MORPHOLOGICAL AND BIOCHEMICAL CHANGES IN BRAIN CELLS OF MICE INFECTED WITH INFLUENZA VIRUS

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Clinical, morbid anatomical, and epidemiologic investigations have demonstrated the aggravating effect of influenza infection on the course of diseases of the cardiovascular and nervous systems. Hence the urgency of the study of the mechanisms of action of influenza virus on nerve tissue in cases when a primary virus lesion develops, associated with changes in the natural reactivity of the host or with increased permeability of the blood-brain barrier.

The important role of membrane lipids (phospholipids, glycosphingolipids) in interaction between virus and cells has been noted in the literature [5, 9]. A high concentration of polysialogangliosides, facilitating virion reception, the specific nature of the immune mechanisms of defense, and the possibility of secondary autoimmune involvement of cell membranes, all determined the particular features of development of infectious processes in nerve tissue [2, 10]. It has been found, for example, that during infection of mice with neurotropic coronavirus JHM, marked demyelinization and activation of lipid peroxidation (LPO) take place in the animals' brain [1].

It was accordingly decided to determine the concentrations of LPO products, in addition to virologic and morphologic investigations to study the action of influenza virus on nerve tissue after intracerebral infection of mice, for we know that activation of LPO is one of the universal mechanisms of modification of the lipid composition, of structural change, and damage to cell structures [3], and of regulation of activity of enzyme systems [11].

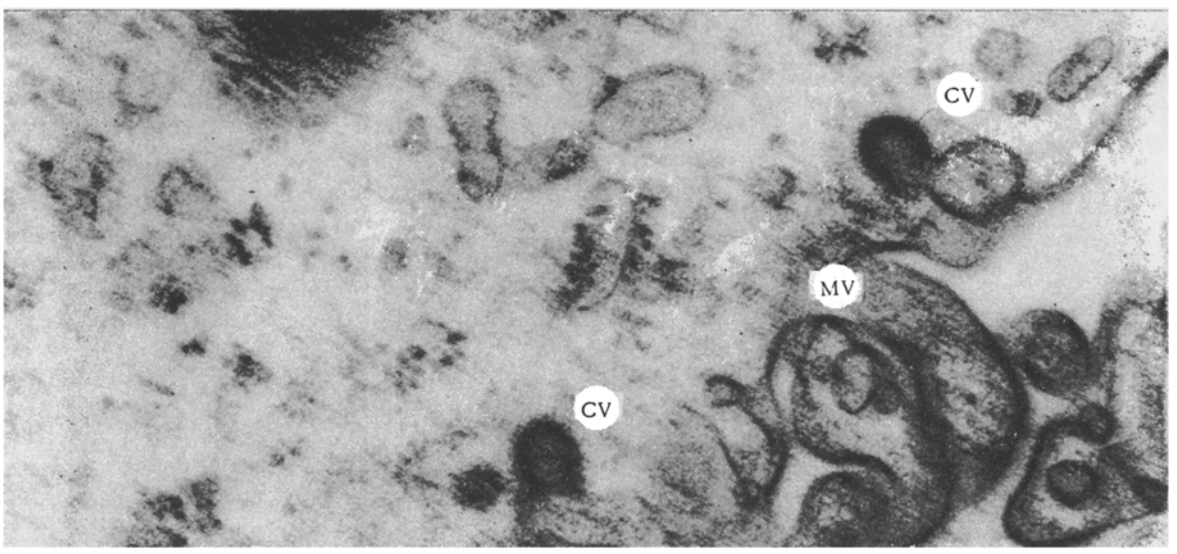

Fig. 1. Ependymal cells of chloroid plexus after intracerebral infection of mouse virus. MV) Microvillus; CV) coated vesicles; 70,000 ×.

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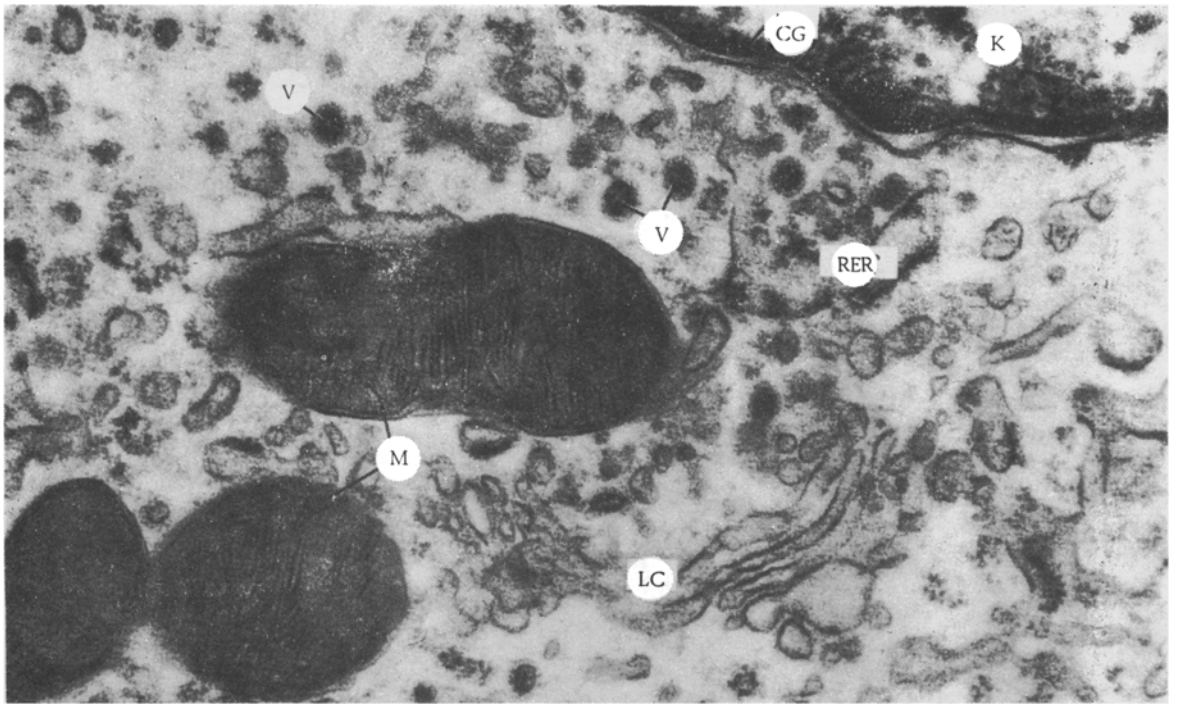

Fig. 2. Influenza virions in an ependymocyte 5 days after intracerebral infection of mouse with influenza virus. V)Virion; RER) rough endoplasmic reticulum; LC) Golgi lamellar complex; M) mitochondrion; K) karyoplasm; CG) layer of chromatin granules. 40,000 ×.

EXPERIMENTAL METHOD

Experiments were carried out on BALB/c mice weighing 8-10 g (aged 4-5 weeks), which were infected with a pathogenic strain of influenza virus A/PR/8/34 (HONI) in a dose of 7 log EID_{50}/0.2 ml. The experimental animals were given an intracerebral injection of 0.02 ml of virus-containing material into the temporal region, whereas the controls received a corresponding injection of 0.02 ml of physiological saline. Virologic, biochemical, and morphologic investigations were carried out on the 5th day after infection, five or six experimental animals and the same number of controls being used for each of the above methods. There were three series of experiments.

For virologic investigation the animals' brain was homogenized with electrolytically produced corundum, the concentration of virus in a 10% suspension was determined by titrating the virus in chick embryos. For electron-microscopic investigations pieces including the dorsal hippocampus and part of the lateral ventricle with the choroid plexus were excised from the animals' brain and fixed in 1% OsO_4 solution in 0.1 M phosphate buffer (pH 7.3) with the addition of 4.5% sucrose. The material was embedded in epoxide resin (Epon). Semithin sections (1-10 μ) were stained with a 1% solution of methylene blue, made up in a 1% solution of borax, and used to prepare ultrathin sections and to study pathological changes. Ultrathin sections were strained with uranyl acetate and lead citrate (after (Reynolds) and were investigated in HU-118 (Hitachi, Japan) and JEM-100 (JEOL, Japan) electron microscopes. Activation of LPO was judged by the accumulation of fluorescent products in lipid prepared from analogous regions of the brain of experimental and control animals. The fluorescence investigations were conducted on the MPF-2A spectrofluorometer as described previously [1].

EXPERIMENTAL RESULTS

After intracerebral injection of the pathogenic strain of influenza virus A/PR/8/34 into mice, it proliferated in the animals' brain tissue, in agreement with data in the literature [6]. On the 5th day after infection the quantity of virus in the animals' brain was very small, and on average, based on the results of three experiments, it was 1 log EID_{50}/0.2 ml. The presence of influenza virus at this time was confirmed by the results of electron-microscopic investigations, which showed the presence of virions in the cytoplasm of the ependymo-

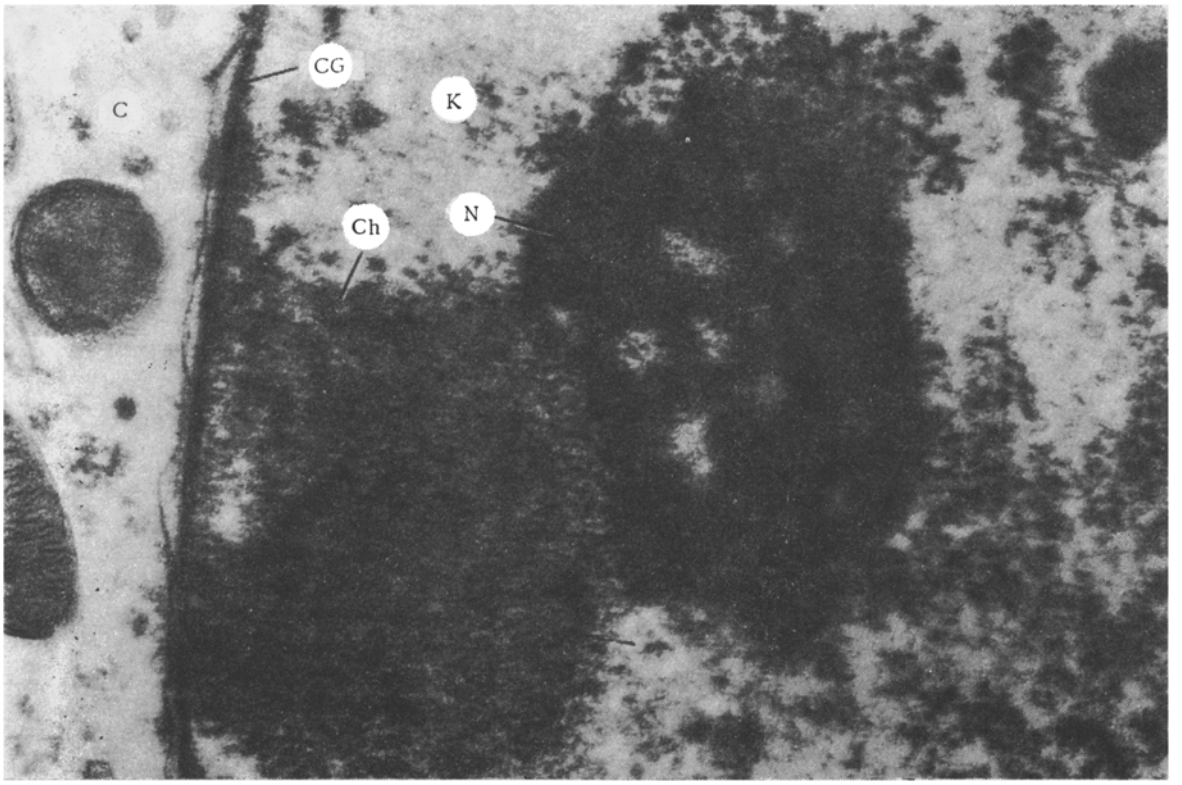

Fig. 3. Changes in ultrastructure of nucleus and nucleolus of an ependymocyte after intracerebral infection of mouse with influenza virus. C) Cytoplasm; K) karyoplasm; N) nucleolus; Ch) chromatin leaving nucleolus; CG) layer of chromatin granules near inner nuclear membrane. 40,000 ×.

cytes, and, in particular, in the ependymal cells of the choroid plexus in the lateral ventricles. The virions were spherical in shape, about 100 nm in diameter, and consisted of a dense core and very thin fibrils bordering it. In their structural organization they resembled the coated vesicles, which are ordinary cytoplasmic components of the body cells. According to our own observations, coated vesicles differ from virions of influenza virus in their larger size and their clearly defined surface membrane (Fig. 1). Virions are distributed throughout the cytoplasm of the ependymocytes — from its surface as far as the nuclear membrane in the rough endoplasmic reticulum, close to the Golgi complex and mitochondria (Fig. 2). The ultrastructure of the nucleus and cytoplasm is greatly altered in ependymal cells containing virions. The outlines of the nuclei are often deformed by invaginations of cytoplasm. Two types of changes in ultrastructure of the nuclei were observed. Large masses of chromatin, which in the control animals were located near the nuclear membrane, disappeared and the nuclear chromatin became more homogeneous. Near the inner nuclear membrane a layer of chromatin granules appeared.

Marked changes in nucleolar ultrastructure were observed in the ependymocytes containing virions. Large accumulations of chromatin, usually bigger than nucleoli, were often formed near them (Fig. 3). The formation of these chromatin "clouds" is generally regarded as departure of chromatin from the nucleolus. This is one manifestation of segregation of the nucleolus, which may be caused by the action of actinomycin D, an inhibitor of RNA synthesis, and by certain viruses [8, 12, 13]. During segregation, physical separation of DNA and RNA takes place in the nucleolus, with the result that RNA synthesis and protein synthesis become impossible.

Changes in the mitochondria and rough endoplasmic reticulum were observed in the cytoplasm of the ependymal cells. The mitochondria became electron-dense, some of their cristae were often destroyed, and at the site of the lesion an osmiophilic granular mass appeared (Fig. 2). Fusion of the modified mitochondria with phagolysosomes was observed.

Consequently, ultrastructural changes in the nucleus and cytoplasm of the ependymocytes, in close connection with influenza virus virions, show that they are the result of the cyto-

pathic action of the virus injected into the brain. The morphology of the cytopathic changes is evidence of inhibition of protein synthesis.

As the results of this investigation showed, the content of fluorescent LPO products in the lipid extracts of the above-mentioned regions of the brain of infected animals was four times higher than in the control: 2.7 ± 1.5 and 0.7 ± 0.2 relative units respectively ($p < 0.02$, $n = 10$), evidence of activation of LPO.

The increase in concentration of LPO products in the infected animals observed in these experiments was not large enough for any definite conclusion to be drawn regarding the degree of involvement of LPO processes in the damage to cell membranes produced in the brain region studied. A detailed discussion will therefore be in order.

It was shown previously [7] that the initial stage of interaction between influenza virus and the cell is accompanied by generation of active forms of oxygen by the oxidation-reduction system of the cytoplasmic membrane. On the one hand, activation of LPO processes leads to a substantial change in the physicochemical properties of the cell membranes (rigidity, surface charge, etc.), which in the first stage may hinder fusion of the virion with the cell. In that case activation of LPO can be regarded as a protective process. On the other hand, accumulation of LPO products leads to increased ionic permeability of the membrane, modification of the properties of membrane-bound systems and, as a result, to a change in metabolic processes in the cell. At the stage of adhesion of the virion, damage to the target cells is therefore possible.

The infected cell is distinguished by enhanced metabolic activity, which [4] disturbs its plastic properties and depresses its antioxidative activity. This last phenomenon may be yet another factor leading to the accumulation of LPO products.

Multiplication of the virus in the brain was thus demonstrated by virologic methods after intracerebral infection of mice in vivo with influenza virus A/PR/8/34. The morphologic and biochemical investigations of pieces of tissue from the region of the cerebral ventricles of the animals under these circumstances revealed both important structural changes and an increase in the concentration of LPO products in the CNS cells of the infected animals, indicating that this virus has a damaging action on nerve tissue cells.

The writers would point out that similar results were obtained in a preliminary experiment with intranasal infection of mice with the same strain of influenza A virus.

LITERATURE CITED

1. N. V. Gorbunov and Kh. N. Annanepesov, Neirokhimiya, 4, No. 3, 290 (1985).
2. G. V. Konovalov and A. M. Dobykin, Arkh. Patol., No. 11, 92 (1981).
3. E. M. Kreps, V. A. Tyurin, N. V. Gorbunov, et al., Dokl. Akad. Nauk SSSR, 286, No. 4, 1009 (1986).
4. A. A. Kubatiev and S. V. Andreev, Metabolism of the Myocardium [in Russian], Moscow (1981), pp. 251-262.
5. Yu. G. Molotkovskii, E. M. Manevich, V. I. Shvets, et al., Vopr. Virusol., No. 2, 182 (1983).
6. B. A. Osetrov, "Pathogenesis of lesions of the nervous system in influenza," Author's Abstract of Dissertation for the Degree of Doctor of Medical Sciences, Leningrad (1981).
7. Yu. M. Sudnik, Yu. E. Kligner, S. N. Cherenkevich, et al., Byull. Éksp. Biol. Med., No. 12, 688 (1985).
8. Yu. S. Chentsov and V. Yu. Polyakov, Ultrastructure of the Cell Nucleus [in Russian], Moscow (1974).
9. A. M. Haywood, J. Gen. Virol., 29, 63 (1975).
10. A. M. Haywood and B. P. Boyer, Proc. Natl. Acad. Sci. USA, 82, 4611 (1985).
11. M. G. Murphy, Neurochemistry, 44, 50 (1985).
12. C. I. Schoefl, J. Ultrastruct. Res., 10, 224 (1964).
13. H. Shinozuka, The Pathology of Transcription and Translation, ed. by E. Farber, New York (1972), pp. 73-99.