ENGLISH VERSION: THE EFFECT OF THE POLYMORPHISM OF THE INTERLEUKIN-28 GENE ON THE EFFECTIVENESS OF THE ANTIVIRAL THERAPY IN PATIENTS WITH CHRONIC EPSTEIN-BARR VIRUS INFECTION*

Sorokina O.G., Popov M.M., Liadova T.I.

V.N. Karazin Kharkov National University, faculty of medicine

Department of general and clinical immunology and allergology,

Mechnikov Institute of microbiology and immunology, Kharkiv, Ukraine

Among many factors that directly affect the immune system, herpes viruses deserve special attention. Currently, Epstein-Barr virus (VEB) is recognized as an etiological agent of not only infectious mononucleosis, limphoadenopathy syndrome, but also oncological processes such as nasopharyngeal carcinoma, Burkitt lymphoma, T- and B-cell lymphoma in HIV-infected people. The aim of this work was to study the effect of the polymorphism of the interleukin-28 gene on the effectiveness of the antiviral therapy in patients with chronic VEB-infection. We examined 92 patients with chronic forms of the VEB: 38 patients (41.3%) had genotypes CC (locus rs12979860 IL-28B); 32 patients (34.7%) with the CT genotype; 22 patients (23.9%) with the TT genotype. As a result of this work, it was found that in patients with chronic VEB-infection who had the genotype CC and CT, the high effectiveness of antiviral therapy is much more often noted. Given the high effectiveness of valacyclovir in these patient groups, it is advisable to further recommend patients to adhere to the treatment regimen used. In patients with the TT genotype, in most cases had low effectiveness of antiviral therapy and it is possible to think that to improve the effectiveness of therapy of such patients it is necessary to supplement the used treatment with immunomodulating medications.

Key words: infectious mononucleosis, Epstein-Barr virus infection, antiviral therapy, genotype, interleukin-28, gene polymorphism.

Currently, the infectious disease prevails in human pathology and, as WHO predicts, the role of infection in the structure of the overall morbidity will increase every year. [1, 4, 11, 15, 17]. The new century is a century of opportunistic infections, due to the growing influence of environmental factors on the body and, above all, on the immune system [2, 7, 8, 16]. Among the numerous factors that directly affect the immune system, the viruses of the herpes family deserve special attention [3, 5, 10, 13, 14]. The achievements of laboratory and, above all, molecular diagnostics have increased the probability of detection of this infection and indicate a steady increase in the number of infected adults and children [6, 12, 19]. The incidence of infectious mononucleosis (IM) over the past decade has increased 4-fold [20].

Epstein-Barr virus (VEB) is now recognized as an etiological agent not only for IM, lymphoadenopathy syndrome, but also for oncological processes such as nasopharyngeal carcinoma, Burkitt's lymphoma, T- and B-cell lymphomas in HIV-infected patients [17, 21].

Until recently, IM was considered a self-limiting disease, as the development of a clinico-pathogenetic manifestation of this infection is a benign lymphoproliferative process [18]. However, at the moment there is already a sufficient number of works covering the issues of immunopathology in IM, which made it necessary to revise the attitude towards this disease as to an absolutely benign one and to prove the possibility of its protracted and chronic course [14].

It is now known that IL28A, IL-28B and IL-29, also called interferon-lambda (INF-λ) 2,3 and 1, respectively, belong to the family of class 2 cytokines and are a newly discovered group of antiviral cytokines. INF-λ induces antiviral, antiproliferative, antitumor and immune effects. In comparison with INF-α, INF-λ family proteins have less antiviral activity in vitro [21]. There have been studies showing that INF-λ3 inhibits viral hepatitis C (HCV), depending on the dose and time of exposure, increases the expression of interferon-stimulated genes, and increases the antiviral activity of INF-α [20].

The IL-28B gene is localized in the chromosome 19q13. Polymorphic loci were found near it (rs12979860, rs8099917). This gene encodes the INF-λ-3 protein, which is a class II cytokine receptor ligand. IL28B triggers JAK (signal transduction and activator of transcription), a signaling cascade that transmits information from extracellular polypeptide signals to target gene promoters, blocking the synthesis of viral proteins [14].

Single nucleotide polymorphism (SNP, from English Single nucleotide polymorphism, SNP) is a DNA sequence of one nucleotide in the genome of one species or between homologous regions of homologous chromosomes.

The region of DNA in the regulatory region of the IL28B gene in which the cytosine (C) nucleotide is replaced with thymine (T) is designated as the genetic marker rs12979860 (designation of the NCBI arrester). There are the following possible genotypes: C/C, C/T and T/T.

At present, there are already studies showing that INF-λ proteins are important for the elimination of HCV, but their role in patients with chronic infection caused by the EBV has not been studied to date. Also, the issues of therapy of IM remain not fully understood. Treatment of patients with herpesvirus infection is a difficult task. The reason for this is a complex strategy of parasitism, opportunistic properties of pathogens, multiple organ failure, the presence of numerous complications and the multifactorial nature of some lesions. The leading place among etiotropic approaches in the treatment of patients

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with chronic VEB-infection (CEBV) is antiviral therapy. The use of acyclic guanosine analogues in herpes virus infections corresponds to the level of evidence A. For the treatment of the patients we examined, we chose valaciclovir as an antiviral drug. This drug is a valine ester of acyclovir. This so-called prodrug, which is converted into an active substance acyclovir under the influence of the intestinal and hepatic enzyme valacyclovir-hydroxylase. Due to the modification of the molecule, an increase in the bioavailability (bioavailability) of the drug is achieved, which is 3-5 times higher than that of acyclovir and is 54-70%. Therefore, valacyclovir can be used less often (2-3 times a day), which makes the therapy for the patient more convenient. The drug is usually well tolerated, side effects are rarely noted [5, 7, 9, 18].

Aim. The aim of this work was to study the effect of the polymorphism of the interleukin-28 gene on the effectiveness of the antiviral therapy in patients with CVEB.

Materials and methods

We examined 92 patients with CVEB, the main clinical manifestations of which were various immunopathological and immunodeficiency states. 38 patients (41.3%) were with the CC genotype (IL-28B rs12979860) (they were included in the I observation group); 32 patients (34.7%) with CT genotype (they made up II group of observation); 22 patients (23.9%) with TT genotype (they made up the III group of observation). The age of the patients was 19 to 65 years (mean age 30 years ± 10.7 years), among them women made up 63.0% (n = 58), men - 37.0% (n = 34).

The work was performed at the Department of General and Clinical Immunology and Allergology of the Medical Faculty of the V.N. Karazin Kharkov National University and clinical bases of the Department of the Regional Clinical Infectious Hospital of Kharkiv and the Urban Polyclinic № 6, as well as on the basis of the Mechnikov Institute of microbiology and immunology in the period 2014-2017. in the framework of the research topic: "The study of the role of immune, autoimmune and metabolic disorders in the pathogenesis and outcomes of the herpesvirus-infectious process", State Registration No. 0112U005911.

The analysis was taken and their technical performance was carried out in the clinical and diagnostic laboratory of the Regional Clinical Infectious Disease Hospital, part of the analyzes were performed in the laboratory of Virola. In the process of confirming the diagnosis, the patients underwent a general blood test, a complex of molecular genetic and serological studies.

To determine the polymorphism of the genes, the method of PLRF (polymorphism of the length of restriction fragments) and the real-time PCR method using the Rotor-Gene-3000 amplifier from Corbett Research and the DT-96 detecting amplifier of the DNA technology firm were used.

To determine the allelic variation of the IL28B gene, a commercial DNA-technology test system was used. To amplify the investigated polymorphisms, amplification of certain sections of the corresponding genes was carried out.

To detect point mutations of SNP 39738787C> T (rs12979860) of the IL-28B gene, the polymerase chain reaction and polymorphism of restriction fragment lengths were used. The material used for the study was DNA obtained from leukocytes using commercial reagents for DNA isolation from the clinical material "Citolysin" by AmpliSens (Russia). Detection of polymorphism of the gene IL-28B rs12979860 was performed by real-time PCR on the detecting DT-96 amplifier of the DNA technology firm. Automatic detection of amplification results was performed by the DT-96 instrument. PCR was performed in a volume of 35 μl in a solution of the following composition: 20 μl primer solution, 10 μl Taq polymerase and buffer mixture, and 5 μl DNA.

All patients received valaciclovir 1000 mg 3 times a day for 14 days as an antiviral therapy, then 500 mg 3 times a day for 14 days. If complete elimination did not occur by this time, patients were recommended to continue taking valacyclovir in maintenance doses (500 mg 1 g / day.) for 3-6 months. After the treatment in cases where there was a complete lack of replicative activity of VEB in both blood and saliva, the effectiveness of antiviral therapy was considered high (HEAVT). The average effectiveness of antiviral therapy (AEAVT) was considered when the replicative activity of the VEB was recorded in the saliva and was absent in the blood. Low effectiveness of antiviral therapy (LEAVT) was considered when both the blood and saliva continued to maintain the replicative activity of VEB, but it tended to decrease.

When writing the article, the international standards recommended by the ethics committee were observed.

Data processing. Statistical processing of the results of the study was carried out using the STATISTICA 10.0 statistical software package. The processing of the results was carried out in accordance with the recommendations for the statistical processing of biomedical data. For each variational series, the arithmetic mean (M), the mean square deviation (σ) and the mean error of the arithmetic mean (m) were calculated. The probability of the difference in the mean values in the compared groups (p) was estimated using the Student-Fisher test (t). To determine the predictive significance of the calculated indicators that were studied, the Pareto analysis was used. To determine the correlation dependencies between certain indicators, the Pearson correlation coefficient (r) and the correlation probability (p) were used. If r = 0, then there is no correlation relation; from 0,1 to ± 0,29 - weak; from ± 0,3 to ± 0,69 - moderate (average); from ± 0,7 to ± 0,99 - expressed (strong), ± 1 - full. For the analysis of qualitative data, we used the Pearson χ2 conjugacy tables. The critical level of statistical significance (p) was 0.05.

Results and discussion

An analysis of the results of the work performed revealed statistically significant differences in patients' adherence to antiviral therapy in the study groups (Table 1). Thus, in patients with the CC genotype (IL-28B rs12979860), the high efficacy of antiviral therapy (HEAVT) was significantly more frequent, in 60.5% of cases (23 patients), which was manifested by rapid improvement of well-being and disappearance of complaints; in this group of patients there was complete reconvalescence and the presence of only the capsid (nuclear) IgG antigen, which indicates the transferred infection. In this group of patients, in the majority of cases, the results of a study on VEB in the blood and saliva were negative in the case of a PCR study.

Table 1

Comparison of the effectiveness of the antiviral therapy in patients with chronic VEB-infection with different genotypes IL-28

IL-28B rs12979860	High efficacy of antiviral therapy		Average level of effectiveness of antiviral therapy		Low efficacy of antiviral therapy		Significance level,
	n	(%)	n	(%)	n	(%)	p
CC	23	60,5	11	29,0	4	14,3	0,001
CT	17	53,1	10	31,3	5	15,6	p>0,05
TT	7	31,8	6	27,3	9	40,9	p>0,05

In 29% of cases (11 patients) - in patients with the CC genotype, the average level of effectiveness of antiviral therapy (AEAVT) was noted, complaints and symptoms of the disease went less rapidly than in patients with HEAVT. In some patients, a PCR test revealed a high level of EBV in the saliva, but the blood was not detected in all the patients, which indicates a low level of viremia, which is not detected in some patients by the amplification system."

Relatively less frequently in patients with the CC genotype, there was a low effectiveness of antiviral therapy (LEAVT) - 4 patients (14.3%); In such patients, complaints and symptoms of the disease persisted for a long time, a complex of early antigens was detected in the blood; as a result of a laboratory test using the PCR method, antibodies most often showed a high level in the blood and saliva. Patients with HEAVT genotype were registered in 17 patients (53.1%), AEAVT in 10 patients (31.3%), and LEAVT in 5 patients (15.5%)

In patients with the TT genotype, HEAVT was significantly more frequent in 40.9% of cases, whereas AEAVT and LEAVT were significantly less frequent in 31.8% (7 patients) and 27.3% (6 patients), respectively.

Conclusions

In patients with chronic VEB-infection who had the genotype of CC and CT, HEAVT is more reliably detected. Given the high effectiveness of valacyclovir in these patient groups, it is advisable to further recommend patients to adhere to the treatment regimen used. Patients with the TT genotype in most cases had LEAVT and it can be assumed that to improve the effectiveness of therapy of such patients it is necessary to supplement the used treatment with immunomodulating medications

Prospects for further research

The next direction of our research will be work on optimization and increasing the effectiveness of antiviral therapy in patients with CVEB, depending on the patient's genotype.

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