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THE IMPORTANCE OF sCD40 AND sCD40L CONCENTRATION IN PATIENTS WITH CHRONIC HCV INFECTION AND HIV CO-INFECTION

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ABSTRACT

CD40 receptor is activated by ligand CD40L (CD154) which is synthesized in inflammation by NK cells, monocytes and lymphocytes B. TRAF proteins are activated in cells by CD40 stimulation and next they stimulate different enzymatic pathways. High concentrations of CD40L stimulate CD40, and consequently STAT enzyme system inhibits the expression of nonstructural proteins of HCV NS3 and NS5A and E2 core in infected human hepatocytes.

PURPOSE. The aim of the study was to evaluate the concentration of soluble components of the complex: sCD40 and sCD40L in the serum of patients infected with HCV and HCV/HIV-1 co-infected. The effect of HCV genotype, HIV and HCV viral load and rs12979860 polymorphism on serum sCD40 and sCD40L was established among the patients. The influence of the number of CD3 +, CD4 + and CD8 + on the concentrations of sCD40 and sCD40L was evaluated in the HIV-1 infected group

MATERIALS AND METHODS. Serum concentrations of sCD40 and sCD40L were determined using ELISA in 68 HCV infected patients including 39 HCV monoinfected and 29 HCV/HIV-1 co-infected.

RESULTS. Serum concentration of sCD40 and sCD40L was significantly higher in HCV and HCV/HIV co-infected patients compared to healthy subjects (25.7 and 23.2 v. 8.5 pg/ml and 12.7 and 7.3 v. 0.79 ng/ml). The concentration of sCD40L in patients with genotype CC rs12979860 was significantly higher compared to patients with Non-CC genotypes (11.8 v. 7.6 ng/ml, $p<0.018$).

CONCLUSIONS. High levels of sCD40 and sCD40L were detected among patients with chronic HCV and HCV/HIV-1 infection The high concentration of sCD40L correlates with CC rs12979860 genotype.

Key words: *sCD40, sCD40L, HCV, HIV*

INTRODUCTION

CD40 is a transmembrane protein receptor classified as a type I TNF receptor group. This receptor is present in many cells, including B and T lymphocytes, plasma cells, monocytes, dendritic cells and cells which occasionally present the antigen. It is stimulated by the CD40L ligand (CD154) which is synthesized by CD4 + cells or by NK cells, monocytes and lymphocytes B in case of inflammation. In the blood, particularly in the course of inflammation, the sCD40L in the not connected form is present. CD40 ligands are polypeptides that are included in the TNF family. Stimulation of CD40 by the ligands activates the metabolism of TRAF proteins which then stimulate enzymatic pathways, such as MAPK, JNK, MEKK1 and STAT (1). Activation of CD40 plays an important role in immune regulation, apoptosis and antiviral response. Experimental studies have shown that the activation of the CD40 receptors stimulates inflammatory reactions and autoimmune diseases (2). Lymphocytes B are activated and converted following the CD40 receptor stimulation (3). These cells play an important role in complement activation, phagocytosis and cytotoxicity induction of the antibody-dependent cell. Moreover, the strong wake up of the CD40 in B cells induces the expression of Fas receptor, which stimulates apoptosis. Activation of macrophages with the CD40 receptor stimulates the synthesis and secretion of cytokines such as IL1, IL6, IL8, and IL10 and IL12, TNF-α, matrix metalloproteinase's, autocrine and paracrine growth factors. The CD40 receptor which connects to the CD40L might be the second signal (co-

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receptor) in the course of HCV and HIV infection (3). Rau et al. have shown that CD40L inhibits the expression of non-structural proteins NS3 and NS5A and E2 core of the HCV in human hepatocytes (Huh7.5) (1).

AIM

The aim of the study was to evaluate the concentration of soluble components of the sCD40 and sCD40L complexes in the sera of HCV monoinfected and HCV/HIV-1 co-infected patients. We also evaluated the effect of single nucleotide polymorphism at site rs12979860 on serum concentration of sCD40 and sCD40L.

Additionally, a dependency between serum sCD40 and sCD40L levels versus CD4 counts or HIV viral load was analyzed in HCV/HIV co-infected patients.

The relationship between the concentration of sCD40 and sCD40L, and the number of CD3 +, CD4 +, CD8 + lymphocytes was determined among HCV / HIV- patients.

MATERIALS AND METHODS

The study comprised 68 HCV infected patients including 39 HCV monoinfected and 29 HCV/HIV-1 co-infected, mean age 39 years (range: 19-70 years) (Tab. 1). All the individuals were both diagnosed with chronic hepatitis C and evaluated prior to the anti-HCV antiviral therapy. None of the patients had liver cirrhosis as well as no symptoms of liver decompensation were observed.

Table 1. Characteristics of patients

	HCV monoinfected	HCV/HIV-1 co-infected	all patients
N	39	29	68
women	12	7	19
men	27	22	49
mean age in years (range min-max)	43 (19 - 70)	34 (27 - 50)	39 (19 - 70)
HCV genotype 1; n (%)	32 (82)	14 (48)	46 (68)
HCV genotype 3; n (%)	7 (18)	7 (24)	14 (21)
HCV genotype 4; n (%)	0	8 (28)	8 (11)
rs12979860 CC; n (%)	10 (26)	13 (45)	23 (34)
rs12979860 CT; n (%)	22 (56)	13 (45)	35 (51)
rs12979860 TT; n (%)	7 (18)	3 (10)	10 (15)

All HIV-1 infected patients had clinical A category and received HAART from 1 to 5 years.

The control group consisted of 15 healthy individuals, 6 females and 9 males aged from 21 to 53 years (mean age 35 ±2.7 years).

HCV-RNA in blood serum was marked by PCR method with reaction starters specific for non-coding 5'-final viral genome area (5'-UTR). Viral genotype was determined with the method of a direct sequencing obtained in PCR product reaction.

HIV-1 infection was diagnosed based on the detection of anti-HIV antibodies in the blood using ELISA method as well as a positive result of Western-blot[1] test (Cambridge Biotech Corporation, USA). HIV viral load was determined by RT-PCR method using Cobas Amplicor HIS 1.5 (Ultra Sensitive)[2]. Subpopulation and CD3+, CD4+ and CD8+ lymphocyte count were determined in the blood by means of flow cytometer and Becton Dickinson apparatus[2].

IL-28B promoter genotyping

DNA from blood specimens was isolated using automatic magnetic extraction method. In brief, 100 ml of whole blood was incubated with Proteinase K for 2 hours in 56^0C for protein digestion. Nucleic acids from deproteinated lysate were then extracted automatically with magnetic beads on EasyMag machine (Biomerieux, France) according to the producer's protocol. The resulting 100 ml of DNA solution in Buffer 3 was obtained.

Standard polymerase chain reaction was used to amplify a fragment of IL-28B gene containing a single nucleotide polymorphic (SNP) site rs12979860 CT. The primers for amplification were created based on Homo sapiens chromosome 19 clone CTC-246B18 sequence from NCBI nucleotide database (accession AC011445.6) with the Primer3 plus software and were as follows: forward primer 5'-gct tat cgc ata cgg cta gg-3', reverse primer 5'-agg ctc agg gtc aat cac ag-3'. Direct sequencing of PCR products was performed using BigDye Terminator Sequencing Kit (v.3.1) and ABI PRISM 3500 Sequencer (Applied Biosystems, Foster City, CA). To specify the IL-28B genotype the obtained sequences were compared with SNP rs12979860-containing sequence provided in the NCBI SNP database.

Serum concentrations of sCD40 and sCD40L were assayed in duplicates by use of ELISA technique. The measurements were performed according to manufacturer eBioscence, Austria.

The study was approved by the Bioethical Committee of the Medical University of Bialystok and all patients and controls provided informed consent for participation in the study.

STATISTICAL ANALYSIS

Statistical analysis was performed using U Mann -Whitney and Spearman correlation coefficient tests. Values $p< 0.05$ were considered as statistically significant.

1 Western-blot testing was performed in Institute of Venereology, Medical University of Warsaw, Poland. Head: Dr Z. Seliborska.

2 Flow cytometry analysis was performed in Molecular Diagnostics Laboratory in Hospital of Infectious Diseases, Warsaw, Poland. Head: Dr J. Stańczak.

RESULTS

The sCD40 concentration of HCV/HIV-1 infected and HCV was significantly higher compared to healthy subjects (25.7 and 23.2 v. 8.5 pg / ml, p <0.0002 and p <0.001). Also, the concentration of sCD40L was significantly higher in the patients as compared to the control group (12.7 and 7.3 v. 0.79 ng / ml, p <0.0004 and p <0.02) (Fig. 1, Fig. 2). There was no significant difference in the concentrations of sCD40 and sCD40L between the HCV and HCV/HIV-1 infection. The concentrations of sCD40 and sCD40L did not depend on the type of HCV genotypes (Tab. 2). The lowest HCV viral load was observed in patients with genotype CC rs12979860. HCV viral levels differed significantly between patients with genotype CC and CT (p = 0.003) and between CC and TT (p = 0.004) (Fig. 3).

Table 2. The concentration of the sCD40 and sCD40L depending on the HCV genotypes

G1 (n=46)		HCV genotypes	
		G3 (n=14)	
sCD40 pg/dl	x± SE	27.1 3.9	16.6 2.6
sCD40L ng/dl	x± SE	9.7 1.8	5.0 1.6

No correlation was found between HCV viral load, and the sCD40 and sCD40L concentration.

The assessment of the levels of sCD40 and sCD40L with respect to gene polymorphism rs12979860 showed a statistically significant higher levels of sCD40L in patients with the CC genotype compared to patients with Non-CC genotypes (Tab. 3).

Among HCV/HIV-1 infected patients the individuals with rs12979860 CC genotype had lower HIV viral load compared to patients with Non-CC genotypes, yet

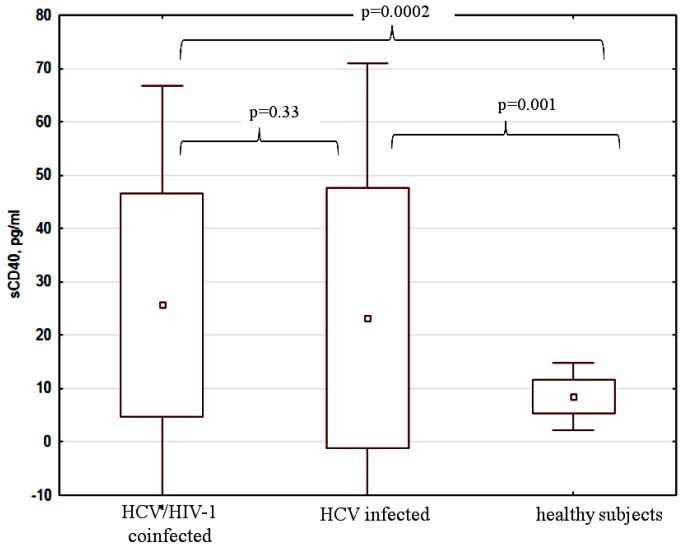

Fig. 1. The sCD40 concentration in serum of the patients and the control group

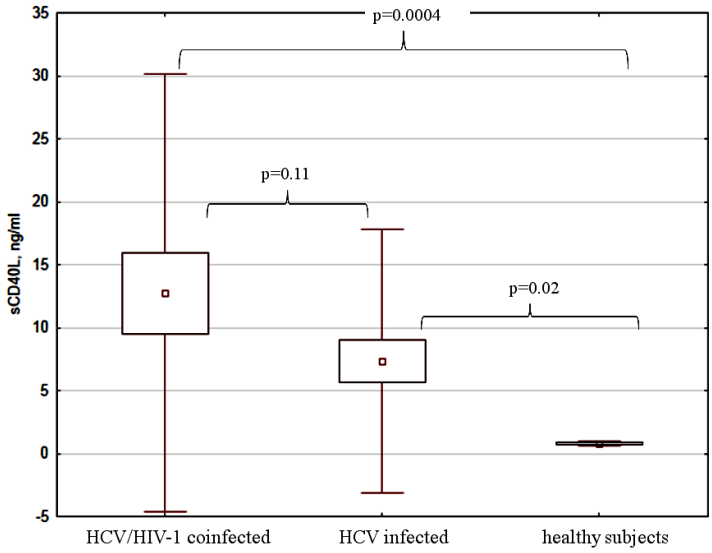

Fig. 2. The sCD40L concentration in serum of the patients and the control group

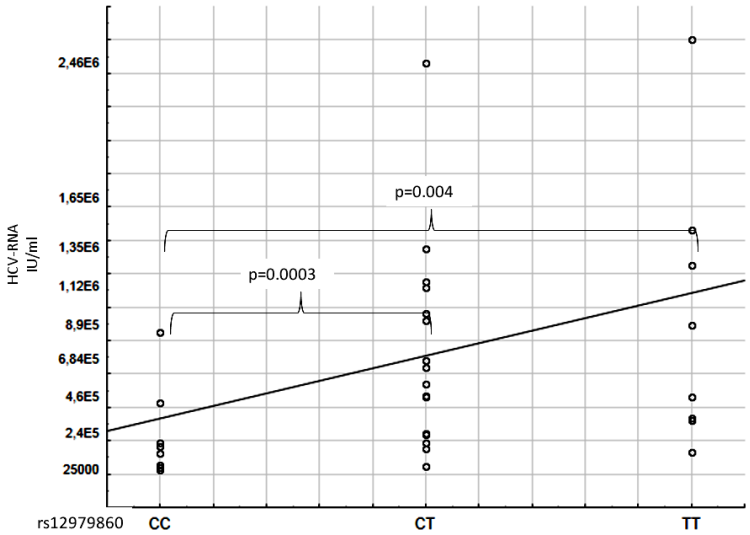

Fig. 3. The HCV viral load depending on the rs12979860 polymorphism

the difference was not significantly greater (CC: 1906 IU / ml v. Non-CC: 6534 IU / ml, p = 0.253).

No correlation between serum sCD40 or sCD40L and the number of CD3+, CD4+, CD8+ was observed

Table 3. The concentration of the sCD40 and sCD40L for the rs12979860 polymorphism

CC (n=23)		gen rs12979860	
		Non-CC (n=45)	
sCD40 pg/dl	x SE±	22.3 3.8	25.3 3.7
sCD40L ng/dl	x SE±	11.8 2.6	7.6 1.5

DISCUSSION

High concentrations of sCD40 and sCD40L of patients infected with HCV and HCV/HIV-1 that were observed in the study indicate that the synthesis of the analyzed proteins might be stimulated bythese viruses, which corresponds with the observations of other authors (4, 5). There was no difference in the concentrations of sCD40 and sCD40L between the HCV or HCV/HIV-1 infection. This result indicates that co-infection with HIV-1 in patients with HCV infection does not affect the higher concentration of sCD40 and sCD40L. No correlation between HIV viral load and sCD40 and sCD40L was observed. This result differs from the observation by Donhauser et al. who observed a decrease in sCD40L levels proportionally to HIV viral load among patients with HIV-1 infection (6). According to our study, no effect of the polymorphism rs12979860 on HIV viral load was noted. Sipsas et al. demonstrated a correlation between the concentration of sCD40L and the number of CD4+ determined at intervals of greater than 500 cells/ml, between 200-500/ml and below 200/ml (5). However, our study showed no correlation between the level of sCD40, sCD40L, and the number of CD4+. Also, the study conducted by Kalayjian et al. on HIV-infected patients demonstrated no correlation between the number of CD4+ and the concentration of sCD40L (7). Furthermore, Davidson et al. showed that HIV-1 can stimulate the increase in sCD40 and sCD40L but viral load does not correlate with the concentrations of the analyzed proteins (8).

Also, Möller et al. observed high levels of sCD40L among HCV infected patients. Ligand concentration decreased during PEG-IFN therapy with RBV and a correlation between CD40L and HCV-RNA levels was observed (4). However, our study showed no correlation of sCD40L with HCV-RNA and HIV-RNA levels. The correlation between sCD40 and sCD40L concentrations, and the level of HCV RNA with respect to the level of HIV-1-RNA at various times of HAART was analyzed prior to the treatment . Our study was performed under different conditions than the test conducted by Möller et al. (the latter evaluated these parameters before, during and after treatment) (4). In our study, the level of HIV-1-RNA was typically very low or undetectable. This is the most probable reason why no difference between the levels of sCD40L and sCD40, and the level of HCV-RNA and HIV-1-RNA was observed.

The studies by Möller et al. showed a high correlation between the concentration of sCD40L and the effectiveness ofthe chronic hepatitis C therapy (4). According to our study, the concentration of sCD40L was significantly higher among patients with the CC genotype compared to patients with genotype non-CC gene rs12979860. The CC genotype is considered to be a predicting factor for the effective treatment of HCV infected patients. In our study, a high concentration of

sCD40L was significantly higher among patients with the CC genotype rs12979860 but did not correlate with the level of HCV-RNA. This result may indicate that CD40L synthesis during agitation related to HCV infection or HCV/HIV-1 is higher in patients with CC rs12979860 compared to the patients with non-CC genotype. There is an indication that a high concentration of sCD40L may be considered a good index of a response to the treatment of chronic HCV infection. However, this result suggests that further trials on the correlation of sCD40L before and after the treatment of HCV infected patients with respect to the rs12979860 polymorphism would be highly useful.

HCV infection affects the increase in sCD40 concentration which may inhibit the synthesis of endogenous interferons. However, sCD40L activates the CD40 receptor that induces STAT system which is independent of IFN antiviral defence (1). High concentrations of CD40L activate antiviral activity and may lead to a spontaneous elimination of HCV (1, 9).

Low viral load HCV in patients with genotype CC rs12979860 may lead to a spontaneous elimination of HCV, particularly in HCV/HIV infected patients (10).

Stimulation of CD40 receptor is also crucial in the activation of autoimmune processes (11). Found in our study, high levels of sCD40 and sCD40L in patients infected with HCV may explain the frequently observed autoimmune reactions in this group.

CONCLUSIONS

High levels of sCD40 and sCD40L can be observed in the serum of patients chronically infected with HCV and HCV/HIV-1 High concentrations of sCD40L correlate with the genotype CC rs12979860. The concentration of sCD40, sCD40L depends neither on the HCV genotypes, HCV and HIV-1 viral load nor on the number of lymphocytes CD3+, CD4+ and CD8+.

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