while the lowest ALT levels were found in patients with chronic hepatitis C.

The increased values of sIL-2R showed a significant positive correlation with ALT activity in both groups of patients ( $r = 0.654$  and  $r = 0.452$ ,  $P < 0.05$ , respectively). A significant positive relationship was revealed also between serum IFN- $\gamma$  and IL-10 levels ( $r = 0.849$ ,  $P < 0.05$ ) in patients from group 2, although there was no correlation between the cytokines studied and the presence of HBV DNA or HCV RNA.

## DISCUSSION

In this study, we aimed to assess the serum concentrations of different cytokines in patients with chronic viral hepatitis B and C. For this purpose, we measured cytokine levels in serum samples from two groups of patients with well-defined clinical conditions as well as from a group of healthy uninfected control individuals. All the patients were also divided into three subgroups according to etiology diagnosis – chronic hepatitis B, chronic hepatitis C and chronic mixed hepatitis (HBV + HCV).

While T-helper type 1 cytokines (IL-2, IFN- $\gamma$ , sIL-2R) are required for host antiviral immune response and are involved principally in cell-mediated immunity, T-helper type 2 cytokines (IL-10) mostly regulate humoral immune response (5).

The data regarding IFN- $\gamma$  and IL-10 in the context of HBV and HCV chronic infection gave conflicting results. A significant decrease of IFN- $\gamma$  levels was reported in patients with viral hepatitis C (6) and hepatitis B (7). At the same time it was shown (2) that in the case of chronic HCV infection the levels of IFN- $\gamma$  did not significant change on the background of a relevant increase of IL-10 concentration. According to the data of the same authors,

in chronic hepatitis B a simultaneous increase of IFN- $\gamma$  and IL-10 levels was observed. Cacciarelli et al. (8) and Piazzolla et al. (9) revealed an analogous dependency also in hepatitis C, but in this case there wasn't any correlation between these cytokines and the activity of ALT. The detected contradictions may be caused by the level of liver injury, the activity of the infectious process, by its severity and duration in the observed patients.

In our study, the serum IFN- $\gamma$  and IL-10 levels were significantly higher during the chronic viral hepatitis apart from etiology. These results may suggest that IFN- $\gamma$  and IL-10 are involved in the pathogenesis of chronic hepatitis B and C virus liver disease.

It is known that the insufficient IL-2 production can promote development of chronic hepatitis B and especially hepatitis C infection. In the present study, serum IL-2 levels were significantly lower only in cases with chronic HCV mono-infection, however, they were close to normal in chronic hepatitis B and in mixed hepatitis cases. Similarly, conflicting results have been reported for serum IL-2 measurements. According to the data of (10), there were no significant changes in serum IL-2 levels in patients with chronic hepatitis C. On the other hand, a decrease in the serum levels of IL-2 has been observed in patients with chronic HBV infection (11).

High serum sIL-2R levels were shown both for patients with chronic hepatitis B (12) and hepatitis C (13). Their levels are related to activity of the disease rather than to virus replication, and this may be a useful marker of T-cells immune response.

ALT is the main and accessible marker for cytolytic syndrome identification. A significant relationship was noticed by Jia et al. (14) between serum sIL-2R and ALT levels in patients with chronic HBV and HCV infection. According to our data, amongst the cytokines studied, only sIL-2R levels showed a significant positive correlation with ALT concentrations. The presence of viral nucleic acids (HBV DNA or HCV RNA) in serum showed no associations with either cytokine or transaminase levels regardless of whether the chronic infected groups were considered as a whole or segregated according to etiology agents.

Thus, serum sIL-2R levels should be an important marker for assessing the phase of active chronic hepatitis and the degree of liver damage.

## CONCLUSIONS

The present data and our previous study (15) give evidence of an imbalance in the production of Th1 and Th2 cytokines in chronic viral hepatitis patients depending on disease etiology and the activity of the infectious process. The most valuable of the cytokines studied as an indicator of not only the current chronic process but also of the activation of cell-

mediated immunity in viral hepatitis B and/or C proved to be the soluble receptor of interleukin 2.

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