Chapter

Recombinant Human Interferon-Gamma: Prospects for the Treatment of Chronic Epstein-Barr Viral Infection

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Abstract

Infection of Epstein-Barr virus (EBV) is about 90% among people over the age of 40. The EBV causes a chronic infection that is characterized by chronic or recurrent symptoms and persists for a long time. Recombinant interferon-gamma (IFN-γ) has high clinical and antiviral efficacy in the treatment of herpesvirus infections. 110 patients with chronic EBV infection were examined. The patients were divided into three groups for different treatment regimens: Group 1—IFN-γ therapy (15 injections of Ingaron i/m, 500,000 IU every other day); Group 2—valaciclovir (Valtrex 500 mg × 2 times/day, orally for 2 months); Group 3—valganciclovir (Valcyte 450 mg × 2 times/day, orally for 2 months) and IFN-γ (10–20 injections of Ingaron i/m, 500,000 IU every other day). The best results were obtained in group 3–73.07% negative PCR. In this group, the combination of valganciclovir + IFN-γ was different. We showed that the efficacy of therapy in patients with chronic EBV is determined by the duration of INF-γ administration. We also determined spontaneous and induced production of IFN-α and -γ cytokines in serum and in lymphocyte culture. We demonstrated that in patients with an initially low level of induced IFN-γ, the production of this cytokine significantly increased in three months after the end of therapy.

Keywords: Epstein-Barr virus, immunity, recombinant human interferon-γ, treatment, herpesvirus

1. Introduction

The development of immunodeficiency leads to the spread of persistent and/or chronic herpesvirus infection, in which the pathogen is not eliminated from the host's body for months, years, or even throughout life. Each herpesvirus in the host organism has its own target tissue, where the virus persists with the ability to enter and exit the tissue using a developed strategic mechanism, which consists of the minimum expression of viral genes in a small number of infected cells or their elimination at the protein level. This allows the virus to evade the immune response and remain in very small quantities (1 infected cell per 5 ml of blood) with minimal impact on the patient's body, remaining in it for the rest of its life. In this case, the immune response is unable to eliminate the infectious pathogen from the body.

1.1 Epstein-Barr virus

Epstein-Barr virus (EBV) or infectious mononucleosis virus, together with other herpesvirus infections, is a prototype of persistent viral infections characterized by latency. In the mid-1980s, the problem of chronic EBV infection or "chronic mononucleosis" attracted the attention of researchers. It has been shown that EBV causes the development of chronic or recurrent infectious mononucleosis-like symptoms in immunocompetent individuals, which persist for a long time and are characterized by constant fatigue, headaches, myalgias, lymphadenopathy, subfebrile fever (37.1–37.3°C), hepatosplenomegaly. Additionally, gastrointestinal diseases may develop hematological, neurological, and skin lesions [1].

EBV infection is accompanied by high production of IgG antibodies to viral capsid (VCA) and early antigens (EA) when compared with the control group, as well as very low production or absence of antibodies to Epstein-Barr virus nuclear antigen (EBNA) [2]. That is why it was suggested that the cause of the syndrome is a chronic EBV infection [3]. However, some patients may not have abnormally high antibody titers associated with EBV [4]. According to data published by Kanegane et al., the severity of the disease directly correlates with the level of EBV DNA copies number in serum or plasma [5]. In later studies, it was shown that an increased level of EBV DNA in the blood is a more specific criterion for chronic EBV than the levels of antibodies to EBV [6]. In 1983, Hellman et al. first proposed an abbreviation for this syndrome "Chronic Active EBV infection" (CAEBV) [7]. As a result of the analysis of the literature, including work on chronic EBV as well as work on herpesvirus infections, immunodeficiencies, and three types were identified based on clinical, pathological, and virological data characteristic of this syndrome [8, 9]:

1. Chronic fatigue syndrome of unknown etiology (CFS), which is characterized by profound, debilitating fatigue and a combination of symptoms leading to a significant decline in occupational, personal, social, and educational status;

2. CAEBV;

3. Severe CAEBV (SCAEBV).

EBV is known to spread through contact with saliva and penetrate the epithelium that lines the nasopharynx. Waldeyer's ring, which includes the adenoids, tonsils, and the lymphoid system surrounding the nasopharyngeal region, forms a continuous structure called lymphoepithelium [10]. The virus infects epithelial cells, replicates, then is released, followed by infection of resting naive B cells in nearby areas by activating latent proteins (six EBV nuclear antigens (EBNAs: EBNA1, EBNA2, EBNA3A, EBNA3B, EBNA3C, EBNA-LP), two latent membrane proteins (LMPs: LMP1, LMP2) and viral anti-apoptotic protein BHRF1) encoded by the growth program. As a result, the cell becomes a proliferating lymphoblast (lymphoblastic burst). This program leads to the expansion of EBV-infected B cells in the lymphoid tissue of the oropharynx and the appearance of infected B cells in the blood. In some infected B cells, EBV inhibits its growth transformation program, allowing the cells to enter the memory B cell pool, while the virus persists as a truly latent infection devoid of viral gene expression. Therefore, the target of the incoming virus is a resting naive B cell, which becomes infected as it passes through the epithelium. Naive B cells are continuously recirculated throughout the body, extravasating from the peripheral circulation to secondary lymphoid tissue through specialized structures called high endothelial vesicles (HEVs) located in the lymphoepithelium. Naive B cells migrate to the mantle zone of the follicles below

the epithelium and remain there for several days, and then return to the circulation. Infection of new naive B cells occurs in the intraepithelial layer, and not in the mantle zone [11], that is, when an infected B cell enters the follicle, it is already a blast and cannot migrate to the mantle zone. EBV infects cells through the interaction of viral glycoproteins gp350/220 with CD21 and gp42/gH/gL with class II HLA in a B cell. Thus, memory B cells are a place of long-term viral persistence, where the virus can remain throughout the patient's life, because immunological memory is formed, and the virus ceases to be pathogenic for the host since the genes that induce cell proliferation and contribute to the development of neoplastic disease are disabled. However, EBV can infect T and NK (natural killer) cells of the tonsils [12] and peripheral blood [13]. Expression of CD21 on T cells [14] and NK cells can be induced by trogocytosis and the formation of an immunological synapse that occurs when EBV contacts B cells, which leads to a possible EBV infection [15].

It has been shown that the level of infected B cells in the population ranges from 5 to 3000 in every 107 memory B cells, both in peripheral blood (on average 110/107) and in Waldeyer's ring (average value 175/107), then there is a virus evenly distributed throughout the ring. Thus, 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) [16]. The total body load in humans amounts to 104–107 (on average 0.5/106) infected memory B-cells, representing a small, stable, and most critically "safe" pool of infected cells, which guarantees long-term persistence. Only about 1% of these cells are found in the peripheral blood. One latently infected memory B cell in the amygdala can differentiate into a plasma cell and secrete a virus that infects epithelial cells. The virus constantly seeps into the oral cavity, where it mixes with saliva for about 2 minutes before swallowing. Thus, the oral cavity is a reservoir for EBV flow and not a static reservoir. About 250 cells begin to replicate in the Waldeyer ring at any given time. The oral cavity and peripheral blood are important anatomical sites for the localization and persistence of EBV infection. These two compartments are connected by oropharyngeal lymphoid tissue such as the lingual, palatine, and pharyngeal tonsils. EBV-infected B cells can re-enter the tonsils, where memory lymphocytes express characteristic sets of adhesion receptors, through which they are able to return to target organs, where they first encountered antigens. Thus, EBV-infected cells can release viral particles through lytic replication, reinfect cells in lymphoepithelial tissue, and subsequently release viruses into the oral cavity [17].

The release of viruses into plasma from different anatomical sites indicates that different viral strains can persist in different tissue compartments. Therefore, EBV can be detected in the tissues of various anatomical structures of the human body.

Despite the fact that EBV is an oncogenic virus, the vast majority of EBV-infected people do not suffer from any long-term consequences. This is due to the antiviral immune response that develops during primary infection with EBV, and further supports subsequent lifelong control to ensure the mutual coexistence of the virus and its host. Early control of EBV infection is associated with the expansion of innate immune cells (primarily NK cells) and CD8+ and CD4+ T cells, specific for a wide range of EBV proteins expressed during the lytic and latent stages of viral infection. Patients with persistent EBV infection develop a specific CD8+ T-cell response to antigens of the lytic and latent cycles, the former being more frequent. An individual lytic epitope-specific response can account for up to 2% of the total population of CD8+ T cells. The response to immediate-early antigens dominates the response to early antigens, and the response to late expressed antigens rarely develops [18]. CD8+ T cells play a major role in the formation of responses to proteins EBNA3A, 3B, and 3C. A less specific immune response develops against EBNA1, EBNA2, and LMP2. Individuals expressing HLA-B*3801 have been shown

to have strong responses to the EBNA2 epitope, and carriers of HLA-A*0203 have strong responses to the epitope from EBNA-LP. In persistently infected individuals the EBV-specific T cell pool contains resting antigen-expressed T cells that are not active and do not proliferate. Lymphoid markers CCR7 and CD62L, specific for the latent antigen, are expressed on T cells. The phenotype, functional profile, and clonotypic composition of TCRs specific for CD8+ T cells remain stable for many years [19]. The EBV-specific CD4+ T cell response in healthy carriers is much weaker and may be 10 times less pronounced than the CD8+ T cell response to the same antigen. EBV-specific CD4+ memory T cells share the same phenotype regardless of whether they are specific for latent or lytic antigens. CD4+ T cells do not express perforin and granzyme, and upon ex vivo stimulation, the cytokine polyfunctionality of cells increases, and TNF-alpha production predominates [20]. NK-T cells are a conserved population of congenital T cells expressing the semi-invariant Va24-Ja18/Vβ11 T cell receptor. Only one study evaluated the frequency of NK T cells in the blood during EBV infection, and it was shown that the number of NK T cells was increased in the first month of infection. A change in cellular phenotype and function was noted with an increase in the content of CD56 (bright) with a high ability to destroy EBV-infected cells. NK-T cells play an important role in the control of primary EBV infection by eliminating infected B cells and increasing the antigen-specific response of T cells through the release of immunomodulatory cytokines [21]. It has been shown that patients with primary immunodeficiency are predisposed to the development of EBV-associated disease. The presence of NK T cells reduces the EBV transformation of B cells in vitro. With EBV infection of blood lymphocytes, the previous depletion of NK T cells leads to both an increase in the number of B cells infected with EBV and an increase in the total viral load in culture. It has been suggested that NK T cells play a role in the early immune recognition of newly EBV-infected B cells [22].

1.2 Interferon-γ

Interferons (IFNs) are important biological regulatory proteins called cytokines and mediators of cellular homeostatic reactions that are produced in response to viral infection inhibiting the replication of a wide range of DNA and RNA viruses, thereby creating negative feedback. Inhibition of the viral replication cycle is carried out with the help of the synthesis of viral polypeptides [23]. When IFNs are administered in vivo, the level of viremia decreases, that is, interferons can be used as antiviral drugs, and the antiviral effect is mediated both by the immune system itself and by intracellular antiviral mechanisms. All types of IFNs inhibit more than one step in the viral life cycle: viral entry and decay, viral mRNA transcription, viral protein synthesis, viral genome replication, and progeny assembly and isolation of virions. According to the amino acid sequence, IFNs are divided into three types: I, II, and III.

IFN-γ is the only representative of type II IFN. It is structurally unrelated to type I IFNs, binds to another receptor and is encoded by a separate chromosomal locus. Type II IFN or immune IFN—IFN-γ is a highly pleiotropic cytokine, secreted not in response to viral infection, but indirectly by mitogen-activated T cells and NK cells, which are the primary producers of IFN-γ during the innate and adaptive phases of the immune response to viral infection. Other cells such as B cells, NK T cells, and professional antigen-presenting cells (APCs) have now been shown to secrete IFN-γ. The production of IFN-γ by monocytes/macrophages and dendritic cells acting locally is important in cell activation [24].

IFN-γ plays an important role in the activation of macrophages for the production of tumor necrosis factor-α (TNF-α), increases macrophage phagocytosis and microbicidal activity, the formation of active nitrogen and oxygen intermediates,

including superoxide radicals - nitric oxide and hydrogen peroxide, which are powerful cytotoxic effectors, stimulates the Th1-T cell response and has a strong inflammatory activity. IFN-γ is the main product of Th1 cells and further shifts the immune response towards the Th1 phenotype. IFN-γ achieves this by stimulating characteristic Th1-effector mechanisms: innate cell-mediated immunity (through the activation of NK cell effector functions), specific cytotoxic immunity (through the interaction of T cells with APC), and macrophage activation. IFN-γ increases the content of lymphocytes and leads to their long-term persistence in the tissue, induces activation of the complement cascade and acute phase response, plays a role in the switch of IgG class production, and has a direct antiviral effect [24]. Normally, in the early stages of the host's immune response, IFN-γ production by NK cells, CD4+ T (Th1) cells, and CD8+ T cells is aimed at improving antigen recognition in APCs such as macrophages and dendritic cells. IFN-γ is one of the key cytokines that differentiate naive CD4 cells into Th1 effector T cells, which produce the main mediators of cellular immunity against viral and intracellular bacterial infections [25]. Together, IFN-γ and IL-12 generate a very strong Th1 response. Th1 cell-mediated immunity and Th2 cell-mediated humoral immunity are modulated by IFN-γ, which affects the differentiation of naive T cells into Th1 or Th2 cells.

When activated, almost all CD8+ T cells, NK cells, and Th1 lymphocytes produce IFN-γ, which stimulates cytokine activity and increases the expansion of low avid NK cells. Of all the interferons/cytokines of the Th1 response, IFN-γ is most strongly correlated with the Th1 response [26]. The effects induced by IFN-γ lead to increased immune surveillance. In addition, IFN-γ blocks the production of IL-4, an inducer of Th2 cell differentiation and proliferation. The synergistic effects of IL-21, IL-18, and IL-15 increase IFN-γ production. The most potent regulator of IFN-γ production is IL-15 compared to IL-21 in human NK and T cells. The cytokines IL-15 and IL-18 are produced by macrophages, while IL-21 is mainly produced by activated T cells. IFN-γ increases the expression of the HLA (major histocompatibility complex) class I and II antigen by increasing the expression of subunits, increasing the expression and activity of proteasomes, resulting in increased sensitivity of the host to an infectious pathogen and an increased ability to identify and respond to this pathogen [26]. Thus, IFN-γ has many important immunostimulatory and immunomodulatory effects.

With the development of inflammation, a high level of IFN-γ leads to the activation of both canonical and non-canonical pathways. In the canonical signaling pathway, IFN-γ dimerizes and binds to two IFN-γ receptors, which are composed of two different ligand-binding chains: high-affinity IFNGR-1 (α) with high expression and two signal-transforming low-affinity IFNGR-2 (β),—with related signaling mechanisms. The IFNGR1 and IFNGR2 chains belong to the class II cytokine receptor family. The IFNGR2 chain limits sensitivity to IFN-γ and the IFNGR1 chain is usually in excess. But the expression level of IFNGR2 can be tightly regulated depending on the state of cell differentiation or activation. Receptors are expressed on the surface of almost all cell types. The expression level is determined by the cell type and its activation status. Initially, IFN-γ binds to IFNGR1, and the formed IFN-γ*IFNGR1 complex facilitates its binding to IFNGR2, then downstream signaling pathway events are initiated [27]. IFN-γ gene transcription is induced through several mechanisms. The most studied response to IFN-γ, mediated by STAT-1-containing transcription factor GAF (gamma-activated factor), which is activated by tyrosine kinases Jak1 and Jak2 and binds to the gamma-activating sequence GAS (Gamma Activating Sequence), which is present in the promoter regions of many genes. As a result of gene activation, the formation of cellular immune response to a viral infection begins [28]. The JAK/STAT pathway is the main signaling pathway initiated by IFN-γ stimulation. Further, IFN-γ, together with one of its receptor

subunits IFNGR1 and pSTAT1, is translocated into the cytoplasmic domain in combination with endocytosis and induces gene expression by binding to GAS elements in the promoter region of inducible IFN genes [29]. When viruses inhibit the functions of STAT1 molecules, IFN-γ can independently induce a noncanonical signaling pathway [30]. That is, IFN-γ is capable of inducing gene expression in STAT1−/− bone marrow macrophages, suggesting that IFN-γ acts independently of STAT-1 or in an alternative non-canonical manner. Typically, activation of noncanonical pathways occurs later, after STAT1 activation. However, there is evidence that noncanonical pathways can be activated in the absence or presence of STAT1 in a dependent manner [31]. The IFN-γ and IFN-α/β signaling pathways intersect at several levels, partially overlap, which allows cross-interaction of certain functions within the cell. This crossover mechanism is relevant because in vivo cells are not stimulated in isolation by a single cytokine, but rather a cytokine cocktail that induces gene expression through the integration of multiple signaling pathways.

When infected with a virus, IFN-γ can induce apoptosis by regulating Fas ligands to remove virus-infected cells, enhancing the expression of type I IFNs, pro-inflammatory cytokines, and chemokines by endothelial, epithelial cells and fibroblasts to attract macrophages, neutrophils, and T cells to the sites of infection [32]. IFN-γ can also initiate the expression of dsRNA-specific adenosine deaminase (ADAR), which inhibits viral replication by editing or disrupting the translation of viral proteins [33].

Virus infection of a cell begins with the attachment of the virus to the surface of the host cell through a receptor and/or through cell membrane molecules such as glycans. Viruses can release their genomes directly into the cell after fusion of its membrane with the plasma membrane, while other viruses enter cells through cellular endocytosis, which allows the virus to release the core virion containing the viral genome directly into the cytoplasm [34]. The isolated genome, either naked or associated with viral proteins, moves to certain regions of the cytoplasm or nucleus for its replication [35]. IFN-γ can inhibit the entry of the virus from the endosome into the cytoplasm.

Virus replication is the primary goal of the virus life cycle [36]. Suppression of any stage of the life cycle can lead to suppression of viral genome replication during viral infection. IFN-γ is a potent antiviral cytokine that interferes with various stages of the viral life cycle in stimulated cells using the following mechanisms [35]:

1. Inhibition of the viral penetration, both at the extracellular and intracellular stages, by controlling the expression and/or distribution of the receptors necessary for the penetration of the virus;

2. Inhibition of the viral replication by disrupting the replication niche of the virus;

3. Disruption of gene expression, interfering with translation;

4. Violation of stability by interfering with nucleocapsid assembly;

5. Violation of virus shedding by breaking the disulfide bond of the required participant in cellular interaction;

6. Modified reactivation by suppressing the main regulator of viral transcription;

7. IFN-γ can also inhibit the penetration of the virus at the stage of transfer of the invading virus from the endosome into the cytoplasm [35].

1.3 The use of interferon-γ in the treatment of herpesvirus infections

In recent years, numerous works have been published in the world on the treatment of herpesvirus infections with recombinant IFN-γ, showing high clinical and antiviral efficacy [27, 37–39]. IFN-γ demonstrated a 7–10 times more potent antiviral effect than IFN-α or -β. When IFN-γ is added at the 3–4th days after infecting, there is a decrease in EBV-induced B cell proliferation and immunoglobulin secretion, while the addition of IFN-α and -β has an effect only within 24 h. Lotz et al. Found that EBV-infected cells can be regulated by all IFNs at an early stage. Subsequently, there comes an intermediate period when only IFN-γ is able to directly influence EBV-induced B-cell responses. In the third phase, B-lymphocytes become insensitive to the direct action of all IFNs and are exposed only to cytotoxic cells [40]. In 2002 the introduction of recombinant IFN-γ, as well as IFN-β, showed high efficiency of inhibition of replication of the herpes simplex virus type 1 (HSV-1) [41]. That is, the high level of inhibition achieved by the administration of exogenous IFN-γ was the result of a synergistic interaction with endogenous IFN-α/β, which is produced locally in response to HSV-1 infection. Other researchers revealed that IFN-β and IFN-γ interact synergistically, blocking viral DNA synthesis and nucleocapsid formation in HSV-1 infected cells, without affecting the viability of the host cells. Thus, the authors concluded that IFN-mediated suppression of HSV-1 replication plays the role of the main mechanism by which the host immune system limits the spread of infection in vivo [42]. In a double-blind, placebo-controlled study, it was shown that the introduction of recombinant IFN-γ three times a week subcutaneously reduces the incidence of severe infections in patients with various genetic types of chronic granulomatous disease [43].

In the Russian Federation, the only IFN-γ preparation has been registered under the trade name Ingaron, developed by SPP PHARMACLON Ltd. via the microbiological synthesis in a recombinant E. coli strain and purified by column chromatography. The drug consists of 144 amino acid residues, devoid of the first three of them (Cys-Tyr-Cys), replaced by Met.

The purpose of this study is to evaluate the efficacy of IFN-γ therapy for the content of the number of EBV DNA copies in saliva samples by the Real-time PCR method, for the dynamics of INF-α and INF-γ production (spontaneous, serum, and induced levels) and the clinical picture in patients suffering from chronic Epstein-Barr virus infection (CEBVI) one and three months after the end of therapy.

2. Material and methods

2.1 Schemes of therapy

All patients were divided into three groups for different therapy regimens.

- The first group consisted of 51 patients (from 22 to 49 years old) who received IFN-γ therapy (500,000 IU every other day, intramuscular injections (i/m)). The total course was 15 injections;

- The second group consisted of 42 patients (from 22 to 48 years old) who received prolonged therapy with a drug from the group of acyclic natural nucleosides - valaciclovir (Valtrex 500 mg × 2 times/day, orally) for two months;

- The third group consisted of 46 patients (from 19 to 52 years old) who received prolonged therapy with a synthetic nucleoside analog of guanosine—valganciclovir

(Valcyte 450 mg × 2 times a day, orally) for 2 months in combination with IFN-γ (10–20 intramuscular injections of Ingaron 500,000 IU every other day). Previously, all patients in this group, as prescribed by a doctor or independently (often repeatedly), received therapy with drugs from the group of acyclic natural nucleosides, including valaciclovir for short courses (7–10 days). There was no pronounced clinical and laboratory positive effect from the previous therapy, for this reason, these patients have prescribed valganciclovir in combination with IFN-γ.

To assess the efficacy, a comparative analysis of the amount of EBV DNA in saliva samples was carried out one month after the end of the treatment course. Clinical complaints were compared for the patients of Group 1 in one and three months after the treatment course.

Patient groups and therapy are presented in **Table 1**.

Patient group	Therapy in the main groups	Subgroup of patients	Therapy in subgroups
1st group (n = 51)	Ingaron 500,000 IU every other day, i/m. Course of 15 injections	—	—
2nd group (n = 42)	Valtrex 500 mg × 2 times/day, 2 months	—	—
3d group (n = 46)	Valcyte 450 mg × 2 times/day, 2 months + Ingaron	3A group (n = 22)	Valcyte 450 mg 2 times/day (2 months) + Ingaron 500,000 IU every other day, i/m. Course of 10 injections
		3B group (n = 24)	Valcyte 450 mg 2 times/day (2 months) + Ingaron 500,000 IU every other day, i/m. Course of 20 injections

Table 1.
Characteristics of therapy in patient groups.

The study procedures were in accordance with the Good Clinical Practice (GCP) guidelines and ethical principles of the Declaration of Helsinki. The study was approved by the Ethics Committee of Fresenius Medical Care (Dialysis Center St. Petersburg, Russia). Written informed consent was obtained from all participants before the study was initiated.

2.2 Survey methods

Clinical research methods included the collection of anamnesis, data on previous immuno- or antiviral therapy, and the presence of concomitant diseases. The clinical condition of patients was assessed according to the generally accepted method, including objective data and registration of patient complaints at the time of examination. The severity of patient complaints was recorded using a subjective assessment scale on a 3-point scale (0—no symptoms, 1—mild symptoms, 2—moderate severity of symptoms, 3—significant severity of symptoms).

Diagnosis of CEBVI was based on clinical data and positive results of EBV DNA studies in saliva samples conducted by polymerase chain reaction (PCR) with real-time hybridization-fluorescence detection. The test systems "AmpliSens EBV/CMV/HHV6-screen-FL" (FBSI "Central Research Institute of Epidemiology", Russia) were used. The unit of measurement used to estimate the viral load during DNA extraction from saliva is the number of copies of EBV DNA per ml of sample. According to the instructions, this indicator is calculated using the formula: Number

of DNA copies = CDNA × 100, where CDNA is the number of copies of the viral DNA in the sample. The analytical sensitivity of the test system is 400 copies/ml.

We studied the dynamics of IFN-α and -γ production before the initiation of IFN-γ therapy and 1 and 3 months after the end of the course. Determined the level of IFN-α and IFN-γ in the blood serum, as well as the spontaneous and induced production of these cytokines in the culture of blood lymphocytes. Newcastle disease virus (NDV) (obtained at the L.A. Tarasevich State Institute of Culture, St. Petersburg) with an infectious titer of 8 lg EID/0.2 ml in a volume of 8 μl/hole and Phytohemagglutinin (PHA-P) (PanEko, Russia) at the dose of 10 μg/ml were used as inducers of IFN-α and -γ production respectively. The quantitative content of cytokines was determined in the serum and supernatant of a 24-h whole blood culture by solid-phase ELISA using the test systems "alpha-Interferon-IFA-BEST" and "gamma-Interferon-IFA-BEST" (JSC "VectorBest", Russia). Reference values for spontaneous, serum, and induced production of IFN-α and IFN-γ are provided by the manufacturer of the test systems.

2.3 Statistical analysis

Statistical analysis of the obtained results was carried out using the statistical software package IBM SPSS Statistics, version 26 (Armonk, NY: IBM Corp.). Group results are presented as mean ± standard error of the mean (SEM). Statistical processing of the results was carried out using parametric (Pearson's method) and nonparametric (Kendall's tau (τ)) criteria. To check the condition of independence of observations, linear regression analysis (with the calculation of the coefficient of determination (R Square) and the Durban-Watson criterion) and analysis of variance (ANOVA Analisis of Variance) with the calculation of the Fisher criterion (F) were carried out to test the significance of the model. The standardized coefficient ß was calculated with 95% Confidence Interval (95% CI). The critical level of significance of the difference in indicators was taken equal to 0.05.

3. Results

The examination was carried out in 139 patients with CEBVI: 86 women and 53 men with average age of 35.27 ± 1.28 years. The duration of CEBVI from the appearance of the first complaints to laboratory confirmation and the diagnosis was 2.23 ± 0.21 years. 98 (7.720%) and 27 (24.54%) patients suffered in childhood from chronic tonsillitis with no response to antibiotic therapy and infectious mononucleosis respectively. All patients underwent differential diagnosis of CEBVI with other viral infections (HIV, viral hepatitis, cytomegalovirus infection, toxoplasmosis), helminthic invasions, autoimmune diseases associated with EBV infection.

CEBVI is characterized by a long course and frequent relapses with clinical and laboratory signs of viral activity, described in detail in the literature [44–46]. Patients worried about prolonged subfebrile condition (37.1–37.3°C), weakness, unmotivated fatigue, excessive sweating (especially at night), a constant feeling of discomfort and/or pain in the throat, lymphadenitis, swelling of the nasal mucosa with abundant drainage mucus, stomatitis. Some patients have a cough, skin rashes, arthralgia, pain in the muscles of the trunk and extremities are possible. There may be manifestations of conjunctivitis, otitis media. Neurological disorders develop headaches, memory and sleep disorders, decreased concentration, irritability, tearfulness, a tendency to depression. Perhaps an increase in internal organs (according to ultrasound, hepato- or splenomegaly) and feeling of heaviness in the right hypochondrium. Also, patients complain of frequent colds, the addition of other herpesviral infections. In the history of such patients, long-term stressful situations,

psychoemotional and physical overloads often take place, against the background of which the patient's condition worsens.

3.1 Comparative analysis of the efficacy of antiviral therapy

In all patients (N = 139) CEBVI was confirmed by PCR reaction in saliva samples. **Table 2** shows the results of a comparative analysis of the dynamics of the number of EBV DNA copies in groups of patients who received different therapy regimens.

Patient group	Number of copies/ml before therapy	Number of copies/ml after therapy
1st group IFN-γ (N = 51)	294630.59 ± 72210.69	154786.97 ± 18671.15 (N = 36) in 15 patients—0.00 copies (29.41%)
2nd group valaciclovir (2 months) (N = 42)	278857.24 ± 44608.15	47108.18 ± 25928.62 (N = 30) in 12 patients—0.00 copies (28.57%)
3d group valganciclovir (2 months) + IFN-γ (N = 46)	425250.00 ± 62697.09	35934.50 ± 33764.56 (N = 13) in 33 patients—0.00 copies (71.74%)

Table 2.
Dynamics of the number of EBV DNA copies one month after the end of antiviral therapy in patients with CEBVI.

From the presented data in the 1st group after Ingaron therapy, only 15 (29.41%) patients had negative PCR results in saliva samples. In the 2nd group of patients taking valaciclovir negative PCR results were obtained in 12 (28.57%) patients. In group 3, one month after taking the combination therapy valganciclovir + Ingaron, 33 (71.74%) patients obtained negative PCR results in saliva samples.

The patients of the 3rd group, according to the combination therapy were distributed as follows:

- 3A subgroup—22 patients received Valcyte 900 mg/day (2 months) + Ingaron 10 injections of 500,000 IU every other day;
- 3B subgroup—24 patients received Valcyte 900 mg/day (2 months) + Ingaron 20 injections of 500,000 IU every other day.

Table 3 shows the results obtained in these subgroups.

Therapy scheme (Group 3)	Number of DNA copies/ml before therapy	Number of DNA copies/ml after therapy
Subgroup 3A: Valcyte 900 mg/day (2 months) + Ingaron 500,000 IU × 10 (N = 22)	334086.00 ± 95214.02	In 12 patients—0.00 copies; (54.54%) in 12 patients—6285.57 ± 2823.61
Subgroup 3B: Valcyte 900 mg/day (2 months) + Ingaron 500,000 IU × 20 (N = 24)	381745.32 ± 161946.09	In 21 patients—0.00 copies; (87.50%) in 3 patients—123469.51 ± 46615.32

Table 3.
Dynamics of the number of EBV DNA copies one month after the end of combined antiviral therapy in the 3rd group of patients.

From the data presented in **Table 2** one can see that in the total 3rd group of patients, there is a reliably positive dynamics of the number of DNA copies the decrease in a month after combination therapy. In subgroup 3A, negative PCR results were obtained in 54.54% of patients. The best result was observed in the patients of subgroup 3B (in 87.50% of patients) who received 20 injections of IFN-γ 500,000 IU every other day, in combination with valganciclovir. That is, the positive result of this therapy regimen is due not so much to the combination of medicines, but to the amount and duration of IFN-γ administration.

3.2 Dynamics of INF-α and INF-γ production

After the comparative analysis of the efficacy of different regimens of CEBVI therapy, we analyzed the dynamics of INF-α and INF-γ production (spontaneous, serum, and induced) in the culture of lymphocytes in the first group of patients (N = 51) before the start of therapy with IFN-γ, after one and three months after the end of therapy. We also assessed the dynamics of clinical complaints in these patients after IFN-γ therapy. **Tables 4** and **5** present the data obtained.

Research indicator	**IFN-α level (pg/ml)before therapy**	**IFN-α level (pg/ml) after 1 month of therapy**	**IFN-α level (pg/ml) after 3 months of therapy**	**p**
	1	**2**	**3**	
Spontaneous IFN-α	3.76 ± 0.58	5.80 ± 4.02	3.85 ± 19.24	P1,2 = 0.345 P2,3 = 0.435 P1,3 = 0.359
Serum IFN-α	5.09 ± 1.47	4.21 ± 0.70	5.57 ± 1.20	P1,2 = 0.289 P2,3 = 0.202 P1,3 = 0.380
Induced IFN-α	296.78 ± 127.43	578.154 ± 129.46	294.78 ± 60.67	P1,2 = 0.284 P2,3 = 0.360 P1,3 = 0.145

Table 4.
Dynamics of IFN-α production before the start, one and three months after therapy in the 1st group of CEBVI patients (N = 51).

Research indicator	**IFN-γ level (pg/ml) before therapy**	**IFN-γ level (pg/ml) after 1 month of therapy**	**IFN-γ level (pg/ml) after 3 months of therapy**	**p**
	1	**2**	**3**	
Spontaneous IFN-γ	2.07 ± 0.26	2.57 ± 0.75	2.00 ± 0.57	P1,2 = 0.34 P1,3 = 0.36 P2,3 = 0.57
Serum IFN-γ	1.85 ± 0.14	5.57 ± 1.20	2.10 ± 0.68	P1,2 = 0.024 P1,3 = 0.21 P2,3 = 0.38
Induced IFN-γ	1862.72 ± 624.52	2487.96 ± 437.73	4308.12 ± 3053.77	P1,2 = 0.38 P1,3 = 0.38 P2,3 = 0.27

Table 5.
Dynamics of IFN-γ production before the start, one and three months after therapy in the 1st group of CEBVI patients (N = 51).

One month after the end of therapy with IFN-γ, a tendency to an increase in the spontaneous production of IFN-α was revealed (statistically insignificant), but after three months the values returned to the initial values. Serum IFN-α production did not change after one and three months, remaining within the normal range. There was a tendency to an increase in the induced production of IFN-α one month after the end of therapy, followed by a normalization of the level after three months. Thus, IFN-γ had no significant effect on IFN-α production in the general group of patients after one and three months of therapy.

From the data presented in **Table 4**, it follows that in the group of patients a month after the end of therapy with IFN-γ, the serum ($p = 0.024$) production of IFN-γ increased, and after three months the serum level practically returned to the initial value ($p = 0.57$). The level of spontaneous production one and three months after the end of therapy did not change significantly. Induced production of IFN-γ also tended to increase one and three months after the end of therapy without significant dynamics.

When analyzing the initial data of the level of induced IFN-γ, it was found that these values sharply differed in patients, i.e. from the lower to the upper limit values of the reference (281-4335 pg/ml).

In this regard, the group of patients ($N = 51$) was divided into 2 groups in accordance with the induced production of IFN-γ before the start of therapy:

- 1st subgroup ($N = 30$)—the level of induced IFN-γ closer to the upper limit of the reference values (2706.00 ± 1058.94 pg/ml);

- 2nd subgroup ($N = 21$)—the level of induced IFN-γ closer to the lower limit of the reference values (287.20 ± 64.65 pg/ml).

Figure 1 shows the data on the dynamics of the induced IFN-γ production in these groups of patients.

The results of the study showed that after the course of therapy with IFN-γ in the 1st subgroup, the content of induced IFN-γ had a tendency to a gradual decrease, while in the 2nd subgroup there was a significant increase in the level of induced IFN-γ three months after therapy ($p = 0.027$). At the same time, the values of IFN-γ levels in both groups remained within the reference values. **Figure 2** shows the results of the dynamics of the spontaneous level of IFN-γ before and after IFN-γ therapy in both subgroups.

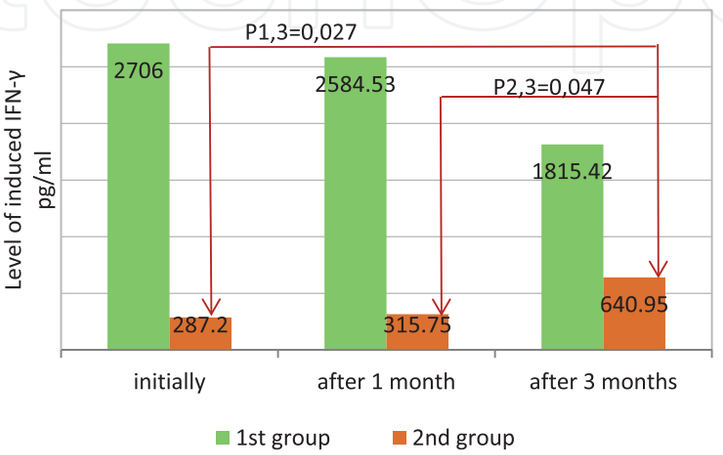

Figure 1.
Dynamics of the level of induced IFN-γ before the start, one and three months after IFN-γ therapy in patients with CEBVI in the subgroups 1 and 2.

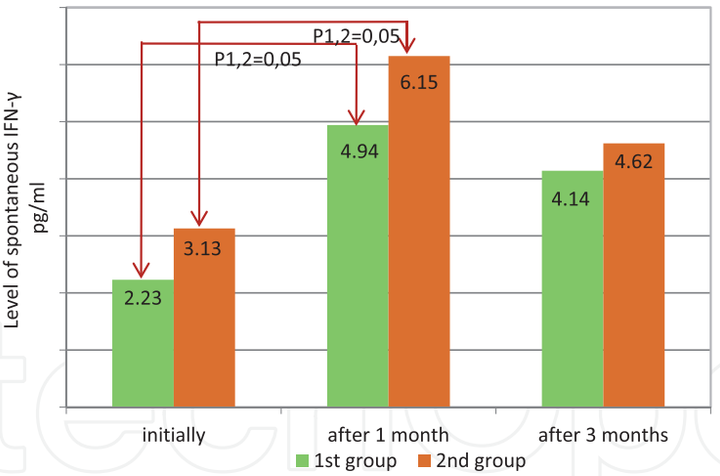

Figure 2.
Dynamics of the level of spontaneous IFN-γ before the start, one and three months after IFN-γ therapy in patients with CEBVI in subgroups 1 and 2.

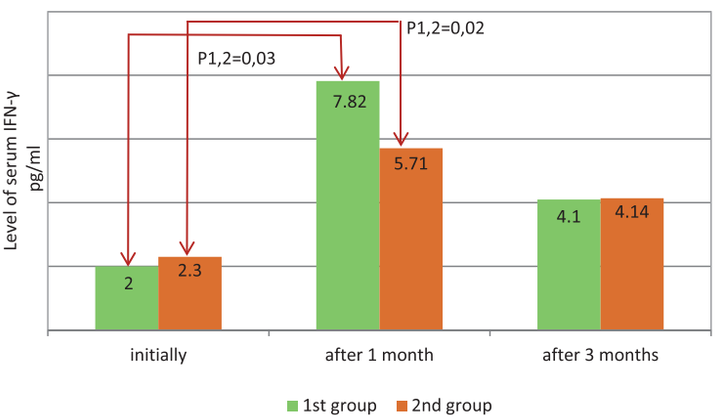

Figure 3.
Dynamics of the level of serum IFN-γ before the start, one and three months after IFN-γ therapy in patients with CEBVI in subgroups 1 and 2.

The presented data show that the values of the spontaneous level of IFN-γ in both groups significantly increased after one month. After three months, there was a trend towards a decrease in the spontaneous level of IFN-γ in both groups, while remaining above the baseline values. However, these values in both groups did not differ from the reference values (0–6 pg/ml). **Figure 3** shows the dynamics of serum IFN-γ levels after therapy in both groups of patients.

The results of the research showed that in both subgroups the increase in serum IFN-γ production was significant one month after the end of therapy ($p = 0.03$ and $p = 0.02$, respectively). In three months after the treatment course, there was a tendency to a slight decrease in serum IFN-γ, while the data obtained did not differ from the baseline (before the start of therapy levels) and from the reference values provided by the manufacturer of the test system (0–10 pg/ml).

3.3 Dynamics of clinical complaints

The next stage of the work was the analysis of the frequency of the main clinical complaints in patients of both subgroups before the start, one and three months after IFN-γ therapy (**Table 6**).

From the data in **Table 6**, one can see that in one and three months after the therapy with IFN-γ in patients of the 1st group there was a significant decrease in

Frequency of complaints (%)	Before therapy (n = 30)	after 1 month of therapy	after 3 months of therapy	p
	1	2	3	
Subfebrile temperature	83.33	30.76	30.76	P1,2 = 0.004 P1,3 = 0.004 P2,3 = 0.000
Lymphadenitis	53.33	43.33	26.66	P1,3 = 0.047 P2,3 = 0.05
Sore throat	93.33	43.33	36.66	P1,2 = 0.001 P1,3 = 0.001
Weakness	76.66	66.66	53.33	P1,3 = 0.001
Chills	70.00	13.33	20.00	P1,2 = 0.001 P1,3 = 0.001
Sweating	93.33	53.33	46.66	P1,2 = 0.001 P1,3 = 0.001
Runoff of mucus	33.33	13.33	16.66	P1,2 = 0.05
Decreased concentration of attention and memory	56.66	40.00	36.66	P1,3 = 0.050

Table 6.
Frequency of main clinical complaints (%) in patients before the start, one and three months after IFN-γ therapy in patients of the 1st group (1st subgroup—with the level of induced IFN-γ closer to the upper limit of the reference values).

subfebrile temperature, sore throat, chills, sweating, and decreased concentration. The rest of the complaints tended to decrease or remain unchanged. The dynamics of clinical complaints in the patients of the 2nd subgroup is presented in **Table 7**.

Frequency of complaints (%)	Before therapy (n = 21)	After 1 month of therapy	After 3 months of therapy	p
Lymphadenitis	66.66	14.28	19.04	P1,2 = 0.002 P1,3 = 0.05
Sore throat	33.33	23.80	19.04	P1,3 = 0.002 P1,3 = 0.002
Chills	47.67	28.57	23.80	P1,2 = 0.001 P1,3 = 0.001
Sweating	61.90	52.38	47.67	P1,2 = 0.029 P1,3 = 0.001
Runoff of mucus	21.05	10.52	10.52	P1,2 = 0.029 P1,3 = 0.029
Stomatitis	15.78	10.52	9.52	P1,2 = 0.004 P1,3 = 0.001
Joint pain	15.78	10.52	9.52	P1,2 = 0.004 P1,3 = 0.001
Decreased concentration of attention and memory	33.33	23.80	26.31	P1,2 = 0.002
Sleep disturbance	15.78	14.28	10.52	P1,3 = 0.004 P2,3 = 0.046

Table 7.
Frequency of clinical complaints (%) in patients before the start, one and three months after IFN-γ therapy in patients of the 1st group (2nd subgroup—with the level of induced IFN-γ closer to the lower limit of the reference values).

In the 2nd subgroup of patients, one and three months after IFN-γ therapy, a significant positive dynamics of the main clinical complaints were observed, in particular, a decrease in lymphadenitis, sore throat, chills, sweating, mucus drainage along the back of the throat, stomatitis, joint pain, decreased concentration attention, sleep disorders. That is, patients with an initially reduced level of induced IFN-γ before starting IFN-γ therapy have a more pronounced response to the therapy.

When analyzing the clinical picture, we revealed that in the group of patients with a higher level of induced IFN-γ production at the time of initiation of therapy complaints were more intensive and occurred with higher frequency (**Figures 4** and **5**).

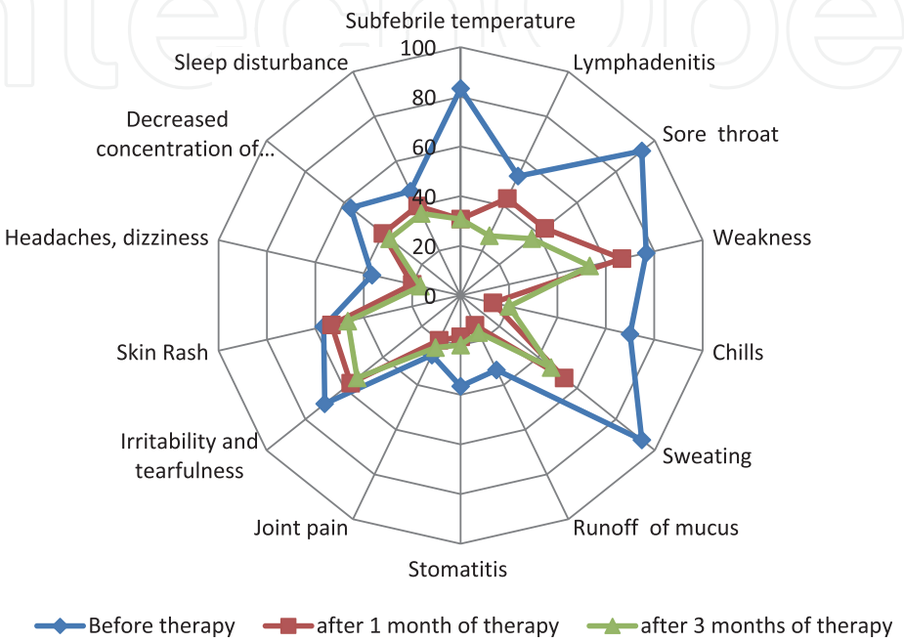

Figure 4.
Frequency of main clinical complaints (%) in patients before the start, one and three months after IFN-γ therapy in patients with CEBVI in the 1st subgroup.

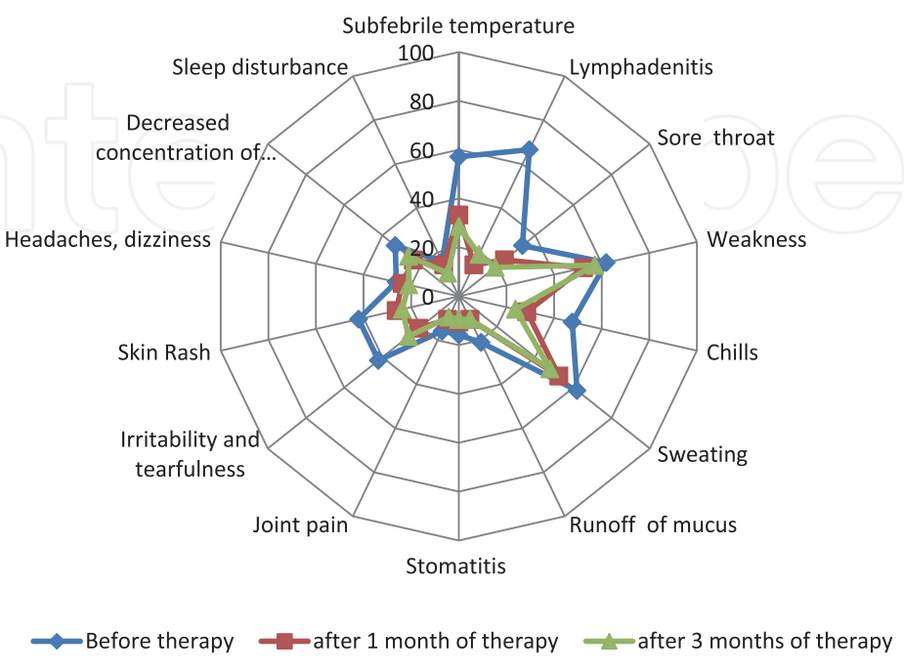

Figure 5.
Frequency of clinical complaints (%) in patients before the start, one and three months after IFN-γ therapy in patients with CVEBI in the 2nd subgroup.

3.4 Relationship between the number of EBV DNA copies and clinical complaints

Further, a correlation analysis was carried out to reveal the relationship between the numbers of EBV DNA copies in saliva samples before the start of IFN-γ therapy with the severity of clinical complaints in patients. The results are shown in **Table 8**.

Complaints	Correlation coefficient
Weakness	$\tau = 0.473$; $p = 0.026$ $r = 0.553$; $p = 0.026$
Sore throat	$\tau = 0.629$; $p = 0.002$ $r = 0.741$; $p = 0.001$
Joint pain	$\tau = -0.413$; $p = 0.052$ $r = -0.521$; $p = 0.039$

Table 8.
Influence of the number of EBV DNA copies on the severity of clinical complaints in patients with CEBVI (N = 51).

Thus, the number of copies of EBV DNA in saliva samples affects the development of weakness, sore throat, and arthralgia in patients with CEBVI.

3.5 Prognostic significance of the number of copies of EBV DNA

In order to clarify the prognostic significance of the number of EBV DNA in saliva samples, a linear regression analysis was carried out with the calculation of the coefficients of determination R2 (R Square) and using the Durban-Watson test and analysis of variance (ANOVA "Analysis of Variance") using criterion F and the calculation of the standardized coefficient beta (ß) with a 95% confidence interval. The results of the F criterion and the ß coefficient, indicating the significance of the obtained regression models, are presented below.

- The number of EBV DNA copies in saliva samples before the initiation of IFN-γ therapy affects the level of induced IFN-α production one month after the end of therapy ($F = 12.166$; $p = 0.002$; $\beta = 0.615$; CI: 75.999; 264.975; $p = 0.002$);
- The number of copies of EBV DNA in saliva samples before the initiation of IFN-γ therapy affects the level of induced IFN-γ production one month after the end of therapy ($F = 3.852$ $p = 0.061$; $\beta = -0.365$; CI:-0.011; −0.001; $p = 0.061$).

3.6 Influence of the induced IFN-γ level on clinical complaints

The next stage of the work was the correlation analysis of the influence of the initial level of induced IFN-γ on the clinical picture of the disease in patients of the 1st and 2nd subgroups (Group 1). It was shown that in the 1st subgroup, a high level of induced IFN-γ inversely influences the development of sweating in patients ($r = -0.506$; $p = 0.023$; $\tau = -0.419$; $p = 0.021$). In subgroup 2, the initially low level of induced IFN-γ inversely influences the development of weakness ($r = -0.405$; $p = 0.045$; $\tau = -0.419$; $p = 0.037$). It was not possible to identify other significant correlations in the subgroups.

4. Discussion

Currently, there is no single approach to the treatment of CEBVI, despite the fact that there are a number of specific antiviral drugs. In particular, acyclic nucleosides are widely used, such as acyclovir, valaciclovir (Valtrex), famciclovir (Famvir), and synthetic nucleoside analogs of guanosine: ganciclovir (cymevene), valganciclovir (Valcyte). In most cases, antiviral therapy is ineffective, which has been confirmed in numerous studies. In 2016, the results of efficacy analysis of infectious mononucleosis treatment were published according to the WHO World Register of Clinical Trials, both completed and ongoing. It was shown that the effectiveness of antiviral drugs (acyclovir, valaciclovir) in acute infectious mononucleosis is doubtful. Acyclovir and valaciclovir reduce EBV replication by inhibiting viral DNA polymerase and decreasing the oral secretion of EBV in patients with infectious mononucleosis. Balfour HH, et al. showed that taking the drug in a dose of 1 g every 8 h for 14 days leads to a decrease in clinical complaints in infectious mononucleosis [46]. In the case of viral shedding, shedding was observed to suppress the shedding against the background of antiviral therapy, but this effect ceased after the end of antiviral therapy [47]. The authors did not obtain a statistically significant difference between the groups of patients receiving antiviral drugs and the control groups. Most of the studies processed were unclear or at high risk of bias. Experimental studies in vitro have shown that EBV thymidine kinase has a variable affinity for antiherpetic antiviral drugs, that is, acyclovir and dihydroxypropylmethylguanine are relatively weak substrates for EBV thymidine kinase [48]. In our study, in the group of patients receiving therapy with Valtrex (valaciclovir) at a dose of 1 g per day for 2 months, suppression of EBV DNA replication in saliva samples was obtained in 28.57% of patients. This is confirmed by literature. The effectiveness of the use of valganciclovir in suppressing EBV replication and reducing the severity of clinical complaints in patients has been shown [49]. Taking valganciclovir leads to a decrease in the amount of EBV DNA from an average of 4.3 $\log_{10}$ copies/ml to 1.2 $\log_{10}$ copies/ml by 0.77 logs (95% CI, .62–.91 logs; $P < .001$) [50]. Valaciclovir and famciclovir suppress EBV DNA replication by 18% and 30%, respectively [51], while valganciclovir reduces EBV DNA secretion by 46% [52]. That is, valganciclovir can be used in the treatment of CEBVI.

The research by Kure S. et al., devoted to the study of the inhibitory effect of pure recombinant human (rh) IFN-α and IFN-γ on EBV infection in B-cell lines, BJAB lines, and in normal mature B-lymphocytes, showed that the pretreatment of cells within 24 h with rhIFN-α and rhIFN-γ suppressed the production of EBV specific nuclear antigen (EBNA-1) in BJAB cells 24 h after EBV infection. Both rhIFN types also effectively inhibited EBV infection in normal mature B lymphocytes, as evidenced by a decrease in [3H] thymidine incorporation 6 days after EBV infection and the total number of proliferating cells 21 days after infection. The authors showed that rhIFN-α exhibited a more pronounced inhibitory effect than rhIFN-γ. None of the rhIFNs showed a pronounced inhibitory effect on EBNA expression in covert EBV-infected Raji and Daudi cell lines. These results indicate that rhIFNs act predominantly at the early stage of EBV infection [53]. In our work, it was shown that in group 1 (N = 51) one month after rhIFN-γ therapy, 15 (29.41%) patients had negative PCR results in saliva samples, and 36 (70.59%) patients had copies of EBV DNA decreased. That is, rhIFN-γ can completely inhibit viral replication in 29.41% of patients. However, in this group of patients, a pronounced reliable dynamics of clinical complaints were obtained after the end of therapy. In 2002, it was shown that treatment of Vero cells with IFN-β or IFN-γ inhibits HSV-1 replication by less than 20-fold, while co-treatment with IFN-β and IFN-γ inhibits

HSV-1 replication by ~1000 times [41]. The authors suggested that the high level of inhibition achieved by the administration of exogenous IFN-γ is the result of a synergistic interaction with endogenous IFN-α/β, which are locally produced in response to HSV-1 infection. Our results confirm these in vitro data. If we compare the results we obtained in the groups of patients who received rhIFN-γ and monotherapy with valganciclovir in terms of the dynamics of the number of DNA copies in saliva samples, then no difference was obtained between these groups, that is, the effectiveness of monotherapy with rhIFN-γ or valaciclovir has similar efficacy (29.41% and 28.57% respectively). Our results are consistent with previously published data, in particular, the Russian literature describes the results of the study of rhIFN-γ (Ingaron) and presents evidence of the high efficiency of its use in the treatment of herpesvirus infections [54]. The authors showed that the drug has a direct antiviral effect, and the clinical effect is manifested through the activation of cellular immunity, which controls the viral antigen. In group 3, a month after taking the combination therapy valganciclovir+rhIFN-γ, a negative PCR result was obtained in 19 (71.74%) patients. The effectiveness of the therapy did not depend on the combination of drugs but on the duration of the course of rhIFN-γ administration. The best result from therapy was in patients who received 20 injections of rhIFN-γ in combination with valganciclovir. It was in this group that the number of copies of EBV DNA in saliva samples was negative in 87.50% of patients. Thus, a positive result on the number of EBV DNA copies during this treatment regimen is due not so much to the total combination course, but to the amount and duration of rhIFN-γ administration.

In 2003, an open, randomized, controlled, multicenter clinical study was conducted to study the anti-fibrotic effect of rhIFN-γ in 153 patients with chronic viral hepatitis B. RhIFN-γ was introduced i/m daily at a dose of 1 MU for three months and 1 MU every other day for the following six months. As a result, it was shown that rhIFN-γ has a pronounced anti-fibrotic effect in patients with chronic hepatitis B [55]. The effectiveness of treatment was 66% in the group of patients versus 16.2% in the control group. Later in 2011, the results of the study of rhIFN-γ monotherapy in 25 HBsAg-positive patients with stage 2-4 fibrosis who received long-term rhIFN-γ therapy were published [56]. The authors also showed that long-term therapy for nine months leads to pronounced positive dynamics of inflammation and fibrosis of the liver tissue. Our results with long-term administration of rhIFN-γ confirm these data.

With herpes viral infection, the secretion of cytokines is altered, modulating a strong and effective antiviral immune response against infected host cells. After primary infection, herpes viruses persist in the host organism for a long time [57]. One of the factors contributing to the persistence of herpes viruses is their ability to adopt two different modes of the life cycle: latent and lytic. After primary infection, herpes viruses pass into a latent, transcriptional-translational suppressed state, which can often be interrupted by lytic episodes. During the latency phase, transcripts were identified, in particular, such as microRNAs (miRs), which play a role in the mechanism of evasion of the virus from the host's immune response, including impaired interferon signaling [58].

It has been shown that the early EBV protein BZLF1 can block IFN-γ production by inhibiting the downstream IFN-γ signaling pathway. Essentially, BZLF1 stops the transcription of all expressed HLA class II molecules and, therefore, the activation of T-helper cells required for the induction of an immune response, inhibits IFN-γ-induced tyrosine STAT1 phosphorylation and nuclear translocation of BZLF1, reduces the expression of the IFN-γ receptor, stimulating the mechanism, with the help of which EBV can avoid the antiviral immune response during primary infection [59]. In addition, the EBV lytic transactivator Zta suppresses the production of IFN-β,

the EBV protein LMP1 inhibits TNF-α and induces the production and secretion of IL-10, and the miR-BHRF1-2-5p EBV blocks the proinflammatory signaling of IL-1 [60]. Cytokine signaling is a very early response to viral infection and explains the presence of corresponding inhibitory viral factors in the tegument. Thus, the dysregulation of the production of proinflammatory cytokines is based on the fact that virions already contain molecules that directly target the proper cytokine signaling. After infection of host cells and transcription of viral DNA leading to translation of viral miRs into viral peptides, other mechanisms of proper immune surveillance are targeted, including, in particular, presentation of HLA class I antigen, as well as decreased expression of NKG2D ligands [61].

INF-γ plays not only an important role in modulating T-cell immunity but also, having a direct antiviral activity is used as an effective therapeutic agent in the treatment of viral infection [62]. Okano et al. conducted a study of the efficacy of therapy with recombinant IFN-γ in a patient with infectious mononucleosis and X-linked lymphoproliferative syndrome (XLP). EBV-determined nuclear antigen and EBV DNA have been found in various tissues of the patient. After therapy with recombinant IFN-γ, there was positive dynamics in the reduction of virus-infected cells and a linear increase in the content of IFN-γ in the blood serum. NK cell activity remained within normal limits throughout the course of therapy. The authors suggested that cytotoxic cells can produce endogenous IFN-γ [63]. A. Linde et al. also revealed an increase in serum IFN-γ levels 24 h ($p = 0.05$) and 48 h ($p = 0.01$) after EBV infection, subsequently, the level of IFN-γ returned to baseline values [64]. In another study, in patients with acute infectious mononucleosis, an increase in the level of serum IFN-γ was shown only during the first week from the moment of infection, later the level of IFN-γ returned to normal [65]. Interesting data were obtained when studying the dynamics of IFN-γ level production in patients with tuberculosis, who showed a decrease in the average IFN-γ level over time ($p = 0.001$), but this decrease occurred during the first 8 weeks from the start of specific therapy ($p = 0.019$). When comparing baseline susceptible ($N = 55$) and drug-resistant patients ($N = 18$), there was no difference in the change in IFN-γ levels over time. Since the production of IFN-γ and secretion from T cells increase in response to an increase in antigenic load and then stabilize over 24 weeks, a decrease in the concentration of IFN-γ may indicate a positive response to the therapy and play the role of monitoring the response to therapy [66].

Our data indicate the absence of a significant increase in the production of the induced, serum, and spontaneous level of INF-γ three months after the end of therapy with INF-γ in the general group of patients, which is fully consistent with the previously published results of other authors. But when analyzed separately in each group of patients, it was shown that in the group with an initially low level, the administration of INF-γ led to a significant increase in the level of induced INF-γ three months after the end of therapy ($p = 0.027$). This is probably due to the initial low level of induced INF-γ and a more pronounced response to INF-γ therapy, which manifested itself in a significant positive dynamics of the main clinical complaints. Thus, we demonstrated that the dynamics of the production of the initially low level of induced INF-γ can be a marker of the positive effect of the therapy with INF-γ.

The absence of positive dynamics of the increase in the production of induced INF-γ in the general group of patients one and three months after the end of therapy with INF-γ indicates the absence of the effect of the drug on the level of production of endogenous INF-γ, which was previously demonstrated in studies by other authors. At the same time, INF-γ has a pronounced antiviral effect, which was shown earlier, and does not cause the increase of INF-γ production to the levels that would exceed the reference values.

When analyzing the clinical picture, we revealed that in the group of patients with a higher level of induced IFN-γ production at the time of initiation of therapy, complaints were more pronounced and more frequent. This is probably due to a more intensive inflammatory response in this group of patients. This conclusion is supported by previously published data that these inflammatory reactions are enhanced by the presence of IFN-γ, which dramatically increases the production of inflammatory mediators by macrophages [67].

5. Conclusions

1. RhINF-γ has a pronounced antiviral effect, which is expressed in a significant decrease in the number of EBV DNA copies in patients with CEBVI.

2. The introduction of rhINF-γ leads to positive dynamics of the clinical picture of the disease. The most pronounced positive dynamics were found in patients with an initially low level of induced INF-γ.

3. The positive dynamics of the production of the initially low level of induced INF-γ can be a marker of the effectiveness of the therapy with rhINF-γ in patients with CEBVI.

4. The efficacy of therapy in patients with CEBVI is determined by the duration of the introduction of rhINF-γ: 500,000 IU every other day at least 20 injections shows the best result.

5. In the group of CEBVI patients with an initial level of induced IFN-γ at the lower limit of reference values, therapy with rhINF-γ leads to a significant increase in the level of induced INF-γ three months after the end of therapy.

6. Therapy with rhINF-γ one and three months after the end of treatment of patients does not cause changes in the production of INF-α to levels that would exceed the reference values in patients with CEBVI.

Conflict of interest

The authors declare that they have no potential conflicts of interest.

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