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THE LEVEL OF CYTOKINES IN PATIENTS WITH CHRONIC VIRAL HEPATITIS C, DEPENDING ON THE IL-28B GENOTYPES

Adilov D. G., Aripova T. U., Ismailova A. A., Rozumbetov R. J.* and Rahimdjanov A. A.

Institute of Immunology and Human Genomics of the Academy of Sciences of the Republic of Uzbekistan, Tashkent.

***Corresponding Author: Rozumbetov R. J.**

Institute of Immunology and Human Genomics of the Academy of Sciences of the Republic of Uzbekistan, Tashkent.

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ABSTRACT

The imbalance in the of cytokines plays a pivotal role in pathogenesis of various diseases, and chronic viral hepatitis C is not an exception. Various cytokines, namely IL-2, IL-4, IL-6, TNF-α, IFN-α and IFN-γ, are studied in chronic viral hepatitis C patients in accordance to their IL-28B gene polymorphisms. Obtained data showed reliable differences between polymorphisms, thus these cytokines profiles can be used to elucidate pathology of CHVC and help in development of novel diagnostic tools.

KEYWORDS: Viral hepatitis C, interferons, interleukins, immunity, interleukin-28 genotypes, disease prognosis.

INTRODUCTION

In the pathogenesis of the immunoinflammatory process, chronicization and progression of hepatitis C virus infection in chronic viral hepatitis C (CHVC), the imbalance in the production of cytokines plays a crucial role, which are key mediators of the inflammatory process, regulate the development of the local immune response and control the general response of the organism to the pathogen.[1,2] Cytokines are directly involved in the development of inflammation, immune reactions and regenerative processes of the liver, and damage to the liver tissue is accompanied by an imbalance in the production of proinflammatory and anti-inflammatory cytokines, which explains the great interest in the study of cytokines, especially in the prognosis of the course of the disease and response to antiviral therapy (AVT). It should be noted that the inactivation of blood cytokines occurs in the liver, therefore, in its pathology, a violation of this mechanism can also cause imbalance of cytokines and immune disorders.[1,3] Numerous data on changes in various cytokines in CHVC and in the dynamics of the cytokine profile on the background of AVT are presented in the literature, but these studies are contradictory. For example, it has been suggested that the breakdown of the liver structure with the development of necrotic and fibrotic changes is associated with the level of production of pro-inflammatory cytokines - interleukin-6 (IL-6) and tumor necrosis factor alpha (TNF-α).[4-6] According to other data, the content of TNF-α significantly increased in 83% of patients with CHVC, regardless of the severity of clinical and biochemical signs.[7] Interleukin-4 (IL-4) is a natural inhibitor of inflammation that suppresses the release of pro-inflammatory cytokines. The lack of consensus on the direction and severity of changes in cytokines dictates the need to study the state of the main cytokines of the immune system, as well as their changes before and on the background of AVT. In this regard, the aim of the study was to assess the levels of the main cytokines of the immune system depending on the expression of IL-28 genotypes in patients with CHVC. It was studied that the IL-28B genotype directly correlated with the differentiated expression of intrahepatic interferon-stimulated genes in patients with CHVC.[9] Also, serum IL-28A / B levels were significantly higher in patients with CHVC with a "favorable" allele of the IL-28B genotype.[13] In 2009, D. Ge et al. found in 19 chromosome a single nucleotide substitution in IL-28B, which, taking into account the localization, was designated as rs12979860. Depending on the nitrogenous base located in this locus, two alleles were isolated: rs12979860 C (cytosine) and rs12979860 T (thymine). It was proved that the frequency of a positive response to antiviral therapy is higher in patients with genotype rs12979860 CC (70.5%) and lower in patients with genotypes rs12979860 CT and TT (32.0% and 23.3%, respectively).[10] The carrier of the T allele, which increases the probability of a negative response to antiviral therapy, is more important than the "protective effect" of the C allele. However, the CC genotype contributes to the elimination of the virus. Determination of the polymorphism of the IL-28B gene made it possible to predict the probability of achieving a sustained virologic response with a sensitivity of 65% and a specificity of 78% for the marker rs12979860 of this gene.[6, 11] The definition of the genetic polymorphism of this marker is most significant for patients with HCV genotype 1, given the lower response rate to standard antiviral therapy. Determination of the genotype IL-28B

is of great importance for evaluating the potential response to antiviral therapy and selecting patients who may have shorter courses of treatment. In general, the polymorphism of IL-28B is one of the factors that makes it possible to individualize the treatment of chronic hepatitis C.[10]

MATERIALS AND METHODS

Collection of material was carried out at the Institute of Immunology of the Academy of Sciences of the Republic of Uzbekistan from 2012 on an outpatient basis on the basis of the Scientific-Diagnostic Center "Immunogen-test" within the framework of the fundamental grant FA-F6-T093 "Immunogenetic mechanisms of resistance to antiviral therapy and their relationship with phenotypic changes in patients with chronic viral hepatitis B and C "(2012-2016). The main group of patients were 84 patients with an average age of 39.5 ± 0.9 years. All patients were with a replicative stage of the disease, previously patients did not receive AVT. A comparable control group included 48 practically healthy (donors) individuals with an average age of 36.8 ± 4.7 years who did not have liver disease. Serum concentrations of IFN-α , Antibodies against IFN-αand IFN-γ , IL-2, IL-4, IL-6, and TNF-alpha were studied. The studies were carried out using the ELISA method using the Vector-Best reagent kit, Russia. The study of IL-28 genotypes was carried out by PCR using DNA technology systems, Russia.

RESULTS AND DISCUSSION

The analysis of the results of the main interferons in the serum of patients with CHVC, depending on the genotypes of the IL-28B gene of CC and non-CC, and TT and non-TT, showed that the serum content of IFN-αin patients with CVH in all groups was significantly lowered. The analysis showed that when comparing IFN-αvalues between groups, we found that the level of IFN-αin the group of patients with genotype CC was 12.5 ± 1.4 pg / ml, in the group of non-CC genotypes - 9.2 ± 1,8 pg / ml, and in the control group - 16,85 ± 3,8 pg / ml. As can be seen, in both groups of patients with chronic hepatitis C, the level of IFN-αwas significantly lower than in the control group, and in the non-CC group, the serum concentration of IFN-αwas the lowest and significantly different from those of patients with the CC genotype. Consequently, in non-CC, a significant decrease in IFN-α was revealed in comparison with CC in 1.4 times. With non-TT, a significant 1.43 fold decrease in IFN-αwas found in comparison with the value of TT. In the non-CC and non-TT patients, serum concentrations of IFN-α were the lowest. According to the level of antibodies to IFN, significant differences were revealed between the groups of patients with CC and non-CC, TT and non-TT genotypes, the highest values of antibodies to IFN-αwere detected in groups of non-CC and non-TT patients, 1,2 and 1,4 times, respectively. IFN-α levels were also significantly lower compared to the control group, and were significantly different. Thus, the serum concentration of IFN-αon the average in the group of patients with CVHC TT genotype was 13.9 ± 1.4 pg / ml in the genotype of the IL-28B gene, while in the group of patients with CHVC, non-TT genotypes were 9.82 ± 0.7 pg / ml, a 1.43 fold difference. Apparently, the trend towards a decrease in serum concentrations of IFN-α in the study groups was quite clearly observed, both in the CC and non-CC patients, and in the groups of patients with TT and non-TT. As mentioned above, in the non-CC and non-TT patients, the serum concentration of IFN-α was the lowest. Unfortunately, we did not find such data in the literature, but there are data that in groups of patients with homozygous alleles a more favorable picture is observed in the study of hematological and immunological features. Further, the level of antibodies to IFN-α was studied. The analysis showed that in the group of patients with CC and non-CC genotypes, the greatest value of antibodies to IFN-α was observed in the group of non-CC patients with genotypes of the IL-28B gene, whereas the analysis of antibodies to IFN-α when studying in groups of patients TT and non-TT, the greatest value was observed in the group of non-TT patients. Thus, in the groups of patients with CC and non-CC values were as follows: 3.8 ± 0.2 pg / ml and 4.4 ± 0.9 pg / ml, respectively, at a control value of 3.4 ± 0.7 pg / ml. In the groups of patients with TT and non-TT: 2.8 ± 0.8 pg / ml and 3.9 ± 1.3 pg / ml, respectively, at a control value of 3.4 ± 0.7 pg / ml. Significant differences were revealed between groups of patients with TT and non-TT genotypes, therefore, analysis showed that the highest values of antibodies to IFN-αwere detected in the group of non-CC and non-TT patients. As is known, different interferons differ in cellular origin. So, IFN-αis produced by monocytes, macrophages, neutrophils and B-lymphocytes.[9,15] The IFN-αinducers are mainly viruses (RNA and DNA-containing).[12,17] It's general knowledge, that the antiviral effect of interferons is associated with their ability to suppress the processes of transcription and translation of the viral genome. The effect of IFN-α on the immune response is manifested by the induction of the cytokines production (in particular, pro-inflammatory).[6,10] Again, it is important to note that in sufficiently high doses IFN suppresses both humoral and cellular immune response, however, in more moderate concentrations have an immunoregulatory effect.[4,5,8,11] Analysis of IFN-γ in the groups of patients with CHVC, depending on the different genotypes of the IL-28B gene, revealed that in the groups of patients with CC and TT, the level of serum IFN-γ was increased in comparison with the value of the control group and with the values of groups of non-CC patients and -TT. We found that in the group of patients with CC and non-CC genotypes, the values of IFN-γ were as follows: 14.2 ± 0.9 pg / ml and 8.82 ± 1.4 pg / ml, respectively. And in the groups of patients with TT and non-TT: 10.6 ± 1.1 pg / ml and 9.2 ± 1.2 pg / ml, respectively, at a control rate of 8.4 ± 0.6 pg / ml. It was established, that activated T-lymphocytes and NK act as a source of IFN-γ (antigens and mitogens act as activators). Among T-lymphocytes, the producers of IFN-γ are primarily cytotoxic CD8 +

and helper CD4 + cells; however, when the latter are differentiated by Th1 and Th2, only TH1 cells retain the ability to produce IFN-γ.[3,4,13,14] It is also important to note that the duration of the synthesis of IFN-γ is greater than that of IL-2. The formation of IFN-γ can be associated with the induction of viruses themselves, as well as the production of IFN-γ is enhanced by it, as well as by IL-2.[8] Among cytokines, the production of IFN-γ is enhanced by the influence of IL-1, in turn, the synthesis of IFN-γ and the differentiation of T-helpers in the direction of Th1 cells are enhanced, as a result of all these humoral interactions, IFN-γ enhances the development of cellular immunity and suppresses manifestations of humoral immune response. Therefore, in the implementation of its effector properties, IFN-γ, promoting a cellular immune response, it plays an important role, especially in the implementation of the cytotoxic effect.[2,4,8,15,16,18], while being a humoral product of cytotoxic T-lymphocytes and NK . Therefore, the analysis of IFN-γ in the groups of patients with CHVC, depending on the different genotypes of the IL-28B gene, revealed that the level of serum IFN-γ was increased in the groups of patients with CC and TT compared to the value of the control group and the values of the non-CC patients groups and non-TT. It was revealed that in the group of patients with CC and non-CC genotypes the difference was 1.6 times, and in groups of patients with TT and non-TT, 1.3 times. So, we studied IL-2, IL-4, IL-6, TNF-alpha. The data obtained will be presented below in Table 1. The analysis showed that the serum concentration of IL-2 normally averaged 4.26 ± 0.5 pg / ml, whereas in the patients with the CC and non-CC genotypes, the level of IL-2 was increased when compared with the control group, but a reliable the difference was observed in the group of patients with CHVC with the CC genotype. Thus, the level of IL-2 in the group of patients with CC was 8.6 ± 1.2 pg / ml, while in the group of non-CC genotypes - 5.4 ± 1.2 pg / ml. As can be seen, the greatest value of IL-2 was observed in the group of patients with the CC genotype. With regard to the study of IL-2 in the genotypes of TT and non-TT, it can be seen that the same pattern was observed here. Thus, IL-2 in the group of patients with TT and non-TT genotypes was 9.4 ± 0.8 pg / ml and 6.54 ± 1.3 pg / ml, respectively. Apparently, there was no significant difference between the groups, but there was a tendency for IL-2 to increase in the group of patients with the CC genotype. Analysis of serum concentration of IL-4 revealed a significant increase in its level in the groups of patients with non-CC and non-TT genotypes. A significant difference was revealed between the groups of patients with CC and non-CC genotypes, and no difference was found in TT and non-TT groups. It was revealed that IL-4 in the group of patients with CHVC in the group of patients with the CC genotype was increased 1.2 times, and in the group of patients with non-CC genotypes - 1.9 times. Also, it was revealed that in the group of patients with TT genotype the level of IL-4 was increased by 1.3 times in relation to the control group, and by 1.7 times in the group of patients with non-TT genotypes. It is known that IL-4 belongs to anti-inflammatory cytokines. Speaking about proinflammatory and anti-inflammatory cytokines, it should be said that this division is rather relative. But despite this, it is commonly believed that the normal functioning of the immune system is built on the balance of Th1 and Th2-lymphocytes. Thus, the activation of Th1-lymphocytes promotes the production of IFN-g, TNF-α, etc., which leads to stimulation of T-lymphocyte and macrophage functions and the development of a cellular immune response that plays an important role in antiviral protection. And Th2 lymphocytes produce IL-4, IL-10, etc., which stimulate the predominantly humoral immunity.[3,6,9,12,]

Table 1: The state of the main cytokines of the immune system in patients with CHVC, depending on the genotypes of the IL-28B gene, pg / ml.

Cytokines	Control group	rs 12979860		rs 8099917	
		CC	Non-CC	TT	non-TT
IL-2, pg/mL	4,26±0,5	8,6±1,2 *	5,4±1,2	9,4±0,8	6,54±1,5
IL-4, pg/mL	3,8±0,6	4,38±0,7	7,2±0,5*	4,8±1,2	6,4±0,7
IL -6, pg/mL	3,6±0,45	9,8±0,8	12,7±1,1*	10,2±1,2	14,6±0,9*
TNF-α, pg/mL	4,5±0,8	12,5±0,6	13,5±0,82	11,9±0,7	15,6±1,1*

Note: * - the reliability of differences between the groups of CC and non-CC, TT and non-TT ($p < 0.05$)

Analysis of IL-6 in the serum of peripheral blood showed that its level was increased in all groups of patients with chronic hepatitis C, regardless of genotypes of the IL-28B gene. But in the analysis it was revealed that the highest values of IL-6 were detected in the groups of patients with CHVC non-CC and non-TT. And in the groups of patients with CC and TT, the concentration of IL-6 was also increased and significantly differed from the values of non-CC and non-TT patients. It was revealed that IL-6 in the group of patients with CHVC in the group of patients with CC genotype was increased 2.7 times, and in the group of patients with non-CC genotypes - 3.5-fold. Also, it was revealed that in the group of patients with TT genotype, the level of IL-6 was increased 2.8-fold in comparison with the control group, and 4-fold in the group of patients with non-TT genotypes. Analysis of IL-6 concentrations between groups of patients with CC and non-CC, TT and non-TT showed that the serum IL-6 concentration in the group of patients with CHVC with CC genotypes was increased 1.3 times in relation to the value of patients with the CC genotype. A serum concentration of IL-6 in the group of

patients with CHVC with TT genotypes was increased by 1.4 times with respect to the value of patients with non-TT genotypes. It is described that IL-6 contributes to exacerbation of chronic and chronic inflammatory processes. IL-6 is related to cytokines completing inflammatory reactions.[10] In the immune system, the main target of IL-6 is B-lymphocytes which promote the differentiation of cytotoxic T-lymphocytes.[16,17] Further, the serum concentration of TNF-alpha in the groups of patients with CHVC was studied depending on the genotypes of the IL-28B gene. The analysis showed that the serum concentration of TNF-alpha was significantly increased in all the study groups of patients with CVH. The results obtained are presented in Table 1. Thus, we found that the level of TNF-alpha was elevated in the group of patients with the CC genotype in comparison with the level of the control group by 2.8 times, and in the non-CC group - by 3-fold. In the group of patients with the TT genotype of the IL-28B gene, TNF-alpha was increased 2.6-fold, in the group of non-TT patients by genotypes - 3.5-fold. As can be seen, the largest value of TNF-alpha in the serum was observed in the group of patients with non-CC and non-TT genotypes. An important effect of TNF-alpha in the study of the pathogenesis of CHVC is that it is able to lyse virus-infected cells[17], with cytolytic action. Thus, the study of the state of the main interferons and cytokines of the immune system made it possible to reveal that a significant decrease in IFN-α was detected in non-CC, compared with 1.4-fold in CC. Also, with non-TT, a significant decrease in IFN-α was found in comparison with the value of TT in 1.43 times. In the non-CC and non-TT patients, serum concentrations of IFN-α were the lowest. In the level of antibodies to IFN, significant differences were found between groups of patients with CC and non-CC, TT and non-TT genotypes. Thus, the highest values of antibodies to IFN-α were revealed in groups of non-CC and non-TT patients in 1,2 and 1,4 times, respectively. Analysis of IFN-γ in groups of patients with CHVC, depending on the different genotypes of the IL-28B gene, revealed that the level of serum IFN-γ was increased in groups of patients with CC and TT compared to the values of the groups of non-CC and non-TT patients. It was revealed that in the group of patients with CC and non-CC genotypes the difference was 1.6 times, and in the groups of patients with TT and non-TT, 1.3 times, respectively. The level of IL-2 in the group of CC patients was increased 1.6 times as compared with the value of IL-2 in the non-CC group. As can be seen, the greatest value of IL-2 was observed in the group of patients with the CC genotype. IL-2 in the group with the TT genotype was increased 1.44 times compared with the value of the non-TT group. Analysis of serum concentration of IL-4 revealed a significant increase in its level in the groups of patients with non-CC and non-TT genotypes. It was revealed that IL-4 in the group of patients with CHVC CC genotype was increased 1.6 times in comparison to the non-CC group. And when comparing between TT and non-TT groups, no significant difference was found. The maximum value of IL-6 was detected in the groups of patients with CHVC non-CC and non-TT. Thus, IL-6 was increased in the non-CC group by 1.3 times, and in the TT group - by 1.4 times with respect to the CC and TT groups. The analysis showed that the serum concentration of TNF-alpha was increased in all the study groups of patients with CHVC. It was revealed that the level of TNF-α was increased in the group of patients with the non-TT genotype by 1.3 times in comparison with the value of TT. When comparing TNF-α data between the CC and non-CC, no significant differences were found.

CONCLUSION

Since each ethnic group has immunological specificities, it's important to conduct researches in home population. Here we analyze various serum cytokines, namely IL-2, IL-4, IL-6, TNF-α, IFN-α and IFN-γ, in CHVC patients according to IL-28B gene polymorphisms. Obtained data showed reliable differences between polymorphisms, thus these cytokines profiles may be used to elucidate pathological mechanism of CHVC and help to work out novel diagnostic tools.

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