IMMUNOMORPHOLOGICAL CHANGES IN THE MOUSE BRAIN AFTER INTRACEREBRAL INJECTION OF A NEUROTROPIC STRAIN OF INFLUENZA VIRUS

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The idea that polysystemic regulatory disorders, determining the pathogenesis of chronic diseases of the CNS, are based on infection by influenza virus has been expressed by Academician N. P. Bekhtereva. Documentary confirmation of this idea has now been obtained. In particular, epidemiologic data show that neurological and mental disorders such as the Reye syndrome, encephalitis, and parkinsonism, can develop in persons recovering from influenzal infection [5]. It has been suggested that intrauterine contact with influenza A virus can increase the risk of development both of schizophrenia [4] and of parkinsonism [3]. This phenomenon is evidently based on that discovered by Laing and co-workers [2]. They showed that immunization of rabbits with neurotropic strains of influenza A virus (NWS/33, WSN/33) can induce the formation of autoantibodies to brain-specific 37 kDa protein. Autoantibodies to 37 kDa protein persist after the end of influenzal infection. This protein behaves as a neurotropic antigen and antibodies against it can be involved in the pathogenesis of nervous and mental disorders with an autoimmune basis [2].

Since much of this problem remains unclear we have undertaken a first series of experiments with the aim of making a virological and morphological analysis of the fate of influenza A virus injected directly into the CNS.

EXPERIMENTAL METHOD

A model of influenzal infection was created by intracerebral infection of 150 noninbred albino mice weighing 8-12 g, aged 3 weeks. The animals were infected with the neurovirulent reference strain A/WSN/33, obtained from the Museum of the Laboratory of Etiology of Influenza, Influenza Institute, Ministry of Health of the USSR. The virus was cultured in the allantoic cavity of 10-day-old chick embryos for 48 h at 34°C. Virus-containing allantoic fluid with infectious activity of 8.0 log EID_{50} was injected into the right parietal region in a volume of 0.03 ml [1]. The degree of infection of the mice was tested by infection of brain homogenates (tenfold dilution, volume 0.2 ml) of 10-day chick embryos [1]. The hemagglutinating activity of the virus-containing material was revealed by the hemagglutination test with a 1% suspension of chick erythrocytes by the standard method. Mice of the control series were given an intracerebral injection of allantoic fluid from chick embryos not infected with influenza virus.

To study the dynamics of reproduction of the virus in the brain, tissue from the choroid plexus and lateral cerebral ventricles, taken from two or three mice in each of nine series of experiments, 6, 12, 24, 48, and 72 h and 4, 7, 15, and 30 days after intracerebral injection of the virus, was investigated immunohistochemically. Targeted pieces of brain tissue were fixed in 6% paraformaldehyde. Subsequent treatment and embedding of the material in paraffin wax followed the standard technique. Sections 4-5 μ thick were treated with 1% hydrogen peroxide solution to inhibit endogenous peroxidase. The

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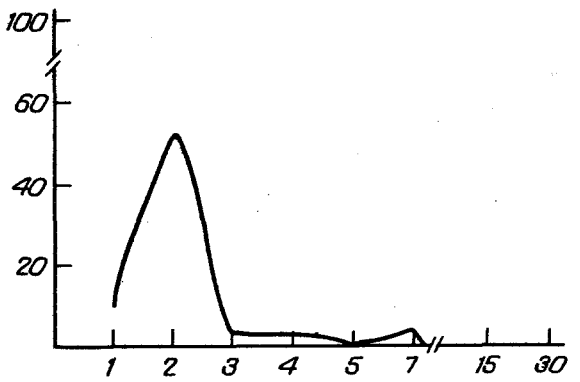

Fig. 1. Dynamics of mortality among mice after intracerebral infection with influenza A/WSN/33 virus. Abscissa, duration of observation (in days); ordinate, mortality (in %).

sections were then rinsed in physiological saline and rabbit serum against influenza A viral antigen applied to them (dilution 1:20), after which they were rinsed 3 times in 0.01M phosphate buffer, pH 7.2, and treated with preparations of goat antiserum (Sigma, USA) against rabbit IgG, labeled with horseradish peroxidase. The localization of the bound peroxidase label was revealed by treatment of the sections with 3,3-diaminobenzidine (Sigma, USA) with the addition of 0.1% hydrogen peroxide solution, followed by postfixation in 6% paraformaldehyde solution. The cell nuclei with stained with hematoxylin. After mounting in balsam the sections were examined in a light microscope (×1000). Control sections were treated with intermediate heterologous serum and serum against human IgG, labeled with horseradish peroxidase, and suppression of endogenous peroxidase was verified by an appropriate control.

EXPERIMENTAL RESULTS

After intracerebral infection of the mice mortality reached a peak on the 2nd day, at 51% of the total number of infected animals (Fig. 1). Later in the course of the experiment mortality among the animals fell to 1%, and 7 days after the beginning of injection of the virus, no further deaths were observed among the animals.

Infectivity of the mouse brain suspension was greatest 6 h after infection (3.2 log EID_{50}), and it fell to 1.3 log EID_{50} after 12 h of incubation and increased to 2.0-2.3 log EID_{50} during the subsequent 3 days (Table 1). After 4 days and at all subsequent times of observation, influenza virus could not be found in the brain of the infected mice by culture in chick embryos.

The results of the virological investigation were completely confirmed by immunohistochemical analysis of the brain tissue. Six hours after intracerebral injection of the neurotropic strain A/WSN/33 of influenza virus, it was distributed in nearly all the ependymal cells lining the cavity of the ventricle (Fig. 2a) and also in individual epithelial cells of the choroid plexus. Vacuolar degeneration of the epithelial cells was observed in these animals, with displacement of the hyperchromic nucleus. In the zone of vacuolar degeneration the largest concentrations of antigenic material were observed (Fig. 2a, b), indicating possible reproduction of the virus.

After 12 and 24 h changes also were observed only in cells of the ependyma and epithelium of the choroid plexus. Destruction of epithelial cells containing the virus was accompanied by their desquamation into the lumen of the ventricle. The leukocytic response, as at the previous time of investigation, was weak. The greatest cell destruction in the ependyma and epithelium was observed 48 h after injection of the virus. Destructive changes were linked with a decrease in thickness of the ependyma and with the release of disintegrating cells containing material stained with hematoxylin and eosin and possessing peroxidase positive particles, into the extracellular space (Fig. 2c). Meanwhile foci of two or three swollen epithelial cells containing a large quantity of antigenic material were observed.

TABLE 1. Infectivity of Brain Suspension of Mice Infected Intracerebrally with Influenza A/WSN/33 Virus

Time of taking material		Infectivity, log EID_{50}	Hemagglutinating activity (HA units/0.2 ml)
6	h	3,2	512
12		1,3	256
24		2,3	256
48		2,1	256
72		2,0	128
4	days	—	—
7		—	—
15		—	—
30		—	—

Legend. —) Virus in brain suspension from infected mice could not be detected during subculture in 10-day-old chick embryos.

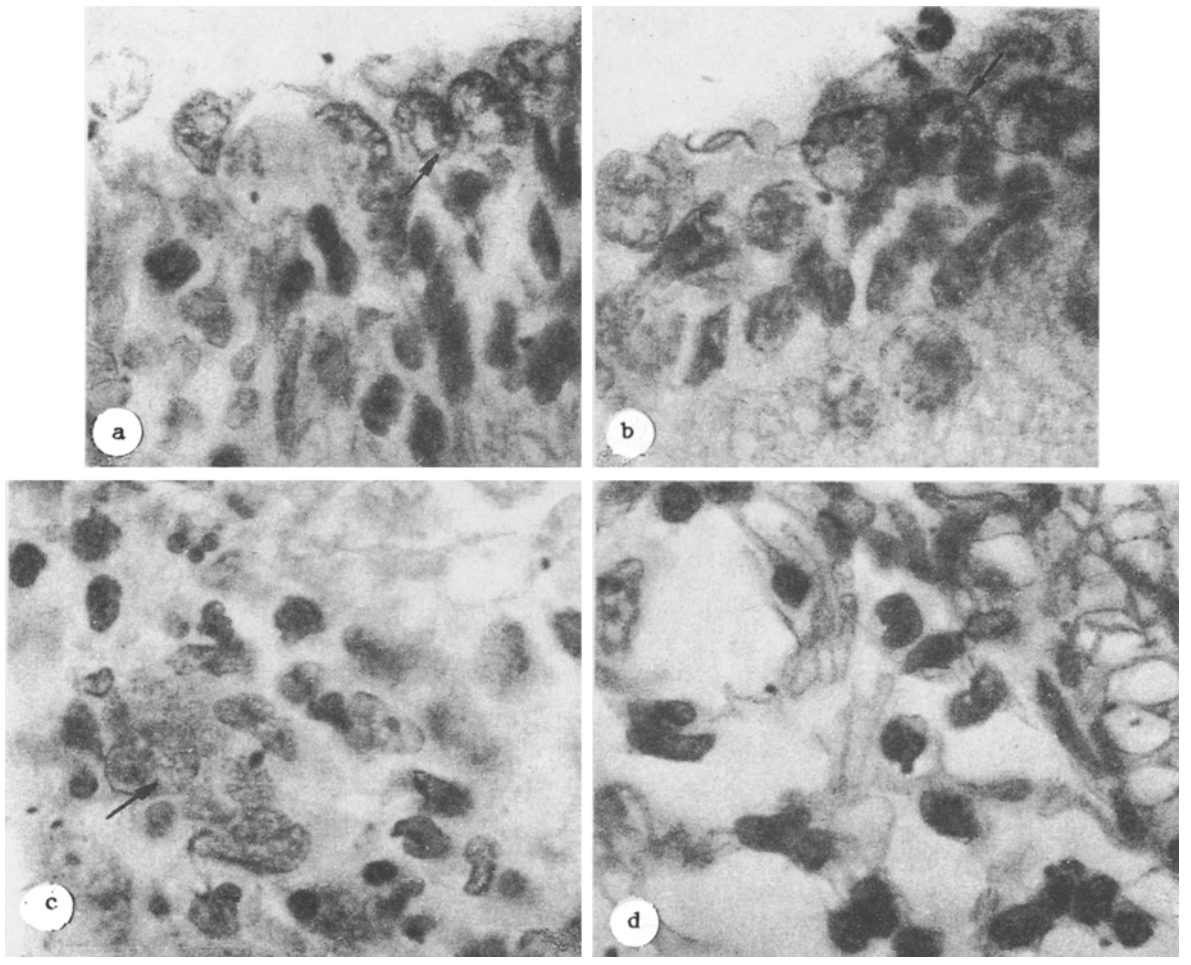

Fig. 2. Morphological changes in cells of the ventricular ependyma. a, b) Localization of virus (arrows) in epitheliocytes (6 h after infection); c) destruction of epitheliocytes with release of contents, including peroxidase-positive material (arrows), 24 h after infection; d) absence of ependyma, tissue macrophagal reaction, 3 days after infection. Immunohistochemistry, ×1000.

On the 4th day after intracerebral injection of influenza virus no ependyma could be seen over a wide area Peroxidase-positive material could not be found immunohistochemically (Fig. 2d). In areas of destruction of the epithelium of the choroid plexus concentrations of macrophages were observed, with antigenic material visible in some of them. At all subsequent times of the experiment, including the 30th day after injection of the virus, gradual restoration of the epithelial and ependymal cells was observed. However, these cells no longer contained antigenic material. After the 4th day proliferation of the glia took place beneath the ependyma of the ventricles, and reduction of the number of epithelial cells in the choroid plexus was accompanied by considerable connective tissue development. Incidentally, in all series of the experiment, only congestion and dilatation of capillaries and precapillaries, with negligible edema, developed in the nerve tissue of the mouse brain.

Intracerebral injection of neurotropic influenza virus strain A/WSN/33 into mice is accompanied by the development of influenzal infection, a particular feature of which in the brain is that the virus is localized in the ependymal cells of the lateral ventricles and the epithelium of the choroid plexus. Reproduction of the virus in the ependymal cells and the epithelial cells of the choroid plexus is accompanied by destruction of the cells and by ingestion of the breakdown product by macrophages. By the 4th day after infection the brain tissues are free from the virus, but this does not mean the end of the process. Since influenzal infection is accompanied by the appearance of numerous macrophages, involved in expression of the genes of the major histocompatibility complex, the development of an autoimmue reaction with the formation of autoantibodies to brain-specific antigens cannot be ruled out.

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