SUNTEXT REVIEWS

SunText Review of Medical & Clinical Research
ISSN: 2766-4813

Open Access
Research Article
Volume 6:1

Anti-Inflammatory Effect of Alloferon in Patients with Chronic Epstein - Barr virus Infection

Rakityanskaya IA[1,*], Ryabova TS[2] and Kalashnikova AA[3]

[1]*Immunologist, Doctor of Medical Sciences, Professor, Department of Allergology, Immunology and Clinical Transfusiology, Municipal Outpatient Hospital no. 112, 190000, Saint Petersburg, Russia*

[2]*Doctor of Medical Sciences, Associate Professor, S.M. Kirov Military Medical Academy, Russia*

[3]*Candidate of Biological Sciences, Laboratory Diagnostics Doctor, A.M. Nikiforov Russian Center of Emergency and Radiation Medicine, Russia*

[*]**Corresponding author:** Irina A Rakitianskaya, Clinical Immunologist, Professor, consultant immunologist Outpatient Unit of Allergology, Immunology, and Clinical Transfusiology, City Outpatient Clinic Number One Hundred and Twelve, St. Petersburg, Academician Baykov Street 25, Russia; E-mail: tat-akyla@inbox.ru

Received date: 03 March 2025; **Accepted date:** 07 March 2025; **Published date:** 13 March 2025

Citation Rakityanskaya IA, Ryabova TS, Kalashnikova AA (2025) Anti-Inflammatory Effect of Alloferon in Patients with Chronic Epstein - Barr virus Infection. SunText Rev Med Clin Res 6(1): 222.

DOI: https://doi.org/10.51737/2766-4813.2025.122

Abstract

Background: Epstein-Barr virus (EBV) belongs to the family of human γ-herpesviruses, which affects about 90% of the adult population in the world and is the etiological factor of various lymphoproliferative, malignant neoplasms, as well as many autoimmune diseases. Currently, there is no single approach to the treatment of chronic Epstein-Barr virus infection in the world and none of the antiviral drugs have been licensed for the treatment of EBV infections. From 1939 to the present, new antimicrobial peptides (AMPs) have been developed in the world - this is a group of oligopeptides with different amounts (from 5 to 100 or more) of amino acids, which are essential components of innate immunity and are candidates for the creation of new anti-infective drugs. One of the AMPs is alloferon, which has antiviral and antitumor activity, suppresses the expression of proinflammatory cytokines.

Methods: The study was conducted in 110 patients suffering from chronic Epstein-Barr virus infection (EBVI). The group consisted of 72 women and 38 men. The average age was 38.45 ± 1.08 years. The duration of EBVI was 3.65 ± 0.20 years. All patients underwent EBV DNA testing using PCR in saliva samples and tumor necrosis factor-α production in lymphocyte culture medium.

Results: TNF-α production depends on the number of EBV DNA copies and the duration of the disease. Alloferon were shown to have an anti-inflammatory mechanism of inhibition of TNF-α production.

Conclusions: Alloferon inhibits the production of proinflammatory TNF-α in patients with EBV.

Keywords: Epstein-Barr virus infection; TNF-α; Therapy; Alloferon

Introduction

Epstein Barr virus (EBV) belongs to the family of human γ-herpesviruses, which affects about 90% of the adult population in the world and is the etiologic factor of various lymphoproliferative, malignant neoplasms, as well as many autoimmune diseases [1-4]. Epstein–Barr virus (EBV) was first isolated and identified by electron microscopy by Anthony Epstein and Yvonne Bar in a culture of tumor cells brought from an expedition to Equatorial Africa (Uganda) and obtained as a result of a biopsy of a patient with Burkitt's lymphoma (BL) in 1964 [5]. EBV was registered as the first oncogenic human virus with a high degree of infection of the population worldwide [6]. The World Health Organization recognized EBV as an oncogenic virus of class I [7-9]. Primary human infection is often asymptomatic and depends on socioeconomic factors, such as overcrowding and poor sanitation. The main way a virus spreads

is through direct contact with saliva from a carrier. Infection can also be transmitted through sexual contact, organ transplantation, and blood transfusion [10]. More than 90% of adults and 50% of children worldwide are seropositive for EBV [11]. Primary EBV infection, transmitted through saliva, is usually acquired in childhood. Most EBV infections in children are asymptomatic. However, approximately 50% of teenagers and adults may develop infectious mononucleosis syndrome [12], which is rarely fatal [13-15], chronic active infection [16,17], hemophagocytic syndrome [18], or neoplasms such as Burkitt lymphoma, undifferentiated nasopharyngeal carcinoma, gastric carcinoma, Hodgkin lymphoma, T-cell lymphoma, NK/T-cell lymphoma of the nose, aggressive NK/T-cell lymphoma/leukemia, leiomyosarcoma, and lymphoproliferative disorders in immunocompromised hosts, including AIDS-associated lymphomas [19,20]. Following primary infection, EBV can persist in the host for life [21]. In high income countries, EBV seroconversion peaks in children aged 2–4 years and 14–18 years, peaking in late adolescence and declining after age 35 [22], reaching infection rates of over 90% with age. Therefore, almost all children become seropositive by the age of 6. Infection rates of over 90% with age. Therefore, almost all children become seropositive by the age of 6 [23]. In low income countries, most children become infected with EBV in early adolescence [24].

Most previously published studies have shown that guanine nucleoside analogues, acyclovir (ACV) and ganciclovir (GCV), are capable of inhibiting the lytic phase of EBV replication [25,26]. Acyclovir is phosphorylated by virus-specific thymidine kinase to its monophosphate form, which inhibits viral DNA synthesis by inhibiting viral DNA polymerase. Thus, the drug stops the synthesis of viral DNA. Under experimental conditions, it was shown that virus-specific thymidine kinase and DNA polymerase are jointly responsible for the action of acyclovir in virus-infected cells. That is, inhibition of EBV DNA polymerase is the main mechanism of inhibition of replication in lytically infected cells expressing viral polymerase [27]. Latent EBV replication in proliferating B cells does not require viral DNA polymerase, which is why antiviral therapy is usually ineffective in this case. Other antiviral agents include penciclovir, famciclovir, ganciclovir, valganciclovir, cidofovir, and foscarnet [28]. The drugs inhibit viral replication by inhibiting viral DNA. The mechanisms of GCV and/or ACV phosphorylation in Epstein-Barr virus (EBV)-infected cells were studied experimentally. The authors showed that EBV-encoded protein kinase (EBV-PK) plays a role in susceptibility to ACV and GCV. EBV-encoded thymidine kinase (EBV-TK) is less involved in this mechanism. This mechanism explains the low efficiency of ACV and GCV [29]. However, other authors have shown that expression of EBV thymidine kinase in cells increases GCV phosphorylation and sensitivity to the cytotoxic effects of GCV and ACV, as well as to the destruction of the virus by penciclovir, a drug similar to acyclovir [30]. In 2016, the results of an analysis of the effectiveness of infectious mononucleosis therapy for the period from 1981 to 2016 according to the WHO World Clinical Trials Registry were published [31]. The authors of the analysis showed that against the background of therapy with antiviral drugs, the number of virus-infected cells practically does not decrease, and the level of EBV DNA copies in the peripheral blood decreases only during the therapy; after the end of therapy, the number of DNA copies returns to the original level. Currently, there is no unified approach to the treatment of chronic Epstein-Barr virus infection in the world and none of these antiviral drugs have been licensed for the treatment of EBV infections [32].

The development of resistance to antibacterial and antiviral drugs is becoming a serious problem worldwide [33]. That is why the Infectious Diseases Society of America has introduced an initiative to develop new antimicrobial drugs. Antimicrobial peptides (AMPs) are a group of oligopeptides with varying numbers (from 5 to 100 or more) of amino acids that are essential components of innate immunity and are candidates for the creation of new anti-infective drugs. The term AMPs refers to antibiotics, antivirals, and antimalarials [34,35]. The first antimicrobial agent was isolated from a soil strain of bacilli in 1939 by Dubos RJ et al. [36]. The authors of the work showed that the obtained extract, under experimental conditions in mice, has a protective function against pneumococcal infection. In 1940, Hotchkiss R.D. and Dubos R.J. [37] divided this extract into fractions and isolated an AMP, which was called gramicidin. The first registered AMP of animal origin is defensin, which was isolated from rabbit leukocytes in 1956 [38]. Currently, more than 5,000 AMPs have been discovered and synthesized [39]. Natural AMPs can be isolated from prokaryotes (e.g., bacteria) and eukaryotes (e.g., protozoa, fungi, plants, insects, and animals) [40,41]. The antiviral action of AMPs consists of neutralizing the virus by integrating either into the viral envelope or into the host cell membrane. They can destroy both RNA and DNA-containing enveloped viruses [42-44]. They can also integrate into the viral envelopes, making the membranes unstable and preventing the viruses from infecting host cells [45]. Additionally, they can reduce the binding of viruses to host cells [46]. Antiviral AMPs can inhibit the entry of viral particles into host cells, bind to heparan sulfate receptors on the cell surface, penetrate the cell membrane and localize in the cytoplasm and organelles. This results in a change in the gene expression profile of host cells, which helps the host immune system block the expression of viral genes [47]. During intracellular interaction of AMP with the virion capsid, decapsidation occurs. Therefore, the viral nucleic acid cannot be released and transcribed [48]. AMP can induce the following mechanisms:

- expression of toll-like receptors that interact with viral nucleic acid;
- production of cytokines that stimulate the action of T-cytotoxic cells and NK cells;
- expression of major histocompatibility complex molecules in infected cells for the purpose of presentation of viral peptides to other cells of the immune system [49];
- Activation of innate restriction factors encoded by the infected cell [50].

Alloferon is a biologically active cationic peptide, originally isolated from the hemolymph of the larvae of the dipterous fly Calliphora vicina infected with firefly bacteria. The cationic peptides alloferon 1 and 2, consisting of 12 and 13 amino acid residues, were isolated. The identified amino acid sequences for alloferon are H-His-Gly-Val-Ser-Gly-His-Gly-Gln-His-Gly-Val-His-Gly-OH (alloferon I) and H-Gly-Val-Ser-Gly-His-Gly-OH (alloferon II). Alloferon 2 corresponds to the N-terminally truncated form of alloferon 1. A database search revealed very few identifiers with known large functionally significant proteins [51,52]. The name of the peptide "alloferon" was chosen due to the similarity of physiological function with interferon and origin from different species of invertebrates (Allo) [53]. Alloferon does not have cytotoxicity, immunogenicity, carcinogenicity, embryotoxicity and does not have reproductive effects [54]. Alloferon is used in the treatment and prevention of various infectious and tumor diseases. The antiviral activity of alloferon is carried out through NK cells [51], enhances the cytotoxic activity of NK cells [55], and it is also advisable to use it in combination with other antiviral drugs. The introduction of alloferon suppresses the expression of proinflammatory cytokines such as TNF-α, IL-1β, IL-6 and IL-18 [52,56]. However, we did not find any data in the literature on the anti-inflammatory effect of alloferon in patients with chronic EBV infection. The aim of this study is to investigate the effect of alloferon on the production of TNF-α in lymphocyte culture in patients with chronic Epstein-Barr virus infection.

Materials and Methods

Patients

A total of 110 patients suffering from chronic Epstein-Barr virus infection (chronic mononucleosis) were included in the randomized study. The group consisted of 72 women and 38 men. The average age of the patients was 38.45 ± 1.08 years. (95% CI: 36.35 - 40.58). The duration of the course of EBV from the onset of the first complaints in the patient to laboratory examination and confirmation of EBV infection, diagnosis was 3.65 ± 0.20 years (95% CI: 3.24 - 4.04). It is known from the anamnesis that 72 patients (65.45%) in the general group under the age of 14 suffered from chronic recurrent tonsillitis, 54 patients (49.09%) under the age of 16 suffered from acute infectious mononucleosis. All patients complained of frequent colds. The diagnosis of "chronic EBV infection" was made on the basis of the patient's complaints, anamnesis and confirmed by laboratory tests during a full examination by relevant specialists at the previous stage.

Participants in the study were selected according to the inclusion criteria

- age 25–50 years;
- presence of clinical complaints caused by EBV
- number of DNA copies
- signed voluntary informed consent before the study

Exclusion criteria were

- presence of autoimmune diseases associated with EBV infection;
- presence of other viral infections (cytomegalovirus infection, viral hepatitis, human immunodeficiency virus);
- presence of toxoplasmosis, borreliosis, staphylococcal and streptococcal infection;
- presence of helminthic invasions, parasitic infections;
- presence of pregnancy in women;
- presence of acute inflammatory diseases of any organs;
- presence of mental illness;
- regular alcohol consumption;
- taking any antiviral drugs in the last 6 months.

After the described examination, confirmation of the diagnosis and detection of chronic EBV infection, the patients were referred for treatment to an immunologist. The course of therapy with alloferon and control studies were carried out in the Department of Allergology-Immunology and Clinical Transfusiology of Municipal Outpatient Hospital no. 112, Saint Petersburg, Russia from December 2022 to December 2024. The clinical research carried out in compliance with the WMA Declaration of Helsinki - Ethical Principles for Medical Research Involving Human Subjects, 2013) and the Protocol of Council of Europe Convention on human rights and biomedicine 1999 and articles 20, 22, 23 of the Act “On the basics of healthcare for the Russian Federation citizens” dated November 21, 2011 Fed. Statute №323-FZ (May 26, 2021 edition). The clinical study was conducted in accordance with a procedure approved by the local ethical committee under LLC “Center of Dialysis of St. Petersburg” FRESENIUS MEDICAL CARE. All patients were treated with Allokin-alpha - 12 subcutaneous injections of 1.0 mg every other day. Subcutaneous administration of the drug was well tolerated, did not cause allergic reactions, did not have a hepato-nephrotoxic or toxic effect on the hematopoietic organs. To assess the effectiveness of the treatment, 4 weeks after the end of the course of therapy, we analyzed the number of EBV DNA copies in saliva samples, the level of serum production,

spontaneous and induced tumor necrosis factor-α (TNF-α) in lymphocyte culture, and the dynamics of clinical symptoms.

Clinical methods of patient examination included: 1). collection of anamnesis; 2). data on the presence of concomitant diseases; 3). data on previously administered therapy. The clinical condition of patients was assessed using a generally accepted method, including objective data and patient complaints at the time of examination. Registration of patient complaints was carried out using a subjective assessment scale on a 3-point scale (0 - no symptoms, 1 - mild symptoms, 2 - moderate symptoms, 3 - severe symptoms). Patients who received antiviral or immunomodulatory therapy during the last 6 months were not included in the study.

Methods

To confirm the viral etiology of the disease, all patients were tested for the virus using PCR in saliva samples. According to numerous studies, in chronic forms of Epstein-Barr virus infection, the analysis of EBV DNA in a saliva sample is more informative [55-58]. The patients were tested for the virus DNA by PCR method (in saliva samples via polymerase chain reaction (PCR) with hybridization-fluorescent detection in real-time mode). The employed test-systems "AmpliSence EBV/CMV/HHV6-screen-FL" from the Central Scientific Institute of Epidemiology (Russia). Measurement units, used for assessing the viral load during extraction of DNA from saliva – number of copies of the EBV DNA per 1 ml of the sample (NCDNA). According to the manual, this indicator is calculated by a formula: NCDNA=CDNA x 100, where CDNA number of copies of virus DNA in the sample. The test-system analytical sensitivity is 400 copies/ml.

Production of tumor necrosis factor-alpha (TNF-α) in the culture environment (spontaneous, induced production) and in blood serum.

Whole heparinized blood diluted fivefold with RPMI-1640 nutrient environment with L-glutamine (Biolot, Russia) was cultured in round-bottomed plates in the presence or without an inducer of cytokine synthesis. Pyrogenal was used as a synthesis inducer (Salmonella typhi lipopolysaccharide 100 μg/ml, N.F. Gamaleya Research Center for Epidemiology and Microbiology, Russia). Cultivation was carried out for 24 hours at 37°C in a CO2 incubator. At the end of incubation, the supernatant was transferred to Eppendorf tubes and frozen for further work. Spontaneous production of cytokines was assessed in the supernatant of blood cell culture without the addition of an inducer, and induced production with the addition of pyrogenal. Determination of the content of cytokines in the serum and in the supernatant liquid of the blood cell culture was carried out by enzyme-linked immunosorbent assay using reagent kits for ELISA (Vector-Best JSC, International Certificate ISO 13485) according to the manufacturer's instructions. The sensitivity of the test kit for TNF-α did not exceed 1.0 pg/ml.

Statistical treatment of acquired data was conducted by means of a software package IBM SPSS Statistics, 26 version (Armonk, NY: IBM Corp.). Group results are presented in form of mean arithmetic M ± Standard Error. For the statistical treatment we employed parametric (Pearson's method) and nonparametric (Spearman's method, tau (τ) Kendall) methods. To verify compliance with condition of independence of observations we conducted linear regression analysis (with computation of coefficient of determination (R Square) and criterion of Durban-Watson) and dispersion analysis (ANOVA Analysis of Variance) with computation of criterion of Fisher (F) for verification of model significance. Standardized rate ß with 95% confidence intervals was calculated. Critical significance level of difference of indicators was taken to be equal 0.05.

Result

Clinical complaints

Chronic Epstein-Barr viral infection is characterized by a long course and frequent relapses of the clinical and laboratory picture of the viral infection, which manifests itself with mononucleosis-like symptoms [59-62]. Patients complain of prolonged subfebrile temperature (37.1-37.3°), which appears only during the daytime, severe weakness and fatigue, increased sweating (especially at night), lymphadenitis (cervical and submandibular lymph nodes, less often axillary and inguinal), a constant feeling of discomfort and / or pain in the throat, frequent exacerbations of aphthous stomatitis, gingivitis, a burning sensation of the mucous membrane of the tongue, severe postnasal drip, development of arthralgia of large and small joints, stiffness of small joints, frequent colds. During the examination of patients by a rheumatologist, no pathology was detected. Typical complaints were headaches, memory impairment, decreased concentration, sleep disorders, irritability, and tearfulness. Patients were examined by a neurologist, consulted psychiatrists, and attended psychotherapy sessions. As prescribed by psychiatrists, patients received antidepressant therapy. Typically, the occurrence of the described complaints was aggravated by long-term stressful situations, psychoemotional and physical overload. We analyzed the frequency of occurrence of the described complaints in the general group of patients (Table 1).

TNF-α production in lymphocyte culture. In the general group of patients, a study of tumor necrosis factor-alpha (TNF-α) production (serum, spontaneous, induced) was conducted (Table 2). When analyzing the level of induced TNF-α in the culture medium of patients with EBV infection, it was found that these values differed sharply in patients, i.e. they were either

significantly lower or within the reference values or significantly higher than the values of healthy donors (2810 – 5700 pg/ml, reference values provided by the manufacturer of the test systems) (Figure 1).

***Table 1**: Frequency of clinical complaints (%) in patients with EBV infection in the general group.*

Complaints	Frequency of clinical complaints % (n=110)
Subfebrile temperature	81.81
Lymphadenitis	50.00
Sore throat	80.00
Weakness	77.27
Physical fatigue	75.45
Chills	56.36
Sweating	81,81
Postnasal drip	50.00
Stomatitis	43.63
Arthralgia	36.36
Irritability and tearfulness	59.09
Headaches	36.36
Decreased concentration	47.27
Memory loss	45.45
Sleep disturbances	52.72
Daytime sleepiness	46.36

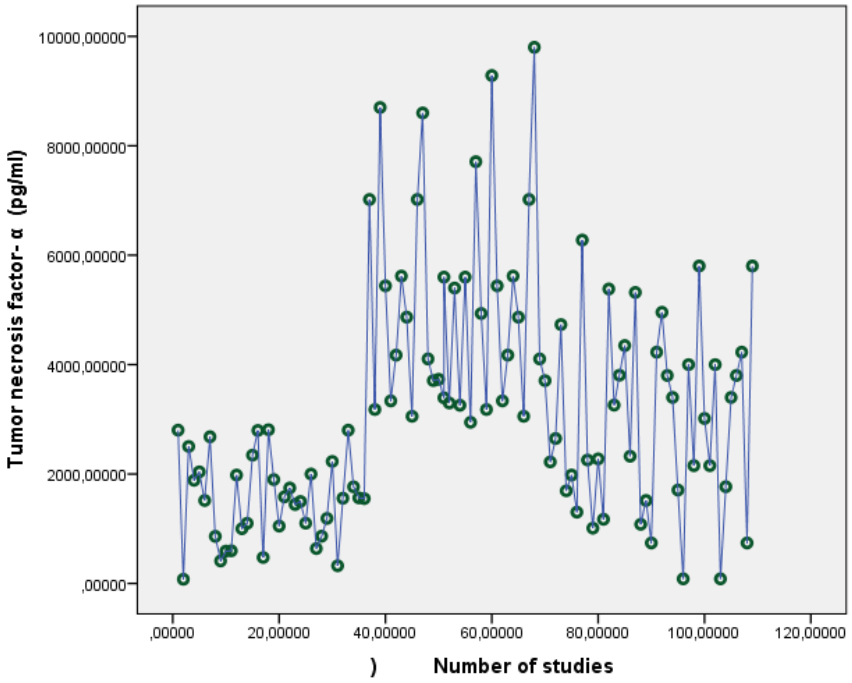

Figure 1: *Level of induced TNF-α production in the culture medium of patients with EBV infection in the general group.*

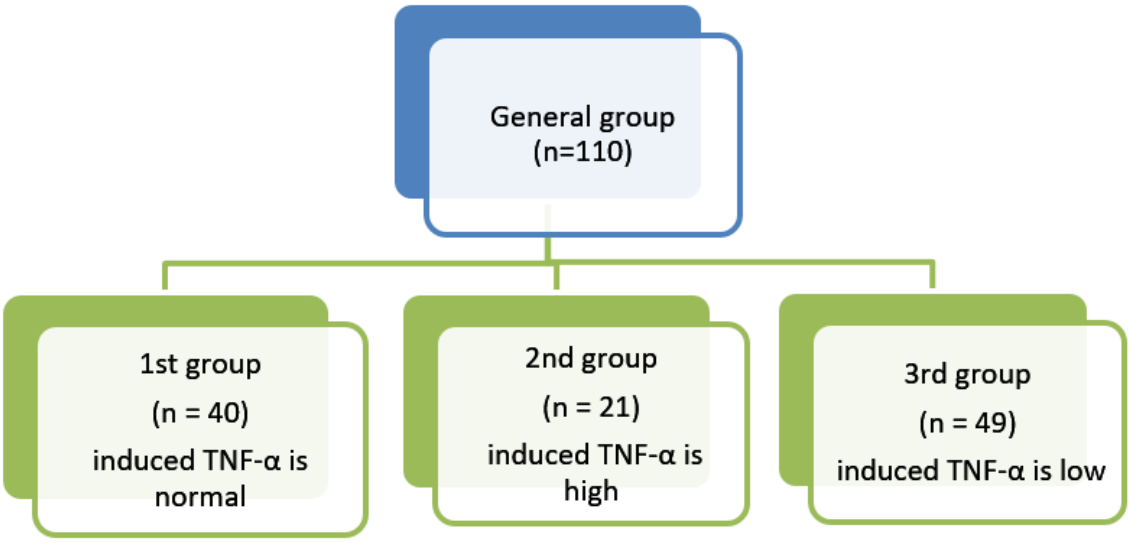

Figure 2: *Scheme of distribution of patients into groups for subsequent analysis.*

Table 2*: Production of TNF-α in the culture medium in the general group of patients with EBV infection (n=110).*

Parameter	TNF-α level (pg/ml)	Reference values (pg/ml)
Serum level	6,21 ± 1,56 95% CI: 3,31 – 9,42 (1,0 – 87,0)	0 - 6
Spontaneous level	46,50 ± 17,61 95% CI: 19,63 – 86,66 (1,0 – 1675,0)	7 - 30

Induced level	3268,73 ± 242,20 95% CI: 2813,40 - 3754,42 (76,0 – 12,826)	2810 - 5700

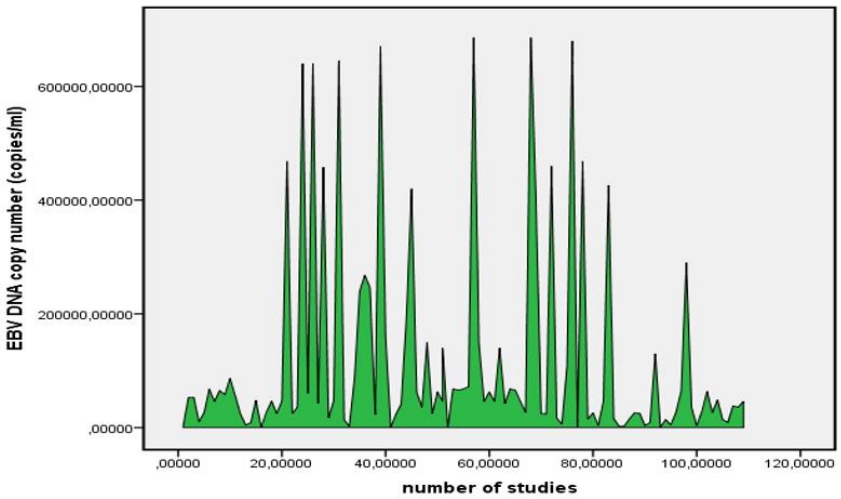

Figure 3: *Number of EBV DNA copies in saliva sample from patients in the general EBV infection group before the start of therapy.*

Therefore, all patients in the general group were divided into three groups according to the initial level of induced TNF-α (Figure 2).

1st group (n = 40) - the level of induced TNF-α is normal within the range of - 2940.0 - 6276.0. M ± m = 4197.47 ± 173.14 (pg / ml) (95% CI: 3848.07- 4546.87);

2nd group (n = 21) - the level of induced TNF-α is high within the range of - 5719.0 - 10826.00. M ± m = 7881.76 ± 507.39 (pg / ml) (95% CI: 6993.55 - 8968.61);

3rd group (n = 49) the level of induced TNF-α is low within the range of - 10.0 - 2407.00. M±м= 1541.46 ± 92.24 (pg/ml) (95% CI: 1347.00 – 1711.81).

The Table 3 presents the data of the comparative analysis of these groups before the start of therapy. The data in the table show that groups with different levels of induced TNF-α production differed in the duration of the disease and the number of EBV DNA copies in saliva samples.

Dynamics of induced TNF-α production 4 weeks after the end of the alloferon therapy course

Next, an analysis of the effectiveness of alloferon therapy on the level of TNF-α production in patients in groups 1, 2, 3 was conducted (Table 4). The analysis of the results shows that therapy with alloferon reliably leads to a decrease in the level of production of serum, spontaneous and induced TNF-α 4 weeks after the end of the therapy course. The maximum decrease in induced TNF-α 4 weeks after the end of the alloferon course was in the 2nd group with an initially high level of induced TNF-α production.

Dynamics of the number of EBV DNA copies 4 weeks after the end of therapy

All patients (n=110) underwent a study of the number of EBV DNA copies using PCR in saliva samples before the start of therapy, which was 125120.48.±19112.09 copies/ml (95% CI: 87635.57 - 164902.45) (Figure 3). In each group of patients with EBV infection, before the start of the course of therapy and 4 weeks after the end of therapy with alloferon, the dynamics of the number of copies of EBV DNA were analyzed (Table 5 and Figure 4). The presented data show that the decrease in the number of EBV DNA copies was significant in all three groups. However, when calculating the percentage of decrease, it turned out that the maximum decrease in the number of EBV DNA copies was obtained in the 3rd group of patients with an initially low value of induced TNF-α production: 1st group - 98%; 2nd group - 84% and 3rd group - 99%.

Dynamics of clinical complaints 4 weeks after the end of therapy

The dynamics of clinical complaints 4 weeks after the end of alloferon therapy was then analyzed. The results are presented in (Table 6). The obtained data showed that the maximum positive dynamics of clinical complaints were observed in patients in the 1st group.

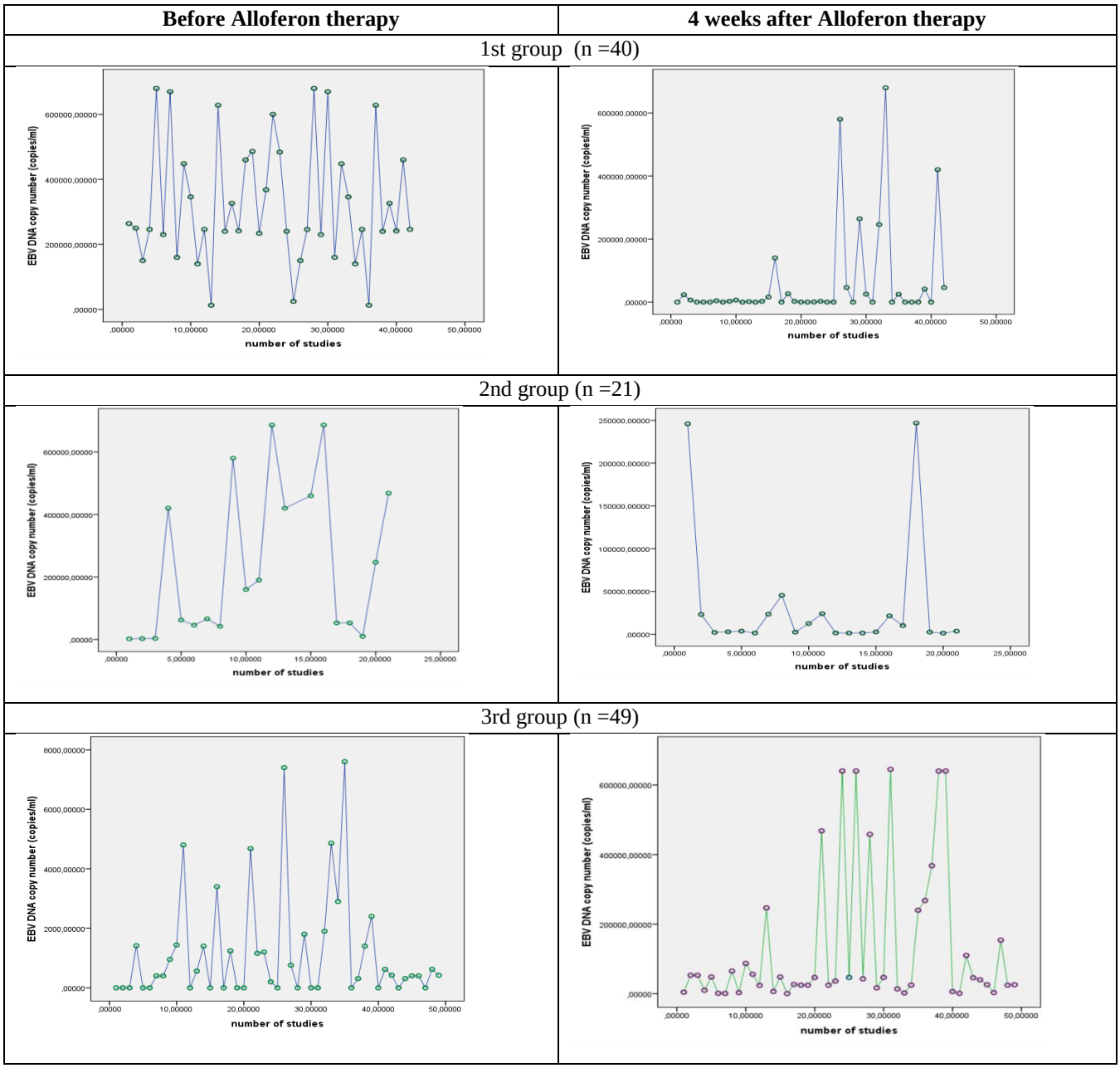

Figure 4:*The number of EBV DNA copies in a saliva sample in patients of the 1st, 2nd and 3rd groups of EBV infection before and 4 weeks after therapy with alloferon.*

Table 3*: Comparative analysis of the 1st, 2nd and 3rd groups of patients with chronic EBV.*

Parameter	**1st group (*n* =40) 1**	**2nd group (*n* =21) 2**	**3rd group (*n* =49) 3**	**P**
Age	37,87 ± 1,67 95%CI:34,51– 41,12	35,75 ± 2,63 95%CI:30,85-40,99	38,02 ± 1,68 95%CI:34,67 - 41,65	1,2 =0,06 1,3=0,08 2,3=0,05
Duration of disease	3,05 ± 0.30 95%CI:2,46– 3,66	3,30 ± 0,43 95%CI: 2,42 -4,14	4,22 ± 0,32 95%CI: 3,59 – 4,86	1,2=0,05 1,3=0,001

				2,3=0,003
Number of DNA copies	183997,17 ± 36673,32 95% CI: 114094,49 - 260426,12	247005,00 ± 2856,43 95%CI: 150253,36 - 357116,89	139903,67 ± 28123,31 95%CI: 89240,59 - 198996,92	1,2=0,001 1,3=0,002 2,3=0,001
Level of induced TNF-α	4197.47 ± 173.14 95% CI: 3934,69-4814,54	7881,76 ± 507,39 95%CI: 6993,55 - 8968,61	1541,46 ± 92,24 (95 % CI: 1347,00 – 1711,81).	1,2=0.001 1,3=0,001 2,3=0,001

Table 4: *Dynamics of induced TNF-α production in the 1st group 4 weeks after the course of therapy.*

Parameter	Before Alloferon therapy	4 weeks after Alloferon therapy	p
	1st group (n =40)		
Serum TNF-α	7,95 ± 4,17 95 % CI: 2,45 - 17,74	3,00 ± 0,43 95 % CI:2,20 - 3,90	0,001
Spontaneous TNF-α	35,40 ± 11,70 95 % CI: 15,50 -60,74	15,75 ± 6,03 95 % CI:6,75 – 29,14	0,001
Induced TNF-α	4197.47 ± 173.14 (95% CI: 3934,69-4814,54)	3882,00 ± 219,21 95% CI: 3548,57– 4449,08	0,03
2nd group (n =21)			
Serum TNF-α	3,40 ± 0,52 95 % CI: 2,20 – 4,40	2,70 ± 0,52 95 % CI: 1,60 – 3,70	0,03
Spontaneous TNF-α	6,80 ± 1,68 95 % CI: 3,80 – 10,50	5,00 ± 0,92 95 % CI: 3,30 – 6.90	0,021
Induced TNF-α	7881,76 ± 507,39 95% CI: 6993,55 - 8968,61	5645,00 ± 348,01 95% CI: 5019,02 - 6406,94	0,001
3rd group (n =49)			
Serum TNF-α	2,80 ± 0,56 95 % CI: 1,80 – 3,96	2,32 ± 0,35 95 % CI: 1,64 - 3,04	0,001
Spontaneous TNF-α	6,96 ± 1,24 95 % CI: 4,56 – 9.36	4,20 ± 0,55 95 % CI: 3,08 – 5,28	0,001
Induced TNF-α	1541,56 ± 92,24 95% CI: 1347,00 – 1711,81	1441,46 ± 92,24 95 % CI: 1237,00 – 1711,81	0,05

Table 5**:** *Dynamics of the number of copies in each study groupin patients with chronic EBV infection.*

Number of EBV DNA copies in saliva samples (copies/ml)		**p**
Before Alloferon therapy	4 weeks after Alloferon therapy	
1st group (n =40)		
115547,48 ± 17196,40 95% CI: 83323,23 - 150973,95	2576,82 ± 827,61 95 % CI: 1187,40 - 4367,31	0,001
2nd group (n =21)		
232878,50 ± 54987,0287822 95%CI: 134291,60 - 349336,27	33879,20 ± 68859,58 95 % CI: 7913,15 - 68859,58	0,0001
3rd group (n =49)		
133132,36 ± 30919,29 95%CI: 78186,14 - 200709,38	1783,91 ± 948,69 95 % CI: 558,72 - 3993,87	0,0001

Table 6*: Frequency of clinical complaints (%) in patients with chronic Epstein-Barr virus infection before the start of alloferon therapy and 4 weeks after its end in each group.*

Clinical complaints	**1st group (n =40)**		**2nd group (n =21)**		**3rd group (n =49)**	
	Before therapy	**After 4 weeks**	**Before therapy**	**After 4 weeks**	**Before therapy**	**After 4 weeks**
	1	**2**	**3**	**4**	**5**	**6**
Subfebrile temperature, p	75.0	52,5 p1-2 =0,001	71,42	47,61 p3-4 =0.001	81,63	77,55 p5-6 =0,064
Lymphadenitis, p	50.0	25,0 P1-2 =0.001	52,38	28,57 p3-4 =0,001	51,02	40,81 p5-6 =0,01
Sore throat, p	75,0	20,0 p1-2 =0,001	71,42	28,57 p3-4 =0,001	77,55	40,81 p5-6 =0,001
Weakness, p	75,0	50,0 p1-2 =0,001	71,42	52,38 p3-4 =0,001	77,55	53,06 p5-6 =0,001
Physical fatigue, p	65,0	55,0 p1-2 =0,01	61,90	42,85 p3-4 =0,001	61,22	59,18 p5-6 =0,084
Chills, p	50.0	20,0 p1-2 =0,001	52,38	23,80 p3-4 =0.001	51,02	38,77 p5-6 =0,01
Sweating, p	75,0	20,0 p1-2 =0,001	76,19	42,85 p3-4 =0,001	77,55	61,22 p5-6 =0,001
Postnasal drip, p	50.0	30,0 p1-2 =0,01	42,85	33,33 p3-4 =0,01	59,18	40,81 p5-6 =0,001
Stomatitis, p	25,0	10,0 p1-2 =0.001	23,80	14,28 p3-4 =0,01	18,36	16,32 p5-6 =0.08
Arthralgia, p	25,0	15,0 p1-2 =0,001	19,04	14,28 p3-4 =0,06	16,32	12,24 p5-6 =0,064
Irritability and tearfulness, p	55,0	32,5 p1-2 =0,001	42,85	38,09 p3-4 =0.07	40,81	38,77 p5-6 =0,064
Headaches, p	25,0	10,0 p1-2 =0.001	23,80	10,04 p3-4 =0,06	24,48	20,40 p5-6 =0,066

Decreased concentration, p	40, 0	25,0 p1-2 =0,01	23,80	19,04 p3-4 =0,08	40,81	32,65 p5-6 =0.052
Decreased memory, p	27,5	25,0 p1-2 =0,08	19,04	14,28 p3-4 =0,06	24,48	20,40 p5-6 =0,06
Sleep disturbance, p	40, 0	32,5 p1-2 =0,05	47,61	42,85 p3-4 =0,08	40,81	38,77 p5-6 =0,07
Daytime sleepiness, p	25,0	20,0 p1-2 =0,054	19,04	14,28 p3-4 =0,06	18,36	16,32 p5-6 =0.08

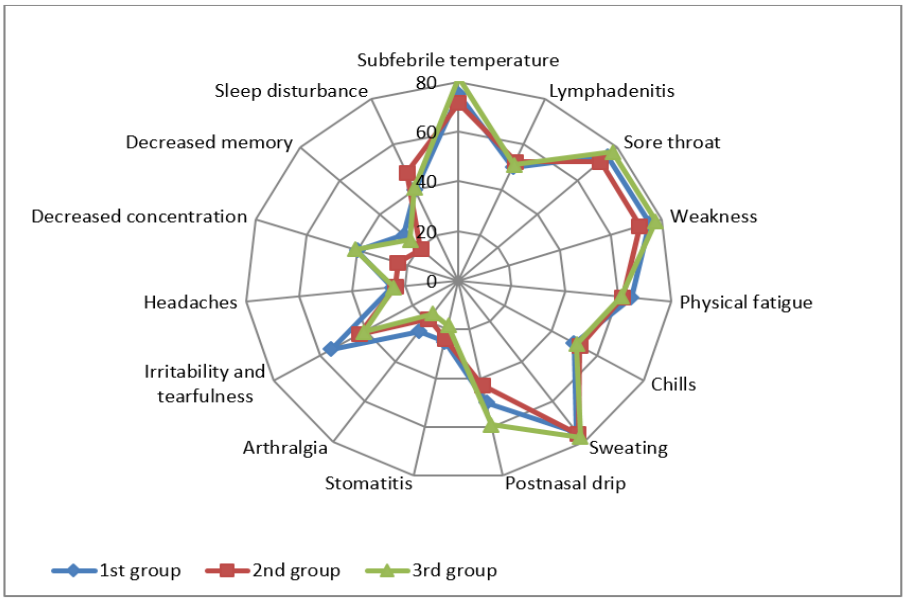

***Figure 5**: Prevalence of clinical complaints in groups of patients with chronic Epstein-Barr virus infection before the start of Alloferon therapy.*

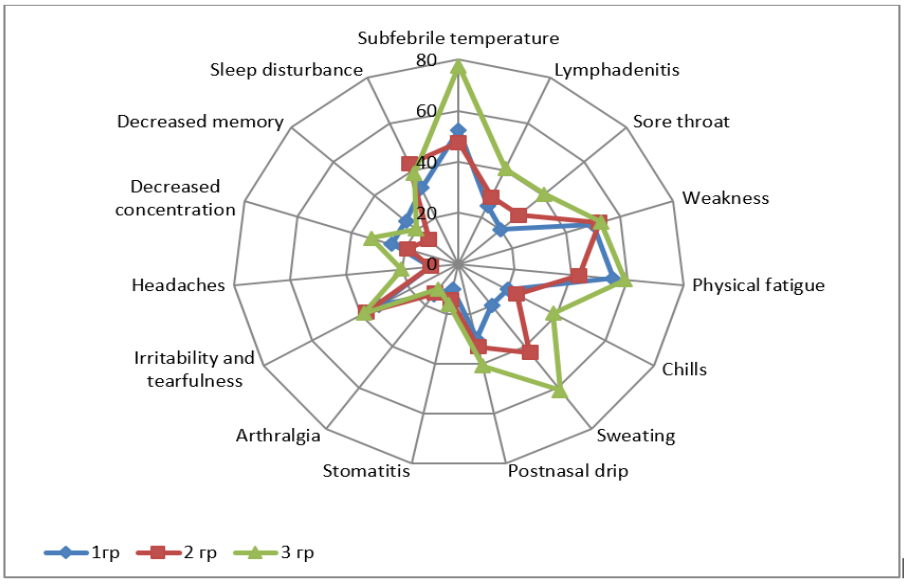

***Figure 6:** Prevalence of clinical complaints in groups of patients with chronic Epstein-Barr virus infection after Alloferon therapy.*

In the 2nd group of patients with an initially high level of TNF-α production, partially positive dynamics of such clinical complaints as subfebrile temperature, lymphadenitis, sore throat, postnasal drip, stomatitis, weakness and fatigue, chills, sweating were noted. The remaining complaints remained without significant positive dynamics. The worst results in terms of the dynamics of clinical complaints were obtained in the 3rd group of patients. Manifestations of lymphadenitis, sore throat and postnasal drip, weakness, sweating and chills decreased. All other complaints did not change after the course of therapy with alloferon. It should be noted that in the 3rd group of patients, a low level of induced TNF-α production and an average content of the number of EBV DNA copies were initially detected. However, the initial clinical complaints in all three groups of patients were the same (Figures 5-6).

Discussion

Alloferon, a linear non-glycosylated oligopeptide, has powerful antiviral, anti-inflammatory and immunomodulatory properties, and is involved in the regulation of cytokine production by immune cells [63]. The antiviral mechanism of alloferon is due to the following: 1) blocking and destruction of viruses and infected cells by producing interferon at the site of infection without causing induction of systemic interferon production; 2) enhancing the cytotoxic activity of NK cells and B lymphocytes responsible for the production of specific antibodies [51]. In 2013, the results of a study of the anti-inflammatory effect of alloferon on skin inflammation caused by ultraviolet radiation were published [64]. A decrease in the levels of proinflammatory cytokines IL-1α/β, IL-6 and IL-18 was shown in HaCaT cells after treatment. The production of proinflammatory cytokines induced by ultraviolet B radiation is known to be due to the activation of mitogen-activated protein kinases (MAPKs) [65]. And alloferon suppresses the activation of p38MAPK and NF-κB induced by ultraviolet B irradiation, preventing skin hyperplasia in HR-1 hairless mice [66]. Later, the results of an experimental study on the model of acute paw edema in mice induced by λ-carrageenan to study the anti-inflammatory mechanism of alloferon were published [52]. The authors of the work showed that the introduction of alloferon slightly suppressed the expression of TNF-α in the inflamed tissues of the paws of mice. It was suggested that alloferon suppresses the inflammatory reaction mediated by NK cells. However, the authors were unable to fully explain the anti-inflammatory mechanism of alloferon, which requires further research. In the work of Qiao et al., the anti-inflammatory effect of alloferon was demonstrated in a mouse model of osteoporosis caused by estrogen deficiency, in the form of an effect on the production of proinflammatory cytokines (IL-1β and IL-18) [56]. Alloferon suppressed inflammatory signals such as the NLRP3 inflammasome, caspase-1 and proinflammatory cytokines (IL-1β and IL-18). The anti-inflammatory effect of alloferon was shown in the treatment of endometrial inflammation caused by lipopolysaccharide (LPS), alloferon inhibits the NLRP3/CASP1/IL-1β/IL-18 signaling cascade [63].

Our work shows the inhibitory effect of alloferon on the production of TNF-α in patients with chronic EBV infection. We conducted a study of this group of patients, since we did not find any data in the literature on the effect of alloferon on the production of proinflammatory cytokines in patients with long-term chronic mononucleosis associated with the Epstein-Barr virus. After receiving the results, it turned out that the level of induced TNF-α production in these patients differed before the start of therapy. That is, the level of TNF-α production was either normal, high or reduced. The severity and frequency of clinical symptoms in these groups of patients did not differ significantly. However, there were differences in the content of the number of EBV DNA copies in saliva samples. That is, the minimum number of copies was detected in patients of the 1st group with a normal level of induced TNF-α, and the maximum number of EBV DNA copies was detected in patients of the 2nd group with a high content of TNF-α. Also, all three groups differed in the duration of EBV infection, the maximum period of infection was observed in patients of the 3rd group with a minimum level of TNF-α (4.22 ± 0.32 years). Thus, initially, before the start of therapy with alloferon, the 3rd group of patients differed in all indicators. It was in the 3rd group that after 4 weeks of therapy there was a minimal dynamics of clinical complaints. The most pronounced effect of alloferon therapy on clinical complaints was obtained in the 1st group of patients with a normal level of TNF-α, a minimum content of the number of EBV DNA copies in saliva samples (115547.48 ± 17196.40) and an average duration of EBV infection of patients (3.05±0.30 years). Thus, our study demonstrated the anti-inflammatory effect of the drug on TNF-α production, which depends on the initial parameters in patients before therapy. Analysis of the obtained data showed that the maximum decrease in induced TNF-α 4 weeks after the end of the alloferon course was in the group with an initially high level of induced TNF-α production (before 7323.30 ± 674.57, after 5645.00 ± 348.01; p=0.001).

The data we obtained confirm the previously published results of the antiviral effect of alloferon on the example of another pathology and in experimental works [56-66].We attempted to explain our data based on the mechanism proposed in the work of Kim, Y. et al. [64]. The authors of the work showed that the proinflammatory cytokine TNF-α increases the phosphorylation of IkB and activates NF-κB for the transcription of many proinflammatory genes (Hayden and Ghosh). And alloferone therapy inhibited TNF-α-induced degradation and phosphorylation of IkB in cells, which was shown in the example

of colon cancer Colo205. In the work of Gosselin J., et al., the mechanism of EBV influence on the production of TNF-α mRNA in the supernatants of PBMC cultures infected with EBV at different times from the moment of infection was described [67,68]. The authors of the work showed that EBV is able to inhibit the production of TNF-α, reduce its secretion and thus evade the inhibitory effect of this cytokine. We performed a correlation analysis between the content of the number of EBV DNA copies and the level of induced TNF-α in the overall group of patients and separately in each group. The obtained results of the correlation analysis were statistically insignificant. To determine the prognostic significance of the number of EBV DNA copies and induced TNF-α, we performed a linear regression analysis with the calculation of the coefficient of determination (R Square) and the Durbin–Watson criterion (Durban–Watson) and variance analysis (Analysis of Variance, ANOVA) with the calculation of the Fisher criterion (F) to test the significance of the model. The acceptable values of the criterion were from 1.020 to 1.268. All possible obtained values of R2 were less than 50%, which indicates the absence of a statistical relationship between the number of EBV DNA copies and the level of induced TNF-α production, since the obtained regression models have a low value. Also, no reliable significant results of the F criterion and the ß coefficient were obtained, indicating the significance of the obtained regression model. Thus, our data do not coincide with the previously published results of other authors [69], who showed that TNF-alpha and TNF-beta have antiviral activity and induce resistance to RNA and DNA viral infection. It was suggested that TNF production is induced by viruses under conditions of an antiviral immune response. Under experimental conditions, when recombinant TNF-α is added to a culture of EBV-infected cells, inhibition of EBV replication, proliferation and differentiation (Ig secretion) of EBV-infected B cells occurs [70]. In 2017, Onozawa E. et al. demonstrated the expression of TNF-α mRNA in EBV-infected cells and healthy cells in their study [71]. The authors, in an experiment on the EBV-infected T cell line MOLT4 and uninfected cells, showed that the expression of TNF-α mRNA was significantly higher in infected cells than in healthy cells. It was concluded that TNF-α is produced by infected and uninfected cells, and EBV induces TNF-α production in infected cells. The data obtained by these authors are applicable to EBV-infected peripheral blood cells, while in our patients the viral load was assessed in saliva samples. This was due to the fact that at the preliminary examination stage these patients had negative PCR results in peripheral blood mononuclear cells. This can probably be explained by the long course of chronic mononucleosis syndrome, the same initial number of EBV DNA copies within 105. Perhaps this can explain the data obtained by our correlation and regression analysis. In our previously published works, a pronounced antiviral mechanism of alloferon on the number of EBV DNA copies in patients with EBV infection was shown [55,72].

As a result of the study, it was shown that alloferon has a pronounced anti-inflammatory mechanism and inhibits the production of proinflammatory TNF-α in patients with chronic Epstein-Barr virus infection.

Data Sharing Policy

The statistical code, dataset used in support of the findings of this study are included within the article.

Funding

The study had no sponsorship

Conflict of interest

The authors declare no conflict of interest.

References

1. Young LS, Rickinson AB. Epstein - Barr virus: 40 years on. Nature Reviews Cancer. 2004; 4: 757-768.
2. Hui Yu, Robertson ES. Epstein - Barr Virus History and Pathogenesis. Viruses. 2023; 15: 714.
3. Houen G, Trier NH. Epstein - Barr virus and Systemic Autoimmune Diseases. Front Immunol. 2020; 11: 587380.
4. Robinson WH, Steinman L. Epstein-Barr virus and multiple sclerosis. Science. 2022; 375: 264-265.
5. Epstein MA, Achong BG, Barr YM. Virus particles in cultured lymphoblasts from Burkitts lymphoma. Lancet. 1964; 1: 702-703.
6. Ward BJH, Schaal DL, Nkad EH, Scott RS. EBV Association with Lymphomas and Carcinomas in the Oral Compartment. Viruses. 2022; 14: 2700.
7. Thorley-Lawson DA, Allday MJ. The curious case of the tumour virus: 50 years of Burkitt's lymphoma. Nat Rev Microbiol. 2008; 6: 913-924.
8. Young LS, Yap LF, Murray PG. Epstein-Barr virus: more than 50 years old and still providing surprises. Nat Rev Cancer. 2016; 16: 789-802.
9. Bakkalci D, Jia Y, Winter JR, Lewis JE, Taylor GS, Stagg HR, et al. Risk factors for Epstein Barr virus-associated cancers: A systematic review, critical appraisal, and mapping of the epidemiological evidence. J Glob Health. 2020; 10: 010405.
10. Tonoyan L, Vincent-Bugnas S, Olivieri CV, Doglio A. New viral facets in oral diseases: The EBV paradox. Int J Mol Sci. 2019; 20.
11. Macsween KF, Crawford DH. Epstein-Barr virus-recent advances. Lancet Infect Dis. 2003; 3: 131.
12. Costa C, Elia M, Astegiano S, Sidoti F, Terlizzi ME, Solidoro P, et al. Quantitative detection of Epstein-Barr Virus in bronchoalveolar lavage from transplant and nontransplant patients. Transplantation. 2008; 86: 1389.
13. Vince A, Lepej SZ, Kurelac I, Barsic B, Kozic S, Klinar I, et al. Virological and immunological characteristics of fatal Epstein-Barr virus mononucleosis in a 17-yearold Caucasian male presenting

with meningoencephalitis and hemophagocytic syndrome. J. Neurovirol. 2007; 13: 389.

14. Kasprzak A, Mrowczynska M, Wysocki J, Jurczyszyn D, Wozniak A, Spachacz R, et al. Morphological and molecular diagnosis of a fatal form of EBV infectious mononucleosis in a child. Pol J Pathol. 2006; 57: 117-130.
15. Satoh N, Koike T, Takato H, Fujiwara M, Emura I, Kaneganed H, et al. Fatal primary Epstein-Barr virus infection due to clonal CD8T-lymphocyte proliferation in an immunocompetent adult. Int J Hematol. 2005; 82: 169.
16. Kimura H, Hoshino Y, Kanegane H, Tsuge I, Okamura T, Kawa K, et al. Clinical and virologic characteristics of chronic active Epstein-Barr virus infection. Blood. 2001; 98: 280.
17. Kimura H. Pathogenesis of chronic active Epstein-Barr virus infection: Is this an infectious disease, lymphoproliferative disorder, or immunodeficiency? Rev Med Virol. 2006; 16: 251.
18. Vince A, Lepej SZ, Kurelac I, Barsic B, Kozic S, Klinar I, et al. Virological and immunological characteristics of fatal Epstein-Barr virus mononucleosis in a 17-year old Caucasian male presenting with meningoencephalitis and hemophagocytic syndrome. J Neurovirol. 2007; 13: 389.
19. Callan MF. The immune response to Epstein-Barr virus. Microbes Infect. 2004; 6: 937.
20. Novelli M, Merlino C, Ponti R, Bergallo M, Quaglino P, Cambieri I, et al. Epstein-Barr virus in cutaneous T-cell lymphomas: Evaluation of the viral presence and significance in skin and peripheral blood. J Investig Dermatol. 2009; 129: 1556.
21. Swinnen LJ. Immune-cell treatment of Epstein-Barr-virus-associated lymphoproliferative disorders. Best Pract Res Clin Haematol. 2006; 19: 839-847.
22. De Paor M, O'Brien K, Fahey T, Smith SM. Antiviral agents for infectious mononucleosis (glandular fever). Cochrane Database Syst Rev. 2016; 12: CD011487.
23. Yu H, Robertson ES. Epstein - Barr virus History and Pathogenesis. Viruses. 2023; 15: 714.
24. Straus SE, Cohen JI, Tosato G, Meier J. Epstein-Barr virus infections: biology, pathogenesis, and management. Annals Internal Medicine. 1993; 118: 45-58.
25. Datta AK, Colby BM, Shaw JE, Pagano JS. Acyclovir inhibition of Epstein-Barr virus replication. Proc Natl Acad Sci USA. 1980; 77: 5163-5166.
26. Lin JC, DeClercq E, Pagano JS. Novel acyclic adenosine analogs inhibit Epstein-Barr virus replication. Antimicrob Agents Chemother. 1987; 31: 1431-1433
27. Cohen JI. Optimal Treatment for Chronic Active Epstein - Barr virus Disease. Pediatr Transplant. 2009; 13: 393-396.
28. Kimberlin DW, Whitley RJ. Antiviral therapy of HSV-1 and -2. In: Arvin A, Campadelli-Fiume G, Mocarski E, et al. editor(s). Human Herpesviruses: Biology, Therapy, Immunoprophylaxis. Cambridge: Cambridge University Press, 2007.
29. Meng Q, Hagemeier SR, Fingeroth JD, Gershburg E, Pagano JS, Kenney SC, et al. The Epstein - Barr virus (EBV)-Encoded Protein Kinase, EBV-PK, but Not the Thymidine Kinase (EBV-TK), is required for Ganciclovir and Acyclovir Inhibition of Lytic Viral Production. J Virol. 2010; 84: 4534-4542.
30. Moore SM, Cannon JS, Tanhehco YC, Hamzeh FM, Ambinder RF. Induction of Epstein-Barr virus kinases to sensitize tumor cells to nucleoside analogues. Antimicrob. Agents Chemother. 2001; 45: 2082-2091.
31. De Paor M, O'Brien K, Fahey T, Smith SM. Antiviral agents for infectious mononucleosis (glandular fever). Cochrane Database Syst Rev. 2016; 12: CD011487.
32. Clercq ED. Antivirals and antiviral strategies. Nat Rev Microbiol. 2004; 2: 704-720.
33. Salam MA, Al-Amin MY, Salam MT, Pawar JS, Akhter N, Rabaan AA, et al. Antimicrobial resistance: a growing serious threat for global public health. Health. 2023; 11: 1946.
34. Zhao X, Wu H, Lu H, Li G, Huang Q. Lamp: A database linking antimicrobial peptides. PLoS One. 2013; 8: e66557.
35. Bellotto O, Semeraro S, Bandiera A, Tramer F, Pavan N, Marchesan S. Polymer Conjugates of Antimicrobial Peptides (AMPs) with d-Amino Acids (d-aa): State of the Art and Future Opportunities. Pharmaceutics. 2022; 14: 446.
36. Dubos RJ. Studies on a bactericidal agent extracted from a soil bacillus: II. Protective effect of the bactericidal agent against experimental Pneumococcus infections in mice. J Exp Med. 1939; 70: 11-17.
37. Hotchkiss RD, Dubos RJ. Fractionation of the bactericidal agent from cultures of a soil Bacillus. J Biol Chem. 1940; 132: 791-792
38. Hirsch JG. Phagocytin: A bactericidal substance from polymorphonuclear leucocytes. J Exp Med. 1956; 103: 589-611.
39. Zhao X, Wu H, Lu H, Li G, Huang Q. Lamp: A database linking antimicrobial peptides. PLoS One. 2013; 8: e66557.
40. Conlon JM, Sonnevend A. Antimicrobial peptides in frog skin secretions. Methods Mol Biol. 2010; 618: 3-14.
41. Radek K, Gallo R. Antimicrobial peptides: natural effectors of the innate immune system. Semin Immunopathol. 2007; 29: 27-43.
42. Peters BM, Shirtliff ME, Jabra-Rizk MA. Antimicrobial peptides: Primeval molecules or future drugs? PLoS Pathog. 2010; 6: e1001067.
43. Bastian A, Schafer H. Human alpha-defensin 1 (hnp-1) inhibits adenoviral infection in vitro. Regul Pept. 2001; 101: 157-161.
44. Horne WS, Wiethoff CM, Cui C, Wilcoxen KM, Amorin M, Ghadiri MR, et al. Antiviral cyclic d,l-α-peptides: Targeting a general biochemical pathway in virus infections. Bioorg Med Chem. 2005; 13: 5145-5153.
45. Sitaram N, Nagaraj R. Interaction of antimicrobial peptides with biological and model membranes: structural and charge requirements for activity. Biochim Biophys Acta. 1999; 1462: 29-54.
46. Belaid A, Aouni M, Khelifa R, Trabelsi A, Jemmali M, Hani KJ, et al. In vitro antiviral activity of dermaseptins against herpes simplex virus type 1. Med Virol. 2002; 66: 229-234.
47. Song BH, Lee GC, Moon MS, Cho YH, Lee CH. Human cytomegalovirus binding to heparan sulfate proteoglycans on the cell surface and/or entry stimulates the expression of human leukocyte antigen class I. J Gen Virol. 2001; 82: 2405-2413.
48. Bahar AA, Ren D. Antimicrobial peptides. Pharmaceuticals (Basel). 2013; 6: 1543-1575.

49. Altfeld M, Gale M. Innate immunity against HIV-1 infection. Nat Immunol. 2015; 16: 554-562.
50. Meije Y, Tonjes RR, Fishman JA. Retroviral restriction factors and infectious risk in xenotransplantation. Am J Transplant. 2010; 10: 1511-1516.
51. Chernysh S, Kim S, Bekker G, Pleskach VA, Filatova NA, Anikin VB, et al. Antiviral and antitumor peptides from insects. Proc Natl Acad Sci USA. 2002; 99: 12628-12632.
52. Zhang X, Retyunskiy V, Qiao S, Zhao Y, Tzeng CM. Alloferon-1 ameliorates acute inflammatory responses in λ-carrageenan-induced paw edema in mice. Sci Rep. 2022; 12: 16689.
53. Appiah C, Chen S, Pori AI, Retyunskiy V, Tzeng C, Zhao Y. Study of alloferon, a novel immunomodulatory antimicrobial peptide (AMP), and its analogues. Front Pharmacol. 2024; 15: 1359261
54. Czarniewska E, Urbański A, Chowański S, Kuczer M. The long-term immunological effects of alloferon and its analogues in the mealworm. Tenebrio molitor. Insect Sci. 2018; 25: 429-438.
55. Rakityanskaya IA, Ryabova TS, Kalashnikova AA. Effects of alloferon versus valaciclovir for treating chronic epstein-barr virus infection. Explor. Res. Hypothesis Med. 2023; 8: 202-214.
56. Qiao S, Zhang X, Chen Z, Zhao Y, Tzeng CM. Alloferon-1 ameliorates estrogen deficiency-induced osteoporosis through dampening the NLRP3/caspase-1/IL-1β/IL-18 signaling pathway. Int Immunopharmacol. 2023; 124: 110954.
57. Bryceson YT, March ME, Barber DF, Ljunggren HG, Long EO. Cytolytic granule polarization and degranulation controlled by different receptors in resting NK cells. J Exp Med. 2005; 202: 1001-1012.
58. Griffiths GM, Tsun A, Stinchcombe JC. The immunological synapse: a focal point for endocytosis and exocytosis. J Cell Biol. 2010; 189: 399-406.
59. DuBois RE, Seeley JK, Brus I, Sakamoto K, Ballow M, Harada S, et al. Chronic mononucleosis syndrome. South Med J. 1984; 77: 1376-1382.
60. Jones JF, Straus SE. Chronic Epstein-Barr virus infection. Annu Rev Med. 1987; 38: 195-209.
61. Straus SE. The chronic mononucleosis syndrome. J Infect Dis. 1988; 157: 405-412.
62. Kimura H. Pathogenesis of chronic active Epstein–Barr virus infection: is this an infectious disease, lymphoproliferative disorder, or immunodeficiency? Rev Med Virol. 2006; 16: 251-261.
63. Chen S, Zhu L, Fang X, Appiah C, Ji Y, Chen Z, et al. Alloferon Mitigates LPS-Induced Endometritis by Attenuating the NLRP3/CASP1/IL-1β/IL-18 Signaling Cascade. Inflammation. 2024.
64. Kim Y, Lee SK, Bae S, Kim H, Park Y, Chu NK, et al. The anti-inflammatory effect of alloferon on UVB-induced skin inflammation through the down-regulation of pro-inflammatory cytokines. Immunol Lett. 2013; 149: 110-118.
65. Karin M, Hunter T. Transcriptional control by protein phosphorylation: signal transmission from the cell surface to the nucleus. Curr Biol. 1995; 5: 747-757.
66. Kim AL, Labasi JM, Zhu Y, Tang X, McClure K, Gabel CA, et al. Role of p38 MAPK in UVB-induced inflammatory responses in the skin of SKH-1 hairless mice. J Investigative Dermatol. 2005; 124: 1318-1325.
67. Gosselin J, Menezes J, D'Addario M, Hiscott J, Flamand L, Lamoureux G, et al. Inhibition of tumor necrosis factor-alpha transcription by Epstein-Barr virus. Eur J Immunol. 1991; 21: 203-208.
68. Gosselin J, Flamand L, D'Addario M, Hiscott J, Menezes J. Infection of peripheral blood mononuclear cells by herpes simplex and Epstein-Barr viruses. Differential induction of interleukin 6 and tumor necrosis factor-alpha. J Clin Invest. 1992; 89: 1849-1856.
69. Wong GH, Goeddel DV. Tumour necrosis factors alpha and beta inhibit virus replication and synergize with interferons. Nature. 1986; 323: 819-822.
70. Janssen O, Kabelitz D. Tumor necrosis factor selectively inhibits activation of human B cells by Epstein-Barr virus. J Immunol. 1988; 140: 125-130.
71. Onozawa E, Shibayama H, Imadome KI, Tsuzura A, Koyama T, Miura O, et al. Inflammatory cytokine production in chronic active Epstein-Barr virus infection. Rinsho Ketsueki. 2017; 58: 189-196.
72. Rakityanskaya IA, Ryabova TS, Kalashnikova AA. Allokin-alpha - new approaches in the treatment of chronic virus Epstein-Barr infections. Problems Virology Russian J. 2019; 64: 118-124.