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Anti-Inflammatory Cytokinin's In Blood Serum Of Patients With Recurrent Genital Herpes.

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ABSTRACT

The concentration of the main pro-inflammatory cytokines (IL-1ß, IL-6 and TNF) in the blood serum of 85 patients with recurrent chronic genital herpesvirus infection was studied. A significant increase in the level of these cytokines with maximum parameters during the peak of the disease was established. During the period of extinction of clinical symptoms, a decrease of their concentration with incomplete normalization during the convalescence is observed. Changes in the level of IL-1ß, IL-6 and TNF in blood serum of patients with recurrent genital herpes depended on the period of the disease and severity of the pathological process.
Keywords: recurrent genital herpesvirus infection, cytokines, IL-1ß, IL-6, TNF

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INTRODUTION

Herpes simplex virus (HSV) type 1 and type 2 are ones of the most common causative agents of viral infections, giving precedence in this regard only to influenza virus [1,3,5]. Genital herpes is caused by HSV-2 in about 80% of cases, HSV-1 - in 20%. More than 25 million people suffer from chronic herpesvirus infection in Russia and CIS countries. The number of people with newly diagnosed genital herpes is increasing more than 10% annually in the Russian Federation [6,7]. HSV infection poses a serious threat to the reproductive health of the population. Primary infection or relapse during pregnancy is very dangerous for the foetus, as it can lead to spontaneous miscarriage, stillbirth, developmental malformations. Infection of the fetus or newborn is most often observed in women with asymptomatic course of genital herpes. With herpes viral infection, as with other chronic diseases with the persistence of the virus, immunodeficiency states develop due to the inadequacy of various parts of the immune system and its inability to eliminate the virus from the body [8].

The profile of cytokines in blood serum can be considered as an important characteristic of the immune system, which allows a deeper understanding of the mechanisms of the pathogenesis of many infectious diseases, as well as one of the key criteria for the effectiveness of etiotropic and immunotropic drugs [2,6]. In the case of a chronic recurrent course of a viral infection, in particular genital herpes, cytokine production cells, in addition to pathogen-associated immunostimulatory signals, are susceptible to various immunosuppressive influences.

The foregoing suggests the practicability of a dynamic study of the level of main cytokines that provide antiviral activity and possess pro-inflammatory and immunoregulatory activity, in particular IL-1β, IL-6 and TNF, in patients with recurrent genital herpesvirus infection (RSHI).

MATERIALS AND METHODS

85 patients with recurrent genital herpes (45 women and 40 men) aged 18 to 60 years were monitored. According to the severity of the clinical course, a mild form of infection (1-2 relapses per year) was diagnosed in 20 patients, a moderate form (3-5 relapses per year) - in 35, severe form (more than 6 relapses per year) - in 30 patients. The main complaints of patients were: pain, itching and burning in the area of herpetic rashes, general weakness, indisposition, headache, decreased performance efficiency. Signs of herpesvirus infection: the presence of vesicles, ulcers, cracks, erythema, swelling of the affected tissues, increased body temperature, increased regional lymph nodes, was revealed in the objective research. The control group consisted of 40 healthy donors that did not have antibodies to HSV-1 and HSV-2 in blood serum and did not contain HSV-1/2 DNA and causative agents of other sexually transmitted infections in urethra scrapings, and from the vagina and the cervix in women.

The diagnosis was made on the basis of the clinical picture, DNA isolation of HSV-1 and HSV-2 by polymerase chain reaction (PCR) in scrapes directly from the rash area, as well as in the detection of specific antibodies to HSV by the solid-state enzyme immunoassay.

All patients were examined clinically and laboratory during the period of exacerbation and clinical remission. From the onset of exacerbation, patients received standard antiviral therapy with oral and topical application of acyclic nucleosides.

The concentration of IL-1ß, IL-6, and TNF in blood serum was determined by solid-state enzyme immunoassay using ProCon IL1 beta, ProCon IL6 and ProCon TNF alpha reagents (OOO Protein Contour, St. Petersburg) according to the manufacturer's instructions. The results of the studies were processed using computer programs Microsoft Exel and Statistica using the Mann-Whitney and Wilcoxon criteria for comparing independent and related samples, as well as correlation analysis.

RESULTS AND DISCUSSION

A significant increase in the level of proinflammatory cytokine TNF in blood serum was observed in patients with RSHI during the exacerbation of the disease (Table 1). In parallel with the positive dynamics of the disease (regress of cutaneous manifestations) with etiotropic therapy with acyclic nucleosides on the background, a gradual decrease in the concentration of this cytokine was observed. Nevertheless, during the

early convalescence, the level of TNF as a whole remained at a level significantly higher than that of healthy individuals. In patients with moderate and severe course of the disease during the inter-recurrent period, the concentration of the cytokine was significantly higher than the reference values in healthy individuals (Table 2). A complete normalization of TNF content during the remission period was observed only in patients with mild form of RSHI. A direct correlation between the concentration of TNF in blood serum and the severity of the disease was found.

Table 1: The content of proinflammatory cytokines in blood serum in patients with recurrent genital herpesvirus infection, depending on the study period.

Indicator under study	Study period	n	X_{min}–X_{max} (pg/mL)	M±m (pg/mL)	P	P1
IL-1ß	Healthy	40	32–61	46±1,9	–	–
	I	85	91–186	132±9,8	<0,001	–
	II	80	77–152	102±5,2	<0,001	<0,001
	III	70	41–112	78±2,1	<0,001	<0,001
IL-6	Healthy	40	28–39	27±1,6		
	I	85	61–89	67±1,2	<0,001	-
	II	80	29–71	47±2,3	<0,001	<0,001
	III	70	30–68	39±1,4	<0,05	<0,05
TNF -α	Healthy	40	15–51	42±2,1	–	–
	I	85	49–298	178±14,1	<0,001	–
	II	80	37–148	109±2,4	<0,001	<0,001
	III	70	34–122	81±2,5	<0,001	<0,001

During the exacerbation of the examined patients, an increase of IL-1ß concentration in blood serum with maximum parameter is at the height of clinical signs, which, as in the case of TNF, was quite expected (Table 1). During the period of remission, despite the absence of herpetic eruptions and suppression of viral replication in places of typical localization, the investigated indicator as a whole remained higher than that of healthy individuals, although it decreased almost twice from the peak level during exacerbation.

The content of IL-1ß in blood serum depended on the severity of the disease. Thus, higher concentrations of this cytokine were determined in patients with a severe and moderate course of the disease. The least signed changes of the investigated parameter were detected in patients with mild course of the RSHI, where the normalization of the IL-1ß level was observed during the inter-recurrent period (Table 2).

The absence of a decrease of IL-1ß level in examined patients at the stage of remission to reference parameters in healthy individuals can be considered natural, considering the biological role and mechanisms of induction of this cytokine, as well as the absence of complete eradication of HSV in the inter-recurrent period. IL-1ß enhances both nonspecific protective responses due to activation of dendritic cells, monocytes / macrophages and neutrophilic leukocytes, and a specific immune response. The mechanism of IL-1ß is associated with stimulation of differentiation of Tnaive-cells in the direction of Th1 and an increase of their functional activity, specifically, an increase of lymphocyte synthesis by IFN-γ and IL-2, and an increase of IL-2-dependent proliferation. In turn, IL-2 activates not only T- and NK-cell antiviral reactions, but also antibody formation. In addition, IL-1ß indirectly induces the synthesis of IFN I, II and III types in the host organism.

Thus, an elevated background level of TNF and IL-1ß in blood serum of patients with RSH in the inter-recurrent period reflects, on the one hand, incomplete eradication of HSV-1/2, and on the other hand, the implementation of important, if not the main, antiviral immune response units.

The dynamics of IL-6 content in blood serum of patients with RSHI as a whole correlated with that of IL-1ß and TNF: during the exacerbation of IL-6 concentration, IL-6 level decreased, but did not reach normal values (Table 1). As well as the concentration of IL-1ß and TNF, the level of IL-6 was in direct correlation with the severity of RSHI (Table 2).

Here and in the Table 2:

I - the period of the peak of the disease; II - period of early convalescence; III - the period of late convalescence; P - reliability of differences from the parameters of a healthy group; P1 - reliability of differences from the previous period, n - the number of observations, M - the arithmetic mean, m - the standard mistake of the arithmetic mean.

Table 2: Dynamics of the content of proinflammatory cytokines in blood serum in patients with recurrent genital herpesvirus infection, depending on the severity of the disease

Degree of severity	Study period	n	IL -1ß			IL -6			TNF –α		
			M±m (пг/мл)	P	P_1	M±m (пг/мл)	P	P1	M±m (пг/мл)	P	P_1
Healthy		40	46±1,9	-	-	27±1,6	-	-	42±2,1	-	-
Lung	I	20	99±2,1	<0,001	-	58±1,7	<0,001	-	98±5,0	<0,001	-
	II	17	76±2,8	<0,001	<0,001	39±3,4	<0,001	>0,05	76±3,1	<0,001	<0,05
	III	14	49±1,4	>0,05	<0,01	26±1,2	>0,05	>0,05	43±2,1	>0,05	>0,05
Medium-heavy	I	35	121±3,2	<0,001	-	65±1,9	<0,001	-	138±4,3	<0,001	-
	II	33	92±1,2	<0,001	<0,001	48±1,2	<0,001	<0,05	110±4,1	<0,001	<0,05
	III	29	80±2,8	<0,001	<0,001	34±1,1	<0,05	>0,05	76±3,2	<0,05	<0,001
Heavy	I	30	178±2,9	<0,001	-	69±1,7	<0,001	-	187±2,2	<0,001	-
	II	30	136±3,9	<0,001	<0,001	52±2,9	<0,001	<0,001	129±5,7	<0,001	<0,001
	III	27	91±2,7	<0,001	<0,001	39±1,7	<0,001	<0,001	98±5,3	<0,001	<0,001

The significance of increasing of IL-6 concentration at different stages of the RSHI is yet to be revealed. At least in part, this cytokine performs protective, antiviral functions, activating the function of neutrophils, antibody formation, production of acute phase proteins. However, its ability to shift the balance of Th1 / Th2 towards the latter and suppress the production of other pro-inflammatory cytokines (IL-1 and TNF) under certain circumstances makes its role in the RSHI dual. The relationship between the reactivation of latent herpesvirus infection and chronic IL-6 expression has been described earlier [12]. If the immunoregulatory effects of this cytokine in the acute phase of the disease to a certain extent can have a protective value, then the lack of normalization of the concentration of IL-6 in convalescence is fraught with the potential for reactivation of the viral infection.

Phase changes of the immune system in cases of herpesvirus infection in vivo and in vitro, which primarily affect the components of innate immunity, namely dendritic cells and monocytes / macrophages, are well known. A bright reflection of these changes is the production of immunocompetent cells of cytokines. In particular, it was found that in the first hours after infection, activation of the production of interferon (IFN) I type and tumor necrosis factor (TNF) occur. Somewhat later, there was an increase in the production of interleukin-12 (IL-12), IL-18 and IFN, type III (IL-28 and IL-29), and in cultures with the presence of NK-cells and in vivo models - also IFN-γ [9]. All of the above cytokines have, to varying degrees, direct and / or mediated antiviral activity. The issue concerning the production of other pleiotropic cytokines - IL-1β and IL-6 - in herpesvirus infection remains open in many aspects, in particular in the aspect of their protective significance and pathogenetic role.

Various in vitro test systems demonstrated the ability of HSV to inhibit antiviral reactions, in particular, to inhibit the functional activity of NK-cells, the production of IFN, type I and II, and the realization of an adaptive cellular response [10]. Some molecular mechanisms of immunosuppressive effects of HSV-1 and HSV-2 are disclosed. Thus, the viral protein ICP34.5 neutralizes the effect of IFN-α / β, reversing the phosphorylation of eukaryotic initiating factor-2α, mediated by protein kinase R. The latter enzyme is the central component of the induction of TNF, IL-6 and RANTES chemokine in herpesvirus infection. Another HSV protein, ICP27, blocks the activation of the transcription factors IRF3 and NF-κB and suppresses the expression of IFN, I type and other pro-inflammatory cytokines. Another viral protein, ICP47, binds to the transporter

associated with antigen processing (TAP), and thus inhibits the presentation of CD8 + antigens to T-lymphocytes in the context of the main histocompatibility complex of the first class [10].

The data from studies of cytokines, including TNF, IL-1β and IL-6, in plasma or blood serum samples in patients with various herpesviral infections are more controversial than those obtained in experimental models. Thus, in young women with peri-menstrual exacerbations of chronic orofacial herpes, there was a decrease in IL-2 and an increase in the concentrations of TNF and IL-6 in blood serum [11]. The levels of TNF and IL-1β in blood serum of patients with infectious mononucleosis caused by the Epstein-Barr virus in the acute phase of the disease did not differ from those in healthy individuals, and the concentration of IL-6 was significantly increased [14]. In the case of prolonged flow, HSV is induced in the orgasm of the virus carrier by the generation of clones of regulatory T cells (Treg), which modulate innate and adaptive defense responses and alter the production of cytokines [13].

The results of studying of the content of proinflammatory cytokines IL-1ß, IL-6 and TNF with genital herpes testify to the developed imbalance of the immune system in patients with genital herpes. Changes in the level of cytokines studied in the blood serum of patients with herpesviral infections depended on the period of the disease and the degree of severity of the pathological process.

The preservation of elevated concentrations of IL-1ß and TNF in patients during remission suggests that secondary immune deficiency, the clinical marker of which is a recurrent herpesvirus infection, is not associated with suppression of the production of these cytokines. Probably, the defect of antiviral protection in patients with chronic recurrent genital herpes is at the level of effector cells (cytotoxic T-lymphocytes and NK cells) and processing / presentation of viral antigens, as discussed above. It is possible that the recurrent nature of the course of herpesvirus infection is associated, to some extent, with the activation of IL-6 production.

The results of this study allow us to conclude that the determination of the content of proinflammatory cytokines in blood serum of patients with genital herpes can be used as an additional criterion for the severity and prognosis of the course of the disease, as well as the effectiveness of the treatment.

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