BIOCHEMICAL AND MORPHOLOGICAL CHANGES IN THE NEUROGLIA IN EXPERIMENTAL HERPES SIMPLEX VIRUS ENCEPHALITIS

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Histopathological and, in particular, biochemical changes in the brain in herpetic infection have not yet been adequately studied [1-3, 12]. For instance, analysis of the state of the neuroglia has been confined to a description of the gliosis response. The possibility of investigating enriched fractions of neuroglia and neurons, whereby the role of each cell population in the pathogenesis of herpes simplex virus encephalitis (HSVE) can be evaluated, has not been used.

The aim of this investigation was to study activity of lysosomal enzymes (deoxoribonuclease – DNase, and ribonuclease – RNase) in fractions of neuroglia and neurons and also the morphological changes in the neuroglia.

EXPERIMENTAL METHOD

CBA mice weighing 10-12 g were used. As the model of experimental HSVE, the animals were given an intracerebral injection of herpes simplex virus type 1 (HSV1, strain L-2) in a dose of 100 TCD_{50}/0.02. Physiological saline was injected in the control. For neurohistological investigation, paraffin brain sections were stained with Einarson's gallocyanin, Nissl's thionine, and Brachet's pyronine methods. Fractions of glial cells and neurons were isolated from the cerebral cortex by the method in [8], and activity of DNase and RNase in them was determined by the method in [6]. The ultrastructure of the aortic endothelium was investigated as in [7]. The virus was isolated both by the usual methods and also by methods of coculture [5] and hybridization [4]. The results were subjected to statistical analysis by Student's test.

EXPERIMENTAL RESULTS

The infected mice developed symptoms of encephalitis toward the 3rd day. In the acute stage of the disease (until the 8th day) 25% of the animals died, and in the rest the infection became chronic in form, as shown by isolation of the virus from various organs and tissues until the 29th day after infection. Maximal accumulation of virus in the mouse brain was discovered on the 7th day of the experiment (Fig. 1). In the fraction of neurons isolated 24 h after infection of the animals, DNase activity was sharply reduced. On the 3rd day it reached the control level, at which it remained until the end of the acute phase (8th day). RNase activity in these cells also was reduced 24 h after infection, and did not reach the control level until the 7th day. A significant increase in DNase activity in the glial cells was observed on the 3rd day, and was followed by a fall toward the 7th day below the control level, but on the 8th day activity again rose sharply (Fig. 2). RNase activity fell only on the 3rd and 5th days. The study of activity of these enzymes in the chronic stage of infection revealed a fall of DNase activity in the neurons on the 15th and 26th day and of RNase activity on the 15th day, whereas in the glial cells, there was an increase of DNase activity on the 15th day (Fig. 2) and a decrease of RNase activity. These data

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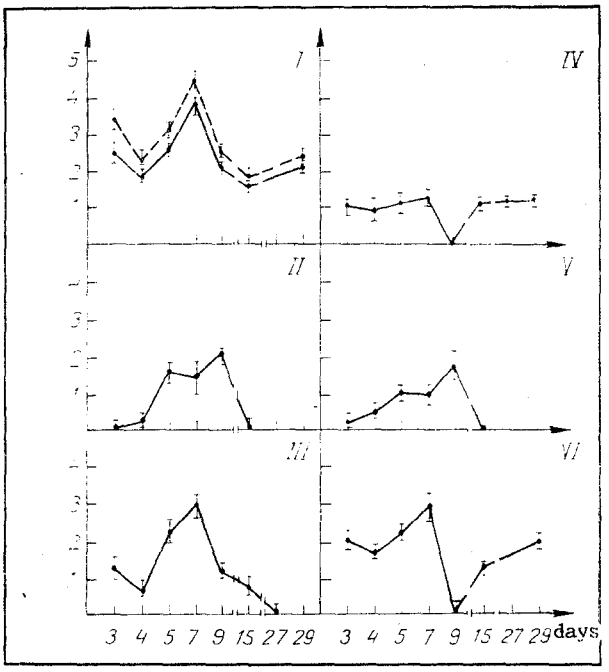

Fig. 1

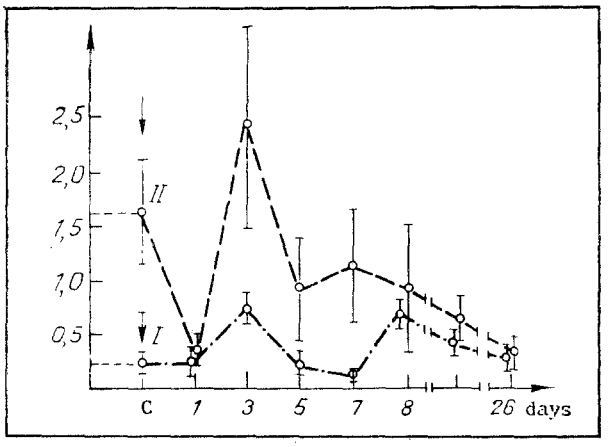

Fig. 2

Fig. 1. Time course of isolation of HSV from mouse organs and tissues. Abscissa, days of experiment; ordinate, virus activity (in log TCD_{50}). Continuous line – isolation of virus by coculture of organ cells with cells of sensitive strain; broken line – isolation of virus by hybridization of organ cells with cells of a sensitive strain. I) Brain, II) kidneys, III) blood, IV) lungs, V) spleen, VI) liver.

Fig. 2. Time course of changes in DNase activity in fractions of glial cells and neurons in mice with HSVE. Abscissa, days of experiment, C) control; ordinate, enzyme activity (in mmoles/mg protein). I) Neuroglia, II) neurons; arrow indicates time of injection of virus.

indicate restructuring of the lysosomal apparatus of the cells in the course of HSVE. The increase in DNase activity in the glial cells suggests a role for this enzyme in the arrest of the infectious process, in agreement with data in the literature on the ability of DNase to inactivate the HSV nucleotide [10].

Morphological investigation revealed no appreciable changes on the 1st day. On the 3rd day infiltration by neutrophils and monocytes was observed in the meninges at the base of the brain. An increase in the number and size of the astrocytes was observed close to the blood vessels. By the 5th day of the experiment, signs of progressive changes in the astrocytes were observed in the cortex at the base of the brain, especially near massive foci of infiltration in the pia mater. These changes consisted of an increase in the frequency of discovery of areas of cytoplasm, an increase in the size and number of chromatin granules, and increased basophilia of the nuclei of the astrocytes. Marked hyperchromatosis of the oligodendrocyte nuclei was conspicuous. A characteristic feature was the formation of glial complexes, consisting mainly of astrocytes, lying on the border with the pia mater and in the cortex at the base of the brain (Fig. 3a). Neurons with signs of chromatolysis and polymorphic inclusions in the nuclei were found near the glial complexes.

These signs of activation of astrocytes preceded intensive inflammatory infiltration of the brain substance. For instance, on the 6th day of the experiment the characteristic changes included marked dilatation and congestion of the vessels, swelling of the endothelial cells, and marked neutrophilic infiltration of all regions of the brain. Macrophages and destroyed neurons and glial cells also could be seen here. Larger pathological foci were observed at the base of the brain. A different picture of changes was seen on the 7th day of the experiment. Neutrophils and macrophages were absent. Activity of the neuroglia was increased in the cortex at the base of the brain, as shown by the formation of glial complexes. Just as on the 5th day these formations consisted mainly of astrocytes, but condensation of chromatin in them was less marked, and the nuclei appeared paler (Fig. 3b). In pathological foci in the white matter, however, redistribution of chromatin and coarse-brain hyperchromatosis of the nuclei were found in the astrocytes.

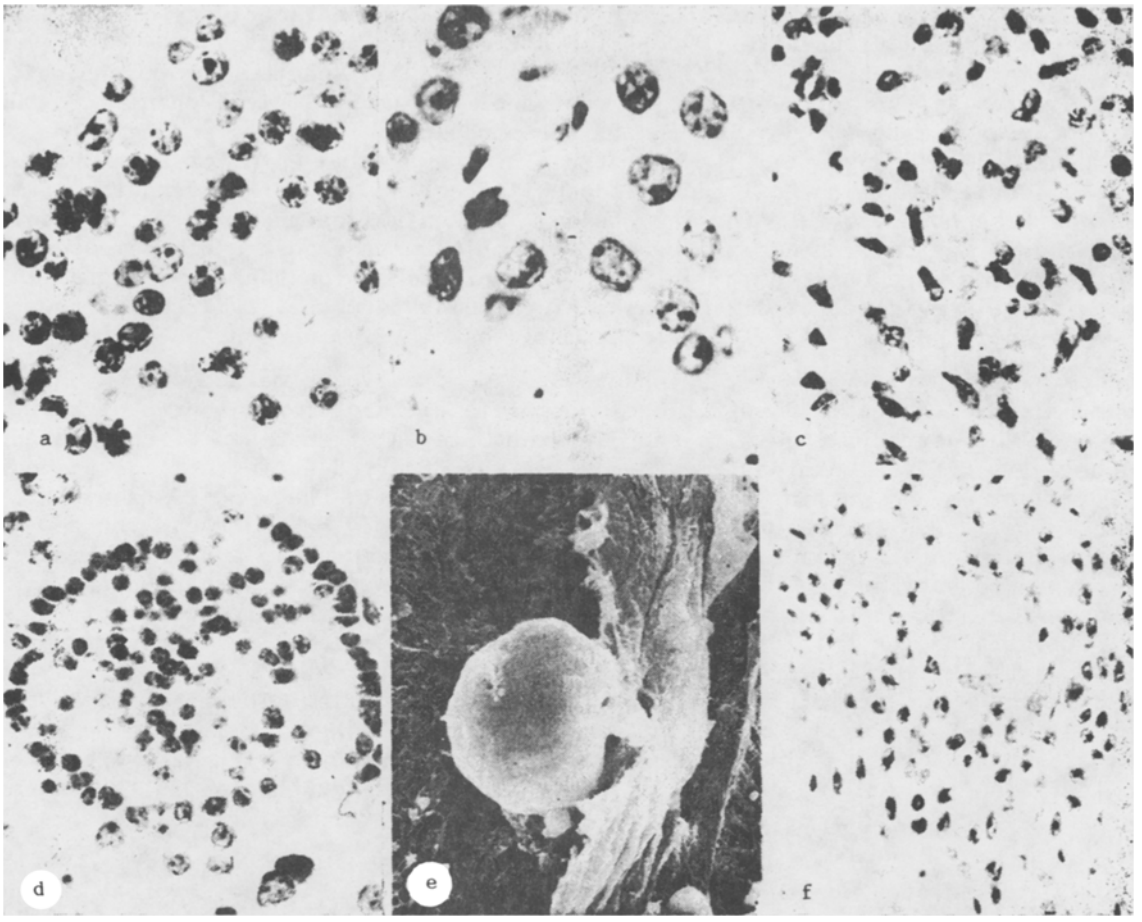

Fig. 3. Changes in astrocytes and aortic endothelium in experimental HSVE. Staining by Einarson's gallocyanin. a) Glial complex consisting mainly of astrocytes in cortex at base of brain. Increase in size of chromatin granules, increased basophilia of astrocyte nuclei (5th day of experiment). 320 ×; b) Formation of glial complex from astrocytes in cortex at base of brain on 7th day of experiment. 400 ×; c) Proliferation of astrocytes and microgliocytes in focus of virus infection of white matter of hemispheres (8th day of experiment). 100 ×; d) Glial complex from astrocytes in white matter of hemispheres. Damaged structures can be seen in center (13th day of experiment). 160 ×; e) Intricate glial complex consisting mainly of astrocytes in cortex at base of brain. Increased basophilia of astrocyte nuclei (21st day of experiment). 180 ×; f) De-endothelization of zone or aorta. Fixation of monocyte. Scanning electron microscopy. 4400 ×.

Activation of astrocytes during this period preceded the outflow of mononuclear cells. For instance, on the 8th day massive foci of infiltration of macrophages appeared around blood vessels in the temporal lobe, mingled with a few lymphocytes. Meanwhile marked proliferation of the microglia was observed. On the 10th day, most cells in the foci of infiltration were lymphocytes . After 13 days, proliferation of astrocytes and microgliocytes was found in the remaining pathological foci (Fig. 3c). Glial complexes were formed from astrocytes in the white matter of the hemispheres also. Nuclei of cells forming these complexes were pale, nearly all contained one or two large basophilic granules (Fig. 3d). Here also a few oligodendrocytes and microglial cells could be seen. No foci of inflammatory infiltration were present near the glial complexes.

On the 21st and 26th days numerous glial complexes, circular in shape, were found in the cortex at the base of the brain. The cells were mainly astrocytes, in which marked condensation of basophilic granules was observed, making the nuclei darker in appearance (Fig. 3e). Foci of inflammatory infiltration were absent in all parts of the brain. Not only structural changes, but also the localization of the glial complexes at different stages of

HSVE, incidentally, reflect the character of increased functional activity of the astrocytes. Since the herpes simplex virus affects cells of different organs and systems [1], as the results of our virologic investigation (Fig. 1) confirmed, it was decided to study morphological changes caused by the virus in the aorta. The time course of the morphological changes in the aorta was found to correspond to the development of the pathological process in the animals' brain. It was not until the 3rd day after intracerebral infection that edema of the subendothelial matrix of the aorta, damage to individual endothelial cells, and marked fixation of monocytes in these zones were observed. By the 5th-7th day, intensive destruction of the endothelial barrier and the formation of zones of de-endothelization with exposure of the subendothelial matrix of the intima were observed (Fig. 3f). The formation of extensive defects in the endothelial monolayer is evidence of disturbances of vascular permeability. Later, toward the 20th day, only isolated damaged cells of the aortic endothelium were noted.

By a combination of virologic, biochemical, and morphologic methods of investigation it was thus possible to obtain a sufficiently complete picture of the course of HSVE in mice. The reaction of the astrocytic glia to the different stages of HSVE was discovered and described in detail. Activation of astrocytes, which was observed during periods preceding diapedesis of leukocytes and outflow of mononuclear cells, and in the repair stage, is a feature of herpetic inflammation in the brain which has not previously been noted. The new data obtained on the dynamics of changes in DNase activity in the neuroglia and the formation of glial complexes, representing the morphological expression of functional strain on the astrocytes, can be regarded as processes reflecting the barrier and eliminative functions of the neuroglia in HSVE. For instance, on the 7yth day, when initial signs of clinical recovery were appearing in the brain of the mice, corresponding signs appeared of activation of the astrocytic glia, and by the 8th day, DNase activity was increased in the neuroglia. The titer of HSV at this period was quite high, and did not fall until the 9th day of the experiment. We know from the literature that astrocytes in a culture of CNS tissue infected with HSV, exhibited stimulated proliferation [9], unlike neurons and oligodendrocytes, which died in the course of 24-48 h. High resistance of astrocytes also has been found under conditions of cerebral hypoxia [11].

It can be postulated on the basis of these observations that the severity of the course and the outcome of herpes simplex virus encephalitis depend to a decisive degree on the state of the astrocytic glia and on its potential ability to exhibit barrier and eliminative functions.

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