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Recombinant Interferon Gamma in the Treatment of Chronic Epstein-Barr Virus Infection

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Abstract

In immunocompetent individuals, Epstein-Barr virus (EBV) causes the development of a chronic infection that is characterized by chronic or recurrent infectious mononucleosis-like symptoms and persists for a long time. 93 patients with chronic Epstein-Barr virus infection (EBVI) were examined (mean age 35.56 ± 2.11 years). The patients were divided into three groups for different treatment regimens: 1 group (n = 34) - Ingaron therapy (500.000 IU intramuscularly, every other day, № 10); Group 2 (n = 33) - Valtrex (500 mg x 2 times/day, oral, 2 months); Group 3 (n = 26) - Valcyte (450 mg x 2 times/day, oral, 2 months) and Ingaron. In the 1st and 2nd groups, negative results of PCR during therapy were obtained in 29.41% and 27.27% of patients, respectively. In group 3, a negative PCR result was obtained in 73.07% of patients. In group 3, the combination Valcyte + Ingaron was different: Group 3a received Ingaron 500,000 IU intramuscularly, every other day, №10; Group 3b - Ingaron 500,000 IU intramuscularly, every other day, №20; 3c group - Ingaron 500,000 IU intramuscularly, every other day, №10 + 100,000 IU intramuscularly, every other day, №15. The best result was obtained in group 3c in 90% of patients.

The maximum effect of therapy is due to combination of Valcyte and Ingaron preparations, the duration of Ingaron administration.

Keywords: *Chronic EBV Infection; Monotherapy; Combined Antiviral Therapy; Antiviral Immune Response; Interferon-Gamma; Ganciclovir; The Number of Copies of DNA*

Abbreviations

EBV: Epstein-Barr Virus; CEBVI: Chronic Epstein-Barr Virus Infection; DNA: Deoxyribonucleic Acid; IFN-γ: Interferon-γ; IFN-α: Interferon- α; PCR: Polymerase Chain Reaction; TK: Thymidine Kinase

Introduction

With the development of immunodeficiency in a patient, a persistent and/or chronic herpes virus infection develops, in which the pathogen is not eliminated from the host's body for months, years, or even the whole life. Each herpesvirus in the body has its own target tissue, where the virus persists with the ability to enter and exit tissue using the strategy developed by it, which consists in minimal expression of viral genes in a small number of cells or their elimination at the protein level. This allows the virus to evade the immune

response and persist in a very small amount (1 infected cell per 5 ml of blood) with minimal impact on the patient's body, remaining for the rest of his life. In this case, the immune response is not able to eliminate the infectious pathogen from the body. It also depends on the persistence of the virus through the latent phase or the rapid mutation with the transition to less virulent forms that persist for a long time in the host organism. In EBV infection, reactivation is accompanied by the resumption of viral replication and, finally, shedding of the virus for a long time, which induces the development of the chronic viral disease.

In 1988, Straus S.E. described in immunocompetent individuals the development of the chronic Epstein-Barr virus infection (EBV infection), which is characterized by recurrent infectious mononucleosis-like symptoms for a long time, accompanied by high production of IgG antibodies to capsid (VCA) and early antigen (EA), low production or absence of antibodies to the nuclear antigen (EBNA) [1]. However, according to other authors, abnormally high antibody titers associated with EBV may be absent [2]. Earlier in 1983, Hellman D., *et al.* for the first time they proposed an abbreviation for this syndrome, "chronic active VEB infection" [3].

EBV spreads through contact with saliva and penetrates the epithelium that lines the nasopharynx. Waldeyer's ring, which includes the adenoids, tonsils and lymphoid system surrounding the nasopharyngeal region, forms a continuous structure called the lymphoepithelium [4]. After infection of epithelial cells, the virus replicates and further infects resting naive B cells in nearby areas through the activation of latent proteins encoded by the growth program. As a result, the cell enters a state of proliferating lymphoblast (lymphoblastic explosion)**.** During primary infection, EBV attaches to B-cells by binding gp350/220 EBV to CD21 and gH/gL/gp42 to HLA class II molecules on the cell membrane. Binding of two viral proteins to receptors allows EBV to penetrate into B-cells. As a result, a lifelong infection occurs, that is, EBV is latently stored in memory B-cells at a low viral level (~ 1 per 10000-100,000 B-cells). To understand the complex biology of EBV, two biological models were proposed: germinal center (GC) model [5] and direct infection model [6]. The GC model remains the only consistent model for explaining the varied biology of EBV and suggests that EBV persists in latently infected naive lymphoid tissue B cells of Waldeyer's ring, which undergo differentiation stages, each of which uses a program of discrete viral gene transcription. At the first stage, the virus expresses all nine latent proteins, this is called a program of latency 3 or growth transcription. These cells then move to the GC, where EBV expresses a more limited structure of hidden proteins, called latency 2 or the default program. Over time, these cells remain as latently infected memory B cells, either express only the viral genomic protein EBNA1 (known as the EBNA1 program or latency 1), or do not have any viral proteins at all. The latency 0 or the latency program further develops. The B-cell compartment of memory is considered to be the site of long-term EBV persistence, which is dormant and invisible to the host's immune response. At any time, a small subpopulation of latent EBV-infected B-cells of memory initiates lytic reactivation in combination with terminal differentiation signals [7]. Reactivation of the virus is divided into three discrete phases: 1) immediate early, when transcription factors are expressed during the formation of proteins involved in viral DNA replication; 2) late, when viral DNA and structural proteins are assembled into virions [8]; 3) final, which leads to the release of the virus, infection of new naive B cells, thereby completing the cycle. The second model [6] suggests that EBV directly infects B memory cells. However, evidence to explain the mechanism of this model has not yet been received. The extravasation of naive B cells proceeds continuously from the peripheral circulation to the secondary lymphoid tissue through the upper endothelial vesicles that are in the lymphoepithelium, then migrate to the mantle zone of the follicles below the epithelium and remain there for several days, and then return to the circulation, and subsequent infection of the new epithelium B cells occur in the intraepithelial layer [9]. Thus, B memory cells are the site of long-term viral persistence, where the virus can remain whole the patient's life. This happens because immunological memory is formed, and the virus ceases to be pathogenic for the host by inhibiting genes that induce cell proliferation and the development of a neoplastic disease. The oral cavity and peripheral blood are important anatomical sites of localization and persistence of EBV infection. In peripheral blood and Waldeyer's ring, the level of infected B-cells in the population ranges from 5 to 3000 in every 107 B-cells of memory (110/107 and 175/107, respectively). That is, the level of infected cells is similar between peripheral blood and Waldeyer's ring, but 20 times lower than in other lymphoid tissue (spleen and mesenteric lymph node) [10]. The virus constantly seeps into the oral cavity, which is a reservoir for EBV flow and where the virus mixes with saliva for about 2 minutes before swallowing.

Interferon-γ (IFN-γ) is the most powerful pleiotropic cytokine, produced by many immune cells in response to interleukin-12 (IL-12), as well as microbial antigens, stimulates the immune response by regulating the production of several cytokines and chemokines. Interleukin-12 enhances production and increases the expression of IFN-γ by activating a signal converter and the transcription activator 4 (STAT4). With the development of viral infection, IFN-γ stimulates various antiviral mechanisms: induces the synthesis of antiviral proteins, including IFN type I, with direct antiviral activity, dsRNA-specific adenosine deaminase (ADAR), inhibiting viral replication by editing viral RNA and causing disruption of viral protein synthesis, enhances the expression of FasL, which mediates apoptosis of virus-infected cells. IFN-γ involves both innate and adaptive immunity cells in the immune response, ensuring the effectiveness of virus elimination processes [11]. There are five main ways in which viruses bypass the IFN response, namely [12]: (1) interfere with the expression of the host cell gene and/or protein synthesis; (2) minimize IFN induction by limiting the production of viral PAMP and/or by specifically blocking the IFN induction cascade; (3) inhibit IFN signaling; (4) block the effect of IFN-induced antiviral enzymes; (5) form a replication strategy that is (largely) insensitive to IFN action. Thus, by blocking the transmission of IFN signals and without restricting the production of IFN, the virus can slowly spread from infected cells to neighboring cells, inactivating specific IFN-induced proteins with antiviral activity.

For patients with herpes virus infection, a marked impairment of IFN-α and IFN-γ products is characteristic. Lotz M., *et al.* studied the effect of all 3 classes of recombinant human IFN on EBV infection of purified B-lymphocytes and the secretion of immunoglobulins (Ig) in patients with rheumatoid arthritis. All 3 types of IFN inhibited the dose-dependent EBV-induced activation of B-cells. However, in patients with rheumatoid arthritis, in order to achieve 50% inhibition, a higher dose of each class of IFN is necessary. Introduction of IFN-γ 3-4 days after infection had the strongest effect on reducing EBV-induced B-cell proliferation and spontaneous B-cell activation in patients when compared with IFN-α and IFN-β [13]. The authors concluded that at an early stage EBV-infected cells can be regulated by all IFNs, and further, in the intermediate period, only IFN-γ can directly affect EBV-induced B-cell response, showing 7 - 10 times stronger antiviral effect than IFN-α or -β. During the third phase, B-lymphocytes become insensitive to the direct effects of all interferons and are regulated only by cytotoxic cells [13]. In 1993 and 1996, positive results of treatment of severe EBV infection with the introduction of recombinant IFN-γ were published [14,15].

In the Russian Federation, the only IFN-γ drug registered under the trade name Ingaron, developed by Pharmaclon Ltd. (Russia, 123557, Moscow, Presnensky Val, 17) by microbiological synthesis in recombinant strain E. Coli and purified by column chromatography. Ingaron consists of 144 amino acid residues, devoid of the first three of them - Cys-Tyr-Cys, replaced by Met.

Purpose of the Study

The purpose of this study is to evaluate the effectiveness of Ingaron therapy on the content of the number of copies of EBV DNA in saliva samples by PCR and on the dynamics of clinical complaints one month after the end of therapy in patients with CEBVI.

Patients and Methods

93 patients with chronic Epstein-Barr virus infection (CEBVI) were examined. The average age of patients was 35.56 ± 2.11 years. There were 56 women, 37 men. The duration of CEBVI from the moment the first complaints appeared in the patient to the laboratory confirmation of EBV infection and diagnosis was 2.17 ± 0.24 years. In 62 patients (66.67%) in childhood there was a repeated exacerbation of chronic tonsillitis; 22 patients (23.65%) suffered acute infectious mononucleosis, and 6 patients (6.45%) suffered from exacerbation of chronic rhinopharyngitis, 72 patients (77.42%) had frequent acute respiratory viral infections (5 to 10 times a year), 78 patients (83.87%) associated the appearance of clinical complaints with prolonged stress. All patients had no immunological disorders or other infections that could explain the complaints at the time of the study, as well as any chronic diseases that could affect the results of the study. The study did not include patients who received antiviral therapy for the last 3 months.

CEBVI is characterized by a long course and frequent relapses with clinical and laboratory signs of viral activity [16]. Patients are worried about long subfebrile condition (37.1 - 37.3°), weakness, unmotivated fatigue, increased sweating (especially at night), chills,

constant feeling of discomfort and/or pain in the throat, lymphadenitis, swelling of the nasal mucosa with abundant draining mucus on the back of the pharynx, stomatitis, skin rashes, arthralgia, pain in the muscles of the trunk and extremities, feeling of heaviness in the right hypochondrium. Often develop neurological disorders: headaches, sleep disorders, loss of memory and concentration, irritability, tearfulness, a tendency to depression. According to an abdominal ultrasound in some patients, an increase in the spleen and/or liver is detected. Clinical research methods included anamnesis, data on previously conducted antiviral therapy, the presence of concomitant diseases. The clinical condition of the patients was assessed according to the generally accepted method, including objective data and patient complaints at the time of the examination. Registration of patient complaints was carried out using the subjective assessment scale on a 3-point scale (0 - no symptoms, 1 - mild symptoms, 2 - moderate symptoms, 3 - significant symptoms). All patients were divided into three groups for different regimens. The 1st group consisted of 34 patients (from 22 to 52 years old) who received Ingaron therapy (500.000 IU every other day intramuscularly №10), the 2nd group consisted of 33 patients (from 22 to 49 years old) who received prolonged treatment with a drug from the group of acyclic nucleosides - Valtrex (500 mg x 2 times a day, orally) for two months. The 3rd group consisted of 26 patients (from 19 to 58 years old) who received prolonged therapy with a synthetic nucleoside analogue of guanosine - Valcyte (450 mg x 2 times a day, by mouth) for 2 months in combination with Ingaron. Previously, these patients were prescribed by a doctor or independently (often repeatedly) received therapy with drugs from the group of acyclic nucleosides (Valtrex) with short courses (7 - 10 days). There was no clinical laboratory benefit from previous therapy. For this reason, Valcyte was prescribed to patients in the third group in combination with Ingaron. To assess the effectiveness of the treatment one month after the end of the course of therapy in each group of patients, an analysis was made of the dynamics of EBV DNA in samples of saliva and clinical complaints.

In order to confirm the viral etiology of the disease, the virus was detected by PCR in saliva samples in patients. Quantitative determination of Epstein-Barr virus DNA in saliva samples was performed by the method of polymerase chain reaction (PCR) with real-time hybridization-fluorescence detection. Used test systems "AmpliSense EBV/CMV/HHV6-screen-FL" (FBUN Central Research Institute of Epidemiology, Russia). The units used to assess viral load during DNA extraction from saliva are the number of EBV DNA copies per ml of sample (CPDNA). This indicator is calculated by the formula from the instructions for the set: CPDNA = CDNA x 100 (copies/ml), where CDNA is the number of EBV DNA copies in the DNA sample. The analytical sensitivity of the test system is 400 copies/ml.

Statistical analysis of the results was carried out using the statistical software package IBM SPSS Statistics, version 26. Group results are presented as mean ± standard error of the mean (M ± Standard Error). Statistical processing of the results was carried out using parametric (Pearson method) and non-parametric (Tau (τ) Kendall) criteria. To determine the prognostic significance of the number of copies of EBV DNA, we used a regression linear analysis with the calculation of the coefficient of determination (R Square) and the Durbin-Watson criterion to verify compliance with the independence of observations, analysis of variance (ANOVA "Analisis of Variance") with the calculation Fisher criterion (F) to test the significance of the model. The standardized beta (ß) was also calculated with a 95% confidence interval. The critical level of significance differences of indicators was assumed to be 0.05.

Results

Before the start of therapy, fluctuations in the number of copies of EBV DNA per ml of sample in the total group of patients (93 patients) were from 1.26 x 104 to 9.96 x 105 copies. When analyzing the dynamics of the number of copies of EBV DNA in all groups of patients one month after the end of therapy, the following results were obtained (Table 1).

As can be seen from the data presented in the 1st group with Ingaron therapy, 29.41% of patients received negative PCR results. In the 2nd group of patients while receiving Valtrex, negative PCR results were obtained in 27.27% of patients. In the 3rd group, after receiving combination therapy (Valcyte + Ingaron), a negative PCR result was obtained in 73.07% of patients. However, in the 3rd group, the combination of Valcyte and Ingaron was different. Patients in this group were distributed as follows:

- Group 3a: 10 patients received Valcyte 900mg/day (2 months) + Ingaron 500.000 IU intramuscularly (i/m), every other day, № 10;
- Group 3b: 10 patients received Valcyte 900 mg/day (2 months) + Ingaron 500.000 IU intramuscularly, every other day, №20;

- Group 3c: 6 patients received Valcyte 900mg/day (2 months) + Ingaron 500.000 IU intramuscularly, every other day, №10 + 100.000 IU intramuscularly every other day №15.

Group of patients	Number of copies/ml before therapy	Number of copies/ml after therapy	P
Ingaron (1 group)	252453,57 ± 93108,909 (n = 34)	11318,42 ± 3643,75 (n = 24) In 10 (29.41%) patients - 0.00 copies	0.001
Valtrex (2 group)	253837,25 ± 48202,14 (n = 33)	53109,08 ± 28828,32 (n = 24) In 9 (27.27%) patients - 0.00 copies	0.001
Valcyte + Ingaron (3 group)	425250,00±62697,09 (n = 26)	35934,50 ± 33764,56 (n = 7) In 19 (73.07%) patients - 0.00 copies	0.0001

Table 1: *Dynamics of the number of copies of EBV DNA a month after the end of the antiviral therapy in patients CEBVI.*

The table below presents the results obtained (Table 2).

Treatment regimen (Valcyte + Ingaron)	Number of copies/ml before therapy	Number of copies/ml after therapy
General group (n = 26): Valcyte (2 months) + Ingaron	425250,00 ± 62697,09	35934,50 ± 33764,56 P = 0,0001
3a group (n = 10): Valcyte 900 mg/day (2 months) + Ingaron 500,000 IU i/m, №10	204000,00 ± 96215,03	In 6 patients (60%) - 0.00 copies; in 4 patients 4245,56 ± 2.752,64; P = 0,07
3b group (n = 10): Valcyte 900 mg/day (2 months) + Ingaron 500,000 IU i/m, №20	361733,33 ± 161746,19	In 9 patients (90%) - 0.00 copies; in 1 patient - 140 copies
3c group (n = 6): Valcyte 900 mg/day (2 months) + Ingaron 500.000 IU i/m, №10 + 100.000 IU i m, №15	274533,33 ± 143185,99	In 4 patients (66.67%)- 0.00 copies; in 2 patients 115000,00 ± 112416,48 P = 0,027

Table 2: *Dynamics of the number of copies of EBV DNA a month after the end of the combined antiviral therapy with Valcyte and Ingaron in patients with CEBVI.*

From the presented data it is clear that the best result was obtained in patients who received 20 injection of Ingaron in combination with Valcyte (in 90% of patients). In the group of the standard administration of Ingaron (10 injections) and in the combined administration groups of Ingaron (combinations of 500.000 IU, №10 + 100.000 IU, №15), the results were almost the same (60% and 66.67%, respectively). That is, a positive result in this treatment regimen is due to two things: 1. combination of Valcyte and Ingaron; 2. duration of administration of Ingaron.

In order to identify the effect of the initial number of copies of EBV DNA on the severity of clinical complaints in patients, a correlation analysis was performed and the following results were obtained (Table 3).

Further, to determine the prognostic significance of the initial number of EBV DNA copies on the effectiveness of therapy in both groups, a linear regression analysis was used with the calculation of the coefficient of determination (R Square) and the Durban-Watson criterion to verify the observance independence condition. Valid criteria values were between 2.059 and 2.765. All possible R2 values obtained were less than 50%, which indicates the absence of a statistical relationship between the number of EBV DNA copies and clinical

Complaints	Correlation coefficient
Weakness	τ = 0,462; p = 0,024 r = 0,563; p = 0,028
Sore throat	τ = 0,630; p = 0,002 r = 0,740; p = 0,001
Joint pain	τ = - 0,420; p = 0,052 r = - 0,526; p = 0,040

Table 3: *The effect of the number of copies of EBV DNA on the severity of clinical complaints in the general group of patients with chronic EBV infection (n = 93).*

and laboratory parameters, since regression models have a low value. Then ANOVA "Analisis of Variance" was performed with the calculation of the Fisher criterion (F) to verify the significance of the model. The standardized beta (ß) was also calculated with a 95% confidence interval. The critical level of significance differences of indicators was assumed to be 0.05. There were no reliable results of the F test and the coefficient ß, indicating the significance of the regression model obtained. When analyzing the dynamics of clinical complaints in each individual patient group, one month after the end of the therapy, the following results were obtained (Table 4).

The frequency of clinical complaints (%)	1st group		2nd group		3rd group	
	Ingaron, before therapy (n = 34)	Ingaron, after therapy	Valtrex, before therapy (n = 33)	Valtrex, after therapy	Valcyte + Ingaron, before therapy (n = 26)	Valcyte+ Ingaron, after therapy
Subfebrile temperature	82.35	29.41 (p = 0,001)	76.75	66,67 (p = 0,054)	86.46	57.69 (p = 0,001)
Lymphadenitis	70.58	44,11 (p = 0,02)	54.54	45.45 (p = 0,082)	53.84	30,76 (p = 0,002)
Sore throat	91.18	32,35 (p = 0,001)	81.81	57,57 (p = 0,001)	80.76	30.76 (p = 0,001)
Weakness	91.18	44,12 (p = 0,001)	81.81	66.67 (p = 0,056)	73.07	53,85 (p = 0,005)
Chills	64.70	44,12 (p = 0,001)	66.67	36,36 (p = 0,001)	69.23	30.76 (p = 0,001)
Sweating	85.29	64,70 (p = 0,01)	90.91	54.54 (p = 0,001)	76.92	38.46 (p = 0,001)
Runoff mucus	91.18	64,70 (p = 0,001)	69.69	60,60 (p = 0,052)	76.92	30.76 (p = 0,001)
Stomatitis	47.05	32,35 (p = 0,017)	36.36	33,33 (p = 0,054)	34.61	26.92 (p = 0,045)
Joint pain	58.82	41,17 (p = 0,056)	33.33	27,27 (p = 0,054)	34.61	23.07 (p = 0,01)
Irritability and tearfulness	85.29	79,41 (p = 0,054)	69.69	66,67 (p = 0,058)	76.92	46.15 (p = 0,001)
Skin eruption	52.94	32,35 (p = 0,031)	54.54	45,45 (p = 0,058)	34.61	23.07 (p = 0,01)

Table 4: *The frequency of clinical complaints (%) in patients before the start of therapy and one month after the end of therapy in the 1st, 2nd and 3rd groups of chronic CEBVI.*

In patients with group 1, the majority of clinical complaints had a significant positive trend. In group 2, patients noted a lack of positive dynamics. In group 3 of patients a reliable positive dynamics of all clinical complaints was revealed.

Discussion

For selective chemotherapy of herpes virus infections, the drug must specifically inhibit one or more stages of viral replication when using non-toxic patient concentrations. The main problem is the specificity of the drug in relation to the replication of the virus. The replicative cycle of the virus is controlled by virus-specific enzymes that are structurally and functionally different from the corresponding enzymes of the host cell. At the time the virus evades the host's immune response, it is a potential target for chemotherapeutic intervention. In this case, the higher the selectivity of antiviral drug exposure, the narrower the spectrum of its antiviral activity, since the drugs affect the viral replication steps and do not affect the latent phase. Currently, there are a number of specific antiviral drugs for the treatment of CEBVI, in particular, acyclic nucleosides and the synthetic nucleoside analogue of guanosine, ganciclovir, are widely used. However, a single approach in the treatment of CEBVI does not currently exist. Some studies have shown that antiviral therapy may be effective in treating CEBVI [17], since thymidine nucleoside analogs can inhibit thymidine phosphorylation and act as nucleoside antiviral drugs that affect the enzyme [18]. It is shown that thymidine kinase (TK) EBV has an activity of thymidylate kinase, but may not phosphorylate ganciclovir and acyclovir, since substrate specificity TK EBV is sufficiently narrow, and nucleoside analogs of thymidine have a pronounced ability to inhibit the phosphorylation of thymidine using TK EBV, being most specific nucleoside antiviral agents to affect the enzyme. Two alternative mechanisms can inhibit viral replication or limit the growth of latently infected cells in the absence of viral TK phosphorylation. 1). The protein kinase gene BGLF4, which is expressed in human herpes viruses and resembles serine/threonine kinase, rather than tyrosine kinase, is responsible for the activation of drugs. The minimal phosphorylation of TK in EBV-infected B cells is due to the fact that the kinetics of BGLF4 expression is very similar to the TK EBV kinetics [19]; 2). These drugs can be completely transformed into their active forms with the help of cellular enzymes. Ganciclovir and acyclovir triphosphates are generated in low-level, uninfected cells and cellular enzymes participate in their conversion [20]. To inhibit lytic viral replication, minimal phosphorylation is sufficient due to the sensitivity of EBV DNA polymerase to inhibition by these preparations [21]. In the latent course of EBV, the phosphorylation of ganciclovir in EBV-infected B-cells is a consequence of the increased absorption of nutrients in the immortalized cells. It should be noted that the sensitivity of EBV to ganciclovir is assessed by chemical induction of lytic replication in latently infected cells, since there is no primary lytic replication system for EBV. Immortalized cells activate cellular enzymes responsible for the phosphorylation of ganciclovir and, therefore, phosphorylate the drug to a greater extent than uninfected cells. Most of the published works have shown that antiviral therapy does not have a pronounced effect, since replication of latent EBV in proliferating B cells does not require viral DNA polymerase [22]. The drugs inhibit viral DNA polymerase and, therefore, inhibit the lytic replication of EBV in infected cells that express viral polymerase.

In 2016, the results of an analysis of the effectiveness of treatment of infectious mononucleosis according to the World Health Organization (WHO) International Clinical Trials Registry, which showed the questionable effectiveness of antiviral drugs (acyclovir, valacyclovir), were published. The overall effect of antiviral therapy on viral shedding reported this outcome was that viral shedding was suppressed while on antiviral treatment, but this effect was not sustained when treatment was stopped [23]. That is, against the background of antiviral therapy, the number of infected lymphocytes practically does not change, the level of viral load decreases slightly, but tends to return to the initial level after cessation of therapy [24,25]. Negative results on the number of copies of EBV DNA in the 2nd group (Valtrex 1000 mg/day 2 months) were obtained only in 27.27% of patients, and in 72.72% of patients the number of copies of EBV DNA significantly decreased. However, patients noted a lack of positive dynamics of clinical complaints when taking Valtrex. Thus, our results confirmed previously published data indicating low efficacy of antiviral drugs of the acyclic nucleoside group in the treatment of CEBVI.

In an experiment on B-cell lines, BJAB lines and on normal mature B-lymphocytes, pretreatment of cells for 24 hours with recombinant (re) IFN-α and reIFN-γ suppressed the production of the EBV-1 specific nuclear antigen in cells BJAB 24 hours after EBV infection. However, reIFN-α showed a more pronounced inhibitory effect than reIFN-γ, but none of the reIFN showed a pronounced inhibitory effect of EBNA expression in hidden EBV-infected Raji and Daudi cell lines. These results indicate that reIFNs act predominantly at the early stage

of EBV infection [26]. In 2002, a study was published showing that sharing IFN-β and IFN-γ inhibits HSV-1 replication in Vero cells by ~ 1000 times [27]. That is, a high level of inhibition of replication is the result of a synergistic interaction of IFN-γ with endogenous IFN-α/β, which are locally produced in response to HSV-1 infection. In our work, it was shown that in the 1st group one month after Ingaron therapy in 29.41% of patients negative PCR results were obtained in saliva samples, and in 71.87% of patients the number of EBV DNA copies was significantly reduced. In this group of patients, pronounced reliable dynamics of clinical complaints was obtained. Our results confirm previously published data in the Russian literature on the high efficacy of the drug Ingaron in the treatment of herpes virus infections [28,29].

In the 3rd group of patients, the effectiveness of the therapy depended on both the combination of drugs (Valcyte and Ingaron) and the duration of the course of Ingaron administration. There are reports of a positive result of treatment with CEBVI ganciclovir with marked inhibition of replication [30,31]. The best result from the therapy was obtained in patients who received 20 Ingaron injections in combination with Valcyte. It was in this group that the number of copies of EBV DNA in saliva samples was negative in 90% of patients. A reliable positive dynamics of clinical complaints was detected in all patients (100%) who received the combined therapy Valcyte + Ingaron. Thus, a positive result on the number of copies of EBV DNA during this treatment regimen is due not so much to the total course of Valcyte and Ingaron, but to the number and duration of administration of Ingaron. In clinical trials in patients with chronic viral hepatitis B, monotherapy with reIFN-γ, which lasts for 6 to 9 months, has been shown to lead to a pronounced anti-inflammatory and antifibrotic effect [32,33]. Our results with the long-term administration of Ingaron confirm this data.

Conclusion

1. Ingaron has a pronounced antiviral effect on the number of EBV DNA copies in patients with chronic EBVI.
2. One month after completion of ingaron therapy in patients with chronic EBVI, a significant decrease in clinical complaints was observed.
3. The best effect of Ingaron therapy is achieved in patients with chronic EBVI at a dose of 500.000 IU every other day with a course dose of at least 20 injections.
4. Ingaron can be used in combination antiviral therapy in conjunction with Valcyte.

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Conflict of Interest

The authors declare no conflict of interest.

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