CHEMICAL CHANGES IN SOME LYSOSOMAL ENZYMES AND DEATH OF CELLS IN HERPETIC INFECTION

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UDC 616.988.13-092.9-07:[616-018.11-008.931:577.152+616-003.8-018

Propagation of herpes simples virus in cell cultures is accompanied by increased activity of lysosomal acid phosphatase. A direct relationship exists between the increase in enzyme activity and the degree of the degenerative changes in vivo. The accumulation of a strain of virus inducing a cytopathogenic effect in the cell culture increases the intensity of the reactions for acid phosphatase, deoxyribonuclease II, and esterase, whereas infection by a strain of virus yielding no visible cytopathogenic effect increases the activity of deoxyribonuclease II only.

Results indicating the role of lysosomes in the death of cells during virus infections have recently been obtained [2-8, 16]. Investigations to study the activity of lysosomal enzymes in herpetic infection are limited in number and have been carried out only on cell cultures [12, 18].

The object of the present investigation was to study changes in lysosomal enzymes in experimental infection due to herpes simplex virus (HSV). The following tasks were undertaken: 1) to investigate the activity of lysosomal enzymes during reproduction of HSV in various tissue systems; 2) to study changes in a number of lysosomal enzymes (acid phosphatase, deoxyribonuclease, and nonspecific esterase) during reproduction of HSV strains inducing a cytopathogenic effect in cell culture and accumulating without producing visible changes in the cells; and 3) to detect a possible connection between changes in the lysosomes and the cytopathogenic action of the viruses.

EXPERIMENTAL METHOD

Strains ELA and AS of HSV were used [1]. A virus-containing brain suspension was used for intracerebral inoculation of albino mice weighing 7-8 g, and also to infect a 3-day primary culture of human embryonic fibroblasts (HEF), and a 2-day subculture of L cells. The dose for the animals was 100 LD_{50} (0.03 ml), and for the cultures 0.1 LD_{50} per cell. The investigation was carried out 4, 6, 10, 24, 48, 72, 96, and 120 h after infection.

To detect acid phosphatase by Gomori's method [13, 14], part of the brain was fixed in neutral formalin for 24 h at 4°C. Another part of the brain, intended for cytochemical determination of nucleic acids by the Feulgen and Brachet reactions and for morphological examination, was fixed in Carnoy's solution. Specimens of the cell cultures were treated in the same way. Besides these methods, lysosomal enzymes deoxyribonuclease II and nonspecific esterase were studied in the cell cultures, the first by Aronson's method [9, 20, 21], as modified for cell cultures [3], and the second by Burstone's method [10]. Parallel with the investigation of the mouse brain tissue and the cell cultures infected with HSV, a cytochemical and morphological study was made of control specimens. The virus content was determined quantitatively by intracerebral titration in albino mice, with calculation of the titer by the Reed and Muench method.

D. I. Ivanovskii Institute of Virology, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR V. M. Zhdanov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 71, No. 4, pp. 43-46, April, 1971. Original article submitted February 23, 1970.

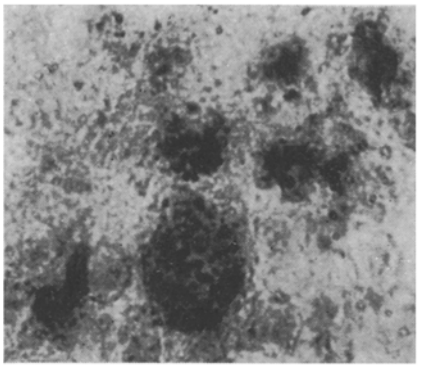

Fig. 1. Increase in intensity of reaction for acid phosphatase in brain neurons. HSV of strain ELA, 48 h after infection, 950 ×.

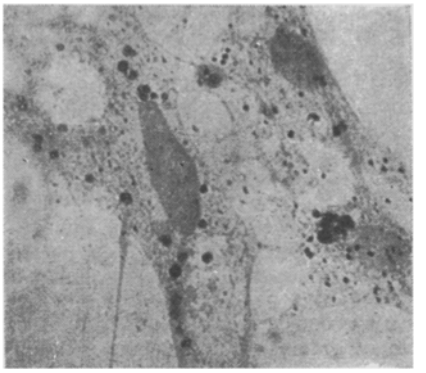

Fig. 2. Increase in intensity of reaction for acid phosphatase in individual cells. HSV of strain ELA, HEF culture 24 h after infection 700 ×.

EXPERIMENTAL RESULTS

Strain ELA of HSV activated acid phosphatase in all types of cells tested (brain neurons, HEF and L cultures). In the brain neurons, intensification of the reaction for the enzyme was observed 48 h after infection (Fig. 1), and in the HEF and L cultures 24 h after infection (Fig. 2). The intensity of the reactions began to diminish in the brain tissue and HEF culture 72-96 h after infection, and in the L cells 48-72 h after infection. The appearance of the changes obeyed the following general rule: initially a certain redistribution of chromatin was observed in the nuclei, after which activity of acid phosphatase increased in the cytoplasm, to be followed by the development of destructive changes in the cells. The increase in acid phosphatase activity was preceded by a decrease in the content of cytoplasmic and nucleolar RNA in the cells. A comparative study of zones of the brain of the infected mice showed a direct relationship between the intensity of the reaction for acid phosphatase and the severity of the generative changes. The lysosomes preceded the appearance of lesions in the cells. For instance, an increase in size of the particles and in the intensity of their reaction for acid phosphatase preceded basophilic degeneration of the cells after infection with strain ELA. The progressive decrease in the number of lysosomes starting from 48 h and continuing until their complete disappearance from the cytoplasm of the cells at 72-96 h (Fig. 3) preceded the appearance of foci of lysis after experimental neuroinfection induced with strain AS.

A study of the dynamics of the lysosomal enzymes in a culture of L cells infected by strain ELA, accompanied by the development of a cytopathogenic effect, revealed activation of all the hydrolases investigated (acid phosphatase, deoxyribonuclease II, esterase) in the overwhelming majority of cells.

During reproduction of strain AS in the cell culture, the activity of deoxyribonuclease II only was increased 3 h after infection of the cells, and it was down to the control level again after 10 h. Activity of nonspecific esterase and acid phosphatase, on the other hand, exceeded the control level starting from 10 h after infection only in a few solitary cells which gradually and completely lost their original shape and became circular. In the overwhelming majority of cells the intensity of the reactions for the enzymes fell in the same way as in the control.

The sequence of changes detected in experimental herpetic infection (redistribution of chromatin, increase in the acid phosphatase content, cell destruction) corresponded to electron-microscopic observations [17] showing that the DNA viruses first penetrate into the nuclei, and then bind themselves secondarily to the lysosomes. The increase in activity of lysosomal enzymes, especially acid phosphatase, and the early decrease in the content of cytoplasmic and nucleolar RNA, with the appearance of histopathological changes both in the brain of the infected mice and in the cell cultures, are evidence of the direct participation of lysosomes in causing death of the cells.

This hypothesis is confirmed by the positive correlation observed between the degree of intensification of the reaction for acid phosphatase and the degree of development of degenerative changes. It has been claimed that the trigger mechanism of cell destruction in virus infections is an increase in permeability

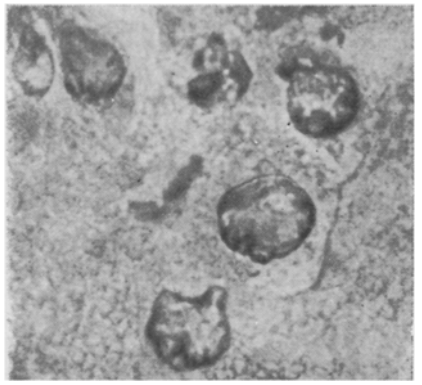

Fig. 3. Absence of reaction for acid phosphatase in focus of lysis in hippocampal neurons. HSV of strain AS, 96 h after infection, 950 ×.

of the lysosomal membranes analogous to the cell injuries induced by administration of various toxic agents or by the action of physical factors [11]. Direct confirmation of this hypothesis was obtained by the work of Thacore and Wolf [19]. Incubation of isolated lysosomes with extracts of cells infected with poliovirus led to the release of hydrolases from the particles.

However, the wide variety of disturbances of lysosomal activity revealed by a study of different pathological states [11, 15] suggests that in virus infections also the release of lysosomal hydrolases is not the only mechanism.

The results of a comparative investigation of several lysosomal enzymes in herpetic infection of cell cultures, accompanied by a cytopathogenic effect but with no visible morphological lesions of the cells, provided grounds for the assumption that in this case only the first stage of activation of the lysosomes took place, viz., an increase in the permeability of their membranes, as Allison [6] suggests.

Another possibility is that increased reproduction of the viruses took place only in single cells. As a result, activity of the enzymes was changed probably in individual cells which subsequently died.

LITERATURE CITED

1. R. M. Bikbulatov, Cytomorphology of Experimental Herpetic Infection Induced by Antigenically Different Strains of Virus. Candidate's Dissertation, Moscow (1967).
2. S. Ya. Zalkind, L. P. Izakova, N. N. Bogomolova, et al., Abstracts of Proceedings of a Symposium on the Role of Lysosomes in Physiological and Pathological Processes [in Russian], Moscow (1968), p. 14.
3. V. V. Malinovskaya, A Study of the Activity of Lysosomal Enzymes in Virus Infections. Author's Abstract of Candidate's Dissertation, Moscow (1969).
4. A. Allison and K. Sandelin, J. Exp. Med., 117, 879 (1963).
5. A. Allison and M. Burstone, Histochemie, 3, 462 (1964).
6. A. Allison and L. Mallucci, J. Exp. Med., 121, 463 (1965).
7. A. Allison, Perspect. Virol., 5, 29 (1967).
8. K. Amako and S. Dales, Virology, 32, 201 (1967).
9. J. Aronson, L. Hempelmann, and S. Okada, J. Histochem. Cytochem., 6, 255 (1958).
10. M. Burstone, J. Histochem. Cytochem., 6, 322 (1958).
11. C. de Duve and R. Wattiaux, Ann. Rev. Physiol., 28, 435 (1966).
12. J. Flanagan, J. Bact., 91, 789 (1966).
13. G. Gomori, Stain Technol., 25, 81 (1950).
14. G. Gomori, Internat. Rev. Cytol., 1, 323 (1952).
15. C. Hug and W. Schubest, J. Cell Biol., 35, 1 (1967).
16. L. Mallucci and A. Allison, J. Exp. Med., 121, 477 (1965).
17. C. Mattern, J. Exp. Med., 121, 3 (1965).
18. A. Newton, Biochem. J., 94, 9P (1965).
19. H. Thacore and D. Wolf, Nature, 218, 1063 (1968).
20. A. Vorbrodt, J. Histochem. Cytochem., 9, 647 (1961).