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EXPERIMENTAL AMYOTROPHIC LEUKOSPONGIOSIS IN GUINEA PIGS WITH RETROBULBAR INFECTION

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Among human neuroinfections a group of slow infection of the CNS caused by nonclassical viruses is distinguished. This group includes kuru, Jakob–Creutzfeldt disease (JCD), amyotrophic leukospongiosis (ALSP), and the Gerstmann–Streissler syndrome [3, 9, 11]. All these diseases are characterized by a long incubation period, measured in months or years, absence of inflammatory reactions in the CNS, and they are inevitably fatal. The agents of slow infections of the CNS are nonclassical viruses, which constitute a new class of subviral pathogens which, as Prusiner has suggested [11], may be called prions.

ALSP, found in the territory of the Belorussian SSR, like the Gerstmann–Streissler syndrome, has been included in the group of slow infections of the human CNS as a result of proof of its infectious nature and of isolation of the causative agent which, in its biological and physicochemical properties, has the features of a nonclassical virus. However, the particular features of the pathogenesis of the disease have so far been inadequately studied, and this is holding up the development of methods of intravital diagnosis, specific chemotherapy, and prevention of this fatal infectious disease in man. The undertaking of such investigations has been retarded by the absence of a convenient laboratory model with a short incubation period. The study of the spectrum of sensitivity of laboratory animals (squirrel, monkeys, rabbits, guinea pigs, hamsters, and rats) to the agent of ALSP has shown that the animal most susceptible to infection is the guinea pig. Nevertheless, the incubation period of the disease after intracerebral or intramuscular infection of the animal, has been found to be 3.5-8.2 and 5.3-11.1 months respectively [2, 4].

For the reasons given above, it was decided to attempt to develop a method of modeling ALSP in guinea pigs with a short incubation period and to study some aspects of the pathogenesis of the disease.

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EXPERIMENTAL METHODS

Experiments were carried out on guinea pigs weighing 250-350 g. The material for infection consisted of a 10% brain suspension (in physiological saline) from patient D., who died from ALSP, and from the victim of an automobile accident. The animals were infected by the retrobulbar route in both eyes as follows: 0.25 ml of brain suspenion was injected at the boundary between the outer and middle thirds of the orbit, through the skin of the lower eyelid at a depth of 0.5-0.6 ml posteriorly and medially, beneath the eyeball. The procedure was repeated on two successive days. In this way 1 ml of infectious and control materials was injected toward the ganglion of the optic nerve. The experimental group consisted of 50 animals, the control group of 10. The animals' eyes were studied histologically by the methods described previously [6, 7]. The following staining methods were used for histological study of the animals' CNS: with hematoxylin and eosin, Nissl's method, and Viktorov's modification of the Klüver–Barrera method [2, 5]. To isolate the ALSP virus from the animals' brain primary cell cultures were prepared and tested as described previously [1, 2].

EXPERIMENTAL RESULTS

During the first week of observation the infected animals remained outwardly healthy, took their food eagerly, and their body weight increased on average by 15 g. Ophthalmoscopic examination of the fundus oculi revealed no pathological changes: it appeared grayish-brown and homogeneous, the optic disk was greyish-pink in color, with blurred, indistinct boundaries, and in the center of the optic nerve four to six blood vessels emerged, whose course could be clearly traced. In 70% of animals 1.5-2.5 weeks after infection ophthalmoscopic examination revealed the first signs of retinopathy, characterized by the appearance of black speckling of the fundus. In some animals the color of the fundus changed from greyish pink to greyish green. Clinical examination of the animals revealed some degree of adynamia, the hair began to lose its natural sheen and was untidy, and the animals gained no more in weight. Three weeks after infection the clinical features of the disease were more marked. Despite a good appetite and a normal diet, the animals became emaciated. The hair was untidy and began to fall out. The animals gave no resistance to examination. Their alerting reflex was absent. On examination of the fundus oculi at this period severe pathological changes were observed in the retina: it was edematous, the course of the retinal vessels in it was lost, and some of them appeared to be thinner than normal and empty. Pallor of the optic disk, which became pale gray in color, was observed. In the 4th week after infection, 27 of the 50 animals of the experimental group suddenly died, and 14 animals developed pareses and paralyses of the hind limbs. During the next 1-5 days these animals also died from asphyxia during disturbance of respiratory function of spinal type. Of the nine animals which remained alive, another four became ill and died 46-59 days after infection. The remaining five animals of the experimental group which survived and the 10 control animals were kept under observation for 6 months. During this time all the animals gained considerably in weight, and no clinical signs of the development of ALSP or pathological changes in the fundus oculi could be discovered. Animals for subsequent histological and virological investigation were killed by exsanguination.

During virological investigations of 20 animals which died with signs of the disease, ALSP virus was reisolated in all 20 cases. It will be noted that the virus was found not only in the brain and spinal cord, but also in the spleen, blood lymphocytes, and retina.

Histological investigations revealed pathological changes characteristic of ALSP in 45 guinea pigs of the experiment group which died. Virtually total death of motoneurons was observed in the CNS and in the anterior horns of the thoracic and cervical portions of the spinal cord of 27 animals, whereas in the remaining 18 animals 40-50% of neurons were preserved. In the neurons which remained lysis of the basophilic material and vacuolation of the cytoplasm were noted. Moderate proliferation of astroglial cells, with signs of satellitosis and neuronophagy, and a spongy state of the white matter of the anterior and lateral columns of the spinal cord also were found. The myelin sheaths of the dying axons were preserved in these cases. Focal disappearance of basket cells and a spongy state of the white matter were found in the cerebellum. Pathological changes in the cerebral cortex were less marked, and disappearance of pyramidal neurons and hyperchromatism of those which remained were noted in only 16 of the 45 animals which died. A spongy state of the white matter was observed even less frequently.

The changes described above were not found in the CNS of the five animals of the experimental group which did not develop the disease or of the 10 control animals. Histological study of the eyes of these animals showed that the retina preserved its layered structure throughout its extent, the 10 layers of the retina were completely differentiated, and the neurons of the retina (the layer of rods and cones, the layer of bipolar and ganglion cells) were intensely stained with eosin.

Meanwhile in animals with experimental ALSP the layers were most marked in the outer nuclear layer – the layer of rods and cones. Uniform reduction of thickness was observed almost to a single layer of both the outer and inner nuclear layer, with shrinking of the nuclear cells in 60% of the cases studied. Dystrophic changes in the retinal neurons (the layer of rods and cones and the inner nuclear layer) were most marked in its central portions, adjacent to the region of the optic nerve. Vacuolar degeneration was seen in the intraorbital part of the optic nerve.

In other tissues and in the visceral organs of the animals no pathological changes were found.

After retrobulbar injection of material containing ALSP virus, experimental amyotrophic leukospongiosis was thus produced in 90% of infected guinea pigs, as was confirmed by the results of clinical-morphological and virological investigations. This is the first time that the disease has been reproduced in 1-2 months without preliminary adaptation of the agent by prolonged passage in the same species of animal. It must be noted that the animals developed clinical signs of the disease similar to those observed when it was reproduced in squirrel monkeys and guinea pigs infected by other methods [2, 4].

In experimental scrapie and in JCD the length of the incubation period depends directly on the site of injection and the dose of infecting material. The principal site of reproduction of nonclassical viruses is nerve tissue. Injection of a large dose (1 ml) of the agent into the region of the optic nerve ganglion, i.e., in the immediate vicinity of the CNS, enabled the incubation period of experimental ALSP to be shortened. In addition, proof was obtained of reproduction of ALSP virus in the tissues of the retina and optic nerve, and this was accompanied by the development of a pathological process in these tissues. Thus the dynamics of development of a slow infection could be observed in an animal in the early stages, for only the retina was accessible for clinical examination and for analysis of the signs of development of the disease. Another important fact is that degenerative changes in the retina developed and could be detected before other features of the disease as described for ALSP.

Several investigators who have studied slow infections of the human and animal CNS have used various methods of infecting animals in their eyes [7, 8]. However, in no case was the incubation period of the disease shortened. This was evidently because of the small dose of material injected (0.01 ml). Infection was carried out, for example, by injecting the agent into the vitreous body, by applying it to the scarified cornea, and also by transplantation of the cornea of an affected animal to a healthy animal [7, 10]. The authors cited also observed that certain pathological processes developed on the retina. For instance, after injection of the agent of scrapie into the anterior chamber of the hamster's eye, a lesion of the retina was observed ophthalmoscopically 3 weeks before the end of the incubation period [7]. Histological changes found in the retina in mice with experimental JCD were similar to those described by us in guinea pigs with experimental ALSP [8].

In conclusion it must be pointed out that our method of modeling ALSP in guinea pigs, with a short incubation period, not only widens the scope of investigations aimed at studying the pathogenesis of this new form of slow infectious diseases of the human CNS, but it also affords definite prospects for the search for ways of early diagnosis of this disease. This particular model of ALSP can be used to study the possibility of specific etiotropic and pathogenetic chemotherapy, and to investigate the theoretical bases of the tactics and strategy of treatment and prevention of slow infections of the human CNS caused by nonclassical viruses. In addition, the results of these invetigations are evidence of involvement of the peripheral part of the visual system in the pathogenesis of the disease in the early stages of its development.

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LOCATION OF BINDING SITES FOR COBRA NEUROTOXIN AND SERUM IMMUNOGLOBULINS FROM MYASTHENIA GRAVIS PATIENTS IN RAT MUSCLES AND BRAIN

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Myasthenia is characterized by increased vascular fatigue and progressive muscular weakness, which can be temporarily relieved by injection of anticholinesterase drugs; in severe cases this disease causes death because of respiratory arrest [2, 3]. According to the generally accepted views, the immediate cause of the disturbances of myasthenia is an autoimmune attack on the nicotinic cholinergic receptors (NChR) of the myoneural junction, which reduces the number of functioning NChR. However, in myasthenia disturbances of the CNS are also observed. These disturbances are connected either with the primary pathological process in the brain and are mediated by dysfunction of the thymus, or with secondary hypoxia caused

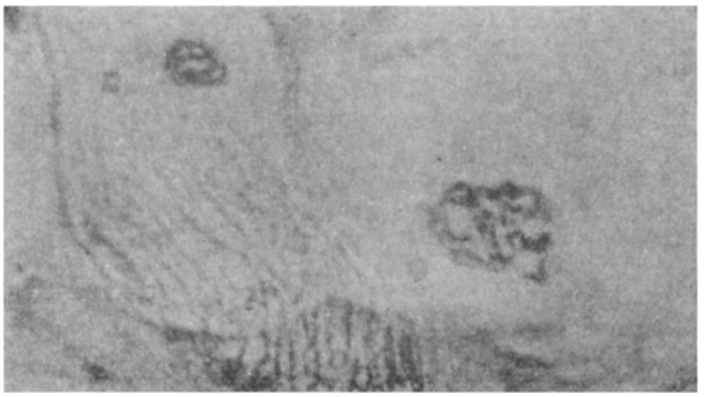

Fig. 1. Location of peroxidase reaction on incubation of sections of rat intercostal muscle with CT-HRP conjugate. Here and in Figs. 2 and 3: objective 40, ocular 10 ×.

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