SEROLOGIC INVESTIGATIONS INTO THE ETIOLOGY OF AMYOTROPHIC LATERAL SCLEROSIS

A. M. Gardash'yan, T. I. Biryulina,
E. A. Lysak, T. L. Bunina, and V. S. Tsvetkov

UDC 616.832.522-02-078.3

The complement fixation, hemagglutination, and passive hemagglutination tests were used to investigate the blood serum of patients with amyotrophic lateral sclerosis, of monkeys with the same disease, and of immunized rabbits for the presence of antibodies. No antibodies could be detected by any of these tests.

Previous investigations [1, 2] showed that if rhesus monkeys are infected with the brains of persons dying from amyotrophic lateral sclerosis (ALS), they develop a disease very similar in its clinical and pathomorphological picture to ALS in man.

In this investigation an attempt was made to detect specific antibodies in the sera of patients with ALS, of infected monkeys, and of immunized rabbits. No data relating to the discovery of antibodies in chronic infections of the central nervous system could be found in the literature, with the exception of one paper [4] describing very briefly the negative results of a search for antibodies in sheep and goats with scrapie, and giving a list of the various serologic tests used.

EXPERIMENTAL

The cerebrospinal fluid and brain tissue from patients with, or dying from, ALS and the brain tissue of monkeys infected with the disease and subsequently sacrificed were used as antigens. The brain tissue of persons dying from trauma was used as the control. To prepare the antigen the brain tissue was ground with sand in a mortar in the cold for 20 min, and suspensions made in physiological saline in concentrations of 1:5 and 1:10 relative to weight of tissue. The suspension was clarified by centrifugation at 2000-4000, 8000, and 13,000 rpm for 10-30 min. The antigen was precipitated and concentrated by centrifugation on a "Spinco" ultracentrifuge at 50,000 rpm for 1-2 h. In addition, V. I. Tovarnitskii's method, used in 1960 [3] for the diagnosis of tick-borne encephalitis was used to prepare the antigen. For this purpose, the brain tissue was frozen and thawed 5 times, and then precipitated with methyl alcohol. This antigen, in which a certain degree of purification of the brain tissue is achieved by precipitation with methanol, was used in the complement fixation test. The culture fluids used as antigens were obtained by cultivation of a suspension of brain tissue from persons dying from ALS, on human embryonic diploid and kidney cells. Specimens of tissue culture fluids were obtained at different periods of cultivation of material from patients with ALS, mostly at intervals of 3-4 days. Tissue culture fluids also were obtained during subcultures.

Sera were obtained from patients with ALS, from monkey infected with the disease, and from rabbits immunized with brain suspensions and cerebrospinal fluid. The sera were heated to 56° for 30 min to destroy thermolabile inhibitors, and diluted with phosphate buffer, pH 7.2. To remove nonspecific agglutinins, the sera were treated with suspensions of the erythrocytes used in the test and a 25% solution of kaolin in borate buffer solution. For the actual test the original suspensions were diluted with borate buffer, pH 9.0, using a series of 1:2 dilutions.

Laboratory of Virology and Division of Virology and Immunology of Tumors, N. F. Gamaleya Institute of Epidemiology and Microbiology, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR O. V. Baroyan.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 69, No. 4, pp. 88-90, April, 1970. Original article submitted October 30, 1969.

Erythrocytes of geese, hens, and chickens of different ages (1, 2, 8, and 10 days and 4 months) were used in the test. The blood was collected into Alsever's solution, and dilutions were made up in phosphate buffer solution. Suspensions of erythrocytes were used in the following concentrations: 2, 1.5, 1, 0.5, and 0.33%. The following salt solutions were used for buffers of different acidities: 1.5 M NaCl, 0.5 M H_3BO_3, 1 N NaOH, 0.5 M Na_2HPO_4, and 1 M NaH_2PO_4.

The complement fixation test (CFT) was carried out in a volume of 0.6 ml with prolonged fixation in the cold, and with one dose of complement. The hemagglutination and passive hemagglutination test were carried out on Plexiglas slabs with wells. The results of the test were read 20 h later at room temperature.

EXPERIMENTAL RESULTS

CFT. To begin with, the sera of patients with ALS of different severity were tested. Suspensions of btain tissue from persons dying from ALS and antigens from the brain of monkeys suspected of having ALS, prepared in different ways, were used as antigens. The results of the CFT were negative, and no antibodies were found in the patients' sera. The search for antibodies in the blood of monkeys with or suspected of having ALS likewise yielded negative results.

Cerebrospinal fluid also was studied as antigen. This was done because of the suggestion that virus may possibly be secreted into the cerebrospinal fluid when present in brain cells. The results also were negative.

Attempts were next made to detect specific antibodies in the sera of rabbits immunized with a suspension of brain tissue and with cerebrospinal fluid of persons with or dying from ALS. Since negative sera gave nonspecific reactions, the sera were absorbed with extract of normal brain to remove tissue antibodies. In this case no difference was found between the sera of rabbits immunized with suspensions of normal brain or with brain of patients with ALS. This series of experiments thus also gave negative results.

The CFT with culture fluids used as antigen and with the sera of patients with ALS and also of the monkey Sobol', also gave negative results. Altogether 380 samples of culture fluids taken at different phases of cultivation of the material were tested. The CFT was carried out with 7 patients' sera and with one monkey's serum. Each serum was tested with 17-80 culture fluids.

Direct Hemagglutination Reaction. For this test a suspension of brain tissue from 3 persons dying from ALS, from 5 monkeys with the disease and subsequently sacrificeed and one control (suspensions of brain tissue from human patients and monkeys were each tested separately) were used in the test. Supernatants obtained after centrifugation under different conditions, and the residue obtained at 50,000 rpm, were used as antigens. The test was carried out at different pH values (6.4, 6.8, 7.2). Suspensions of goose and chicken erythrocytes were used. The results of all these tests were negative.

The direct hemagglutination test was carried out with samples of tissue culture fluids obtained at different times of cultivation of material from patients with ALS and from control subjects. Samples of tissue culture fluids obtained during subcultures also were used. Negative results were obtained, and the conclusion from this section of the work suggests that it is impossible to detect hemagglutinins in the liquid fraction of tissue cultures.

Passive Hemagglutination Reaction. For this test, rabbit sera obtained by immunization of rabbits with the cerebrospinal fluid of patients with ALS were used. The control serum was obtained from a patient with spinal arachnoiditis. Antigens were prepared from the brain tissue of 3 patients, a control patient, and 5 affected monkeys. After centrifugation of the brain tissue suspension at 2000 and 8000 rpm, the supernatant was used as antigen. Goose and chicken erythrocytes were used. The results of these tests were negative.

Thus, none of the tests used revealed antibodies in ALS. This conclusion agrees with the results of investigations to detect antibodies in other chronic infections of the central nervous system.

LITERATURE CITED

1. L. A. Zil'ber, Z. L. Baidakova, A. M. Gardash'yan, et al., Vopr. Virusol., No. 5, 520 (1962).
2. L. A. Zil'ber, Z. L. Baidakova, A. M. Gardash'yan, et al., Vestn. Akad. Med. Nauk SSSR, No. 6, 32 (1963).
3. M. K. Tyushnyakova, M. S. Zagromova, and Yu. V. Fedorov, Vopr. Virusol., No. 2, 204 (1960).
4. R. L. Chandler, Vet. Rec., 71, 58 (1959).