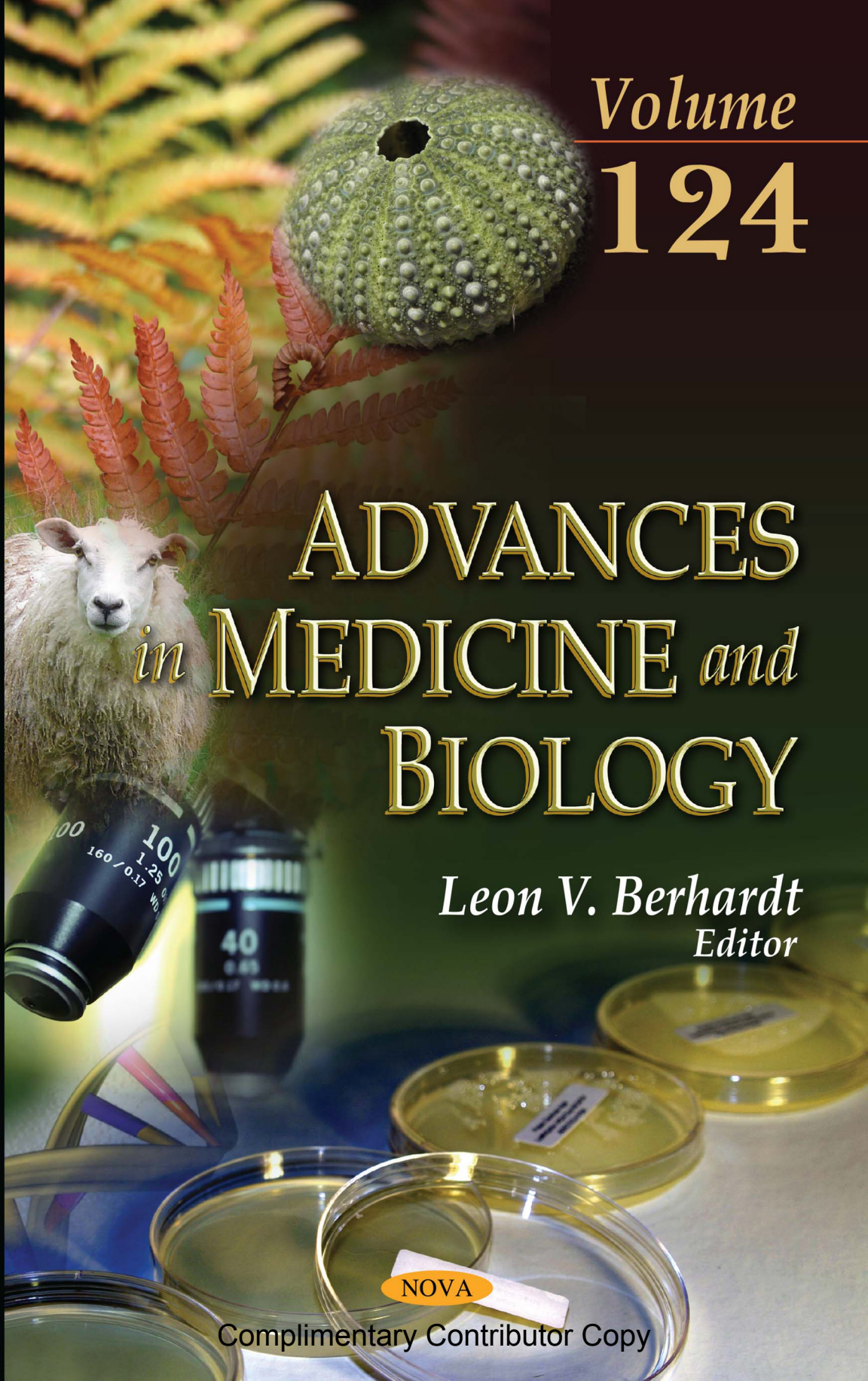
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ADVANCES IN MEDICINE AND BIOLOGY

VOLUME 124

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Chapter 6

GENITAL HERPES IN RUSSIA: THE SCOPE OF THE PROBLEM AND TREATMENT PROSPECTS

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ABSTRACT

Genital herpes (GH) is one of ubiquitously common, socially significant viral diseases. The infection caused by herpes simplex viruses type 1 (HSV-1) and type 2 (HSV-2) is sexually transmitted and is the main cause of GH. There are approximately 530 million people infected with HSV-2 (Looker et al., 2015) and almost 3.7 billion people under 50 years old infected with HSV-1 in the world today (Looker, Magaret, May et al., 2015). These data point to an epidemic of GH.

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In the Russian Federation, the incidence rate of GH in 2014 was 14.2 cases per 100,000 people. However, the true prevalence of HSV-1 and HSV-2 in the Russian Federation remains under researched. The number of people infected with HSV-2 is believed to reach 15-30 million.

Based on the data obtained, this study proposes the inclusion of interferon drug therapy and interferon inducers that normalize the immunity and interferon systems in patients as a new method of GH treatment. These drugs can be used for prevention in the monotherapy of herpes viral infections (during GH remissions) or in complex therapy with basic etiotropic drugs (during relapses).

In general, for treatment of GH patients with frequent relapses (more than 6 times a year), we recommend individualized therapy that combines the well-known etiotropic chemotherapy and additional immunotherapy with IFN drugs and IFN inducers, most likely, with polyprenyl phosphate-based drugs.

Keywords: recurrent genital herpes, HSV, IFN inducers, Fortepren

INTRODUCTION

Genital herpes (GH) is one of ubiquitously common, socially significant viral diseases. Infection of herpes simplex virus type 2 (HSV-2) is sexually transmitted and is the main cause of GH. However, GH may be caused by HSV-1. There are 530 million people infected with HSV-2 in the world today (10-20% of the adult population with GH are in developed countries) (Looker et al., 2015). According to the WHO, almost 3.7 billion people under the age of 50 (67% of the population) are infected with the HSV-1 virus (Looker, Magaret, May, et al., 2015; WHO, 2017). These data point to a real epidemic of GH (Samgin, Haldin, 2002; Barinsky and Makhmudov, 2013; Akovbyan et al., 2003).

SCOPE OF THE GH PROBLEM IN RUSSIA

In Russia, compulsory registration of the GH disease was introduced in 1993. According to statistical data, the GH incidence in the Russian

Federation increased during the period from 1994 to 2005 (from 7.4 to 21.6 cases per 100 thousand people). There was a slight decrease in the incidence rate by 2012 (from 21.6 to 16.8 cases per 100 thousand people), while in a number of regions, such as the Volga Region, Urals, and Far East, an increase in the incidence is a stable trend (Kuznetsova, Khlystova, 2016). It is established that women aged 18 to 39 are in the risk group, where the incidence of genital herpes is higher than usual standing at 135.7 cases per 100 thousand people of the given sex and age (Khryanin, Reshetnikov, 2009).

The relatively low morbidity rates in Russia - 4.2 times lower than in Europe (80 cases per 100 thousand) and 10.5 times lower than in America (200 cases per 100 thousand) - do not appear very plausible. In Moscow, according to the data for 1999, the GH incidence increased from 11.0 (in 1993) to 74.8 cases per 100 thousand people and has practically reached the level of European countries. An important reason for the increase in the GH incidence is the widespread occurrence of asymptomatic and (or) undiagnosed GH forms (Isakov, Arkhipova, Isakov, 2013.): up to 70% of cases of transmission of genital HSV occur in the asymptomatic course of the infectious process in a patient.

In Russia, patients with GH are usually referred to specialized dermatovenerologic dispensaries (DVD) located in large cities that register, diagnose and treat GH. Modern immunomorphological, molecular biological methods of GH detection are widely used. The bulk of patients (70-94% of registered ones) consults physicians on their own. The existing difficulties in the development of rational pharmacotherapy can be explained by the fact that patients with GH often refer to different specialists: neonatologists, pediatricians, infectious disease specialists, virologists, urologists, dermatologists, venereologists, and others. The specific value of active detection of patients with GH by physicians of healthcare institutions of the first level during all types of preventive examinations in Russia is 22.7-27.8%, while in Moscow it is 5.4-7.2%. Obstetrician-gynecologists diagnose 45.1-54.8%, dermatovenereologists - 39.8-43.8% of the total number of actively detected patients with genital herpes; urologists account for no more than 5-12%.

It should be noted that it is very difficult to analyze the statistical data pertaining to the incidence of GH in Russia by regions. Therefore, the true prevalence of HSV-1 and HSV-2 in the Russian Federation remains under researched, since large-scale population epidemiological studies are practically not carried out. According to the data for 2003-2004, in the Moscow region HSV-2 is detected in 76% of examined patients with genital herpes, and HSV-1 - in 24%.

One of the truly informative analyzes is presented in the study by A.A. Khryanin et al. (2015) "Epidemiological Features of Herpesvirus Infection: Long-Term Trends" (http://ngmu.ru/cozo/mos/article/text_full.php?id=1762), where the authors studied the epidemiological features of herpesvirus infection (HSV-2) in the population of Novosibirsk by analyzing the official statistics of GH incidence from 2003 to 2014.

Data of the statistical study show the following dynamics of GH incidence: 2003 - 6 people per 100 thousand (85 cases), 2004 - 6.7 (95 cases); 2005 - 10.5 (148 cases); 2006 - 9.5 (133 cases); 2007 - 8.7 (122 cases); 2008 - 8 (112 cases); 2009 - 17.7 (247 cases); 2010 - 8.6 (127 cases); 2011 - 7,5 (110 cases); 2012 - 7 (106 cases); 2013 - 7.2 (110 cases); 2014 - 5.3 (82 cases) (see Figure 1).

The authors note that "in the entire 11-year period there is a pronounced gender-related trend: women are infected almost twice as often as men. By gender identity, the incidence structure is as follows: 63% – females; 37% – males. In the age group of 0-14 years: males - 50%, females - 50%; 15-17 years old: males - 10%, females - 90%; 18-19 years old: males - 24%, females - 76%; 20- 29 years old: males - 35%, females - 65%; 30-39 years old: males - 43%, females - 57%; over 40 years old: males - 49%, females - 51%. The above data are quite typical given the data on the HSV-2 incidence in the population. According to prospective studies, the infection rate in women in Novosibirsk is 1.5 times higher than in men. Obviously, this is due to a larger surface of the mucous genitalia in women. Men have frequent relapses whose manifestations are less pronounced than in women. The latter does not prevent men from having sex, which contributes to women getting infected in the most contagious period. Finally, the probability of transmission of the virus from a man to a

woman is 6 times higher than from a woman to a man." The authors come to the conclusion on the stability of the epidemiological situation in terms of the GH incidence in Novosibirsk: the average incidence rate is 8.6 cases per 100 thousand people. The highest incidence rate is among 20-29 year-olds in the fall and winter period.

According to the Russian Society of Dermatovenereologists and Cosmetologists (Federal Clinical Recommendations for Management of Patients with Genital Herpes, 2015), the incidence of infection with the newly acquired HSV-2 is 5.1 cases per 100,000 people per year. In the Russian Federation, the incidence rate of GH in 2014 was 14.2 cases per 100,000 people: 0.1 cases per 100,000 people among 0-14 year-olds, 8.5 cases per 100,000 people among 15-17 year-olds, 17.2 cases per 100,000 people 18 years and older.

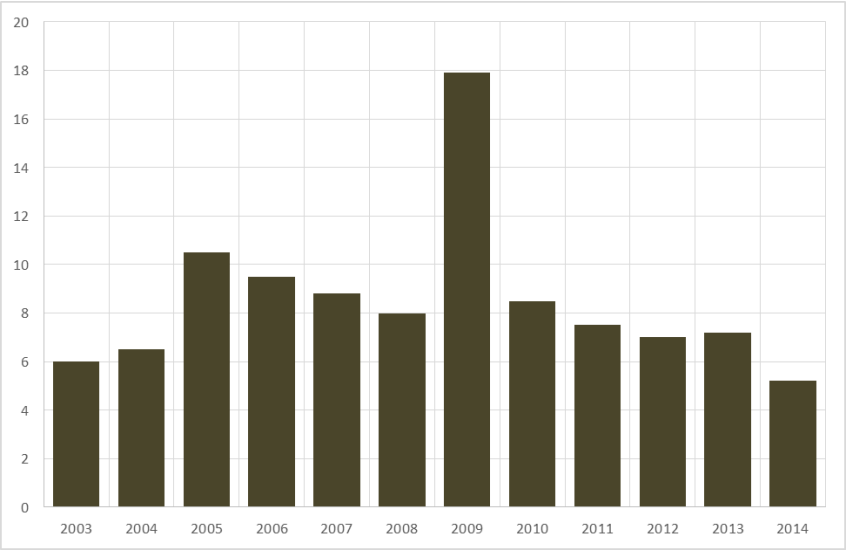

Figure 1. Incidence of herpesvirus infection in Novosibirsk Region from 2003 to 2014 (per 100 thousand people). According to Khryanin et al. (2015).

CLINICAL PICTURE OF GH

Without dwelling on the clinical manifestations of GH that are well described in the medical literature (Isakov, Arkhipova, Isakov, 2013; Barinsky and Makhmudov, 2013), we consider it necessary to point out some features that characterize GH in Russia.

Most of the reported cases of GH have a tendency to a latent lifelong course with a high frequency (50-75%) of relapses. Most GH patients are in a productive period of their life (20-45 years). The overwhelming majority of cases are characterized by an asymptomatic (20%) or atypical course (60%), making it difficult to timely diagnose them and initiate treatment.

In men, the urethra, bladder, prostate, and testes are affected. In case of anogenital contacts, the anus and rectum are affected. In women, herpes viruses can cause infertility (up to 60%), miscarriage (up to 20%), premature birth (up to 80%), neonatal mortality (up to 20%), respiratory distress syndrome (up to 12%) and other pathologies in newborns (Barinsky and Makhmudov, 2013).

IMMUNOLOGY OF HERPES

Herpetic infection is accompanied by the development of specific humoral and cellular immunity. Types of antiherpetic immunity are as follows: non-sterile (the virus is not eliminated from the body), type-specific (mainly against the relevant type of virus), and partially cross-over.

Recognition of the virus by the immune system begins with the interaction of TLR9 with viral DNA and viral glycoproteins with TLR2 (Paludan et al., 2011; Cai et al., 2013).

In addition, viral nucleic acids interact in the cytosol with IFI16 and RIG-I by a mechanism dependent on RNA polymerase III, and the cytosolic DNA binds to cyclic GMP-AMP synthase (cGAS), which leads

to activation of the stimulator of interferon genes (STING) through secondary messengers (Chiu et al., 2009; Sun, 2013; Wu et al., 2013). Finally, HSV activates inflammasomes through cytokine IFI16 induced by the gamma-interferon-inducible protein regardless of the protein that is absent in melanoma 2 (AIM2), which is part of inflammasomes recognizing viral DNA (Rathinam et al., 2010).

In the central nervous system, the main role in the recognition of HSV-2 belongs to TLR3 and inferior signal molecules UNC-93B and TRIF, as their insufficient expression is accompanied by herpetic encephalitis in humans and increased sensitivity to infection of the central nervous system with HSV-2 in mice (Sancho-Shimizu et al., 2011; Reinert et al., 2012; Lafaille et al., 2012). In the peripheral nervous system, HSV-1 infection is controlled, apparently, by autophagy, as in the absence of the Atg5 protein required for the formation of autophagosomes the virus titer is significantly increased (Yordy et al., 2012).

However, activation of only natural immunity is not sufficient for protection against herpesvirus infection. The cellular immunity mediated by T-killers, natural killers, and macrophages plays an important role in preventing herpes recurrence.

A signal transmitted via TLR with MyD88 in hematopoietic and stromal cells leads to activation of Th1 cells and induction of adaptive immunity (Sato, Iwasaki, 2004). Initially, activation of T cells is observed in draining iliac lymph nodes (Iwasaki, 2007). T cells are primed by dendritic cells migrating from the infection zone that present viral antigens (Lee et al., 2009; Heath, Carbone, 2009). Activated Th1 cells migrate to the genital tract during 3 days with a peak on the 6th day after infection. Some time later, in direct dependence on the production of IFNγ by Th1 cells, activated CD8 T cells appear in the vagina. IFNγ produced by Th1 cells induces the synthesis of inflammatory chemokines CXCL9 and CXCL10 by epithelial cells. However, CXCL9 and CXCL10 are not chemoattractants for CD8 T cells (Nakanishi et al., 2009).

Genital HSV-2 infection also results in the activation of Treg cells that regulate the migration of NK cells, plasmacytoid dendritic cells (DC) and $CD11b^+$ DC to the vagina (Lund et al., 2008). Foxp3+CD4+CD25+ nTreg

cells in the conjunctiva inhibit the response of HSV-specific CD4 and CD8 effector T cells. The suppressive effect of regulatory cells is reduced after the arrival of a signal from TLR2 (Chentoufi and BenMohamed, 2012).

In case of a relapse, virus-specific CD4 cells, pDC and other DC populations accumulate in the inflammation zone. The main infection control during this period is CD8ααT lymphocytes that are located on the border of the dermis and epidermis and kill infected epithelial cells (Zhu et al., 2013). The density of CD8α-T lymphocytes determines whether the transition of virions from nerve endings to epithelial cells will be accompanied by ulceration or asymptomatic virus release. In remission CD8αα-T cells accumulate around nerve endings and control the virus upon its subsequent exits from neurons (Schiffer et al., 2010; Zhu et al., 2007). The involvement of CD8 T cells in protection from herpes infection is not limited to their cytolytic activity alone. They also produce IFN γ and release granzymes into neurons that break down the early viral protein ICP4 blocking apoptosis (St. Leger, Hendricks, 2011; Knickelbein et al., 2008). Given the high activity of CD8 T cells, a latent infection develops, which can last an entire life (Shin and Iwasaki, 2013). The T cells of people with symptomatic and asymptomatic herpesvirus infection respond to different epitopes of glycoproteins B and D HSV (Chentoufi and BenMohamed, 2012). In case of asymptomatic infection, only one HSV gene, a latency-associated transcript (LAT) that enhances the expression of PD-1, TIM-1 and LAG-3 on resident CD8 T cells, is transcribed leading to the suppression of their effector functions (Knop and Knop, 2003).

Experiments on mice showed that intravaginal immunization with attenuated strains of HSV-2 fully protects the animals against subsequent infection with a wild strain. The protective effect is mainly associated with the IFNγ-producing CD4 T cells (Iijima et al., 2008). At the same time, resident CD8 T memory cells also provide more effective protection than circulating CD8 T cells when HSV-2 infection occurs via the skin or genital tract (Gebhardt et al., 2009; Shin, Iwasaki, 2012). Although B cells play a lesser role in protection, they enhance the protective effect of T lymphocytes and are necessary at the stage of induction of the primary immune response (Harandi et al., 2001). In addition, passive transfer of a

sufficient amount of IgG antibodies against HSV-2 protects B-deficient mice from vaginal infection due to neonatal Fc receptor-mediated IgG transcytosis into the lumen of the genital tract.

Studies conducted on a model system using induced pluripotent stem cells indicate the key role of TLR3 and IFN in the immunity against HSV-1 in neurons and oligodendrocyte precursor cells and the potential role of these factors in astrocytes. Other mechanisms can act in microglia cells, since they do not express TLR3 on their surface, are not permissive for HSV-1 and do not activate IFN-α and -β when HSV-1 is infected. They produce TNF-α, which can block replication of HSV-1 in human astrocytes, but not in neurons (Lafaille et al., 2015).

Thus, humoral antiherpetic immunity reduces the risk of infection by the virus, development of superinfection, dissemination of the infectious process, and vertical transmission of the virus from the infected mother to the fetus and does not prevent the development of recurrent herpes. Cellular antiherpetic immunity plays a crucial role in preventing herpes relapses.

In recurrent herpes, an important role belongs to local (mucosal) immunity. Exacerbation of herpes is detected in case of a decrease in local immunity after injuries, surgical interventions, sexual contact with mucosal trauma, topical application of corticosteroids, facial treatment with liquid nitrogen for skin rejuvenation, etc.

IMMUNOSUPPRESSIVE EFFECT OF HERPES INFECTION

Reproduction of the virus in lymphocytes, neutrophils and monocytes-macrophages is the basis of T-cell immunodeficiency, which develops in herpes infection. Mechanisms of the immunosuppressive effect are conditioned by suppression of chemotaxis and decreased activity of the phagocytosis process, suppression of the function of natural killers (NK), the reaction of blast-transformation of lymphocytes, and possible direct stimulation of regulatory T cells. In addition, a 50% reduction in the levels of IgG2 immunoglobulins, avidity of immunoglobulins M are observed.

Moreover, the formation of circulating immune complexes (CIC) and their immunopathological effect are noted.

INTERFERON DEFICIENCY IN HERPES INFECTION

An important role in the pathogenesis of herpes is played by interferon deficiency. The ability of lymphocytes to synthesize gamma-interferon and fibroblasts to produce beta-interferon is reduced. The levels of alpha, beta and gamma interferons in patients with recurrent herpetic infection are reduced by 10-20 times compared with the control group. Local suppression of interferon formation in foci of herpetic lesions is also observed (Ershov, 1996; Sen, 2001).

LATENCY MECHANISMS OF HERPES VIRUS IN GH

Innate immunity plays the leading role in neutralizing the herpes virus. The latency of GH viruses is attributed to disorders of the immunological and interferon status, secondary negative immune restructuring associated with the suppression of cellular immunity reactions and interferon-producing ability of leukocytes. Affection of immunocompetent cells is the cause of secondary immunodeficiency (Ershov, 2006; Ershov, Kiselev, 2005).

Typical immunological disorders in patients with recurrent GH are associated with a number of factors, namely:

- decrease in the production of endogenous IFN, Th1 lymphocytes, activity of NK cells and antibody-dependent cellular cytotoxicity,
- decrease in the absolute number and activity of T-lymphocytes ($CD3^+$ and $CD4^+$ cells, $CD8^+$ cytotoxic T lymphocytes),
- decreased neutrophil activity (incomplete phagocytosis),
- increase in the number of immune complexes.

The most common triggers of latent infection activation are stresses, chronic diseases, seasonal acute respiratory viral infections, environmental conditions, socioeconomic factors, malnutrition, etc. (*Isakov V.A., Aspel Yu.V., 1999).*

As a rule, the HSV gene is retained in a latent (non-replicating) state in the sensory ganglia of the lumbosacral spine under the control of two barriers - immune and interferon. The virus penetrates through the damaged skin, spreads through the paraneural tissues within 3-4 hours, and enters a latent state.

It is established that interferons, cytotoxic T lymphocytes, natural killers, humoral immunity factors, mononuclear phagocytes and cytokines play an important role in the reactivation and development of clinical manifestations of HSV. Together with other factors of innate and acquired immunity, they provide a latent state of viruses by blocking the release of herpes viruses from the sensory ganglia.

In general, the latency of the HSV genome is based on the lack of expression of the full set of viral genes responsible for the lytic (reproductive) cycle of the virus.

Disorders of innate and adaptive immunity lead to a change in the synthesis of early and late cytokines. The development of viral latency is also explained by the fact that the virus induces apoptotic effects against the cells of the host's immune system. Reducing the expression of viral genes during the latent phase does not allow the immune system cells to recognize the virus in infected cells, since virus antigens are not represented on the surface of these infected cells.

INF-α, -β, -γ, TNF-α, IL-6 cytokines are constantly synthesized by plasma dendritic cells in the infected ganglia, maintaining the HSV latency. Expression of INF-α/β determines the differentiation of the Th-1 response of T cells, increases proliferation and cytolysis, and maintains the CD8+ cytotoxic lymphocyte population.

TREATMENT OF GH

Treatment of GH, first and foremost, is aimed at suppressing the virus reproduction in the exacerbation period and the formation of an adequate immune response. The purpose of treatment is: 1) relief of acute symptoms of the disease and/or recurrent infection in the shortest time possible; 2) reduction of the re-epithelization duration; 3) prevention of relapses and reduction of their frequency and severity; 4) prevention of virus transmission to sexual partners (Akovbyan et al., 2003; *Gladko, 2005;* Federal Clinical Recommendations for Management of Patients with Genital Herpes, 2015).

Treatment of frequently recurrent forms of GH remains a difficult task (Shulzhenko, Zuykova, 2011; Marchenko, 2011; Budanov, 2011; Silina, Bibicheva, 2011; Budanov, 2004; Gupta et al., 2007; Patel et al., 2010). Despite the array of proposed effective treatment regimens and etiotropic antiherpetic drugs (Akovbyan et al., 2003; Federal Clinical Recommendations for Management of Patients with Genital Herpes, 2015), a radical effect of treatment has not been achieved yet.

The European standards for the treatment of herpes primarily provide for the use of well-known anomalous nucleosides: Acyclovir®, Valacyclovir®, Famcyclovir® and other abnormal nucleosides, such as Ganciclovir®, Valtrex®, Famvir®, Cymevene®, Foscavir®, and others (Ershov, 2006; Leung, Saks, 2000). Unfortunately, the long-term use of this group of classical etiotropic chemotherapy drugs has little effect on the overall reduction in GH incidence, which may be due to irrational pharmacotherapy and development of virus resistance to the drugs used. The treatment is effective against complicated mixed infections that often occur in the event of GH (influenza + herpes, herpes + Chlamydia infection, etc.).

Various immune factors play an important role in the reactivation and development of clinical manifestations of HSV: cytotoxic T lymphocytes, natural killers, humoral immunity factors, mononuclear phagocytes, and cytokines. The detected immunological disorders occur in the recurrence phase and during remission of the disease.

To correct the specific and nonspecific mechanisms of anti-infective protection, various immune drugs of systemic and external action that can prevent reproduction of the virus in the body are used (Ershov, Kiselyov, 2005; Ershov, Narovlyansky, 2011; Isakov et al., 2006; Rakhmatulina, 2007).

Immunomodulators are represented by several classes of drugs: endogenous cytokines (interferons, interleukins, colony stimulating factors, tumor necrosis factor, etc.), exogenous immunomodulators of natural origin (viruses, microorganisms, endotoxins, LPSs, glucans and biologically active substances), synthetic high- and low-molecular drugs (interferon inducers, surface-active substances, pyran derivatives, imidazole, fluorene, etc.) (Marchenko, 2011). In the past few years, Russian scientists have found interferons and their inducers to be very promising in the complex prevention and therapy of GH. Some of them are given below.

MEDICINAL PRODUCTS DEVELOPED IN THE RUSSIAN FEDERATION AND USED IN THE TREATMENT OF GH

IFNs have a universal mechanism of antiviral action and block the transmission of viral mRNAs to ribosomes of infected cells, i.e., at a stage that is intrinsic to all viruses. In addition to their antiviral effect, which is no less important, interferon drugs are among the important factors of innate immunity; they control the latency of herpes viruses, and have a wide range of effects on the mechanisms of the immune system. Alpha, beta and especially gamma interferons are responsible for interaction between lymphocytes and macrophages and regulate the ratio of cellular and humoral components of the immune response (*Rakhmatulina, 2007).*

The drug containing exogenous interferon alfa-2b – Viferon® – and inductors of endogenous interferon – Cycloferon® and Kagocel® – are among the most studied and promising drugs for the interferon therapy of

GH. These drugs are widely used to treat a number of viral diseases (Ershov, 2006; Ershov, Kiselev, 2005; Ershov, Narovlyansky, 2011).

Viferon® is a combination of IFN alpha-2b and two antioxidants – tocopherol acetate and ascorbic acid. The drug in the form of rectal suppositories is used in complex therapy of various infectious and inflammatory diseases. Viferon® is the only drug in Russia permitted for the prevention of viral infections in pregnant women and newborns.

As an example, the results of Viferon® treatment of 74 pregnant women diagnosed with GH are presented (Budanov, 2007). The course of Viferon therapy was carried out from 28-30 weeks of pregnancy. Viferon in a dose of 500000U was administered by 1 suppository every 12 hours (20 suppositories), then 1 suppository 2 times a week (10 suppositories). A similar course of Viferon was repeated at 35-36 weeks of gestation. In the treated women, the clinical course of GH was abortive. A correlation was established between the frequency of relapses before and after the start of treatment. Thus, in 14 patients the frequency of recurrent GH before the beginning of the study was 8 to 11 times a year; after the treatment with Viferon® the relapse rate decreased to 3-4 times per year. Clinical manifestations of GH did not occur in patients with the baseline recurrence rate of 3-4 times per year during the ongoing therapy. Thus, the use of Viferon® allows achieving a 3-6-fold reduction in the frequency of GH recurrences shortly before the delivery, which reduces complications of pregnancy and perinatal morbidity.

As shown in a series of clinical studies, the therapy of GH with Viferon® shortens the duration of the acute period of GH, reduces the severity of clinical manifestations, prolongs the period of remission, and reduces the frequency of relapses by 1.5-2 times (Kagramanova et al., 2008; Shperling et al., 2010).

Cycloferon® is a methylglucamine salt of carboxymethylene acrydon (NTFF Polysan, Saint Petersburg). It is a low-molecular interferon inducer (Figure 2).

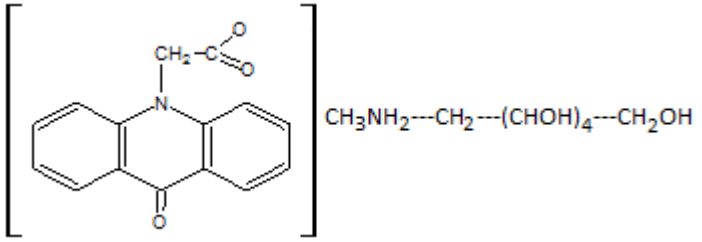

Figure 2. Structural formula of the molecule of Cycloferon® (meglumine acridon acetate).

Cycloferon® has been subjected to long-term mono- and multicenter clinical trials. According to their results, a high level of IFNα synthesis in tissues and organs containing lymphoid elements occurs for at least 72 hours after the administration of Cycloferon®. The direct and indirect immunotropic effects of Cycloferon® are described below.

Cycloferon® activates the interferon system in patients, induces the synthesis of IFNα in B cells, macrophages and neutrophils, increases CD4+, phagocytosis, NK activity and cytotoxic T cells, as well as the synthesis of IgG, IL-2, IL-1, IFNα, and IFNγ, increases the production of high-affinity antibodies, influences the switching of synthesis of classes of immunoglobulins by B lymphocytes (Ershov, 2005; Ershov, Kiselev, 2006; Sokolova et al., 2015).

In the treatment of recurrent herpesvirus infection, 2 ml of 12.5% solution of Cyclophorone® was used for an intramuscular injection once a day on the 1st, 2nd, 4th, 6th, 8th, 10th and 12th days of treatment (as monotherapy or in combination with antiviral agents). Clinical efficacy was observed in 88%, clinical improvement – in 10% of cases. Cycloferon® promoted the onset of persistent clinical and immunological remission (Isakov, Aspel, 1999).

According to the developers of Cycloferon® (https://medi.ru/info/4153/; Isakov et al., 2013), comparative studies were carried out in the course of treatment of 100 GH patients with acyclovir (50 patients) and Cycloferon® (50 patients). Cycloferon® was administered once a day in a dose of 2 ml of 12.5% solution on the 1st, 2nd, 4th, 6th and 8th days of treatment (the main group). Patients of the control group received

acyclovir (AC) in a dose of 200 mg 5 times a day for 5 days. The disease was diagnosed based on the medical history, clinical symptoms, and colposcopy and by the direct immunofluorescence method. All patients (80 women and 20 men) were aged 24-42; the GH recurrence rate was 5-8 times a year; the duration of the disease was 1 to 5 years. Herpetic eruptions in women were localized mainly in the area of the large and small labia, perineum. In men – on the glans penis, foreskin and body of the penis.

The analysis of the dynamics of the main clinical and laboratory symptoms did not reveal any differences, which indicated the high efficacy of Cycloferon® in the treatment of recurrent GH (Tables 1 and 2).

Table 1. Duration of the main clinical manifestations of GH

Symptoms	Duration of symptoms (days)	
	Cycloferon (n = 50)	Acyclovir (n = 50)
Local subjective manifestations (itching, burning, pain)	4.1	3.8
Intoxication	0.8	1.0
Vesicular stage	1.8	1.6
Erosive stage	2.2*	2.1*
Epithelization stage (crust formation)	2.6	2.4
Duration of relapse	6.6	6.4

Note: * development of erosions was noted in 30% of patients in the main group and in 40% of the control group, which is apparently due to a relatively late (on the 2nd day of relapse) administration of these drugs.

Analysis of the dynamics of changes in immunological parameters in the group of patients receiving Cycloferon® (the main group) revealed a significant increase of T lymphocyte subpopulations of general, helper and regulatory T cells and natural killers after treatment compared to patients receiving AC (AC does not affect the immunity indices). The functional activity of lymphocytes restored (reserve capacities of immunocompetent cells increased) in the test of spontaneous and induced RBTL. This is significant, since it is the condition of the T cell link of immunity that largely determines recovery from a viral infection. It should be noted that

after treatment with Cycloferon® the activity of natural killers reached the norm.

Table 2. Dynamics of immunological parameters of GH patients with account for the type of therapy

Parameters (norm)	Parameters	Cycloferon® (n = 50)	Acyclovir (n = 50)
$CD3^+$ (40-60%)	Before treatment	29.8	31.6
	After treatment	47.6*	38.3
$CD4^+$ (30-40%)	Before treatment	19.3	20.2
	After treatment	32.4*	26.4
$CD8^+$ (10-20%)	Before treatment	10.5	11.7
	After treatment	13.8*	14.8
NK** (30-40%)	Before treatment	21.0	19.4
	After treatment	35.2*	27.2
Spontaneous RLBT (200-400 imp/min.)	Before treatment	171	166
	After treatment	296	276
ConA-induced RLBT (4000-12000 imp/min.)	Before treatment	4001	3582
	After treatment	5986*	5485
IgG (7-16 g/l)	Before treatment	5.1	6.8
	After treatment	13.8*	12.3*
IgM (0.5-2.0 g/l)	Before treatment	0.8	0.5
	After treatment	2.9*	2.7*
IgA (0.8-3.6 g/L)	Before treatment	0.9	1.0
	After treatment	2.8*	2.2*

Note: * $P < 0.05$.

As a result of treatment, an increase in the remission duration by 2.0-2.5 times and a 2-fold reduction in the rash resolution were observed; other clinical symptoms subsided. All patients noted a persistent improvement in their well-being and relief of subjective symptoms from the second day of therapy.

Kagocel® is a high-molecular compound synthesized on the basis of carboxymethylcellulose sodium salt and low molecular natural polyphenol – gossypol (Figure 3). It is an interferon inducer that belongs to biologically active polymers (BAP).

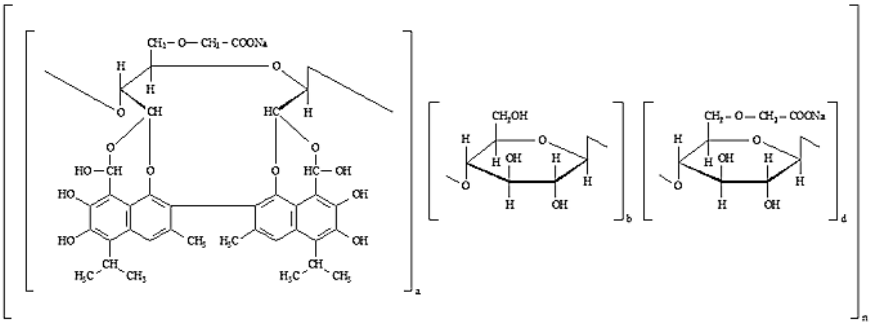

Figure 3. Structural formula of the molecule of Kagocel®.

A detailed study of the targets and mechanisms of action of Kagocel® revealed that exposure to virus-infected cells in *in vitro* systems changes the activity of a number of genes involved in transferring the cell to the apoptotic pathway (i.e., elimination of virus-infected cells by activating special mechanisms of programmed cell death) and, therefore, to a decrease in the number of full-fledged viral particles. The obtained data show the involvement of proteins from the apoptosis system, such as bcl-2 and p53, into the cascade of reactions to Kagocel® (Sokolova T.M., 2012).

Apparently, the specific action of Kagocel® is its ability to transfer cells into an activation state with subsequent full-fledged realization of its effect only in the presence of an infectious agent (the second activation signal). As a result, under the influence of Kagocel®, the normal level of the antiviral immune reactivity and, above all, the activity of the interferon system, which ensures the body's ability to effectively counteract further development of the viral infectious process are restored.

After administration, Kagocel® stimulates production of physiological quantities of endogenous INF-α/β with peak activity after 24-48 hours, followed by circulation of interferons for up to 4-5 days. The drug regulates production of immunocompetent cells of cytokines that have immunomodulatory and immunostimulating effects and are involved in antiviral protection.

Figure 4 presents the effect of Kagocel® used in monotherapy of GH.

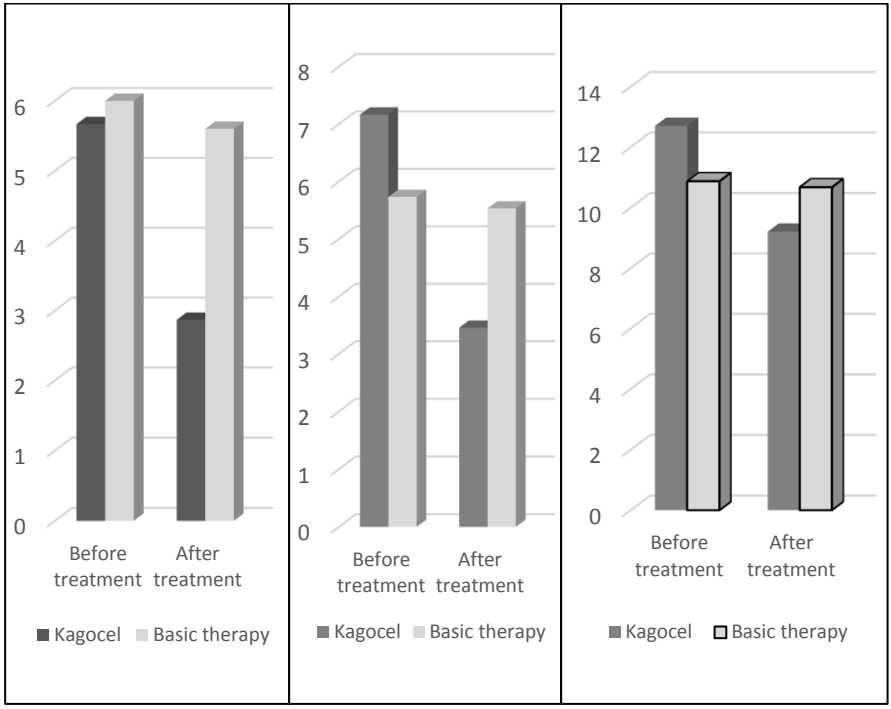

Figure 4. Kagocel® in treatment of GH.

As can be seen from Figure 4, the course of treatment with Kagocel® reduces the frequency and duration of GH relapses and decreases their severity (Tutushkina, Shulzhenko, 2004; Shulzhenko, Ershov, 2004; Shulzhenko, Tutushkina; 2005).

In another study by S.A. Masyukova et al. (2006), 50 GH patients (30 (60%) females and 20 (40%) males aged 18 to 50) were observed. Among them, 8 (16%) patients had a mild form of GH (1-3 relapses per year), 14 (28%) patients had a moderate form (4-6 relapses), and 28 (56%) patients had a severe form (6 relapses and more). The duration of exacerbation in 52% of GH patients was 5-10 days, in 40% - 10-20 days or more, which is also an objective indicator of a severe course of the disease.

In a clinical study of the efficacy and tolerability of Kagocel® patients were divided into three groups:

Group 1 included 20 patients (12 women and 8 men) who received Kagocel® as a monotherapy.

Group 2 included 10 patients (6 women and 4 men) who received a combined therapy (Kagocel® + acyclovir).

Group 3 (comparison) consisted of 20 patients (12 women and 8 men) who received acyclovir as a monotherapy.

The most significant results of treatment with Kagocel® were achieved on the 10th day. Thus, a comparative analysis of the clinical evaluation of the efficacy of the therapy showed that the best results had been achieved in the 1st and 2nd group of patients (5.6 ± 0.31 and 3.2 ± 0.18, respectively), but not in the 3rd group (6.4 ± 0.49). In addition, the treatment reduced the period of rush resolution, relieved itching and local pain, and decreased the duration of relapse.

At the last visit following one month, clinical recovery was observed in all groups of patients. When comparing the efficacy of drugs before treatment and after 3 months, the duration of relapse in patients of the 1st and 2nd groups decreased by 2-2.5 times (in the 1st group - 12.8 and 6.25, in the 2nd group - 13.4 and 5.0) compared to the comparison group (12.5 and 8.7). During the safety assessment of Kagocel® none of the patients had abnormal laboratory indicators, or adverse or serious adverse events.

In general, the therapy of GH with Viferon®, Cycloferon® and Kagocel® revealed that the use of these drugs as a monotherapy or combined therapy of GH patients results in earlier relief from signs of the disease and has a positive effect on the frequency and duration of relapses (Isakov, 2010; Masyukova et al., 2006; Ospelnikova et al., 2004; Tutushkina, Shulzhenko, 2004; Shulzhenko, Ershov, 2004; Shulzhenko, Tutushkina; 2005; Haldin, Ignatyev, 2011; Malinovskaya et al., 2007; Ershov F.I., Romantsov M.G., 2007).

FORTEPREN® AND POLYPRENYL PHOSPHATES

Recently, in addition to the above well-studied drugs Viferon®, Cycloferon® and Kagocel®, we have proposed (currently, the drug is

registered with the Russian Ministry of Health) a new drug Fortepren® whose active substance is one of the products of the mevalonic acid pathway – polyprenols and polyprenyl phosphates. Polyprenols are of great importance in the life cycle of viruses and can affect some stages of the virus-cell interaction (Blanc et al, 2011; Narovlyansky et al., 2007).

Fortepren® is a product of phosphorylation of conifer needle polyprenols (the main active ingredient is sodium polyprenyl phosphate) and an immunomodulating antiviral drug (group J05AX). Recently, Fortepren® has undergone a full cycle of preclinical trials and clinical approbation in GH.

Efficacy of Fortepren® in Treatment of GH

A multicenter clinical study of Fortepren® was conducted at three testing sites. There, 80 patients selected during a screening process underwent a 10-day course of treatment of the acute phase of the disease with the antiviral drug acyclovir in a dose of 400 mg three times a day. At the end of the basic course, patients received Fortepren® according to the following scheme: 8 mg per person at the stage of remission, three times with an interval of 21 days.

The study population included 80 patients: 57 women (72%) and 23 men (28%) aged 18 to 55 years. Distribution of patients in the treatment group was 43 women (72%) and 17 men (28%), in the control group - 14 women (70%) and 6 men (30%). Both among men and women, patients from 25 to 35 years of age prevailed.

The duration of GH infection in patients enrolled in the study was 1 to 35 years, on average 7.31 ± 4.5 years. Patients with a high frequency of exacerbations of herpesvirus infection - 12.81 ± 0.44 times a year for patients in the treatment group and 12.95 ± 0.34 times per year for the control group – were enrolled in the study. In this category of patients, treatment of herpesvirus infection is especially difficult, as in case of relapses secondary immunological failure is developed, and physical and

psychological depletion and poor adherence to therapy occur due to the ineffectiveness of previous treatment.

The clinical efficacy and safety of the therapy (using Fortepren®) and the interferon status parameters were evaluated.

Criteria for the clinical efficacy of the therapy were as follows: increase in the duration of inter-recessive periods (remission); decrease in the frequency of relapses (exacerbations) of herpesvirus infection; decrease in the severity of relapses which was assessed in points and included characteristics of local signs of relapse (itching, burning, soreness, congestion, edema, regional lymphadenitis); general signs of relapse (weakness, headache, fever, chills, neuralgia, myalgia); severity of skin or mucous membrane lesions before and after treatment (number of lesions, affected area, number of vesiculo-erosive lesions); change of immunological indicators based on the evaluation of the interferon status.

Analysis of the obtained results showed that Fortepren® treatment of patients with chronic recurrent herpesviral infection with genital localization leads to an increase in the duration of inter-recessive periods, which is one of the main criteria for evaluating the treatment efficiency (Figure 5).

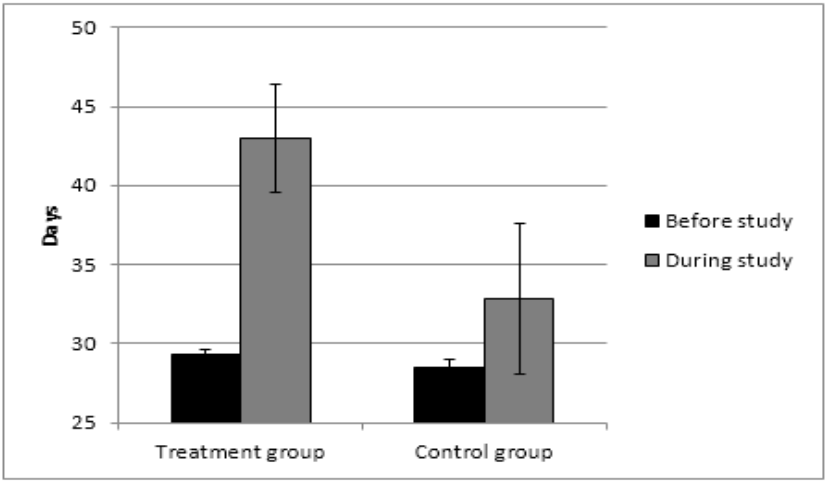

Figure 5. Duration of the inter-recessive period (remission) in patients of the treatment and control groups before and during the study.

In the treatment group of patients treated with Fortepren® the inter-recurrence period increased by 1.5 times from 29.36 ± 2.16 days three months before the study to 42.98 ± 3.29 days (p = 0.0002) during the study. In the control group of patients treated with placebo the inter-recurrent period changed insignificantly from 28.51 ± 1.98 3 months before the study to 32.83 ± 19.12 during the study (p = 0.37).

The criterion "duration of the inter-recessive period" (remission) is directly related to another indicator – "frequency of relapses" (exacerbations). Assessment of the frequency of exacerbations of genital herpes in the treatment group showed a significant decrease in this indicator during treatment by 1.6 times (Figure 6).

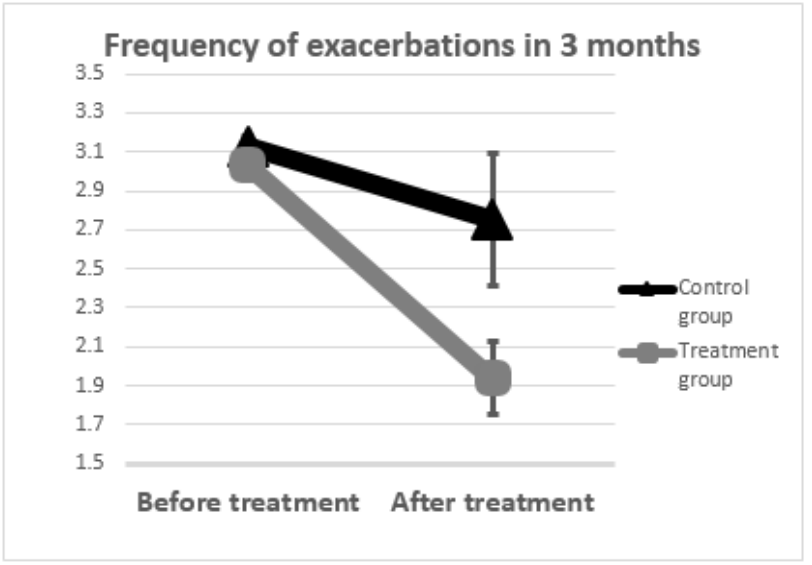

Figure 6. Frequency of exacerbations of GH in patients from the experimental and control groups.

While the recurrence rate of herpes 3 months before treatment was 3.03 ± 0.02, during treatment and 3 months of observation it was 1.94 ± 0.19 (p <0.05). These results differed significantly from those of the control group, where the number of relapses 3 months before treatment was 3.13 ± 0.05 and 2.75 ± 0.34 during the study; the difference was not statistically significant (p = 0.27).

The duration of recurrence (days) of genital herpes before and after treatment showed significant changes both in the treatment group and control group (placebo): in the treatment group, this indicator decreased from 6.54 ± 0.27 to 5.75 ± 0.24 days (P = 0.001), in the control group – from 7.10 ± 0.55 to 5.67 ± 0.55 days (p = 0.004). However, the difference in the duration of relapse between the treatment group and control group was not statistically significant (p = 0.32).

The next criterion for evaluating the efficiency of Fortepren® was reduction in the severity of relapses, which was assessed in points by symptomatic signs of relapse in patients undergoing treatment in the treatment and control groups from the moment the patient with chronic recurrent GH viral infection was enrolled in the study at the screening stage (1st visit before treatment) and during treatment.

Observations showed that the predominant localization of recurrences of herpes was genital: the penile region, the pubic area in men, the labia, the perineum, the pubic, and the perianal region in women. More often rashes were localized on the mucous membrane of the genitals. In addition, some patients had extragenital rashes - in the region of the sacrum, gluteal region, where, as a rule, rash has a large area and longer duration of epithelialization.

Assessment of such local signs of relapse as itching, burning, soreness, hyperemia, edema, and regional lymphadenitis showed a statistically significant decrease by 1.6 times in patients of the treatment group receiving Fortepren®.

Statistically, the total value of the mean score of local signs of relapse in the treatment group significantly decreased from 1.63 ± 0.09 points at visit 1 (screening) prior to treatment to 1.03 ± 0.09 (p = 0.0001) during treatment. In the control group (placebo) changes were not statistically significant: 1.28 ± 0.11 points at visit 1 (screening) and 1.23 ± 0.79 points during treatment (p = 0.79).

The results of the assessment of the common signs of relapse (weakness, headache, chills, neuralgia, myalgia) in the form of mean values in points at visit 1 (screening) before treatment and during treatment in the treatment and control groups suggest that treatment with Fortepren®

leads to a decrease in the severity of the general signs of relapse in patients of the treatment group by 1.5 times.

In the treatment group of patients treated with Fortepren®, the mean value of the common signs of relapse is significantly reduced from 0.62 ± 0.10 to 0.42 ± 0.09 points ($p = 0.049$). In the control group of patients receiving placebo, there is no quantitative change in the sign. It is 0.50 ± 0.18 before treatment; it is 0.53 ± 0.14 points at $p = 0.86$ during treatment.

For the relapse sign "temperature" in both the treatment and control groups from the beginning of treatment at visit 1 (screening) and during treatment, changes are not statistically reliable: from 0.23 ± 0.06 points to 0.17 ± 0.07 points ($p = 0.48$) in the treatment group, and from 0.13 ± 0.09 points to 0.19 ± 0.11 points ($p = 0.65$) in the control group.

Manifestations of recurrent genital herpes can range from asymptomatic viral or mild symptoms to very painful weeping ulcers with clear boundaries. A typical picture of manifestations of genital herpes is characterized by the appearance of single or multiple vesicle elements on the mucous membranes of the genital organs and adjacent skin areas with erythema. Normally, 1-2 days later the vesicles burst open forming erosions or, less frequently, ulcers epithelizing with or without crust formation. In this case, lesions of the skin and mucous membranes can differ both in the number and area of lesions and in the number of vesiculo-erosive elements in the lesion foci.

In this clinical study, the severity of recurrent genital herpes was evaluated by the "number of foci," "lesion area," "number of elements" in the lesion foci as the mean score at visit 1 (screening) before treatment and during treatment in the treatment and control groups.

In the treatment group of patients treated with Fortepren® the number of lesions was significantly reduced by 1.4 times from 1.21 ± 0.07 to 0.84 ± 0.10 points at $p = 0.002$. In the control group (placebo) at visit 1 (screening) the number of lesions was estimated at 1.25 ± 0.14 points before treatment. During treatment, this value was 1.00 ± 0.12 points at $p = 0.06$, i.e., changes in this sign were not statistically significant.

The lesion area was also different in the treatment and control groups at visit 1 (screening) and during treatment. In the treatment group at visit 1

(screening) it was 1.13 ± 0.15 points before treatment. It decreased by 1.9 times to 0.59 ± 0.11 points (p = 0.003) during treatment. Accordingly, in the control group (placebo) at visit 1 (screening) the lesion area was estimated at 1.06 ± 0.21 points before treatment; this value was 0.75 ± 0.16 points at p = 0.24 during treatment. The difference was not statistically significant.

The number of vesiculo-erosive elements in the lesion (the sign of recurrence "number of elements") as the mean score in the treatment group decreased 1.5 times from the moment of evaluation at visit 1 (screening) before treatment (2.53 ± 0.14 points) and (1.71 ± 0.19 points) at p = 0.001 during treatment. In patients of the control group (placebo) at visit 1 (screening), the sign "number of elements" is estimated at 2.69 ± 0.15 points before treatment and at 2.25 ± 0.27 points at p = 0.14 at the time of treatment, i.e., the difference was not statistically significant.

The aggregate relapse severity score based on local (itching, burning, soreness, hyperemia, edema, regional lymphadenitis) and general (weakness, headache, chills, neuralgia, myalgia) signs of relapse, body temperature, number of lesions, lesion area, and number of vesicular-erosive elements in the lesion foci in the treatment group was 7.36 ± 0.35 points at visit 1 (screening) before treatment and decreased to 4.75 ± 0.35 points during treatment at p = 0.0002. In the control group of patients receiving placebo according to the same scheme, the aggregate relapse severity score at visit 1 (screening) before treatment and during treatment was not statistically significant and amounted, respectively, to 6.91 ± 0.42 and 5.95 ± 0.42 points at p = 0.24 (Figure 7).

Thus, in patients of the treatment group receiving Fortepren® intramuscularly in a dose of 2.0 ml (8 mg) three times at intervals of 21 days, a statistically significant decrease in the severity of relapses estimated in scores was 1.6 times compared with the control group of patients receiving placebo according to the same scheme, which demonstrates the efficacy of Fortepren® used to reduce the severity of relapses in the chronic recurrent herpes virus infection of genital localization.

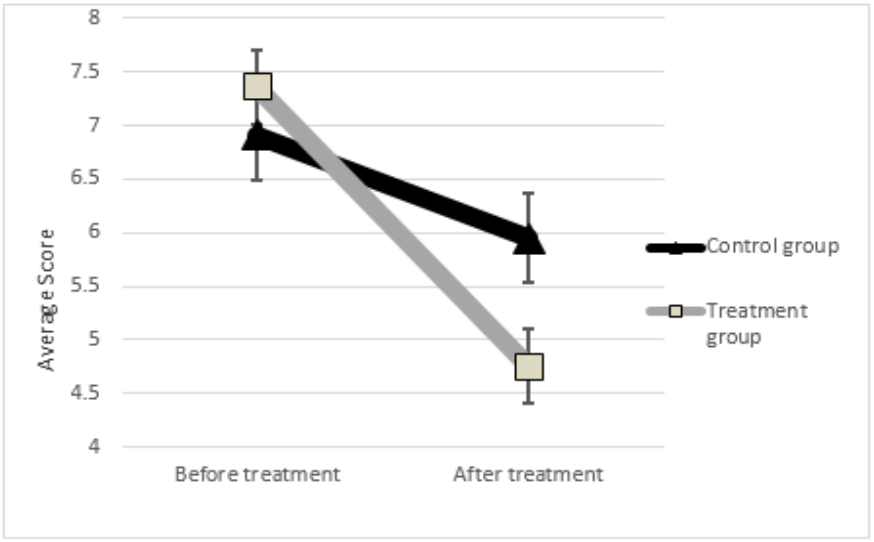

Figure 7. Comparative characteristics of symptoms of recurrent herpes in patients of the treatment and control groups. The aggregate relapse severity score based on local (itching, burning, soreness, hyperemia, edema, regional lymphadenitis) and general (weakness, headache, chills, neuralgia, myalgia) signs of relapse, body temperature, number of lesions, lesion area, and number of vesicular-erosive elements in the lesion foci was compared in the treatment and control groups before and after treatment.

In general, it should be noted that in the treatment group, along with an increase in the duration of the inter-recessive period and a decrease in the frequency of relapses of GH in 70% of patients, the level of leukocyte virus-induced interferon (LVI-IFN) remained unchanged or increased in 64.4% of patients. In the control group, a decrease in the frequency of relapses was observed in 55% of patients; the number of patients with the LVI-IFN level remained unchanged or increased only 36.8%, i.e., the change in the recurrence rate of herpes simplex in patients of both the treatment group and the control group in this clinical study is accompanied by a change in the level of leukocyte virus-induced interferon (LVI-IFN).

According to ICD-10, all patients who participated in this study could be classified by the clinical form of GH to section A60 - Anogenital Herpetic Virus Infection (herpes simplex). According to the clinical and morphological classification, the study involved patients with recurrent GH

(typical and atypical forms). In addition, when classifying the severity of the clinical course of GH, the following forms are distinguished:

- mild - exacerbation of the disease 3-4 times a year, remission of at least 4 months;
- moderate - exacerbation of the disease 4–6 times a year, remission of at least 2-3 months;
- severe - monthly exacerbation, remission from several days to 6 weeks.

All patients who took part in this study were diagnosed with the last form of severity of the clinical course of GH. Such patients with severe disorders of the immune system and monthly relapses of GH are less responsive to antiviral and anti-relapse therapy.

It is known that the herpes simplex virus has a tendency to affect cells of the immune system (Interferon-2011, 2012; Pokrovsky, 1996). Persisting and reproducing in them, it causes a decrease in the functional activity or even death of immunocompetent cells. As a result, suppression of both cellular and humoral immunity occurs. Immunodeficiency develops (Hoffman, Schmitz, 1995; Maksimova, 2005; Paludan et al., 2011; Kumari et al., 2015; Guo et al., 2015). When the immune system is weakened, it becomes impossible to completely eliminate the intracellular virus; favorable conditions are created for its spread in the body and activation of other infections. A typical form of recurrent genital herpes is characterized by severe symptom manifestation with classical development of limited, weakly spreading lesions (erythema, vesicular rashes and their opening with the formation of wet erosion, epigastric epithelialization) and localization on the same area of the skin or mucous membrane. Normally, 12-48 hours before the onset of rashes, local and general prodromal manifestations may appear: itching and burning in the foci, swelling, inguinal lymphadenitis on the affected side, subfebrile condition, weakness, and malaise.

Today, there is a debate about immunological parameters, specifically what parameters should be studied in the treatment of patients with GH

(Aleksandrova, 2004; Isakov, 1999; Shulzhenko, 2006; Shulzhenko, Zuykova, 2011; Marchenko, 2011; Budanov, 2011; Silina, Bibicheva, 2011; Interferon-2011, 2012).

In most cases, virus invasion is controlled by the IFN system. The effect of IFN is not limited to the violation of the virus reproduction. IFNs act as immunomodulators of the effector link of immunity, influencing the direct cytotoxic reaction of T lymphocytes, antibody-mediated lysis of infected cells by T lymphocytes, macrophages, and polymorphonuclear leukocytes. According to numerous authors (Isakov et al., 2013; Ershov, Kiselev, 2005), recurrent herpes reduces the ability of peripheral blood leukocytes to produce interferons by 100 times during relapse and 10 times during remission.

A number of studies demonstrated that more than 90% of patients with recurrent GH showed a decrease in the ability to produce IFNα, an integrative indicator of the state of antiviral protection of the body (Interferon-2011, 2012; Ershov, Kiselev, 2005; Mezentseva, 2006). This is special attention was paid to this indicator during the study. This was all the more important because in all examined patients the authors could not take into account the level of circulating IFN by the biological method of assessing the IFN status due to the toxic effect of serum on indicator cells of transplanted cultures. The production of IFNγ, an indicator of the functional activity of T lymphocytes and ability to activate the immune system, was not informative in this group of patients.

Therefore, the LVI-IFN indicator gave valuable information on the status and functioning of antiviral immunity in the examined patients with severe clinical course of recurrent GH.

This indicator is all the more important, since at present five main ways of virus elimination, including HSV, due to the IFN activity are determined: 1) general interfering with gene expression and protein synthesis; 2) minimizing IFN induction by limiting the production of viral pathogen-associated molecular patterns (PAMPs) and/or specific blockade of the IFN induction reaction cascade; 3) inhibition of IFN signals; 4) blocking the effect of IFN-induced enzymes by antiviral activity; and 5) the acquisition of a replication strategy that is insensitive to the action of

IFN (Randall, Goodbourn, 2008). Thus, HSV has a wide variety of molecular mechanisms that make it possible to evade the action of IFN.

Therefore, this indicator defined by the authors as leukocyte virus-induced IFN (LVI-IFN) suggesting the potential ability of cells to produce IFN in chronic recurrent genital herpes virus infection with a severe clinical course, appears to adequately reflect the state of antiviral immunity of patients treated with Fortepren®.

Thus, the therapy with Fortepren® in a dose of 2.0 ml intramuscularly, three times with an interval of 21 days, in patients with initially high-frequency recurrence of GH leads to a statistically significant ($p < 0,0002$) prolongation of the interrecurrent period by 1.5 times, reduces the frequency of relapses by 1.6 times ($p < 0,05$), decreases the aggregate relapse severity score by 1.6 ($p = 0.0002$) in the treatment group compared with the control group (placebo), which is accompanied by preservation or restoration of the antiviral immunity indicators in more than 64.4% of treated patients compared to the control group during the 3-month follow-up.

Given the limited information obtained by studying the IFN status through the biological method and in order to further justify the possibility to increase the immune response in cells, to establish possible mechanisms that determine the efficacy of treatment with Fortepren®, an evaluation of cytokine production was conducted using the modern multiplex method of analysis in patients with chronic recurrent herpes viral infection of genital localization. Production of the main cytokines involved in protection against herpes virus infection in patients treated with Fortepren® with the optimal dosage regimen was compared with those in the control group of patients who received a placebo solution.

The results of the studies conducted show the following (Tables 3 and 4): IFNα and IFNγ were not detected in the serum of patients from the control group, whereas in patients treated with Fortepren® there was certain dynamics with a decrease at visit 6 (IFNγ) or visit 8 (IFNα) and a subsequent increase at visit 9. These data indicated stimulation of the production of one of the key cytokines involved in the maintenance of antiviral immunity as a result of therapy with Fortepren®.

Table 3. Production of cytokines by blood cells in patients with GH before treatment with Fortepren® (visit 1) and after treatment (visit 9)

Indicator	Visit 1		Visit 9	
	Placebo	**Fortepren®**	**Placebo**	**Fortepren®**
IFNα, NDV	211.28±74.89	241.0±70.88	139.25±47.08	**592.79**±57.9
INFγ, NDV	12.72±7.97	11.56±3.76	20.12±12.65	**40.04**±12.82
IL12p40, NDV	4.42±1.1	4.34±1.13	5.7±2.0	**9.66**±2.21
IL12p40	4.25±0.11	3.15±0.8	14.35±8.12	**25.58**±4.9
IL17a	0.1±0.16	0.37±0.09	**6.46**±4.9	0.77±0.16
IL1β NDV	1.14±0.7	0.95±0.7	40.95±28.96	**181.49**±18.02
IL1β, NDV+fortepren	1.06±0.75	3.22±2.31	24.67±17.44	**227.79**±65.09
IL1β PHA	17.36±0.92	9.56±2.93	53.04±35.06	**203.14**±55.02
IL1β	2.67±1.88	1.16±1.06	71.5±50.56	**223.38**±40.0
IL2	0	0	**6.87**±4.86	0
IL6, NDV	27.07±2.58	15.94±4.52	291.16±129.07	**835.67**±149.06
IL6, PHA	487.07±103.66	297.28±108.04	466.97±204.92	**1837.7**±112.9
IL6	13.29±8.95	7.47±5.17	184.7±82.51	**1045.64**±250.74
MIP1α, NDV	268.86±31.73	209.58±51.98	1143.29±498.02	**2503.95**±149.06
MIP1α, NDV+fortepren	371.34±149.17	380.19±138.49	842.46±369.07	**3946.51**±151.79
TNFα, NDV	22.84±5.71	16.89±5.52	229.88±99.97	**444.42**±71.19
TNFα, NDV	6.19±2.61	3.38±0.68	97.05±68.02	**223.85**±77.02

Table 4. Dynamics of the content of key cytokines in blood serum of patients with GH at different stages of Fortepren® therapy

Indicator	Visit 1		Visit 4		Visit 6		Visit 8		Visit 9	
	Placebo	Fortepren®	Placebo	Fortepren®	Placebo	Fortepren®	Placebo	Fortepren®	Placebo	Fortepren®
IFNα	0	0.49±0.49	0	0.63±0.49	0	0.91±0.71	0	0.31±0.24	0	1.3±0.95
IFNγ	0	0.19±0.16	0	0.17±0.41	0	0	0	0.1±0.08	0	0.4±0.00
IL10	0.87±0.39	0.93±0.26	1.09±0.49	1.77±0.5	0.78±0.35	1.67±0.47	1.14±0.51	2.43±0.6	0.7±0.31	1.47±0.64
IL12p40	2.97±0.78	4.38±3.87	5.94±0.3	5.07±3.41	3.44±0.99	5.18±3.85	2.87±0.27	4.36±3.13	3.6±1.06	3.81±2.94
IL12p70	0.09±0.04	0.24±0.17	0.2±0.09	0.31±0.19	0.09±0.04	0.43±0.29	0.09±0.04	0.4±0.26	0.2±0.09	0.33±0.18
IL15	2.02±0.25	1.43±0.47	2.41±0.31	1.62±0.40	1.61±0.01	1.82±0.51	1.38±0.17	2.13±0.46	1.99±0.02	1.87±0.41
IL2	0	0.43±0.43	0	0.47±0.37	0	0.56±0.42	0	0.5±0.37	0	0.34±0.32
IL4	0	8.55±3.14	1.33±0.59	9.11±3.31	0.18±0.08	5.8±2.40	0	7.14±2.20	0	9.34±3.51
MIP1	3.8±1.7	6.0±4.2	15.4±6.9	22.5±10.4	10.6±4.7	23.9±11.9	18.3±8.2	16.1±10.8	18.3±8.2	20.0±13.6
TNFα	2.15±0.0	1.43±0.4	2.4±0.3	1.53±0.4	1.96±0.2	1.62±0.4	2.24±0.2	1.65±0.4	2.19±0.1	1.77±0.4

IL-10 is an anti-inflammatory cytokine. Its product includes monocytes, macrophages, and activated type 2 T helper. IL-10 inhibits the production of IFN, all proinflammatory cytokines by macrophages, the expression of TNFα and IL-12 receptors on natural killers. Excess IL-10 leads to a decrease in anti-infection protection and development of chronic infections. The ability of IL-10 to inhibit production of IL-1, IL-6, TNFα by macrophages is associated with its ability to inhibit IL-12 production. As a rule, macrophages initially produce and secrete proinflammatory cytokines, including IL-12, and then IL-10, but with the predominance of IL-12. However, sometimes IL-10 production sharply increases, which leads to suppression of the immune response. The data obtained show that the content of IL-10 in the blood serum of patients from the experimental group is slightly increased by visit 8, while the production of IFNα is reduced with a subsequent decrease by visit 9. At the same time, the level of IL-10 does not reach critical inhibitory values.

A significant increase in the concentration of the IL12p40 subunit in the serum of patients treated with Fortepren® was not observed in comparison with the control group. The concentrations of the IL12p70 subunit in the serum of patients of the experimental and control groups varied in the opposite way, which was indicative of stimulation of the active form of IL12 by Fortepren®.

Interleukin 15 (IL-15) regulates the activation and proliferation of T lymphocytes and natural killers. Signals that support the survival of memory T cells in the absence of antigen are also provided by IL-15. In the serum of the control group, the IL-15 content reached the maximum values by visit 4, gradually decreased and returned to the baseline level at the final stage of the study. In contrast, in patients treated with Fortepren®, the concentration of IL-15 in the serum increased by visit 8 with a subsequent return to the baseline values.

IL-2 is required to maintain the proliferation of T cells, including T helper cells and regulatory T cells. This cytokine was not detected in the serum of the control group. In contrast, in patients treated with Fortepren®, it was observed at all study points with a peak at visit 6.

IL-4 produced by type 2 T helpers autocratically regulates their proliferation, provides the growth and differentiation of B lymphocytes. In the control group, this cytokine was observed only at visit 4, whereas in the experimental group it was detected during the entire study period, with a slight decrease by visits 6 and 8, when the maximum activity of Th1 detected by IL12p70 was observed. This is consistent with the information that Th1 and Th2 are activated in the antiphase and suppress each other.

MIP-1α belongs to the CC subfamily of chemokines. It is produced by stimulated leukocytes and provides chemotaxis and increased intracellular calcium concentration. MIP-1α plays an important role in the activation of T cells, enhancing their proliferation, secretion of IL-2 and expression of its receptor. A significant increase in the production of this cytokine in the serum of patients from the experimental group as compared to the control group was noted at visits 6 and 9.

The level of TNFα did not change significantly during the whole period of observation and was somewhat lower in patients of the experimental group.

In the study of spontaneous and in vitro induced production of cytokines by blood leukocytes in patients of the two groups the results were largely similar to those described above. Thus, under the action of NDV, leukocytes in patients of the experimental group more strongly produced IFNα and IFNγ as compared to the cells of patients from the control group. The same pattern was also revealed in the study of IL12p40 production, both after stimulation of NDV cells and without additional stimulation.

In contrast, production of leukocytes IL-17A, the main cytokine of Th17 involved in the development of autoimmune and allergic reactions, as well as in protection from extracellular pathogens, is dramatically increased by visit 9 in patients of the control group, whereas after treatment with Fortepren IL-17A is practically not detected in supernatants of blood leukocytes.

The key cytokine with which the immunomodulatory effect of Fortepren®, IL-1, is associated, was also intensively produced by cells in the experimental group compared to the control group at visit 9. This effect

was observed with non-activated leukocytes and leukocytes activated by NDV or PHA and further increased after addition of Fortepren® to the culture medium.

It should be noted that leukocytes in patients treated with Fortepren® did not produce IL-2 in vitro. At first glance, this is not consistent with the results obtained in the study of serum. The differences may be due to the fact that resident, rather than circulating T cells respond to the production of IL-2 observed in serum.

Cytokine IL-6 is synthesized mainly by activated macrophages, including under the influence of IL-1 and TNF-α, as well as by T cells. It stimulates the immune response, production of liver proteins of the acute phase and serves as one of the most important mediators of the acute phase of inflammation. IL-6 enhances the proliferation and differentiation of B and T cells and leukocytopoiesis. It is not surprising that treatment with Fortepren® leads to a significant increase in the ability of leukocytes to produce IL-6 spontaneously, as well as under the influence of NDV or PHA.

Similar data were obtained in the study of two other cytokines of the same group - MIP1α and TNFα. In the latter case, there are some discrepancies with the data given above. However, it is illusory. It is obvious that TNFα is produced and consumed directly in the inflammatory focus and its entry into the circulation, which results in an increase in body temperature, and, on the contrary, decreases after treatment.

Thus, the studies conducted show that the treatment course with Fortepren® leads to increased production of key cytokines, which provide protection against viral infection. The data obtained suggest that the effect of the drug is aimed, first of all, at the cells responsible for the natural resistance of the organism (macrophages, dendritic cells, etc.). The activation of natural immunity, apparently, is the leading mechanism of protection against herpesvirus infection under the influence of Fortepren® (Pronin et al., 2016).

Treatment with Fortepren® in a dose of 2.0 ml three times in comparison with the control group of patients with high frequency of GH relapses during 3-month follow-up decreases the severity of local relapse

symptoms and the frequency of exacerbations in 60.0% of treated patients, increases the interferon-producing ability of leukocytes, enhances the production of key cytokines that provide protection against viral infections and is, therefore, accompanied by the activation of natural immunity.

CONCLUSION

Recent studies have shown that the interferon system plays a significant, positive role in chronically recurrent GH. Based on the data obtained, the authors propose inclusion of the interferon drug therapy and interferon inducers that normalize the immunity and interferon systems in patients as a new method of treatment of GH. These drugs can be used for prevention in the monotherapy of herpes viral infections (during GH remissions) or in complex therapy with basic etiotropic drugs (during relapses).

The clinical studies conducted clearly show that GH therapy using these drugs decreases the severity of GH, leads to earlier relief from the signs of the disease in comparison with patients who received only conventional therapy. In parallel, normalization of immunological indicators was noted, in particular, restoration of IFNα and IFNγ production. The proposed approach reduces the incidence and duration of GH relapses by 1.5 – 2 times in comparison with patients receiving basic therapy.

In general, as a method of treatment of GH patients with frequent relapses (more than 6 times a year), the authors recommend individual therapy combining etiotropic chemotherapy and additional immunotherapy with the IFN drugs and IFN inducers developed in Russia.

REFERENCES

Akovbyan, V. A., Masyukova, S. A., Vladimirova, E. V., Zudin, V. A. and Pokrovskaya S. V. (2003). (Genital Herpes: Recent problems and their

solution). *Clinical Microbiology and antimicrobial chemotherapy*, 5 (1): 4-18.

Aleksandrova, V. A. (2004). (Fundamentals of immunology and the principles of immunotropic therapy). Saint-Petersburg, SPbMAPO, 41pp.

Barinsky, I. F. and Makhmudov, F. R. (2013). (Herpes). Baku, *"Victory":* 352 pp.

Blanc, M., Hsieh, W. Y. and Robertson K. A. (2011). Host defense against viral infection involves interferon mediated down-regulation of sterol biosynthesis. *PLoS Biol*, 9 (3): 1–19.

Budanov, P. V. (2004). (Problems of recurrent genital herpes therapy). *Questions of gynecology, obstetrics and perinatology*, 3 (4): 94-98.

Budanov, P. V. (2007). (Differential approach to the choice of the method of immunocorrective therapy in obstetrics and gynecology). *Effective pharmacotherapy in obstetrics and pharmacology*, (5): 4 – 11.

Budanov, P. V. (Recurrent genital herpes). (2011). Immunotherapy. A guide for physicians. Ed. by Haitov R. M. and Ataullakhanov, R. I. Moscow, *GEOTAR-Media:* 423-433.

Cai, M., Li, M., Wang, K., Wang, S., Lu, Q., Yan, J., Mossman, K. L., Lin, R. and Zheng, C. (2013). The Herpes Simplex Virus 1-Encoded Envelope Glycoprotein B Activates NFkB through the Toll-Like Receptor 2 and MyD88/TRAF6-Dependent Signaling Pathway. *PLoS ONE*, 8:e54586.

Chentoufi, A. and Ben Mohamed, L. (2012). Mucosal Herpes Immunity and Immunopathology to Ocular and Genital Herpes Simplex Virus Infections. *Clinical and Developmental Immunology*, doi:10.1155/ 2012/149135.

Chiu, Y. H., MacMillan, J. B. and Chen, Z. J. (2009). RNA Polymerase III Detects Cytosolic DNA and Induces Type I Interferons through the RIG-I Pathway. *Cell*, 138:576–591.

Ershov, F. I. (1996). (Interferon System in health and pathology). *M, Medicine:* 238 pp.

Ershov, F. I. (2006). (Antiviral drugs). *Handbook* (2nd ed.). M.: 368 pp.

Ershov, F. I. (2006). (Antiherpetic drugs). *Russian journal of skin and venereal diseases,* (1): 5-11.

Ershov, F. I. and Kiselev, O. I. (2005). (Interferons and their inducers (from molecules to medicines)). M, *GEOTAR-Media,* 368 pp.

Ershov, F. I. and Narovlyansky, A. N. (2011). (Interferons and inducers of interferons. Immunotherapy). A guide for physicians, ed. by Haitov, R. M., Ataullakhanov, R. I. *GEOTAR-Media:* 80-98.

Ershov, F. I. and Romantsov, M. G. (2007). (Medicinal products used in viral infections). A guide for physicians. *GEOTAR-Media,* 363 pp.

(Federal clinical practice guidelines for the management of patients with genital herpes). (2015). *The Russian society of dermatovenerologists and cosmetologists,* Moscow, 14 pp.

Gebhardt, T., Wakim, L. M., Eidsmo, L., Reading, P. C., Heath, W. R. and Carbone, F. R. (2009). Memory T cells in nonlymphoid tissue that provide enhanced local immunity during infection with herpes simplex virus. *Nat Immunol,* 10:524–530.

Gill, N., Chenoweth, M. J., Verdu, E. F. and Ashkar, A. A. (2011). NK cells require type I IFN receptor for antiviral responses during genital HSV-2 infection. *Cell Immunol.,* 269:29–37.

Gladko, V. V. (2005). (Clinical and epidemiological monitoring, control, standardization of the methods of treatment, prevention of STIs and scabies in the soldiers of the Ministry of defense of the Russian Federation and persons of military age). *Thesis Diss. Doct. Med. Sci.* VPO "Military medical Academy," Saint-Petersburg, 188 pp.

Guo, H., Omoto, Sh., Harris, P. A. and Finger, J. N. (2015). Herpes simplex virus suppresses necroptosis in human cells. *Cell Host & Microb,* 17:243-251. http://dx.doi.org/10.1016/j.chom.2015.01.003.

Gupta, R., Warren, T., Wald, A. (2007). Genital herpes. *Lancet,* 370:2127–2137.

Haldin, A. A. (2002). (Herpes simplex). *Russian Journal of skin and venereal diseases,* (2): 42-50.

Haldin, A. A. and Ignat'ev D. V. (2011). (New interferon inducer Kagocel® in the treatment of herpes simplex: opportunities and prospects). Effective pharmacotherapy. *Dermatovenerology and*

dermatocosmetology, (2), http://umedp.ru/articles/novyy_induktor_interferona_kagotsel_v_terapii_prostogo_gerpesa_vozmozhnosti_i_perspektivy.html.

Harandi. A. M., Svennerholm, B., Holmgren, J. and Eriksson, K. (2001). Differential roles of B cells and IFNgamma-secreting CD4+ T cells in innate and adaptive immune control of genital herpes simplex virus type 2 infection in mice. *J Gen Virol.*, 82:845–853.

Heath, W. R. and Carbone, F. R. (2009). Dendritic cell subsets in primary and secondary T cell responses at body surfaces. *Nat Immunol*, 10:1237–1244.

Hoffman, I. F. and Schmitz, J. L. (1995). Genital ulcer disease. Management in the HIVera. *Postgrad. Med*, 98, (3): 67-82.

Iijima, N., Linehan, M. M., Zamora, M., Butkus, D., Dunn, R., Kehry, M. R., Laufer, T. M. and Iwasaki, A. (2008). Dendritic cells and B cells maximize mucosal Th1 memory response to herpes simplex virus. *The Journal of Experimental Medicine*, 205:3041–3052.

(Interferon-2011). (2011). Ed. Ershov, F. I. and Narovlyansky, A. N. Moscow, 512 pp.

Isakov, V. A. (2010). (Efficiency of Kagocel® in the treatment of recurrent herpes simplex). *Terra Medica Nova*, (2).

Isakov, V. A., Arkhipova, E. I. and Isakov, D. V. (2013). (Human herpesvirus infections). *A guide for physicians.* 2-nd edition. Saint Petersburg, Spec Lit., 303 pp.

Isakov, V. A. and Aspel, Yu. V. (1999). (Immunopathogenesis and treatment of genital herpes and chlamydia). *A Guide for physicians*, Saint-Petersburg.

Isakov, V. A., Borisova, V. V. and Isakov, D. V. (1999). (Herpes: pathogenesis and laboratory diagnosis). *A guide for physicians*, Saint-Petersburg, Publishing house "LAN," 192 pp.

Isakov, V. A., Rybalkin, S. B. and Romantsov, M. G. (2006). (Herpes virus infection). *A guide for physicians.* Saint-Petersburg.

Iwasaki, A. (2007). Mucosal Dendritic Cells. *Annual Review of Immunology*, 25:381–418.

Kagramonova, J. A., Malinovskaya, V. V. and Parfenov, V. V. (2008). (Therapy of recurrent herpes infections in women with the drug Viferon-3). *Effective pharmacotherapy in obstetrics and gynecology*, (1): 33.

Khryanin, A. A. and Reshetnikov, O. V. (2009). (Epidemiological aspects of herpes virus infection in Siberia). *Journal of Medicine and education in Siberia*, (3), http://ngmu.ru/cozo/mos/article/text_full.php?id=351.

Khryanin, A. A. Kornienko, M. V. and Titenko, A. V. (2015). (Epidemiological features of herpes virus infection: long-term trends). *Journal of Medicine and education in Siberia*, (3), http://ngmu.ru/cozo/mos/article/text_full.php?id=1762.

Knickelbein, J. E., Khanna, K. M., Yee, M. B., Baty, C. J., Kinchington, P. R. and Hendricks, R. L. (2008). Noncytotoxic Lytic Granule-Mediated CD8+ T Cell Inhibition of HSV-1 Reactivation from Neuronal Latency. *Science*, 322: 268–271.

Knop, E. and Knop, N. (2003). Eye-associated lymphoid tissue (EALT) is continuously spread throughout the ocular surface from the lacrimal gland to the lacrimal drainage system. *Ophthalmology*, 100 (11): 929–942.

Kumari, P., Narayanan, S. and Kumar, H. (2015). Herpesviruses: interfering innate immunity by targeting viral sensing and interferon pathways. *Rev. Med. Virol.*, 25 (3), DOI: 10.1002/rmv.1836.

Kuznetsova, I. V. and Khlystova, A. E. (2016). (Genital herpes: modern approaches to solving the problem (literature review)). Effective Pharmacotherapy. *Dermatovenerology and dermatocosmetology*, Special issue (30), http://umedp.ru/articles/genitalnyy_gerpes_sovremennye_podkhody_k_resheniyu_problemy_obzor_literatury.html.

Lafaille, F. G., Pessach, I. M., Zhang, S. Y., Ciancanelli, M. J., Herman, M., Abhyankar, A., Ying, S. W., Keros, S., Goldstein, P. A., Mostoslavsky, G., Ordovas-Montanes, J., Jouanguy, E., Plancoulaine, S., Tu, E., Elkabetz, Y., Al-Muhsen, S., Tardieu, M., Schlaeger, T. M., Daley, G. Q., Abel, L., Casanova, J. L., Studer, L. and Notarangelo, L.

D. (2012). Impaired intrinsic immunity to HSV-1 in human iPSC-derived TLR3-deficient CNS cells. *Nature,* 491:769–773.

Lafaille, F. G., Ciancanelli M. J., Studer, L., Smith, G., Notarangelo, L., Casanova, J. L. and Zhang, S. Y. (2015). Deciphering human cell-autonomous anti-HSV-1 immunity in the central nervous system. *Frontiers in Immunology*, 6, doi: 10.3389/fimmu.2015.00208.

Lee, H. K., Zamora, M., Linehan, M. M., Iijima, N., Gonzalez, D., Haberman, A. and Iwasaki, A. (2009). Differential roles of migratory and resident DCs in T cell priming after mucosal or skin HSV-1 infection. *J. Exp. Med.*, 206:359–370.

Leung, D. N. and Saks, S. L. (2000). Current recommendationfor the treatment of Genital Herpes. Drugs, 60: 1329-1352.

Looker K. J, Magaret A. S., May M. T., Turner K. M. E., Vickerman P., Gottlieb S. L., et al. (2015) Global and Regional Estimates of Prevalent and Incident Herpes Simplex Virus Type 1 Infections in 2012. *PLoS ONE* 10(10): e0140765.

Looker, K. J., Magaret, A. S., Turner, K. M. E., Vickerman, P., Gottlieb, S. L. and Newman, L. M. (2015). Global Estimates of Prevalent and Incident Herpes Simplex Virus Type 2 Infections in 2012. *PLoS ONE,* 10(1): e114989. pmid:25608026.

Lund, J. M., Hsing, L., Pham, T. T. and Rudensky, A. Y. (2008). Coordination of Early Protective Immunity to Viral Infection by Regulatory T Cells. *Science,* 320:1220–1224.

Maksimova, T. A. (2005). (Optimization of diagnostics and immune correction therapy of genital herpes type II in women). *Abstract. Diss. Cand. Med. Sciences,* Saint-Petersburg, 24 pp.

Malinovskaya, V. V. and Delenyan, N. V., Arinenko, R. Y. (2007). (Interferon). *A guide for physicians.* Publishing house "INKO-TNK" M, 87 pp.

Marchenko, L. A. (2011). (Genital herpes: pathogenesis, diagnostics, treatment). Immunotherapy. A guide for physicians. Ed. by Haitov, R. M. and Ataullakhanov, R. I. Moscow, *GEOTAR-Media:* 408-422.

Masyukova, S. A., Glazko, V. V., Egorov, Yu. and Ustinov, M. V. (2006). (Kagocel in the treatment of genital herpes). *Herpes,* (2): 48-52.

Mezentseva, M. V. (2006). (Patterns of functioning and correction of cytokine regulatory network). *Diss. doct.biol.science*, 280 pp.

Nakanishi, Y., Lu, B., Gerard, C., Iwasaki, A. (2009). CD8+ T lymphocyte mobilization to virus-infected tissue requires CD4+ T-cell help. *Nature*, 462:510–513.

Narovlyansky, A. N., Vasil'ev, A. N., Savoyskaya, S. L., Ivanova, A. M., Izmestieva, A. V., Danilov, L. L., Veselovsky, V. V., Ozerelkov, S. V., Zubashev, I. K., Pronin, A. V., Sanin, A. V. and Ershov, F. I. (2017). (The system of isoprenoids: role in antiviral immunity). *Vedomosti Center Expert. Means Med. Application*, (3): 66-78.

Ospelnikova, T. P., Polonsky, V. O., Narovlyansky, A. N., Tutushkina, T. V., Shulzhenko, A. E. and Ershov, F. I. (2004). (Efficiency of Kagocel at chronic recurrent herpes). *Clinical pharmacology and therapy*, 13 (2):74-76.

Paludan, Sr. R., Bowie, A. G., Horan, K. A. and Fitzgerald, K. A. (2011). Recognition of herpesviruses by the innate immune system. *Nat Rev Immunol.*, 11:143–154.

Patel, R., Alderson, S. and Geretti, A. (2011). IUSTI/WHO Europe. European guideline for the management of genital herpes, 2010. *Int. J. STD AIDS*, 22(1):1–10.

Pokrovskiy, V. I. (1996). (Infectious diseases). M, Medicine.

Pronin A. V., Narovlyansky A. N., Shulzhenko A. E., Sanin A. V., Sedov A. M. (2016) New polyprenyl phosphate based preparation Fortepren® as promising cytokine regulating antiviral remedy. *Cytokine and Growth Factor Reviews*, 30: 119-126.

Rakhmatulina, M. R. (2007). (Interferon-gamma in the treatment of genital herpes). *Infectious diseases*, (3): 76-79.

Randall, R. E. and Goodbourn S. (2008). Interferons and viruses an interplay between induction, signaling, antiviral responses and virus countermeasures. *J. Gen. Virol.*, 89: 1-47.

Rathinam, V. A. K., Jiang, Z., Waggoner, S. N., Sharma, S., Cole, L. E., Waggoner, L., Vanaja, S. K., Monks, B. G., Ganesan, S., Latz, E., Hornung, V., Vogel, S. N., Szomolanyi-Tsuda, E. and Fitzgerald, K. A. (2010). The AIM2 inflammasome is essential for host defense

against cytosolic bacteria and DNA viruses. *Nat Immunol.*, 11:395–402.

Reinert, L. S., Harder, L., Holm, C. K., Iversen, M. B., Horan, K. A., Dagnos-Hansen, F., Ulhшi, B. P., Holm, T. H., Mogensen, T. H., Owens, T., Nyengaard, J. R., Thomsen, A. R. and Paludan, S. R. (2012). TLR3 deficiency renders astrocytes permissive to herpes simplex virus infection and facilitates establishment of CNS infection in mice. *The Journal of Clinical Investigation*, 122:1368–1376.

Samgin, M. A. and Haldin, A. A. (2002). (Herpes simplex: dermatological aspects). M.

Sancho-Shimizu, V., Perez de Diego, R., Jouanguy, E., Zhang, S. Y. and Casanova, J. L. (2011). Inborn errors of anti-viral interferon immunity in humans. *Current Opinion in Virology*, 1:487–496.

Sato, A. and Iwasaki, A. (2004). Induction of antiviral immunity requires Toll-like receptor signaling in both stromal and dendritic cell compartments. *Proceedings of the National Academy of Sciences of the United States of America*, 101:16274–16279.

Schiffer, J. T., Abu-Raddad, L., Mark, K. E., Zhu, J., Selke, S., Koelle, D. M., Wald, A. and Corey, L. (2010). Mucosal host immune response predicts the severity and duration of herpes simplex virus-2 genital tract shedding episodes. *Proceedings of the National Academy of Sciences of the United States of America*, 107:18973–18978.

Sen, G. C. (2001). Viruses and interferons. *Annu. Rev. of Microbiol.*, 55: 255-281.

Shin, H. and Iwasaki, A. (2012). A vaccine strategy that protects against genital herpes by establishing local memory T cells. *Nature*, 491:463–467.

Shin, H. and Iwasaki A. (2013). Generating protective immunity against genital herpes. *Trends Immunol*, 34(10): doi:10.1016/j.it.2013.08.001.

Shperling, N. V., Vengerovskiy, A. I., Shperling, I. A., Engel, E. E. and Romanova, E. V. (2010). (Clinical efficacy of interferon therapy in the treatment of recurrent genital herpes). *Clinical dermatology and venerology*, (1): 69-72.

Shulzhenko, A. E. and Ershov, F. I. (2004). (Efficiency of Kagocel at chronic recurrent genital herpes). *Clinical pharmacology and therapy*, 13(2):74-76.

Shulzhenko, A. E. and Tutushkina, T. V. (2004). (Structure of immune changes in patients with genital form of chronic recurrent herpes infection (HGVI) during therapy with Kagocel). *Clinical pharmacology and therapy*, 13(2):74-76.

Shulzhenko, A. E. and Tutushkina, T. V. (2005). (Kagocel therapy of chronic recurrent genital herpes virus infection). *Cytokines and inflammation*, 4(2):59-65.

Shulzhenko, A. E. (2006). (Standard and alternative approaches to the diagnosis and treatment of herpes viral infections). *Diss. Doctor. Med. Science*, 220 pp.

Shulzhenko, A. E. and Zuikova, I. N. (2011). (Immunotherapy of herpes virus infection). Immunotherapy. A guide for physicians. Ed. by Haitov, R. M. and Ataullakhanov, R. I. Moscow, *GEOTAR-Media:* 398-407.

Silina, L. V. and Bibicheva, T. V. (2011). (Recurrent genital herpes). Immunotherapy. A guide for physicians. Ed. by Haitov, R. M. and Ataullakhanov, R. I. Moscow, *GEOTAR-Media:* 434-450.

Sokolova, T. M., Kolodyazhnaya, L. V., Ospelnikova, T. P. and Ershov, F. I. (2012). (Mechanisms of the drug Kagocel actions in human cells. Part 1. Regulation of transcription of the genes of interferon system and apoptosis). *Interferon* 2011, M.: 389 – 401.

Sokolova, T. M., Shuvalov, A. N., Poloskov, V. V. and Ershov, F. I. (2015). (Stimulation of genes of signal transduction by drugs Ridostin, Cycloferon and Ingavirin). *Cytokines and inflammation*, (2): 26 – 34.

St. Leger, A. and Hendricks, R. (2011). CD8+ T cells patrol HSV-1-infected trigeminal ganglia and prevent viral reactivation. *Journal of Neuro Virology*, 17:528–534.

Sun, L., Wu, J., Du, F., Chen, X. and Chen, Z. J. (2013). Cyclic GMP-AMP Synthase Is a Cytosolic DNA Sensor That Activates the Type I Interferon Pathway. *Science*, 339:786–791.

Tutushkina, T. V. and Shulzhenko, A. E. (2004). (Influence of treatment with Kagocel on indicators of immunograms of patients with genital form of chronic recurrent herpes infection*). Physiology and pathology of immune system,* (4):24-30.

WHO, The herpes simplex Virus. Newsletter January 2017; http://www.who.int/mediacentre/factsheets/fs400/ru.

Wu, J., Sun, L., Chen, X., Du, F., Shi, H., Chen, C. and Chen, Z. J. (2013). Cyclic GMPAMP Is an Endogenous Second Messenger in Innate Immune Signaling by Cytosolic DNA. *Science,* 339:826–830.

Yordy, B., Iijima, N., Huttner, A., Leib, D. and Iwasaki, A. (2012). A Neuron-Specific Role for Autophagy in Antiviral Defense against Herpes Simplex Virus. *Cell Host & Microbe,* 12:334–345.

Zhu, J., Koelle, D. M., Cao, J., Vazquez, J., Huang, M. L., Hladik, F., Wald, A. and Corey, L. (2007). Virus-specific CD8+ T cells accumulate near sensory nerve endings in genital skin during subclinical HSV-2 reactivation. *The Journal of Experimental Medicine,* 204:595–603.

Zhu, J., Peng, T., Johnston, C., Phasouk, K., Kask, A. S., Klock, A., Jin, L., Diem, K., Koelle, D. M., Wald, A., Robins, H. and Corey, L. (2013). Immune surveillance by CD8aa+ skin-resident T cells in human herpes virus infection. *Nature,* 497(7450):494-7. doi: 10.1038/nature12110.

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Publications in the past three years:

1. Ershov F.I., Narovlyansky A.N. (The use of interferon inducers in viral infections). (2015) // Vopr. Virusol. (Rus), 60(2): 5-10.
2. Narovlyansky A.N., Ivanova A.M., Shevlyagina N.V., Didenko L.V., Borovaya T.G., Izmestieva A.V., Sanin A.V., Pronin A.V., Ershov F.I. (Efficiency of Polyprenyl Phosphates in Experimental Model of Genital Herpes) (2015) // Vopr. Virusol. (Rus), 60(4): 9-13.
3. Ospelnikova T.P., Isaeva E.I., Kolodyazhnaya L.V., Kazulina I.S., Andreeva S.A., Poloskov V.V., Ershov F.I.- (Antiviral activity of interferon beta-1a) (2015) // Vopr. Virusol. (Rus), 6: 24-28.

4. Sokolova T.M., Shuvalov A.N., Poloskov V.V., Ershov F.I. (Stimulation of signal transduction genes with Ridostin, Cycloferon and Ingavirin) (2015) // Cytokines and Inflammations (Rus), 2: 26-34.
5. (Respiratory diseases in children who are often ill) (2015). The reference book of the doctor edited by F.I.Ershov. // M., "GEOTAR" (Rus), 176 p.
6. Ospelnikova T.P., Morozova O.V., Isayeva E.I., Andreeva S.A., Ershov F.I. (Interferons of type I, II, III and antiviral protein MxA in blood and washings in patients with Acute respiratory viral infections) (2016) // Russian Immunological Journal (Rus), 10(2): 378-380.
7. Romantsov M.G., Melnikova I.Yu., Ershov F.I. (Medicinal preparations with interferon-inducing activity in children's practice) (2016) // The Symbol of Science (Rus), 4:121-125.
8. Sokolova T.M., Poloskov V.V., Shuvalov A.N., Rudneva I.A., Ershov F.I. (Recombinant avian influenza virus H5N1(A/VIETNAM/1203/04) and its escape mutant m13 (13) induce early signaling immunity responses in human lymphocytes) (2016) // Vopr. Virusol. (Rus), 61(1): 21- 26.
9. Sokolova T.M., Poloskov V.V., Shuvalov A.N., Ershov F.I. (Regulation of the activity of tlr/rlr receptor genes and the synthesis of cytokines in the process of differentiation of THP-1 monocytes into macrophage-like cells under the action of phorbol-myristate acetate (PMA)) (2017) // Medical Immunology (Rus), 19(1): 27-34.
10. Poloskov V.V., Ershov F.I. (Activators of the synthesis of endogenous interferons (Review)) (2017) // Development and registration of medicinal products (Rus), 1(18): 188-192.
11. Respiratory diseases in frequently sick children. A guide for physicians. Ed. by F.I.Ershov (the second corrected and supplemented edition) (2017) // M., "GEOTAR" (Rus), 193 p.

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Research and Professional Experience: Antiviral immunity. Mechanisms of generation and action of interferons and other cytokines, development of immunomodulatory and antiviral drugs. In recent years, he has actively participated in research of the antiviral and cytokine-regulating effects of polyprenyl phosphates. Together with his colleagues, he determined that Fortepren® treatment of patients with chronic recurrent herpes infection after acute phase termination with acyclovir decreased the recurrence rate, as well as the severity of local symptoms. The activation of natural immunity appears to be a leading mechanism of protection against herpes viral infection under the influence of polyprenyl phosphates.

Professional Appointments: Head of Cytokine Laboratory

Honors: Professor

Publications in the past three years:

1. Pronin A.V., Danilov L.L., Narovlyansky A.N., Sanin A.V. Plant polyisoprenoids and control of cholesterol level. (2014) // Arch Immunol Ther Exp (Warsz), 62 (1): 31-39 (DOI 10.1007/s00005-013-0253-y).
2. Borovaya T.G., Narovlyansky A.N. (Apoptosis and herpes simplex viruses (review)) (2014) // Morphological Vedom. (Rus), 3: 3-14.

3. Ershov F.I., Narovlyansky A.N. (The use of interferon inducers in viral infections. (2015) // Vopr. Virusol. (Rus), v.60(2): 5-10.
4. Borovaya T.G., Didenko L.V., Narovlyansky A.N., Shevlyagina N.V., Ivanova A.M., Sanin A.V., Pronin A.V. (Histological Features of Ovary Network in Acute Period of Genital Herpes Virus Infection) (2015) //Morphological Vedom. (Rus), 2: 15-20.
5. Sedov A.M., Pronin A.V., Shulzenko A.E., Narovlyansky A.N., Zuykova I.N., Izmestieva A.V., Parfenova T.M., Zubashev I.K., Sanin A.V.(Clinical Studies of Innovation Drug Based on Polyprenyl Phosphates)(2015)// Med. Immunol. (Rus), 17: 279-280.
6. Narovlyansky A.N., Sedov A.M., Pronin A.V., Shulzenko A.E., Sanin A.V., Zuykova I.N., Shubelko R.V., Savchenko A.Yu, Parfenova T.M., Izmestieva A.V., Izmestieva An.V., Suprun O.V., Zubashev I.K., Kozlov V.S. (Treatment of Patients with Chronic Recurrent Herpes Virus Infection of Genital Localization: a clinical trial of Fortepren®) (2015) // J. Microbiol. (Rus), 4: 111-118.
7. Narovlyansky A.N., Ivanova A.M., Shevlyagina N.V., Didenko L.V., Borovaya T.G., Izmestieva A.V., Sanin A.V., Pronin A.V., Ershov F.I. (Efficiency of Polyprenyl Phosphates in Experimental Model of Genital Herpes) (2015) // Vopr. Virusol. (Rus), 60(4): 9-13.
8. Pronin A.V., Narovlyansky A.N., Shulzhenko A.E., Sanin A.V., Sedov A.M. New polyprenyl phosphate based preparation Fortepren® as promising cytokine regulating antiviral remedy (2016) // Cytokine and Growth Factor Reviews, 30: 119-126 (DOI: http://dx.doi.org/10.1016/j.cytogfr.2016.04.001).

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Research and Professional Experience:

Main research interests: immunology of infectious diseases in humans and animals - mycoplasmosis, legionellosis, brucellosis, listeriosis, Bordetella bronchiseptica, influenza and others. In recent years, he has actively participated in the development of new immunomodulatory drugs, in particular polyprenol-based drugs. Main area of scientific activity: study of mechanisms of activation of lymphoid cells exposed to viral and bacterial superantigens and immunomodulatory drugs.

Professional Appointments: Deputy Director of Center of Scientific Work, Head of Innate Immunity Lab

Honors: Professor

Publications in the past three years:

1. Pronin AV, Danilov LL, Narovlyansky AN, Sanin AV. Plant polyisoprenoids and control of cholesterol level. (2014// Arch Immunol Ther Exp (Warsz), 62 (1), 31-39.
2. Sedov A.M., Pronin A.V., Shulzenko A.E., Narovlyansky A.N., Zuykova I.N., Izmestieva A.V., Parfenova T.M., Zubashev I.K., Sanin A.V.(Clinical Studies of Innovation Drug Based on Polyprenyl Phosphates)(2015)// Med. Immunol. (Rus), V.17, 279-280.
3. Narovlyansky A.N., Sedov A.M., Pronin A.V., Shulzenko A.E., Sanin A.V., Zuykova I.N., Shubelko R.V., Savchenko A.Yu, Parfenova T.M., Izmestieva A.V., Izmestieva An.V., Suprun O.V., Zubashev I.K., Kozlov V.S. (Treatment of Patients with Chronic

Recurrent Herpes Virus Infection of Genital Localization) (2015)// J. Microbiol. (Rus), 4, 111-118.

4. Narovlyansky A.N., Ivanova A.M., Shevlyagina N.V., Lilenko L.V., Borovaya T.G., Izmestieva A.V., Sanin A.V., Pronin A.V., Ershov F.I. (Efficiency of Polyprenyl Phosphates in Experimental Model of Genital Herpes) (20150// Vopr. Virusol. (Rus), v.60, 4, 9-13.
5. Belaya Yu.A., Belaya O.F., Petrukhin V.G., Vakhromeeva M.S., Bystrova S.M., Pronin A.V. (Immunological Monitoring of *Helicobacter pylori*) (2015) //J. Microbiol. (Rus), 4, 106-112.
6. Borovaya T.G., Didenko L.V., Narovlyansky A.N., Shevlyagina N.V., Ivanova A.M., Sanin A.V., Pronin A.V. (Histological Features of Ovary Network in Acute Period of Genital Herpes Virus Infection) (2015) //Morphological Vedom. (Rus), 2, 15-20.
7. Narovlyansky A.N, Pronin A.V., Sanin A.V., Ivanova A.M., Parfenova T.M., Izmestieva A.V., Izmestieva Anastasia V., Gerasimova E.V., Sedov A.M., Zubashev I.K., Amir I. Tukhvatulin, Shulghenko A.E., ZuykovaI.N., Shubelko R.V The cytokine-regulating activity of polyprenyl phosphates: clinical studies of Fortepren (2015)// Cytokine, v76, 80-81.
8. Pronin AV, Narovlyansky AN, Shulzhenko AE, Sanin AV, Sedov AM. New polyprenyl phosphate based preparation Fortepren as promising cytokine regulating antiviral remedy. (2016) // Cytokine Growth Factor Rev, v. 30, 119–126.
9. Ivanova V.T., Garina E.O., Nikolaeva T.N., Suetina I.A., Trushakova S.V., Mezenceva M.V., Byrceva E.I., Sapurina I.Yu., Steyskal Ya., Pronin A.V. (A Sorption of Pathogenic Microorganisms on Composites of Polypirrol and Polyaniline) (2016) // Water: Chemistry and Ecology (Rus), 10, 71-81.

And 22 some more publications.

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Affiliation: N. F. Gamaleya Federal Research Center of Epidemiology and Microbiology of the Ministry of Health of the Russian Federation

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Business Address: Moscow, Russian Federation

Research and Professional Experience:

Main research interests: immunology of viral and transmissive diseases in animals, including babesiosis and haemobartonellosis; agents stimulating hematopoietic stem cells proliferation and migration; development and research of new immunomodulatory drugs. Main area of scientific activity: study of mechanisms of antiviral and anti-inflammatory effects of polyprenyl phosphates and other polyprenol-containing immunomodulatory drugs. Their role in correction and prevention of metabolic disorders, including metabolic syndrome, and pancreatitis.

Professional Appointments: Head of Cell-Mediated Immunity Lab.

Honors: Professor

Publications in the past three years:

1. Pronin AV, Danilov LL, Narovlyansky AN, Sanin AV. Plant polyisoprenoids and control of cholesterol level. (2014)// Arch Immunol Ther Exp (Warsz), 62 (1), 31-39.
2. Sedov A.M., Pronin A.V., Shulzenko A.E., Narovlyansky A.N., Zuykova I.N., Izmestieva A.V., Parfenova T.M., Zubashev I.K., Sanin A.V.(Clinical Studies of Innovation Drug Based on

Polyprenyl Phosphates)(2015)// Med. Immunol. (Rus), V.17, 279-280.

3. Narovlyansky A.N., Sedov A.M., Pronin A.V., Shulzenko A.E., Sanin A.V., Zuykova I.N., Shubelko R.V., Savchenko A.Yu, Parfenova T.M., Izmestieva A.V., Izmestieva An.V., Suprun O.V., Zubashev I.K., Kozlov V.S. (Treatment of Patients with Chronic Recurrent Herpes Virus Infection of Genital Localization) (2015)// J. Microbiol. (Rus), 4, 111-118.
4. Narovlyansky A.N., Ivanova A.M., Shevlyagina N.V., Lilenko L.V., Borovaya T.G., Izmestieva A.V., Sanin A.V., Pronin A.V., Ershov F.I. (Efficiency of Polyprenyl Phosphates in Experimental Model of Genital Herpes) (20150// Vopr. Virusol. (Rus), v.60, 4, 9-13.
5. Borovaya T.G., Didenko L.V., Narovlyansky A.N., Shevlyagina N.V., Ivanova A.M., Sanin A.V., Pronin A.V. (Histological Features of Ovary Network in Acute Period of Genital Herpes Virus Infection) (2015) //Morphological Vedom. (Rus), 2, 15-20.
6. Sanin A.V., Pronin A.V., Narovlyansky A. N., Kozhevnikova T. N., Sosnovskaya O. Yu., Belyaeva M. V., Annikiv V. V. Pharmacological correction of dyslipidemia in dogs: a clinical study. Veterinary Medicine of Kuban (Rus) 2015 N3,15-17.
7. Pereslegina I. A., Sanin A. V., Pronin A.V., Narovlyansky A. N. Pharmacological correction of hyperlipidemia in dogs and cats. Russ. Veterinary Med. J. (Rus) 2015. No. 4, 36-38.
8. Narovlyansky A.N, Pronin A.V., Sanin A.V., Ivanova A.M., Parfenova T.M., Izmestieva A.V., Izmestieva Anastasia V., Gerasimova E.V., Sedov A.M., Zubashev I.K., Amir I. Tukhvatulin, Shulghenko A.E., ZuykovaI.N., Shubelko R.V The cytokine-regulating activity of polyprenyl phosphates: clinical studies of Fortepren (2015)// CYTOKINE, v76, 80-81.
9. Sanin A. V., Pronin A. V., Narovlyansky A. N., Stepanova T.N. et al. The effect of Polyprenyl Phosphates against the feline rhinotracheitis virus. Veterinary medicine (Rus) 2015 No. 11, 17-21.

10. Rudneva S. Yu., Narovlyansky A. N.,Pronin A. V., Stepanova T. N., Sanin A.V. Treatment of papillomatosis of the oral cavity in dogs using Phosprenyl. Russ. Veterinary Med. J. (Rus). 2016 N3, 9-11.
11. Sanin A.V., Narovlyansky A. N., Pronin, A.V., Kozhevnikova T. N., Timofeeva T. Y. et al. Efficiency of Phosprenyl in the prevention of experimental stress in vitro. Veterinary medicine (Rus) 2016 N10 c.11-13.
12. Pronin AV, Narovlyansky AN, Shulzhenko AE, Sanin AV, Sedov AM. New polyprenyl phosphate based preparation Fortepren as promising cytokine regulating antiviral remedy. (2016) // Cytokine Growth Factor Rev, v. 30, 119–126.
13. Konyuschko, O. I., Ozherelkov S. V., Khitrina E. V., Salichev A.V., Kozhevnikova T. N., Sanin A. V. Expression of the interferons genes in the culture of human fibroblasts following exposure to immunomodulators and viruses. Molecular medicine (Rus). 2016. Vol. 14 N5, 43-48.
14. Sanin A.V., Narovlyansky A.N, Pronin A.V., Kozhevnikova T.N. et al. Phosprenyl therapeutic efficiency in the treatment of pancreatitis in dogs. Veterinary Medicine of Kuban (Rus) (2017) // N2, 24-27.