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Correlations among the serum levels of some interleukins and the histopathological aspects in chronic viral hepatitis C

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Abstract

Premises and objectives. The evolution of the infection with the hepatic virus C depends on the defense of the organism, found under the control of a network of cytokines and chemokines. The mechanisms that are causing both viral persistence as well as hepatic pathology are not entirely elucidated. We have proposed to study the amount in which different categories of cytokines are incriminated in the pathogenesis of the chronic liver disease, as well as the eventual correlations between the serum levels of these cytokines and certain histopathological aspects in the chronic viral hepatitis C. *Patients and methods*. Thirty-five patients with chronic viral hepatitis C (persistent – nine, active – 15, cirrhosis – 11) have been studied, constituting group P, and 20 healthy subjects constituting the reference group (R). In both groups have been determined the serum concentrations of some proinflammatory interleukins (TNF-alpha, IL-6, IL-8), and antiinflammatory (IL-10) cytokines, through the immunoenzymatic technique ELISA. *Results*. For the proinflammatory cytokines taken into consideration (TNF-alpha, IL-6, IL-8) increased serum values have been determined to the patients with chronic hepatitis C, the maximal level being observed to the patients active chronic hepatitis and cirrhosis (24/35 patients – 68.57%, 19/35 patients – 54.28% and, namely, 18/35 patients – 51.42%). The serum values of IL-10 are increased in 19/35 patients – 54.28%. The direct relationship among the increased levels of IL-10, the astringency of the inflammation and the hepatic functional insufficiency has been taken into consideration. *Conclusions*. The immune cellular answer has a fundamental role in the pathogenesis of the liver disease in the patients with chronic viral hepatitis C. The disequilibrium between the pro- and antiinflammatory cytokines participates to the installation of hepatic lesions of cytolysis and/or to the progression of fibrosis. The serum concentrations of the studied cytokines (TNF-alpha, IL-6, IL-8 and IL-10) are correlated to the histopathological spoilages of the liver.

***Keywords*:** chronic viral hepatitis C, cirrhosis, pro- and antiinflammatory cytokines.

Introduction

Viral hepatitis C constitutes a major problem of public health, with often-severe evolution and significant death. The hepatic virus C inducing a chronic disease in over 60–70% of the infested cases. The persistence of the hepatic virus C (HVC) in the organism may provoke hepatic inflammation, hepatic fibrosis, presenting an increased risk for cirrhosis and hepatocellular cancer. The evolution of the infection depends on the defense of the host organism (natural and obtained- immune), defense controlled by a network of cytokines and chemokines. The mechanisms that determine both viral persistence as well as hepatic pathology are not completely elucidated. It is well-known the fact that the immune answer is present all along the chronic infection, but it is suggested that it would not be enough in order to control or eliminate the virus [1, 2]. In the immunopathogenesis of chronic viral hepatitis C, is acknowledged the essential intervention of the cellular mediated answer, especially through the intervention, on the one side, of a group of cells, and on the other side, of the system of cytokines and chemokines [3]. The cells involved in the antiviral cytotoxicity are represented by T-lymphocytes cytolytic –cytotoxical (Tc), naturally killing cells (NK), and the macrophages carrying receptors Fc [4–6].

The first category of cells, Tc-lymphocytes, recognize the viral antigens expressed on the membrane of the infested cell in the presence of the molecules of the major histocompatibility complex (MHC) of Ist class. In the absence of these cells, the process may be compensated through the T-helper lymphocytes (Th), with the condition that the cells infested virally to express on the surface the MHC molecules of IInd class. The NK-cells and the macrophages, the second category of cells responsible for the cellulary mediated cytolysis, that recognize through the Fc receptor the antiviral antibodies coupled to the viral antigens from the membrane of the infected cell, realizing the

phenomenon of cellulary mediated cytotoxicity antibody-dependent (ADCC) [2].

There are data according to which, immediately after the infection with the hepatic virus C, the Tc-lymphocytes increase in number, but they present a diminished cytotoxic activity and even an inactivity concerning the synthesis of antiviral cytokines. One of the causes of persistency of the virus in the body, manifested under the chronic form of infection, with an inefficient antiviral immune answer, is the induction of tolerance of Tc-lymphocytes [3, 6].

Many works stipulate the fact that the Tc-lymphocytes, specific VHC, are better represented numerically and have a higher cytotoxic activity in the hepatic tissue than in the peripheral blood [7, 8]. Tc lymphocytes may exert a control over the viral replication, but may be also responsible for the progressive hepatic lesions characteristic to the chronic infection with HVC. Viral antigens are T-dependent, that is why the immunoantiviral answer is controlled by Th-lymphocytes. Thus, the equilibrium or the disequilibrium between Tc-lymphocytes and Th-lymphocytes (Th1 and Th2) plays a major role, expressed through disorders in the synthesis of cytokines. Th1 subpopulation secrets stimulating factors (interleukin-2 – IL-2, IL-12, gamma interferon – IFNγ), while subpopulation Th2 produces inhibitory factors (IL-4, IL-10, IL-13 so on) having as a role the deactivation of the Th1 subpopulation and of the macrophages that secret the TNF-α and IL-8. The modification of the equilibrium between the immunostimulating and inhibitory cytokines has as a result the prolongation of the inflammation, which, in its turn, leads to necrosis, fibrosis and chronic disease of the liver [7].

In the immunopathogenesis of chronic hepatitis C, besides Tc-lymphocytes, NK-cells and macrophages, is being recognized the essential intervention of a complex network of cytokines with "mediating and modulating" role of the inflammatory answer existent in this affection. The cytokines, a very heterogeneous group of protein molecules, have the capacity to undertake a real "dialogue" among leucocytes. They play a central role in all the physiological reactions of cellular growing and differentiation, of tissular repairing and remodeling, but also in the inflammatory and immune reactions. The cytokines, produced in different quantities and moments, by numerous lymphoid and non-lymphoid cells (B- and T-lymphocytes, macrophages, stromal cells from the hematogenous marrow, endothelial cells and so on) are stimulating one another in an autocrine, paracrine or endocrine way, initiating the "cascade" of cytokines, reason for which they can be called real "hormones with local action" [2].

In the inflammatory answer from chronic viral hepatitis C is being established a real "hierarchy" among the participating cytokines in what the producing moment is concerned and the intensity of the biologic effects, the production of one cytokine being able to stimulate or, on the contrary, to block the production of other cytokines [2].

The chronicization is the result of an ineffective antiviral cellulary answer that allows the persistence of the virus. It is being associated to an important destruction of hepatic cells induced by Tc-lymphocytes, destruction that is not accompanied by the depuration of the virus because of the installation of a tolerance towards this one. The viral cytopathic effect over the hepatic cell or over the lymphocytes continues even at a reduced level, but while this one leads to hepatic insufficiency or to the major compromise of the immune answer. Processes may be aggravated through the development of an autoimmune answer, because of the persistent hepatic lesions, with modifications of the hepatic cellular structures [3, 6].

Objectives

The immune answer cellularly mediated from the chronic hepatitis C depends on the cytotoxic activity of Tc-lymphocytes, found under the modulating influence of Th-lymphocytes, as well as of the functions through which cytokines act. These are involved on the one hand, in the defense of the host against the extension of C hepatitis, in the conditions in which the virus avoids the defense mechanisms through T cells, and on the other hand, in the hepatocellular injuries observed in the majority of the patients chronically infested. The mechanisms that cause the viral persistency and the hepatic pathogenesis are not enough known.

We have proposed to study in what measure different categories of proinflammatory (TNF-α, IL-6, IL-8) and antiinflammatory (IL-10) cytokines are incriminated in the pathogenesis of the chronic liver disease, as well as the eventual correlations among the serum levels of these ones and the histopathological aspects in patients with chronic viral hepatitis C.

Patients and methods

Thirty-five patients with chronic viral hepatitis C have been taken into consideration (persistent – nine, active – 15, cirrhosis – 11) came from the Medical Clinics of the Emergency County Hospital of Craiova. The cases included in the study, constituting the group P (proof), have been chosen through biochemical tests that have evidentiated a hepatocytolysis process (increased serum levels of the alanin-transaminase (ALT) and of the gamma-globulins (concentrations that have maintained increased during the last six months), and through immunological tests that have proven the presence of antiviral specific antibodies (antibodies anti-virus C hepatitis). In parallel, has been constituted a reference group (R), containing adults clinically healthy (20 subjects), with limits of age near those of the group of patients. In both groups was determined, through the ELISA immunoenzymatic technique, the serum level of some proinflammatory (TNF-α, IL-6, IL-8) and antiinflammatory (IL-10) cytokines. The following kits for human interleukins have been used: PeliKine^{TM} human ELISA kit (Nederland) for IL-6 and TNF-α; Milenia Biotec GmbH for IL-8 and IL-10. The obtained results in the patients have been compared to those noted in the control group. The serum values of TNF-α, in these healthy individuals, have been under 10 pg/ml,

of IL-6 under 11 pg/ml, of IL-8 under 31 pg/ml, and of IL-10 between 2–24 pg/ml.

The histopathologic exam of chronic viral hepatitis is useful for the affirmation of the hepatic histopathological modifications and the evaluation of the immune answer in these diseases. To point out the hepatic histopathological modifications, it has to be taken into consideration that the fragment of hepatic biopsy to be big enough (1.5–2 cm) and not fragmented. The tissue will be then fixed into formalin and included in paraffin, with ulterior staining with Hematoxylin–Eosin and Van Gieson.

Results

The cytokine system

Proinflammatory cytokines

The most increased serum levels of proinflammatory cytokines have been registered in the patients with chronic viral hepatitis C moderated active by forming fibrous porto-portal bridges (score METAVIR A2F3), for the patients with chronic hepatitis severely active (score METAVIR A3F3) and the patients with cirrhosis, in which lobular architecture has been erased with formation of cirrhotic nodules surrounded by fibrosis.

TNF-α

As compared to the reference group, chronic hepatitis is associated with bigger values of TNF-α. Increased serum levels of TNF-α have been evidentiated in 24/35 patients (68.57%), the most increased values being in the patients with cirrhosis.

IL-6

The serum levels of IL-6 in patients with chronic viral hepatitis C as compared to the reference group have presented increased values (19/35 patients – 54.28%). The serum level of IL-6 in the patients with chronic active hepatitis was higher as compared to that from the patients with persistent chronic hepatitis.

IL-8

The incidence of the increased levels of IL-8 cytokine has been in the proof group of 51.42% – 18/35 patients. The serum levels of IL-8 have been increased in the patients with chronic viral hepatitis C as compared to those found in the reference group. In addition, the values have been increased in the patients with chronic active hepatitis as compared to those from the persistent chronic hepatitis, the higher serum levels being registered in the serum of the patients with cirrhosis.

Antiinflammatory cytokines

IL-10

Serum values of IL-10 in the studied group (proof) have been different both comparatively to the reference group, as well as by affection. Thus, in the majority of the patients with chronic hepatitis found in the active study of the disease (active chronic hepatitis and cirrhosis), the IL-10 levels have been increased (19/35 patients – 54.28%) towards the patients found in a study of persistent hepatitis (16/35 patients – 45.71%).

The maximal level of IL-10 has been observed in the patients with cirrhosis, with severely active chronic hepatitis (score METAVIR A3F3), and in the patients with moderately active chronic hepatitis with formation of fibrous porto–portal bridges (score METAVIR A2F3), demonstrating the direct relationship with the severity of the inflammation and with the hepatic functional insufficiency, as well as its involvement in the pathogenesis of the chronic viral hepatitis C.

The histopathological exam

On the fragments of biopsy have been numbered the portal spaces, evaluated the presence of the necro-inflammatory activity, and of the fibrosis using the METAVIR score [17].

There have been nine cases of *chronic hepatitis minimally active*; in these cases, the lobular architecture has been kept, with the presence of chronic lymphocytary inflammatory infiltrate, with rare plasmocytes, limited to the port case, in seven cases necrosis was absent, and in two cases there have been small focuses of necrosis (Figure 1). Fibrosis was limited to the port space without septus formation (score METAVIR A1F1) [18]. Most of the cases (12) have been of *moderately active chronic hepatitis*, in which the inflammatory infiltrate has been present both in the portal spaces as well as intralobular, interface hepatitis has been present in over half of the perimeter of the portal spaces. In nine cases fibrous septa have been short (score METAVIR A2F2) and in three cases they were with formation of porto-portal fibrous bridges (score METAVIR A2F3) (Figure 2).

Severely active chronic hepatitis has been diagnosed in three cases. The necrotic-inflammatory activity has been marked in both portal spaces and septa as well as intra lobular (Figures 3 and 4). Fibrosis has been presented in numerous septa with formation of porto–portal and porto–central bridges (score METAVIR A3F3) [19]. *Cirrhosis* has been present in 11 cases. In four cases, it has been accompanied also by a very important necrotic-inflammatory activity (Figure 5). In all of the cases of cirrhosis, lobular architecture has been erased with formation of cirrhotic nodules surrounded by fibrosis [20].

Discussions

Chronic viral hepatitis C is tied to the viral persistency, that is being associated to an abundant production of cytokines, especially those of the Th1 and Th2 type. It is known that, while the Th1 profile is necessary to the immune antiviral answers of the host, the Th2 profile inhibits the development of effectors mechanisms, being involved in the pathogenesis of the chronic C hepatitis, as well as in the severity of the chronic liver disease. The modification of the balance between Th1 and Th2 cytokines has as a result the prolongation of the inflammation, which, in time, induces necrosis, fibrosis and, finally the chronic disease of the liver [9–12].

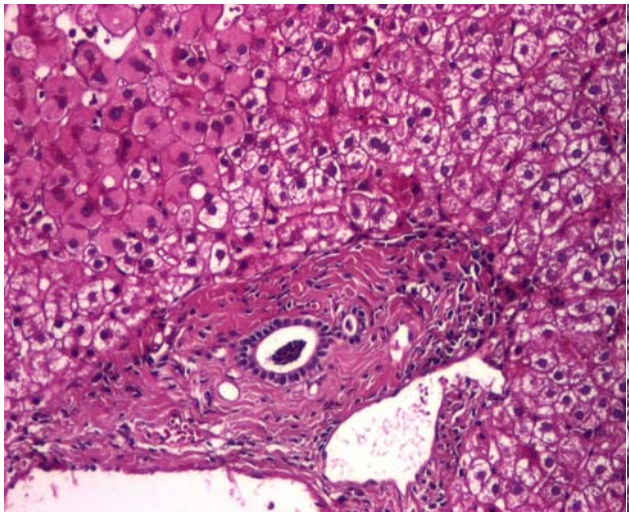

Figure 1 – ***Minimally active chronic hepatitis. Fibrosis and discrete inflammatory infiltrate in the portal space (HE staining, ×200)***

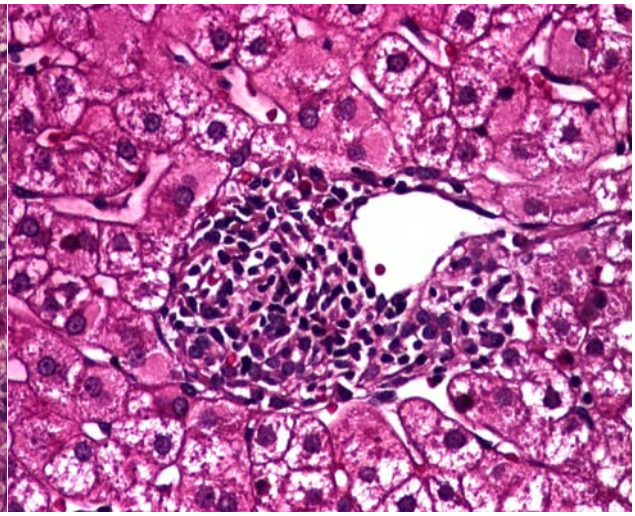

Figure 2 – ***Moderately active chronic hepatitis. Inflammatory pericentrolobular infiltrate (HE staining, ×100)***

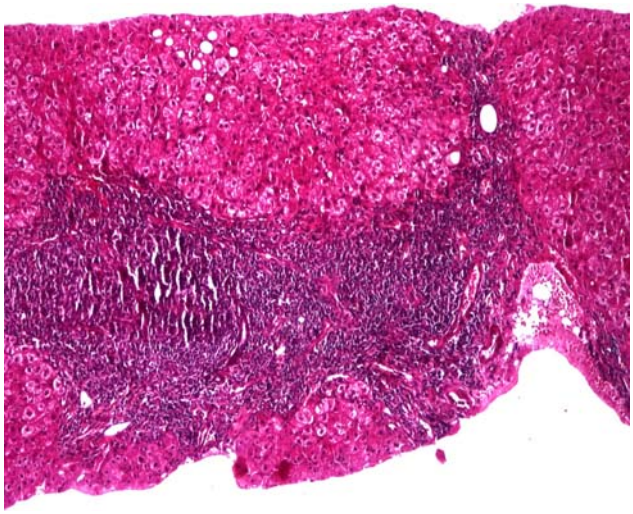

Figure 3 – ***Severely active chronic hepatitis. Porto–portal and lobular inflammatory infiltrate (HE staining, ×40)***

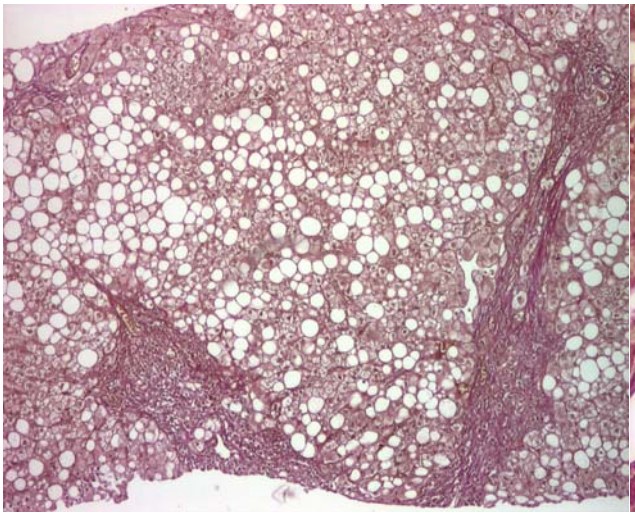

Figure 4 – ***Severely active chronic hepatitis. Fibrosis and porto–portal and porto–central inflammatory infiltrate (VG staining, ×100)***

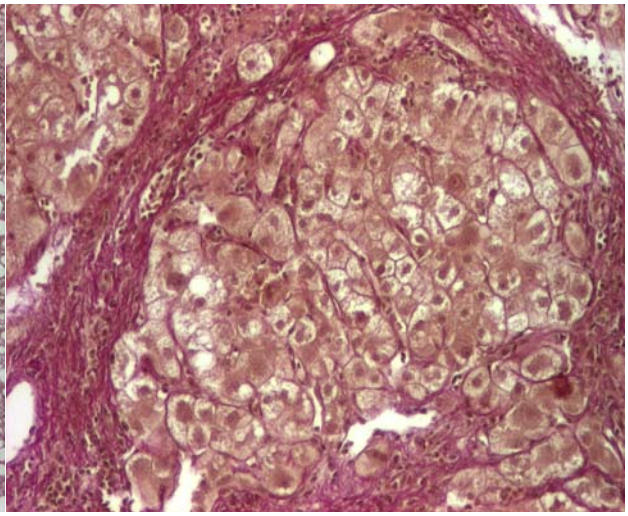

Figure 5 – ***Hepatic cirrhosis. Cirrhotic nodule (VG staining, ×400)***

The analysis of the parameters of the concentration and of the functional activity of serum cytokines endures a more objective evaluation of the mechanism of the disease and anticipates, finally, the prognostic of the disease. For all of the three cytokines of the group studied (TNF-α, IL-6 and IL-8) have been determined increased serum values in the patients with chronic viral hepatitis C. This observation comes as an extra argument concerning the proinflammatory effect of these cytokines, proinflammatory effect specified in other publications of profile that consider that the serum levels of these cytokines in the viral chronic hepatitis depend on the etiological factors (chronic viral hepatitis C, B, D or G), activity (decreased, moderated, increased), stage (chronic hepatitis, cirrhosis) [12].

TNF-α is a proinflammatory cytokine with a benefic role in the physiologic immune answer, but the non-adequate production, excessive of this one determines inflammation, tissue destruction and affectation of an organ. It is one of the first cytokines secreted during an inflammatory process, orchestrating the entire process, being the main releaser and potentate of this one, being responsible of the appearance and maintaining of the biologic nonspecific inflammatory syndrome with the increase of proteins of acute phase [10, 12, 13].

IL-6 is a multifunctional cytokine that acts on numerous target cells, being involved in the immune answer, inflammatory answer and in hematopoiesis. It stimulates the synthesis of the proteins of acute phase, the differentiation of B- and T-activated lymphocytes, the differentiation of Tc-lymphocytes and the cytotoxic function with formation of perforines. There are affirmation that indicate the reversed levels of this cytokine, at the level of the hepatic tissue: an increased level in the patients with persistent chronic hepatitis, towards the decreased level in those with active chronic hepatitis, proving that there are no correlations between the histological modifications and the levels of cytokines in serum and in the hepatic tissue [9, 11, 14].

IL-8, important mediator of the inflammation and of the immune answer, is known as the most powerful chemotactic factor for neutrophiles (in the smallest value for eosinophiles, basophiles) and T-lymphocytes. Its production is stimulated by IL-1, TNF-α, IL-6. It activates the neutrophiles and induces degranulation with release of lactoferine. This type of inflammatory cells has been noticed in the hepatic tissue during chronic viral hepatitis. It seems that IL-8 is in direct relationship with the injuries evidentiated in the patients with such affections [9, 10, 15].

IL-10, cytokine known also under the name of "inhibitor factor of the secretion of cytokines", is an antiinflammatory endogenous mediator through the inhibition of the production of proinflammatory cytokines (IL-6, TNF-α, IL-8). It has an immuno-supressor effect through the inhibition of the function of the macrophage of cell presenting antigen (reduction of the expression of molecules HLA IInd class, of the molecules of adhesion ICAM-1), as well as through the blocking of the capacity of activation of Th-lymphocytes suppressing thus the T-releasing of the immune specific answer. In the same time, the same cytokine has an immunostimulating effect over the population of Tc- and B-lymphocytes.

IL-10, secreted by Th2 cells, may regulate the effective immune mechanisms cellularly mediated, important in the defense of the host from the intracellular pathogens [13, 16]. Besides, the same anti-inflammatory cytokine, which is simultaneously secreted with the proinflammatory cytokines, normalizes the ALT level, limits the hepatic lesions, reduces fibrosis, through the effects of counter attack of the proinflammatory cytokines [11, 12, 16].

Conclusions

The disequilibrium between the pro- and anti-inflammatory cytokines participate at the installation of hepatic lesions of cytolysis and/or to the progression of fibrosis, to the delineation of the pathogenesis of chronic viral hepatitis C.

The prominence of IL-6 high-concentrations as inflammatory factor with a role in the regulation of the functions of the hepatocyte, involve this soluble factor in the pathogenesis of the chronic liver disease.

Our data lead to the sustaining of the idea according to which IL-8 would play a role in the installation of hepatic injuries to the patients with chronic viral hepatitis C.

It has been noticed that the direct relationship between the increased levels of IL-10, the acuity of the inflammation and the functional hepatic insufficiency.

We may conclude that the investigated cytokines (TNF-α, IL-6, IL-8 and IL-10) modulate the hepatic metabolism, the physiologic and pathologic functions of the liver as well as the evolution of the existent inflammation at the level of the liver, hepatic alterations (fibrosis, eventually necrosis).

Taking into consideration the alteration of the cytokines' concentrations (as expression of some disorders in the activity of cellular subpopulations), it becomes obvious the fact that the immune cellular answer is playing a fundamental role in the pathogenesis of the liver disease in the patients with chronic viral hepatitis C.

The serum concentrations of the cytokines taken into study ((TNF-α, IL-6, IL-8 and IL-10) are being correlated to the histopathological alterations of the liver.

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