ETIOLOGY OF AMYOTROPHIC LATERAL SCLEROSIS

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Mice were insensitive to intracerebral injection of brain tissue and CSF from patients with amyotrophic lateral sclerosis.

Two views are held on the etiology of amyotrophic lateral sclerosis (ALS): the hereditary and the toxico-infective. Most investigators [1, 4-6] consider that the disease is due to inherited factors, on the basis of its familial incidence, which is especially high on the island of Guam. The toxico-infective hypothesis of the nature of ALS is based entirely on the analysis of clinical and morphological data. Except papers by Zil'ber et al. [2, 3], no descriptions of experiments to confirm this view could be found in the accessible literature.

The object of this investigation was to study the effect of injecting material from patients with ALS into mice.

EXPERIMENTAL METHOD

The material containing the infective agent consisted of the CSF of patients taken at different stages of the disease and tissue suspensions prepared from affected parts of the brain of persons dying from ALS.

The CSF was tested either in the native state or concentrated by centrifugation at 50,000 rpm. In control tests the CSF was heated for 1 h at 60 and 95°C.

The pieces of brain for testing were ground with sand in a mortar for 15-20 min in the cold. The suspension was made in physiological saline in the ratio of 1:3 or 1:5, with the addition of 10% horse, rabbit, human, or other serum, and clarified by centrifugation at 1500-2000 rpm for 5-15 min.

Antibiotics (penicillin and streptomycin) were added to the supernatent in doses of 100-200 units/ml. The clarified fluid, after heating for 1 h at 60 and 95°C, was used as the control.

Mice (adult, young, and newborn) were infected intracerebrally in the region of the right fronto-parietal lobe in a dose of 0.01 ml for adults and 0.005 ml for newborn mice. Both noninbred mice and inbred mice of lines Af, C57BL/10Sn, CC57BR, and CC57W, obtained from the pure-line nursery of the N. F. Gamaleya Institute, were used.

The method of infecting the mice and the amount of material injected during subculture were indistinguishable from those described for primary inoculation of the animals. The dose for adult mice by subcutaneous injection was 0.5 ml and for newborn mice 0.1 ml. Centrifugation (Spinco ultracentrifuge) was carried out at 50,000-52,000 rpm for 1-2 h.

EXPERIMENTAL RESULTS

To determine the sensitivity of mice to the infective agent of ALS, two series of tests were performed: I) intracerebral inoculation of the animals and subcultures at short intervals (7-15 days) by the method used

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for encephalitis and other infectious diseases of the CNS; II) tests with prolonged observations on the animals by analogy with a chronic infectious disease of the CNS, scrapie in sheep [7].

In the tests of series I, noninbred mice were inoculated and subcultures carried out with CSF and clarified suspension of various parts of the brain from persons dying from ALS. Adult and young mice were inoculated intracerebrally, kept under observation for 7-22 days, and material was then taken from them for inoculation of other mice.

From two to 11 subcultures were carried out with material obtained from five patients; the brain tissue suspension from four patients was prepared from the medulla, and in one case (patient N.) the cerebellum and a mixture of the cerebral cortex and subcortex also was tested.

Observations on the mice inoculated with CSF from patient G. revealed changes in the behavior of one of the ten mice on the fourth day after the fourth subculture (the mouse moved about in a circle around the case or on the spot, with its head tilted to the right). Other mice were inoculated with material from the infected mouse (six successive subcultures). None of these mice showed any pathological symptoms.

One other case of an illness with neurological features was observed in an adult mouse inoculated with material after the sixth subculture of a suspension of the medulla. On the fourth day after inoculation the mouse developed paresis of the hind limbs. The mouse was reluctant to move, its hair stood on end, and it breathed with difficulty. Material for further subcultures was taken from this sick mouse, together with material from other, healthy, mice of the same group. No pathological symptoms were observed in the ten mice successively receiving material from the sick mouse or after subculture.

Subculture of brain material from patient G. by inoculation of adult mice thus revealed no persistent pathological features affecting the nervous system in the inoculated mice.

In all these experiments, slight pathological changes were detected in inoculated adult mice only in two cases, at the fourth and sixth subcultures. These changes did not appear after subsequent subcultures.

To weaken the natural resistance of the mice they were given three injections of cortisone (altogether 2.4 mg). After the second subculture, mice receiving a suspension of brain tissue with and without cortisone remained under observation for over 100 days. No pathological manifestations were found.

Usually in virus diseases of the CNS the disease in mice appears on the fourth to seventh day after intracerebral inoculation. Failure to reproduce the disease in mice in these present experiments may indicate either that these animals are insensitive to the agent of ALS or that the method itself was unsatisfactory.

The first suggestion to arise is that the short intervals decided upon between subcultures were not long enough for the pathogenetic agent of ALS to develop.

On the basis of this suggestion, blind subcultures of the material were eliminated and replaced by prolonged observations on animals receiving original material directly from patients. From 18 to 35 mice were used in each experiment. The CSF of patient Ts. was used to inoculate noninbred mice, which remained under observation for 5 months. No pathological changes were found in these mice.

The next tests were carried out on inbred mice. Newborn mice and mice aged 1-2 months were inoculated by the intracerebral route and kept under observation for 18-31 months.

No matter whether native or concentrated CSF was used for inoculation, no abnormality was observed in the behavior of the mice during observation for 23-31 months.

Similar results were obtained by intracerebral inoculation of adult and newborn mice with clarified suspension of brain tissue from persons dying from ALS when the animals were kept under observation for 18-29 months. There was likewise no difference in tests during which C57BL/10Sn mice aged 2 months were inoculated with a concentrated suspension of brain tissue and then remained under observation for 19 months.

Histopathological investigation of different parts of the brain showed no evidence of pathological changes in the experimental mice, even in those exhibiting pathological behavior.

The mice were thus insusceptible to the disease when attempts were made to reproduce it by injection of the CSF of patients with ALS or of suspensions of the brain tissue of persons dying from ALS.

LITERATURE CITED

1. S. N. Davidenkov, in: The Current State of the Principal Divisions of Neuropathology [in Russian], Moscow (1961), p. 97.
2. L. A. Zil'ber, Z. L. Baidakova, A. M. Gardash'yan, et al., Vopr. Virusol., No. 5, 520 (1962).
3. L. A. Zil'ber, Z. L. Baidakova, A. M. Gardash'yan, et al., Vestn. Akad. Med. Nauk SSSR, No. 6, 32 (1963).
4. R. E. Espinoza, M. M. Okihiro, and D. W. Mulder, Neurology (Minneapolis), 4, 355 (1954).
5. W. Haberlandt, Dtsch. Z. Nervenheilk., 180, 55 (1959).
6. L. T. Kurland and D. W. Mulder, Neurology (Minneapolis), 4, 355, 438 (1954); 5, 249 (1955).
7. J. H. Pattison, W. S. Gordon, and G. C. Millson, J. Comp. Path., 69, 300 (1959).