GENERALIZED EXPERIMENTAL HERPES DEVELOPING IN GUINEA PIGS AFTER RETROBULBAR INFECTION

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UDC 616.98:578.825.11]-031.81-092.9-022.1-032:611.84

KEY WORDS: generalized herpetic infection; herpes simplex virus; clinical manifestations in guinea pigs; visceral and CNS lesions

A generalized infection caused by herpes simplex virus (HSV) together with localized herpetic lesions of the CNS constitutes a comparatively rare, but most severe form of clinical manifestations of herpetic infection in man, and is attended by high mortality (75-95%) [2, 8, 13, 17]. Most efforts of research workers in this connection in recent years have been directed toward the search for effective antiviral agents and the development of a strategy of treatment of generalized herpetic infection and herpetic lesions of the CNS [1, 4, 6]. However, the successful solution to these problems is largely dependent on the availability of experimental laboratory models that are adequate and convenient for routine use, as well as sound ideas on the pathogenesis of general herpetic infection (GHI).

Experimental models of localized or systemic forms of herpes are widely used in virology: examples include ophthalmic and genital herpes, cutaneous herpes, and herpetic encephalitis. In some cases disxmination of HSV has been noted in animals, with the development of generalized infection and lesions of several organs and systems. The highest levels of generalization of the infection and death of the animals (up to 50%) have been described in nude mice with a localized form of experimental cutaneous herpes [9, 11, 15].

Previously, as a model of slow CNS infections (amyotrophic leukospongiosis, Creutzfeldt–Jakob disease), caused by nonclassical viruses (prions), the writers developed an original model which, besides rapid involvement in the pathological process, facilitated dissemination of the infectious agent in the body of the affected animal [3]. For reasons discussed in this paper, we used the retrobulbar method of infection of guinea pigs in order to creat a model and study some aspects of the pathogenesis of GHI.

EXPERIMENTAL METHOD

Herpes simplex virus type I (strain C_I), obtained from the Museum of Virus Strains, D. I. Ivanovskii Institute of Virology, Academy of Medical Sciences of the USSR, having previously undergone seven passages through noninbred albino mice, was used. Experiments were carried out on guinea pigs weighing 250-300 g. The animals were infected by the retrobulbar route in both eyes (0.25 ml of a 10% suspension of mouse brain containing HSV in a titer of 5.5 log LD_{50}/ml) [3]. The experimental group consisted of 37 animals, the control group of eight. A 10% brain suspension from healthy (not infected with HSI) albino mice was injected by the retrobulbar route in the control animals. The titer of HSI in the blood, and suspensions of organs and tissues of the guinea pigs was determined by the standard method on noninbred albino mice weighing 6-7 g [11]. HSV antigens were found in the cells of organs and tissues by the indirect fluorescent antibodies method and indirect immunoperoxidase method. Squash preparations fixed in cold acetone and histological sections were used for this purpose as described previously [6, 7]. The histological investigations were conducted by the usual methods, sections being stained with hematoxylin

Belorussian Research Institute of Epidemiology and Microbiology, Ministry of Health of the Belorussian SSR, Minsk. Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 109, No. 4, pp. 376-379, April, 1990. Original article submitted July 14, 1989.

 0007-4888/90/0004-0492$12.50

TABLE 1. Clinical Manifestations of Disease in Affected Guinea Pigs (n = 30)

Clinical manifestations	Time of appearance of manifestation after infection (days)	Duration of manifestation (days)	Number of animals (%)
Skin eruptions	23±16	15±8	27 (90)
Trophic disorders	32±11	40±15	21 (70)
Neurological manifestations	46±12	5±2	9 (30)

TABLE 2. Results of Virological and Histological Study of Animals with Manifestations of GHI

Clinical manifestations	Number of animals	Brain		Spinal cord		Spleen		Liver		Kidneys		Lungs		Lymph nodes	
		1	2	1	2	1	2	1	2	1	2	1	2	1	2
Herpetic eruptions	4	—	1,0	—	1,0	+	1,5	++	2,5	—	1,5	—	1,8	—	—
Herpetic eruptions and trophic disturbances	17	+	1,4	+	2,0	++	3,4	++	2,8	++	2,0	++	3,0	+	1,0
Herpetic eruptions, trophic and neurological disturbances	6	++++	4,8	++++	4,9	++	4,0	++	3,8	++	2,2	++++	6,7	++	2,7
Neurological and trophic disturbances	3	+++	5,9	++	3,2	++	2,7	++	2,5	+	1,0	++	1,8	+	1,0

Legend. 1) Severity of histological changes in organ; 2) titer of HSV in log LD_{50}/ml; —) no changes present; +) mild changes in parenchyma of organ, predominantly vascular reaction; ++) moderately severe damage to parenchyma of organ, individual cells show severe structural disturbances of components of cytoplasm and nucleus; +++) considerable damage to parenchyma of organ, individual cells in a state of necrobiosis and necrosis; ++++) severe damage to parenchyma of organ, diffuse in character, marked inflammatory reaction of vessels, areas of necrosis.

and eosin, by Nissl's method, by Viktorov's modification of the method of Klüver and Barrera, and with orange—red—blue on a basis of Luxol Fast Blue MSB [5, 10].

EXPERIMENTAL RESULTS

Of the 37 animals infected 30 guinea pigs developed clinical manifestations of the disease, mainly herpetic eruptions, and trophic and neurological disorders (Table 1).

Herpetic eruptions on the skin were the first sign of the disease in 90% of animals, and as a rule they appeared on the 20th-30th day after infection, or in isolated cases, on the 12th-15th day. The vesicles were present in groups of three or four and were located most frequently on the snout in the region of the eyes and ears, less frequently on thp limbs, trunk, around the anus, and in the region of the genitalia. During the next 1 or 2 days their contents became opaque and a dense crust was formed on the surface. As a rule by the 15th day after the appearance of the lesion the crust was shed, and in places where the area of the lesion reached 1 cm^2 or more, the loss of hair was permanent. In four animals which developed the disease, new lesions constantly were formed around cicatrized earlier lesions, the total area affected reached 30% of the body surface, and the animals quickly died. The mean length of survival of the animals of this group was 18 days.

The morphological investigation revealed characteristic injuries to nuclear chromatin in the form of local chromatolysis with hyperchromic masses in the center or total chromatolysis with single hyperchromic masses along the inner layer of the nuclear membrane. Changes of this kind were found in cells of the stratum spinosum of the epidermis, reticuloendothelial cells of the red pulp of the spleen, and in hepatocytes. Detachment of the upper layers of the epidermis was observed on the skin with the formation of vesicles filled with serous fluid; microscopic foci of necrosis were observed in the liver.

HSV was found in minimal numbers in tissues of the CNS and internal organs (Table 2). HSV could be isolated sufficiently easily only from liver tissue and the serous contents of the vesicles on the body surface, in which its titer was 4.8 log LD_{50}/ml.

The next clinical manifestations of the disease in the animals were trophic disorders, which were found in 70% of the infected guinea pigs. They developed not less than 25-27 days after infection and in the early stages consisted of loss of the natural sheen of the fur, with slight shedding of the hair, and after 12-17 days more extensive regions of depilation were observed, mainly on the sides and abdomen, and also where there were abundant skin eruptions. Permanent reduction of the animals' body weight was observed also at this period. The animals became apathetic and adynamic, they responded only weakly to acoustic and nociceptive stimulation, and their respiration and heart rates increased. Death of the guinea pigs with these manifestations occurred 60-70 days after infection.

Morphological investigation of the animals of this group revealed characteristic changes in nuclear chromatin in all the internal organs, accompanied by inflammatory lymphomonocytic infiltration, microfoci of necrosis, and thrombohemorrhagic changes at the microcirculatory level. The changes were most marked in the CNS, where damage to neurons was observed in all parts, with foci of disappearance of neurons and the formation of glial nodules. At the same time, severe ischemic changes in neurons were found in the cortex, with the formation in some cases of microfocal infarcts, which were combined in these areas with microthrombosis of the blood vessels. In the lungs, thrombohemorrhagic changes were more typical, and these created a variegated pattern of morphological changes ranging from atelectases and hemorrhagic infarcts to focal emphysema combined with marked interstitial lymphomonocytic infiltration. The next organs in frequency of involvement were the liver, adrenals, kidneys, spleen, and pancreas. Perivascular infiltration was observed in the optic nerve, just as in all the internal organs. HSV antigens were found in the cells of these organs by immunohistochemical methods. HSV also could be isolated from all these organs, and its titer was 0.8-2.0 log LD_{50}/ml higher in the spleen, liver, and lungs than in the other tissues, in close correlation with the severity of the morphological changes.

The least frequent clinical manifestations of infection in the animals were neurological disturbances, which were observed in only 30% of the affected animals, and lasted between 3 and 7 days. In three animals these disturbances developed against the background of persistent trophic disorders, and their course resembled that of acute meningoencephalitis. The animals became very weak, did not respond to nociceptive stimulation, and developed hyperkinesia and head shaking. In the terminal stage the animals performed circular movements with the anterior part of the trunk, whereas the posterior part remained fixed. After two or three such movements the hyperkinesia intensified greatly. Respiration became quick and superficial. Pelvic functions also were disturbed.

In the animals of this experimental group the CNS was mainly affected, with severe changes in the neurons, proliferative and dystrophic changes in the macroglia, and an inflammatory reaction. The most substantial changes were found in the basal ganglia and, in particular, in the thalamus, and also in the nuclear groups of the brain stem and cerebellum. Groups of hypertrophied astrocytes and oligodendrogliocytes with marked changes in their chromatin, pallor of the myelin sheaths, and spongiform changes were frequently found in the white matter.

These morphological changes correlated with the results of the virological investigations: the highest titer of HSV was found in the brain (5.9 log LD_{50}/ml), and it was 2.7-3.2 log LD_{50}/ml lower in the spinal cord, spleen, and liver.

The severest course of herpetic infection occurred in six guinea pigs. Initially these animals developed a vesicular eruption on the snout, around the eyes, and later spreading to the trunk, the region of the genitalia, and the anus. The eruption disappeared after 15-25 days. The animals ceased to gain weight, their hair lost its color, and began to fall out. Some animals (three) developed unilateral or bilateral paresis of the hind limbs, although this disappeared after 10-14 days. Later, after a further 10-17 days, the emaciation became more marked and hair was absent on the sides of the trunk and abdomen, on 60% of the surface. The animals became adynamic, their pupils did not respond to light, they moved about the cage with difficulty, and were reluctant to eat, but their chewing and swallowing reflexes were intact.

By this time the paresis of the hind limbs had redeveloped in all the animals, and after 1 or 2 days paresis of the forelimbs and paralysis of the hind limbs were observed in three guinea pigs, accompanied by disturbance of the functions of the pelvic organs. In the terminal stage the animals developed convulsions and died.

Histological investigation revealed considerable changes in the basal ganglia, brain-stem nuclei, and spinal cord, with damage to neurons of the anterior and lateral horns and the roots of the spinal cord. Changes in the internal organs were mainly the same as in the previous groups of animals. Virological investigations in the terminal period revealed the highest HSV titer in the tissues of the CNS and lungs.

In the terminal period of the disease, in animals of all four groups the titer of the virus in the blood varied between 3.5 and 4.0 log LD_{50}/ml, evidence that the animals had developed viral septicemia. Additionally, microscopic examination of most animals at the light-optical level revealed, besides microfoci of necrosis of the intima, hyperchromic nuclei, with destruction of the chromatin, and minimal changes in the media, showed that in some animals necrosis with the inflammatory reaction also had spread to the media in blood vessels of the visceral organs and CNS, as well as in the arteries supplying these organs (in particular, small and medium-sized branches of the pulmonary artery, and the suprarenal, peripancreatic, hepatic, and other arteries). By the use of indirect immunoperoxidase and fluorescent antibodies methods, HSV antigens were discovered in the vascular endothelium around the zones of necrosis.

Thus virological and morphological analysis revealed the presence of GHI in four groups of experimental animals, involving individual internal organs in its course, with disseminated lesions of all visceral organs, but with damage predominantly to the deep brain structures, but also affecting the rest of the brain and the spinal cord, and giving rise to marked autonomic disturbances.

The pattern of development and the duration of the disease, together with the results of the virological and morphological investigations, demonstrate the formation of severe GHI in animals infected by the retrobulbar route, and assuming the character not only of a primary acute, but also a recurrent subacute process, in agreement with clinical observations reported by several workers [2, 8, 13, 19]. By the use of this method of infection, 30 of 37 guinea pigs (81%) developed GHI. Animals of the control group remained healthy throughout the period of observation (3 months). The high percentage of successful production of GHI can evidently be attributed to the fact that with retrobulbar injection of HSV the conditions are right for its dissemination in the body in three ways: hematogenous, lymphogenous, and intraneural. This is shown mainly by the fact that HSV could be isolated from the blood and lymph gland tissue. These observations are in agreement with experimental data [15], which showed that the spread of HSV through lymphatic pathways contributes to dissemination of the virus in the body. In turn, the presence of the virus in lymph nodes is accompanied by invasion of sensory ganglia. Morphological changes discovered in the optic nerve (evidence of demyelinization and glial proliferation), combined with the formation of retinopathy and blindness in the majority of the animals, are evidence of involvement of the optic nerve in the pathological process and its role in dissemination of the virus in the CNS. Since the optic neuropathy, although it may be isolated, is most frequently associated with generalization of infection of the CNS [16], its discovery must evidently be used as a diagnostic and prognostic test for GHI.

Our data showing predominant involvement of the spinal cord in some animals do not rule out the possibility of the involvement of nerves, plexuses, and ganglia of the sympathetic and parasympathetic nervous system, innervating the visceral organs, in the spread of HSV in the CNS, as has been shown on a model of genital infection in Balb/c mice [18]. The ability of HSV to cause destruction of cells of the sympathetic nervous system also has recently been described on a model of newborn rats [12].

However, in the discussion of these results, attention must be paid to the observations of Gerritzen and Schneweis [14], who showed that neutral persistence of HSV requires inhibition of the lymphogenous spread of the infection. This may perhaps be associated also with the fluctuating and prolonged (subacute) character of the course of GHI in guinea pigs infected by the retrobulbar route.

The experimental results thus show that generalized herpes is a severe polysystemic disease, due to dissemination of HSV in the body by different pathways, and characterized by involvement both of the external coverings and mucous membranes of the body and of the internal organs and systems, including barrier systems (CNS and eyes) in the pathological process. The suggested model of GHI offers prospects for the study of the pathogenesis of the disease and mechanisms of formation of its acute, subacute, and chronic versions, including the possible link between HSV infection and the development of acute and recurrent demyelinization of the CNS in experimental animals and in man. Its use also will permit not only the organization of screening of antiviral agents, but also development of the tactics and strategy for etiopathogenetic combination chemotherapy and immunotherapy of this severe herpetic disease, which a high proportion of patients with AIDS also develop.

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DETECTION OF ANTISPERM ANTIBODIES ON THE SURFACE OF LIVING SPERMATOZOA BY FLOW CYTOMETRY

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UDC 612.616.2.017.1:083.33+ 616.697-07:616.153.962.4-097-02:616.69-008.8

Key words: flow cytometry; antisperm antibodies

The detection of antisperm antibodies (ASAB) is an important stage in the diagnosis of sterility. Most methods used to detect antisperm antibodies (sperm agglutination [1], immobilization [2], enzyme immunoassay [3], and radioimmunoassay [4]) are aimed at detecting antibodies present in seminal fluid, cervical mucus, or serum, but they do not enable the quantity of antibodies on the surface of spermatozoa to be estimated; the mixed agglutination reaction test [5] and the IBT test [6] reveal the localization of antibodies of different isotypes actually on the surface of spermatozoa, but cannot be used to determine the quantity of antibodies bound with the cells. The aim of this investigation was to estimate the quantity of antibodies of different isotypes (IgG, IgA, and IgM) on the surface of living spermatozoa by the flow cytometry method (FCM).

EXPERIMENTAL METHOD

To determine ASAB in seminal fluid spermatozoa were washed with phosphate buffer (PB, pH 7.4) and incubated with mouse monoclonal antibodies to human IgG, IgA, and IgM and then treated with FITC-labeled antibodies to mouse Ig (Beckton, Dickinson, West Germany). Propidium iodide (PI), which stains only dead cells [6], was added to the suspension of cells in PB. The "Facscan" cytofluorometer (Becton Dickinson) was used for analysis, 10,000 living cells being studied in each sample (dead cells were eliminated from the analysis by the computer on the basis of their staining with PI). For quantitative assay of the different classes of Ig on the surface of the spermatozoa, the mean value of x, expressing fluorescence in conventional units, was determined for each histogram of distribution of the cells according to their green fluorescence, and compared with the value of

(Presented by Academician of the Academy of Medical Sciences of the USSR A. D. Ado.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 109, No. 4, pp. 379-382, April, 1990. Original article submitted October 20, 1989.

 0007-4888/90/0004-0496$12.50