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The Levels of IL-1β, IL-4 and IL-6 in the Serum and the Liver Tissue of Chronic HCV-Infected Patients

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Abstract. The pro-inflammatory interleukines play a major role in the progress of chronic hepatitis C. Among the patients with chronic hepatitis C virus (HCV) infection, the morphology of the liver was assessed and the levels of serum and liver-tissue IL-1β, IL-4 and IL-6 were determined. The levels of the cytokines were related to the liver-tissue changes. RNA-HCV was measured by the RT-PCR method. Cytokine levels of the serum and liver tissue were measured by the Quantikine® High Sensitivity test. The levels of serum IL-1β, IL-4 and IL-6 (0.221, 0.104 and 1.393 pg/ml) in all HCV patients were higher in comparison with healthy adults (0.188, 0.025 and 0.600 pg/ml). The levels of liver tissue IL-1β, IL-4 and IL-6 (4291.3, $p<0.05$; 1624.6, $p<0.05$; 1158.7 pg/g protein) in all HCV patients were higher compared with patients with liver cirrhosis without HBV or HCV infection (2319.9, 553.6 and 756.2 pg/g protein). Patients with HCV infection demonstrated a significant correlation between serum and liver-tissue levels of IL-1β (Pearson: 0.61, $p<0.05$) and IL-4 (Pearson: 0.51). The level of serum IL-6 in patients with moderate chronic active hepatitis was higher when compared with patients with mild chronic persistent hepatitis. Among the patients with mild chronic persistent hepatitis, the levels of liver-tissue IL-6 were higher compared with those with moderate chronic active hepatitis. There was no correlation between histology changes and the levels of serum and liver-tissue IL-1β and IL-4.

Key words: HCV infection; cytokines; histology changes.

Introduction

Among the numerous infectious diseases hepatitis C infection is besides HIV infection the most serious challenge for medicine today. The long years of studies on the hepatotoxicity of the HCV have not been very successful. There are significant data which point to the large role of the non-specific immunological response in the development of hepatitis[10]. Interleukins, which are among the many agents of the non-specific immunological response, play an active role in the local and general inflammatory reaction. The highest interleukin activity begins at the site of their production however, when entering the circulation they are still biologically active[21]. The morphological changes in the liver among patients with chronic HCV infection partly correlate

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with the viremia. The assessment of the interleukin concentration in the hepatic tissue with respect to the morphological state of the liver may be helpful in understanding the way in which the HCV damages hepatocytes as well as be an indicator for treatment modification in patients with chronic HCV infection[1].

I decided to assess morphological changes in the liver of patients with chronic HCV infection. The interleukin concentrations in the hepatic tissue of patients with chronic HCV infection and patients with liver cirrhosis were measured. The dependence between the interleukins and changes in the morphology of the liver in patients with chronic HCV infection were determined. A comparison was also made of the interleukin concentrations in the hepatic tissue with their concentrations in the serum with respect to the morphological changes in the liver of the HCV-infected patients.

Materials and Methods

Patients. The study included 34 patients, 11 women (aged 22–53 years) and 23 men (aged 18–49 years), infected with HCV and 9 patients, 5 women and 4 men (aged 26–46 years), with liver cirrhosis (post aethylica) without HBV or HCV infection.

Among the 34 patients were 15 subjects with moderate chronic active hepatitis and 19 subjects with mild chronic persistent hepatitis (Table 1).

Reference values. The reference values were determined on 12 healthy control subjects (8 females and 4 males, aged 26–46 years).

In order to qualify for the control group, subjects were required to have 2 tests 3 and 6 months prior to being included in the study, so as to confirm normal alanine aminotransferase (AlAT) activity in the serum and to rule out HBs antigen and HCV antibodies.

Table 1. Prevalence of histology features in 34 liver biopsies from patients with HCV infection

Conventional diagnosis	No. patients	Necroinflammatory activity (0 to 4)		Fibrosis (0 to 4)
		portal/ /periportal	lobular	
Moderate chronic active hepatitis	15	2.9	1.8	1.7
Mild chronic persistent hepatitis	19	1.3	1.0	1.0

Informed consent was obtained from each patient and the study protocol was approved by the Ethics Committee of the Medical Academy of Białystok.

Serological analyses. The initial diagnoses were made by measuring the levels of HCV antibodies and RNA-HCV. The levels of anti-HCV were assessed by micro-enzyme immunological method (MEIA, Abbott, Germany), which included core proteins for HCr43, structural proteins for NS3 and NS4 (c 200), and non-structural proteins for NS4 (c 100-3) and NS5. RNA-HCV was measured by the RT-PCR* method (Ortho, Switzerland). The HCV antibodies were examined in the serum twice in interspecies from 3 to 6 months.

Liver histology. Liver tissue was obtained by biopsy from the right lobe of the liver (with 1.4 mm needles from Hepafix packs by Braun, Germany). One part of the liver tissue was used for estimation of morphology and the other for the level of cytokines. The morphology of the liver tissue was assessed descriptively and scored (for necroinflammatory activity and fibrosis) according to SCHEUER[19] with our own modification (Table 2).

Cytokines measurement The liver tissue obtained from the biopsy was homogenized in 0.9% NaCl. The levels of total proteins and the cytokines in the homogenate liver tissue were determined. The levels of cytokines in 1.0 g of all proteins were analyzed twice.

The cytokine levels in the serum were measured twice.

The cytokine levels of the serum and liver tissue were estimated:

1. IL-1β and IL-4 were measured by Quantikine® High Sensitivity tests (R&D System, Minneapolis, USA).

Sensitivity: IL-1β: 0.05 pg/ml, readings at 450 nm.

Sensitivity: IL-4: 0.02 pg/ml, readings at 490 nm.

2. IL-6 was measured by Quantikine® tests (R&D System, Minneapolis, USA).

Sensitivity: IL-6: 0.4 pg/ml, readings at 490 nm.

Statistical analyses. Values were expressed as their means and standard deviations (±SD). The significance of the difference was calculated by Student's *t*-test and the Pearson correlation test. Values of $p<0.05$ were considered to be significant.

All data were collected and analysed with Microsoft Excel 6.0 software (Microsoft Corporation).

* The HCV-RNA in serum was measured in the Immunopathology Laboratory of the National Institute of Hygiene in Warsaw, Poland, head: Prof. Adam Nowosławski.

Table 2. Scoring system of liver histology* in patients with hepatitis C (proprietary classification according to SCHEUERS[19] with own modification)

Necroinflammatory activity (grade)			Fibrosis (stage)
portal/periportal	score	lobular	
Absent	0	absent	absent
Minimal inflammation Minimal hepatitis < 1	1	one necrosis foci in lobular	fibrosis without occupation of partition
Inflammation < 50% portal area **1 > mild chronic persistent hepatitis < 2**	2	< five necrosis foci in lobular	periportal fibrosis with occupation of partition and P-P bridging
Inflammation > 50% portal area and/or Inflammation near border blade **2 > moderate chronic active hepatitis < 3**	3	five to ten necrosis foci in lobular	P-P and P-C bridging fibrosis
Intensive inflammation near border blade, P-P bridging inflammable **3 > severe chronic active hepatitis**	4	> ten necrosis foci in lobular and/or bridging necrosis	definite cirrhosis

* The material had to contain at least five porto-biliar areas.
P-P – portal-portal, P-C – portal-central.

Table 3. IL-1β, IL-4 and IL-6 serum levels and activity of alanine aminotransferase in patients with chronic HCV infection

Diagnosis	No. patients		IL-1β	IL-4	IL-6	AlAT IU
			pg/ml			
All patients	34	x	0.221	0.104*	1.393*	117
		± SD	0.186	0.114	1.247	88
Mild chronic persistent hepatitis	19	x	0.267	0.115***	1.347**	110
		± SD	0.221	0.148	1.828	100
Moderate chronic active hepatitis	15	x	0.157	0.090*	1.750**	125
		± SD	0.099	0.050	1.112	73
Reference values	12	x	0.188	0.025	0.600	<45
		± SD	0.111	0.012	0.760	<45

x – mean, SD – standard deviation.
* Statistically significant ($p<0.001$), ** ($p<0.01$), *** ($p<0.05$) in comparison to reference values.

Table 4. IL-1β, IL-4 and IL-6 liver tissue levels and activity of alanine aminotransferase in patients with chronic HCV infection

Diagnosis	No. patients		IL-1β	IL-4	IL-6	AlAT IU
			pg/ml			
All patients	20	x	4291.3*	1624.6*	1158.7	165
		± SD	2446.8	1291.8	1202.4	191
Mild chronic persistent hepatitis	11	x	4274.8	1605.9*	1406.8	148
		± SD	2391.2	1175.4	1136.7	158
Moderate chronic active hepatitis	9	x	4307.8	1645.9	784.9	186
		± SD	2666.6	1510.3	1104.1	234
Patients with liver cirrhosis without HCV infection	9	x	2319.9	553.6	756.2	75
		± SD	1698.1	138.6	491.8	34

x – mean, SD – standard deviation.
* Statistically significant ($p<0.05$) in comparison to patients with liver cirrhosis without HCV infection.

Results

The activity of AlAT among the patients with chronic HCV infection was 2.5 times higher than the reference range, and in the group with liver cirrhosis, not infected with the virus, this activity was 2 times higher. We did not find any statistically significant differences in the AlAT activity among the studied subjects (Table 3, 4).

All patients with chronic HCV infection showed elevated levels of IL-1β, IL-4 ($p<0.001$) and IL-6 ($p<0.001$) in comparison with the reference values. Among the subjects with mild chronic persistent hepatitis, the concentrations of IL-4 and IL-6 were statistically markedly higher ($p<0.05$ and $p<0.01$). The patients with moderate chronic active hepatitis showed statistically markedly higher values of IL-4 ($p<0.001$) and IL-6 ($p<0.01$) in comparison to the reference values (Table 3).

The concentrations of IL-1β ($p<0.05$), IL-4 ($p<0.05$) and IL-6 in the hepatic tissue of patients with chronic HCV infection were higher in comparison with patients with liver cirrhosis but not infected with HCV or HBV. The subjects with mild chronic persistent hepatitis and moderate chronic active hepatitis showed significantly higher concentrations of IL-1β, IL4 and IL-6 than to the patients with liver cirrhosis. Among the patients with chronic HCV infection, no differences in the concentrations of the studied interleukins dependent on the histological changes in the liver were observed (Table 4).

While comparing the concentrations of the interleukins in the hepatic tissue and the serum, a consistency was found with respect to IL-1β ($p<0.05$) and IL-4 in tissue and serum (Table 5).

Table 5. The correlation test (Pearson) between serum and liver tissue levels of IL-1β, IL-4 and IL-6

	IL-1β	IL-4	IL-6
Pearson (–1: +1)	0.613*	0.512	–0.169

* Statistically significant ($p<0.05$).

Discussion

Interleukins play a major role in the development of the inflammatory process, fibrosis and the regeneration of the liver[14]. A very important role in the escalation of the inflammatory process is played by the pro-inflammatory cytokines IL-1, IL-4 and IL-6.

IL-1β is synthesised in the liver by the Kupffer cells and the sinus cells of the endothelium[6, 16] as an inactive procytokine[11]. They gain their activity after the action of the cystein protease (IL-1β-converting enzyme – ICE)[4]. Many factors, such as viruses, may induce the synthesis of IL-1β[15]. IL-1β stimulates the proliferation and differentiation of B cells, induces the production of antibodies and activates the chemotaxis of neutrophiles and monocytes. In the liver it activates the sinus cells of the endothelium to active phagocytosis[16]. This cytokine induces the production of the acute phase of proteins in the liver, such as ceruloplasmin and C3 component complement[3].

DELHANTI[3] showed the inhibitory effect of IL-1β on the synthesis of the anabolic, insulin-like factor (IGF-I) in the liver. This factor delays the process of liver cell regeneration. The concentration of cystein protease (ICE) increases with the increase of active IL-1β. ICE is the third kaspase, active in the chain reaction of apoptosis[7]. This stresses the increase of apoptosis in patients with chronic HCV infection who show high levels of IL-1β in the hepatic tissue[2].

GRAMANTIER et al.[9] maintain that high levels of IL-1β result in a lenghtening of the inflammatory processes in the liver. FURUSYO et al.[8] showed the high replication of the HCV in patients with high levels of IL-1. No correlation between the concentration of IL-1 and morphological changes in the liver were observed.

In this study, the concentrations of IL-1β were higher in patients with mild chronic persistent hepatitis, but in the hepatic tissue the concentration of this cytokine did not depend on the histological changes. I observed a correlation between the concentration of IL-1β in the liver tissue and the serum. ZEUZEM et al.[25] showed a higher activity of HCV replication in patients with mild chronic persistent hepatitis than those with moderate chronic active hepatitis. The study by CALABRESE et al.[2] showed no correlation between morphological changes and viremia. It seems that the high concentrations of IL-1β depend on the replication activity of the virus, not on the inflammatory activity in the hepatic tissue. High serum and hepatic tissue concentrations of this interleukin may be a prognostically negative factor in the persistence of the HCV infection.

IL-4 is synthesised mainly in Th2 lymphocytes and induces the expression of MHC particles class I and II on the lymphocytes, which facilitates recognition of the viral antigens. IL-4 stimulates the cytotoxicity and phagocytosis of monocytes and macrophages. This may provoke the incidence of the autoimmunological processes found in HCV infection.

I observed a correlation between the high concentrations of IL-4 in the serum and the hepatic tissue. These results are similar to those of SCHIRREN et al.[20], who showed an increase in the level of IL-4 in the hepatic tissue of patients after liver transplantation with the renewal of the HCV infection. This indicates that there may be a correlation between IL-4 concentrations and the intensity of the inflammation caused by the HCV. TSAI et al.[23] turned their attention to the great importance of Th2 lymphocytes for IL-4 production in the persistence of HCV infection. On the other hand, REISER et al.[17], while showing the high concentrations

of IL-4 in the serum in 49% of HCV infected patients, did not find any correlation between IL-4 concentrations and morphological changes in the liver. He suggests that this interleukin, which is produced in the peripheral T lymphocytes, is responsible for the general, but not the local response to the HCV infection. The degree of liver damage does not influence the IL-4 concentration[17].

TILG et al.[22] showed the stimulating influence of IL-6 on B lymphocyte conversion to plazmocytes and the production of non-specific immunoglobulines in subjects with viral hepatitis. IL-6 activates the proliferation and the differentiation of T lymphocytes. This is an important factor, which activates the hepatocytes to the production of the acute phase proteins, the C-reactive protein, haptoglobin, fibrinogen and C3 compound[24].

MCGUINNESS et al.[13] observed an elevated concentration of the mRNA: IL-1β, IL-6, correlating with an increase in the activity of the macrophages in the hepatic tissue of HCV-infected subjects. Irrespective of the pro-inflammatory qualities, SAKAMOTO et al.[18] and FAUSTO[5] point to the influence of IL-6 on the liver regeneration through the modification of mitogenesis and the inhibition of hepatocyte apoptosis.

Among the subjects studied the IL-6 concentration in the serum and the hepatic tissue was high. In the studies of MALAGUARNERA et al.[12] and HUANG et al.[10] it has been shown that high IL-6 concentration in the serum correlated with histological changes in the livers of HCV-infected subjects. In this study, I observed high IL-6 concentrations in the serum of moderate chronic active hepatitis patients compared with mild chronic persistent hepatitis patients, but the IL-6 concentrations in the hepatic tissue were higher in mild chronic persistent hepatitis patients. This may be due to the correlation of IL-6 synthesis with viral replication and not with the local intensification of the inflammation.

This finding was also confirmed by SHAPIRO et al.[21], indicating a decrease in the number of Th1 cells and a concentration of interleukins produced by these cells, IL-4 and IL-6, in the period of HCV replication inhibition due to the treatment with interferons.

This study shows that levels of IL-1β in the serum and liver tissue and IL-6 in liver tissue are not dependent on the morphological changes in the liver. Exacerbation of morphological changes in the liver are influenced by an increase of IL-6 levels in the serum. However, the high levels of these interleukins seem to be a conditional synthesis of IL-6 in the peripheral macrophages[21]. The levels of IL-1β and IL-4 in the serum correlated with the levels of these cytokines in the liver tissue. It would seem that the pro-inflammatory cytokine correlates with the replication of the virus and not with the exacerbation of the inflammatory changes in the liver.

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