Pathogenetic Aspects of Severe Course of Herpetic Infection

N. A. Didkovskii, I. K. Malashenkova, A. N. Tanasova, I. A. Zuikov, I. N. Zuikova, N. M. Khitrik, Zh. Sh. Sarsaniya

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Supplement 2, pp. 76-81, April, 2007
Original article submitted February 18, 2006

We studied the state of antiviral defense in patients with severe course of herpetic infection of anogenital and labial localization and the frequency of its combination with other herpes virus infections. It was found that severe course of herpetic infection caused by herpes simplex virus occurs against the background of combined secondary immunodeficiency and its complication. We first demonstrated that severe course of the disease is associated with mixed viral infection.

Key Words: *herpetic infection; immune dysfunction; immune status; immune correction*

Increasing growth of recurrent forms of herpetic infections of various localizations (labial, anogenital, oropharyngeal, ophtalmoherpes, cutaneous herpes, *etc*.) caused by types 1 and 2 herpes simplex virus (HSV) is a serious medical problem [1,8,9]. It is known that most humans are infected with HSV; the primary immune response to the infection can be latent or had clinical manifestations (herpetic rash). In individuals with normal antiviral immunity, HSV replication is under immune control and relapses are rare or absent. Various exo- or endogenous factors affecting the immune system [7] can impair the controlling mechanisms of the host, which leads to reactivation of the virus and development of a relapse.

An important pathogenic property of HSV is its invasion into cells of the skin, urogenital and gastrointestinal mucosa, respiratory pathways, central and peripheral nervous system, liver, vascular endothelium, and blood (lymphocytes and others). Paravertebral sensory ganglia are the sites of constant (live-long) persistence of HSV. Moreover, HSV can be transmitted by various pathways: contact, domestic, vertical (from the mother to fetus), transfusion, and parenteral (during surgical and dental interventions, *etc.*).

The antiviral defense of the organism include factors of nonspecific defense eliminating or blocking the viruses: macrophages and other cells producing IFN-α, IFN-β, and IFN-γ, some IL (TNF, IL-6, *etc*.), natural killer cells (NK) and factors responsible for the specific immune response against a certain virus, namely, cytotoxic T cells ($CD8^+$), T killers, and B cells responsible for the production of specific antibodies blocking viral replication and located extracellularly. Adequate functioning of these cells requires production of the corresponding IFN and IL.

Viruses, in turn, produce a versatile immunosuppressive effect under conditions of chronic viral infection. HSV directly damages immune cells (lymphocytes, macrophages, and NK). Viruses can suppress many immune reactions via the synthesis of suppressor and chimeric proteins; they block the effects of IFN, disturb recognition of infected cells, block activation of the complement system, *etc*. High mutability of the virus helps him to escape from the immune control.

Chronic HSV infection (HSVI) can provoke the development of autoimmune states (antiphospholipid syndrome, autoimmune thyroiditis, vasculites, *etc.*) Moreover, integration of HSV into DNA can result in neoplastic transformation of cells. HSVI is

Laboratory of Clinical Immunology, Institute of Physicochemical Medicine, Federal Agency on Health Care and Social Development, Moscow. ***Address for correspondence:*** didkovski@rambler.ru. N. A. Didkovskii.

0007-4888/07/1443-0447

a risk factor for the development of uterine body and cervical cancer and other neoplasms. HSVI can also be responsible for spontaneous abortions, premature labor, and birth of babies with pathologies of CNS and visceral organs. HSVI is associated with various "civilization diseases": atherosclerosis, CHD in individuals under 50 years old, and others. Severe course of HSVI can be a marker of oncopathology and AIDS [6].

HSVI can run a mild course (≤3 relapses per year, each lasts 3-7 days, infection involves a small area and not impairs general state of the individual) and moderate course (4-6 relapses per year, each lasts 7-14 days, several eruption foci, the general state of the individual is impaired). In severe course of HSVI, more than 6 relapses per year are observed (in some cases 2-3 relapses per month), eruption foci are multiple, general intoxication symptoms are observed, physical and psychic state is impaired during both exacerbations and between them. Some patients during relapses note fatigue, fever, myalgia, headache, arthralgia, irritability, sleep disorders, emotional lability, sometimes severe depressive states requiring medical correction develop. According to American Health Organization data, HSVI of anogenital localization is the cause of ~10% depressions and suicidal attempts.

Therapy of patients with severe HSVI is difficult, because episodic courses of combined therapy (antiviral drugs, immunomodulators, and drugs improving general state of the patient) produce no sustained and significant therapeutic effects in most cases. The patients with severe course of HSVI usually change many health-care institutions in their search for adequate medical help. Various adverse reactions to drugs or their intolerance develop with time, in some cases the resistance of HSV to acyclovir, IFN-α, and other preparations is formed. Immunotropic preparations used empirically can alleviate symptoms during treatment, but in many cases they are ineffective and even aggravate clinical course of the disease [2,5]. The infection aggravates secondary immunodeficiency (SID) and other infections can develop in parallel, subdepressive states progress to sustained psychic disorders, the patients lose hope for recovery and do not trust that modern medicine can help them.

We studied the state of various elements of antiviral defense and cytokine system in patients with severe and constantly relapsing course of HSVI of anogenital and labial localization and the frequency of its combination with other herpes virus infections for the development of new approaches to the control over the disease.

MATERIALS AND METHODS

We examined 102 patients (28 men and 74 women, mean age 34.0±1.5 years) with a more than 2-year history of severe HSVI. Of them, 60 patients had 6-11 relapses per year and 42 patients had 12-20 relapses per year, *i.e.* in ~40% patients the disease run an intermittent relapsing course. Anogenital or mixed (in combination with nasolabial) form of HSVI predominated in the majority of patients: anogenital herpes was diagnosed in 50 patients, mixed in 30 patients, and nasolabial in 22 patients. Relapses were accompanied by typical complains on general malaise, headache, weakness, enlargement of lymph nodes, low physical capacity, and other symptoms. More than half of patients complained of depression, irritability, memory impairment, and other physical, psychic, and cognitive disorders. It should be emphasized that mixed (labial and genital) herpes was more incident in patients having 12-20 relapses per year (54.9% *vs.* 11.6% in patients with lower frequency of relapses). The mean duration of the severe course of the disease was 9.3±1.2 years.

The control group for evaluation of immunological parameters comprised 32 healthy individuals (14 men and 18 women, mean age 32.0±1.6 years). In the analysis of some parameters we also used the results of examination of 36 patients with mild course of the disease (10 men and 26 women, mean age 33.4±2.1 years, 2.9±0.8 relapses per year). Nasolabial and anogenital herpes was diagnosed in 20 and 16, patients, respectively, mixed localization was absent.

Examination of the patients with severe HSVI revealed SID markers [5] in the form of chronic infectious inflammatory diseases of various localizations with mixed microflora (in most cases resistant to the therapy with antibiotics).

The majority of patients had repeated courses of antiviral and/or immunotropic therapy, which had ambiguous effects on the course of HSVI (transient improvement or aggravation and development of a relapse against the background of immunocorrection or after its discontinuation). These data reflect the severity of immune incompetence and inadequacy of empiric therapy without evaluation of the immune status and individual choice of immunocorrectors [3,4].

The presence of HSVI was diagnosed on the basis of medical history, clinical picture, PCR detection of HSV DNA in rash scrapes, and measurements of IgG to HSV by ELISA. Evaluation of the immune status included measurements of the main lymphocyte subpopulation ($CD3^+$, $CD4^+$, $CD8^+$,

CD72$^+$, CD16$^+$) and activation markers HLA-DR$^+$ and CD11b$^+$ in the peripheral blood using monoclonal antibodies of the LT-FITC series (F(ab')2 fragments of sheep antibodies to mouse Ig). The cells were counted on an Ortum spectrum flow cytometer.

The content of IgA, IgM, and IgG was measured by radial immunodiffusion in gel, circulating immune complexes in blood serum were also assayed. Functional activity of neutrophils (production of reactive oxygen species determining bactericidal properties) was assayed by the NBT test. Reserve capacity of neutrophils was evaluated by the NBT test in response to *in vitro* incubation with *St. aureus* culture. The state of IFN system was determined: the levels of serum IFN and production of IFN-α and IFN-γ by peripheral blood leukocytes during stimulation with Newcastle disease virus and phytohemagglutinin, respectively, were measured. The contents of cytokines (IFN-α, IFN-γ, TNF-α, IL-1β, IL-2, IL-4, and IL-6) in blood plasma/serum were measured by enzyme-linked immunosorbent assay using ProCon and Vektor-Best test systems.

In parallel, PCR testing of the obtained biological samples for the presence of DNA of HSV and other herpes viruses (cytomegalovirus, human herpesvirus type 6, Epstein—Barr virus) was performed. The amplification protocols used in the present study allowed us to detect viral DNA at the level corresponding to active replication of the virus (but not carriership). Active replication was confirmed by semiquantitative PCR and serological tests (measurement of IgG antibodies by enzyme immunoassay).

RESULTS

Patients with severe HSVI had laboratory signs of SID. Adequate increase in IgG production in response to HSVI relapse was absent in 91% patients.

In the subgroups with HSVI relapse frequency of 6-11 and 12-20 per year, decreased level of IgG (936.00±22.15 *vs.* 1455.1±30.3 mg% in the group of healthy individuals, $p<0.01$) was observed in 25 and 64.9% patients, respectively. The level of specific IgG antibodies to HSV in the majority of patients was also low (2.26±0.15 arb. units) and often decreased before the relapse. In patients with mild course of HSVI, the content of total IgG was significantly higher that in patients with severe HSVI (1480.63±62.30 *vs*. 1127.33±50.40 mg%, respectively; $p<0.05$); the same was true for specific IgG (3.52±0.25 arb. units; $p<0.05$).

In more that 70% patients with severe course of HSVI, the level of circulating immune complexes 1.5-2.0-fold surpassed the upper limit of normal. It is known that their long-term circulation can be a factor underlying the formation of autoimmune pathology.

The content of T helper cells CD4$^+$ was reliably increased in 25% examinees and tended to decrease in more than 50% patients (compared to the corresponding parameter in the control group).

Despite the presence of active infection, 70% patients with severe course of HSVI had disturbances in the system of natural and/or specific cytotoxicity characterized by decreased content of mature activated NK CD16$^+$ below 11% (in 27% cases), cytotoxic lymphocytes (CTL)/T suppressors below 27% (in 18% cases). In general, the content of activated NK CD16$^+$ was 11.81±0.60% (227.58± 12.34 cell/μl) *vs*. 14.18±1.25% (248.15±8.70 cell/μl) in the group of healthy donors. In 52.7% patients, the content of NK CD16$^+$ decreased to 7.40±0.28% (159.6±9.6 cell/μl, $p<0.01$).

In 43.1% patients the content of activated CTL CD8$^+$ considerably decreased to 23.00±0.62% (432.90± 22.15 cell/μl) *vs.* 30.53±1.28% (534.28±13.25 cell/ μl) in the group of healthy donors ($p<0.01$ and $p<0.05$, respectively).

In 24% patients we observed combined incompetence of natural and specific cytotoxicity systems playing the leading role in antiviral defense. For instance, the contents of NK CD16$^+$ and CTL CD8$^+$ cells were reduced to 7.40±0.39% (150.96±10.46 cell/μl; $p<0.01$) and 23.44±0.72% (460.58±28.07 cell/μl; $p<0.01$).

During the relapse, the response to active replication of HSV in the form of increased CTL (CD8$^+$) count was noted in only 30% patients and increased NK (CD16$^+$) count in less than 20% patients. The pronounced decrease in the level of CD8$^+$ cells (including also suppressor cells) in the majority of cases was associated with increased level of CD4$^+$ lymphocytes and CD4$^+$/CD8$^+$ ratio (so-called immunoregulatory index), which can attest to the risk of autoimmune pathology (AIP).

In a half of patients with HSVI, the disturbances in the system of natural and/or specific cytotoxicity were associated with disimmunoglobulinemia and IgG production deficiency. The level of HLA-DR$^+$-lymphocytes (activated lymphocytes) reflecting production of IL-2 and carrying receptor DR$^+$ essential for recognition of virus-infected cells and other immune reactions was low in 39% patients and increased in only 17.4% patients.

The observed disturbances in reactivity of the cytotoxicity systems and their activation markers during HSVI indirectly attest to insufficient production of IL-2, one of the main elements of the

TABLE 1. Parameters of Spontaneous and Stimulated NBT Test in Patients with Severe HSVI (*M*±*m*)

Group	*n*	Spontaneous NBT test, arb. units	Stimulated NBT test, arb. units	Stimulation index
Control group	32	88.22±5.23	146.37±9.50	1.65±0.15
Spontaneous NBT test				
<70 arb. units	16 (32%)	48.00±3.31**	47.53±5.70**	0.99±0.11*
70-110 arb. units	10 (20%)	93.30±4.20	91.56±5.60*	0.99±0.10*
>110 arb. units	24 (48%)	144.00±4.85**	137.00±10.30	0.95±0.05*

Note. *p<0.05, **p<0.01 compared to the control.

cytokine network. As was mentioned above, production of this cytokine is essential for antiviral immune response.

Evaluation of the functional state of neutrophils in patients with severe course of HSVI revealed increased level of spontaneous NBT test in almost 50% cases. However, parameters of stimulated NBT test and index of stimulation were considerably decreased in most patients, which attested to exhaustion of reserve capacities of neutrophils in these patients (Table 1).

Evaluation of IFN status of patients during HSVI exacerbation revealed differences in mild and severe course of the disease (Table 2). For instance, the level of serum IFN in patients with mild course of HSVI increased 2-fold compared to the corresponding parameters in the control group. On the contrary, in severe course of HSVI, the relapse in most patients was accompanied by low level of serum IFN. Stimulated production of IFN-α and IFN-γ was also suppressed, especially in severe course of the disease (by 3 and 2 times compared to healthy donors and patients with mild course of HSVI, respectively).

Thus, severe insufficiency of the interferonogenesis system was found in the majority of patients with HSVI, which agrees with the data on exhaustion of the reserve capacity of neutrophils, deficiency of T helpers and cytotoxicity systems (cells producing various types of IFN) in these patients.

In the serum of patients with severe HSVI, the adequate response of proinflammatory IL (IL-1β, TNF-α, IL-2, IL-4, IL-6) was absent on days 2-3 of relapse. Insufficiency of IL production was observed the majority of patients (90%) and in 10% patients the studied IL were not detected in the blood. Abnormal cytokine profile of the blood persisted during the rash-free period (long-term increase in the content of one or two cytokines).

PCR study detected DNA of lymphotropic viruses of the herpetic group (Epstein—Barr virus and human herpesvirus type 6, Table 3).

Samples from the oropharyngeal area (saliva, throat swab) and peripheral blood lymphocytes most often contained DNA of Epstein—Barr virus and human herpesvirus type 6. Cytomegalovirus DNA was detected in 11.5% patients of this group (in epithelial cells from the saliva, urethra, cervix, and urine sediment). Generally, in severe course of HSVI only 5% patients had HSV monoinfection (*vs*. 72% in mild course of the disease).

Thus, other herpes-group viruses were actively replicated in 95.6% patients with severe course of HSVI, which allows us to consider this form as a severe mixed infection.

Treatment of HSVI should include suppression of virus replication during exacerbation and the formation of adequate immune defense for the prevention of relapses [7].

Patients with frequent relapses of HSVI are prescribed suppressive long-term intake of abnormal nucleotides, which can lead to remission during therapy. At the same time, reduced incidence of relapses after discontinuation of treatment (compared to the period before therapy) was observed in only 20% patients [3]. Long-term or frequent therapy with abnormal nucleotides can be associa-

TABLE 2. Parameters of IFN System in Patients with Severe amd Mild course of HSVI during exacerbation (*M*±*m*)

Group	Serum IFN, U/ml	IFN-α, U/ml	IFN-γ, U/ml
Control (*n*=32)	3.15±0.12	106.3±7.5	42.1±3.3
Mild course of HSVI (*n*=36)	6.30±0.03*	88.0±5.2*	28.8±2.8*
Severe course of HSVI (*n*=52)	2.02±0.02*+	29.0±3.9*+	14.8±2.1*+

Note. p<0.05 compared to *control, +patients with mild HSVI.

TABLE 3. Incidence of Detection (%) of Other Herpesviruses in Patients with Mild and Severe Course of Recurrent HSVI

Detection of other herperviruses in different samples	Mild course of HSVI (*n*=36)	Severe course of HSVI (*n*=52)
Epstein—Barr virus	8.3±2.8	76.5±5.7*
Human herpesvirus type 6	22.2±4.5	63.5±6.6*
Cytomegalovirus	0	11.5±3.3
Epstein—Barr virus and human herpesvirus type 6 simultaneously	2.8±1.7	44.3±5.8*
Percent of patients with mixed infection (2 and more viruses)	27.8±6.0	95.6±2.8*

Note. *$p<0.05$ compared to mild course of HSVI.

ted with side effects (gastrointestinal pains, vertigo, *etc*.), in some cases virus resistance to the preparation develops. The preparations suppressing replication of HSV include also glycyrrhizinic acid (licorice component), IFN-α (reaferon, viferon, realdiron, *etc*.) and inductors of its synthesis (cycloferon, ridostin, poludan, *etc*.). Course monotherapy with these preparations produces insufficient clinical effects in severe and long-tern course of HSVI, well-known side and adverse effects develop. IFN-α is only a part of the antiviral defense of the immune system and it cannot replace its other elements in case of their incompetence. Moreover, herpesviruses can block the action of IFN-α.

Clinical studies and our findings suggest that the use of immunocorrectors without evaluation of the immune status of the patient and individual choice and control of their action can be little effective or aggravate immune dysfunction or lead to hyporeactivity of some immune mechanisms due to inadequate stimulation [4,6]. It should be noted that some advertised preparations for local applications (ointments containing acyclovir, foscarnet, epigen intime (glycyrrhizic acid) and bonaphthone) can be only adjuvant preparations, because papular eruption on the skin is only one of manifestations of severe HSVI. HSV can replicate in other cells and organs (oropharyngeal area, lymph nodes, lymphocytes, rectal mucosa, *etc*.), which requires the corresponding treatment and its control. Our studies showed that severe HSVI is as a matter of fact not a monoinfection, but a mixed infection against the background of SID, which changes our views on the examination and treatment of these patients. It should be noted that the presence of other chronic viral and bacterial infections aggravates SID and increases the risk of autoimmune disorders and neoplasms.

The heterogeneity and severity of disturbances in the immune system during severe HSVI require differentiated approach to their correction and immunorehabilitation. More comprehensive understanding of cytokine response in the dynamics of severe HSVI is of crucial importance. The effects of individually chosen preparation of cytokines IL-2 (ronkoleukin), IFN-γ (gammaferon, ingaron) and others on the course of the disease and duration of their effects of the parameters of the immune status should be investigated.

REFERENCES

1. V. M. Granitov, *Herpesvirus Infection* [in Russian], Moscow, Nizhnii Novgorod (2001).
2. N. A. Didkovskii and I. K. Malashenkova, *Alergol. Immunol.*, No. 9, 2-15 (2006).
3. *European Standards for Diagnosis and Treatment of Sexually Transmitted Diseases* [in Russian], Moscow (2003), p. 102-110.
4. A. A. Levko, R*ole of Individual Choice of Immunocorrectors in Complex Therapy of Chronic Recurrent Prostatitis Caused by Urogenital Infection*. Abstract of Cand. Med. Sci. Dissertation, Moscow (2005).
5. I. K. Malashenkova and N. A. Didkovskii, *Ross. Med. Zh.*, **10**, No. 21, 973-977 (2002).
6. I. K. Malashenkova, N. A. Didkovskii, and A. A. Levko, *Farmateka*, No. 12, 26-33 (2004).
7. I. K. Malashenkova, N. A. Didkovskii, A. N. Tanasova*, et al.,* Doktor. Ru, No. 4, 26-30 (2004).
8. L. N. Khakhalin, *Consilium Medicum*, **1**, No. 1, 18-24 (1999).
9. A. E. Shul'zhenko, G. Kh. Vikulov, and T. V. Tutushkina, *Difficult Patients*, **1**, No. 4, 6-15 (2003).