VIROLOGY

Modeling of Chronic Herpesvirus Infection. Experimental Studies of Infectious Process in Mice

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The dynamics of infectious process was studied by modeling chronic herpesvirus infection in mice. The infectious process ran a wave-like course with periods of relapses and remissions. Clinical manifestations and severity and duration of relapses largely depended on the disease duration. Reactivation of herpesvirus led to systemic involvement, this indicating the dissemination of infection in animals and chronic transformation of the disease.

Key Words: *herpesvirus infection; clinical picture; experimental model*

The representatives of *Herpesviridae* family are highly prevalent in the human population. During the recent decade, the wave of diseases caused by herpes simplex virus (HSV) alone in fact became a sort of an epidemic [2]. The capacity of HSV to latent persistence in the infected host is the cause of periodical relapses. Clinical forms of herpesvirus infection (HVI) are characterized by great variety: from slight hyperemia of the lip mucosa and eye conjunctiva to generalized involvement of systems and organs with irreversible pathological processes [1,2]. The diagnosis of this infection is particularly difficult because of variability, severity, acuteness, and location of the clinical manifestations, depending on the disease duration and individual features of the patient [1,3].

The effects of HSV on the host are studied (in Russia and in foreign countries) on the models of herpetic encephalitis, ophthalmic herpes, genital herpes, herpetic involvement of the skin [3]. However, these models reproduce just certain clinical forms of the disease.

The aim of our study was to reproduce experimental HVI for long-term observation of the development and chronic transformation of the process in different forms.

MATERIALS AND METHODS

Laboratory Balb mice (25 males aged 3 months, 23.5 g) were intraperitoneally infected with HSV (HSV-1, strain L_2, infectious titer 4.5 lg $TCD_{50/ml}$; 0.1 ml).

The antigen was detected by the immunofluorescent test in the urethral mucosal cells, ocular and oral mucosal cells of living mice during the development of clinical manifestations of HVI.

The most informative periods for collection of experimental material were determined in preliminary studies: days 5, 15, 20, 25, 30, and then every 10 days. Hence, a total of 29 samples were collected throughout the study.

The development of clinical picture of was evaluated daily. Infectious activity of HSV in organs of dead animals was evaluated by virus isolation on LECh-4 (81) diploid cell culture.

RESULTS

The first clinical signs of the disease were noted on day 5 postinfection in 16% animals and were charac-

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terized by moderate lesions of the eyes. The peak of acute disease was recorded on days 6-7. During this period, 32% mice developed poor appetite, high salivation, ocular involvement, and signs of intoxication. Study of smears from the urethra, ocular and oral mucosa of animals during this period detected the HSV antigen in 40% cases. The HSV antigen was detected mainly in the ophthalmic mucosa. This was followed by a period of well-being without clinical manifestations, which lasted about 5 days (from day 9 to day 13; Fig. 1). However, on day 14 postinfection the infection progressed and augmented: involvement of the eye was paralleled by the development by motor discoordination. Clinically, the first manifestations of HVI started from slight eyelid edema and constrained hindpaw movements. During the next 4 days, the severity of the symptoms rapidly progressed. In 62% animals, sharp edema of the eye conjunctiva with abundant mucous discharge was associated with pronounced meningeal reaction (convulsive attacks in response to external stimuli, such as sharp sound and/or bright light). Two animals developed hindpaw paralysis. In $^{2}/_{3}$ experimental animals, motor discoordination was characterized by tremor and pulling of the limbs during walking. In addition, the majority of animals exhibited signs of intoxication: the animals lost 30-35% body weight. The HSV antigen was detected not only in the ocular mucosal cells, but also in the oral mucosal cells (in 40% animals) and in the urethral canal cells (in 32%). Isolation of HSV from the organs of dead animals (2 mice with hindpaw paralysis) showed the presence of infectious virus in the cerebral, spinal (lumbosacral compartment), and hepatic tissues. Clinical manifestations of HVI reduced on days 20-21, and by day 25 the animals looked healthy. However, HSV antigen in the form of intranuclear incorporation was detected in the ocular, oral, and urogenital mucosal cells of 30.4% animals.

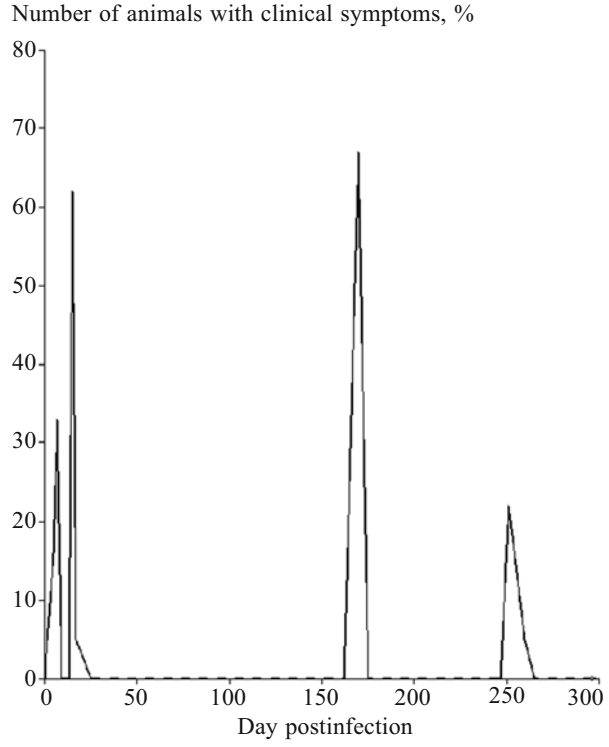

Fig. 1. Dynamics of clinical manifestations of chronic HVI in experimental animals. Interrupted line: no clinical manifestations.

Examination of smears from the ophthalmic, oral, and urogenital canal mucosa on days 30, 40, and 50 after infection failed to detect the HSV antigen. The period of clinical well-being (absence of clinical manifestations of HVI) was rather long (to day 159). However, HSV antigen was detected in the urethral mucosa cells in 17.3% animals on day 60, in 8.6% on day 70, and in oral mucosal cells in 21.7% animals on day 80 under conditions of clinical health. Hence, HVI was latent on days 60-80.

New manifestations of HVI in infected animals were recorded on days 162-175. During this period, 8 animals developed rhinitis and then skin involvement. The development of skin lesions started from the appearance of small ulcers on a narrow strip of nude part of the lip, after which the hairs on the lower lip and neck disappeared, leaving dry scaling skin in sites of lesions, with numerous signs of scratching. The hair was restored after 14-15 days. During this period HSV antigen was detected in the oral mucosa cells in 36% animals and in the urethral canal cells in 21.7%. In 13% animals, the involvement of the oral mucosa and urethra were paralleled by eye involvement. Hindpaw paralysis in 38% animals with combined lesions of the oral cavity, eyes, and skin was paralleled by signs of acute intoxication (a sharp loss of body weight, cyanosis of the nasolabial triangle, hairless parts of the paws, tail, and conchae). Examination of the material from mice dead during this period detected HSV antigen in the cerebral, spinal, hepatic, and renal cells.

The next period of clinical well-being was much shorter than the previous one: about 65 days (from day 180 to day 246 postinfection). Exacerbation of HVI on day 247 in 14.2% experimental animals was characterized by pronounced involvement of the eyes (edema of the eyelids, suppurative mucous discharge). In addition, HSV antigen was detected in the ophthalmic mucosal cells in 9.5% animals without manifest signs of the disease. The duration of exacerbation was 3-5 days on average; the infection ran a stubborn course, eventuating in corneal opacity and formation of a leukoma. Importantly that HSV antigen was not detected in the oral mucosa and urethral canal cells during exacerbation.

Hence, our studies showed that the development of HVI in experimental animals was characterized by a

wave-like pattern, with periods of acute manifestations alternating with periods of clinical well-being and latent course without clinical manifestations. It is also noteworthy that the number of clinical manifestations and their severity augmented with prolongation of the disease. Involvement of the eyes and nervous system was moderate during the early period of infection, while at later stages during reactivation of HSV they were systemic: involvement of the skin, oral mucosa, eyes, urethral canal, and nervous system with development of paralysis and stable pareses.

These clinical forms of HVI in experimental animals on days 5-25 largely coincide with those in humans after primary contact with HSV and are acute [1,2]. The process in the patients with sluggish disease, caused by chronic HVI, is similar to that observed in our experiment: slight short-term manifestations (slight hyperemia of the mucosa, minor discomfort) are associated with the presence of the antigen in the clinical material during a long period [1,2,3]. Presumably, with time the prolongation of the disease promotes dissemination of the virus in the patients and involvement of more and more organs and tissues into the process. Of course, this experimental model of HVI cannot fully reproduce the development of clinical disease in humans, as patients use drugs (often spontaneously), bioactive substances, are vaccinated, and suffer from chronic diseases. However, our findings demonstrate the time course of HVI development and transformation of the acute phase of infection into chronic one and give an idea of the severity of the pathological processes in this infection.

REFERENCES

1. N. P. Glinskikh, A. P. Poryvaeva, T. S. Nekrasova, and I. V. Ustyantsev, *Herpesvirus Infections* [in Russian], Ekaterinburg (2001).
2. *Unknown Epidemic (Herpes)*, Ed. L. N. Khakhalin [in Russian], Smolensk (1997).
3. E. Kita, *Reproductive Health* [in Russian], Moscow (1998), Vol. 2, pp. 224-234.