STUDY OF THE SEROLOGIC REACTIONS IN MICE WITH SCRAPIE

A. M. Gardash'yan, N. V. Nartsissov, and O. V. Bobkova

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Negative results of the complement fixation test were obtained with sera of animals immunized against scrapie and of mice infected with scrapie. The neutralization test in vivo did not reveal neutralizing antibodies against the agent of scrapie adapted to mice. No virus-neutralizing antibodies against scrapie could be found in the sera of patients with amyotrophic lateral sclerosis.

Scrapie is a chronic disease of sheep, which was transferred in 1959 to goats [7] and in 1961 to mice [6].

Serological work on scrapie began in 1959 when Chandler [5] attempted to discover antibodies in sheep infected naturally or experimentally with scrapie and in infected goats. The cerebrospinal fluid and saline extracts of the brain and spleen were tested. Ten different immunological tests were studied and negative results were obtained. Gibbs et al. [3] reported absence of antibodies in the CFT and neutralization test in mice infected with scrapie and immunized animals.

It was decided to study the CFT and neutralization test in vivo in mouse-adapted scrapie, and to compare them with the results of serologic investigations (negative) obtained in amyotrophic lateral sclerosis [1], a disease which probably belongs to the same group.

In the investigation described below an attempt was made to discover scrapie antibodies in mice by means of the complement fixation test (CFT) and the neutralization test in vivo.

EXPERIMENTAL METHOD

Scrapie virus of Compton strain (Bethesda, USA), adapted to Swiss albino mice of line NJH after three passages, was studied during passages through mice of different lines.

To obtain antisera guinea pigs and rats were used. The animals were immunized with the use of Freund's adjuvant. A lyophilized suspension of brain tissue from mice with scrapie and a suspension of the tissue of healthy mice of the same lines, prepared in the same way, were used as the antigen.

The resulting sera were stored in a lyophilized state until required, when they were diluted with distilled water to the original volume and heated on a water bath for 30 min at 56°C. The antisera were absorbed at 4°C for 18-20 h and then centrifuged at 1500 rpm for 20-30 min. The residues of brain tissue suspensions from mice with scrapie and from normal mice, obtained by centrifugation for 30 min at 1500 rpm, were used for absorption. A residue obtained by the addition of ethanol to a mouse brain tissue culture, obtained by trypsinization of the brain tissue of mice of the corresponding lines up to 1 month old, also was used.

The protein concentration in the supernatant from the lyophilized mouse brain tissue suspension, clarified by centrifugation at 1500 rpm, was determined by the biuret method. Dilutions of the antigen with

Laboratory of Virology, Department 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. 71, No. 6, pp. 67-69, June, 1971. Original article submitted November 28, 1970.

a protein concentration of 0.4-0.7 mg/ml, and also the transparent fluid obtained after boiling the brain tissue suspension for 3-5 min and clarification by centrifugation, were used for the tests. No protein could be detected by the biuret test in this supernatant.

Blood serum of guinea pigs, preserved by the method of Kalinin and Ginzburg [2], was used as the complement. The complement was titrated with a hemolytic system in the presence of antigens diluted to known titer.

The hemolytic system consisted of a 4% suspension of the residue of washed sheep's erythrocytes which was mixed with an equal volume of rabbit hemolytic serum diluted to a titer of 1 : 4.

The method of performing the CFT was as follows: 0.05 ml serum + 0.05 ml complement containing 1.2-1.5 hemolytic unit + 0.05 ml antigen, diluted to known titer. The phase of fixation lasted 18-20 h at 4° and 30 min at 37°. The results were recorded as between minus (−), corresponding to complete hemolysis, and 2+ and 3+ (partial inhibition of hemolysis), to 4+ (complete inhibition).

The neutralization test was carried out as follows. Serum was obtained from infected mice in different stages of the disease. A freshly prepared suspension of brain tissue from mice of line BALB/c after the 2nd and 5th passages was taken in dilutions of 10^{-2} and 10^{-3}. Experiments were carried out on BALB/c mice of both sexes, aged 3 weeks and 7 months.

The mixture of virus and serum was kept for 2 h at 37°C and then for 12 h at 4-12°C. The mice were inoculated intracerebrally in a dose of 0.01 ml.

For the crossed neutralization test, experiments were carried out with the sera of patients with amyotrophic lateral sclerosis, at various stages of the disease, and also of healthy persons. The experimental technique was the same as with the mouse sera except that the virus-containing suspension of mouse brain tissue in three of the eight experiments was obtained from the lyophilized state, in which it had been kept for between 1 and 16 months. Inbred mice (C57BL/10-H_2^d; C57BL/10Sn; C57BL/He; CC57W; BALB/c) also were used in the experiments. The results of the neutralization test were recorded as the number of mice developing the disease and the number dying as a result of it.

EXPERIMENTAL RESULTS

Sera of guinea pigs immunized with the brain of mice infected with scrapie virus, the sera of guinea pigs immunized with normal mouse brain tissue, and the sera of all guinea pigs before immunization were tested in the CFT. The titers of the sera in the CFT on the 7th day after the first cycle of immunization reached 1 : 160 and 1 : 320, on the 14th day 1 : 40-1 : 320, and on the 21st day 1 : 40-1 : 320. After the second cycle no further increase in antibody titers was observed. The titers of complement-fixing antibodies were almost identical when tested with antigens obtained from normal mouse brain tissue and brain tissue from mice infected with scrapie; the CFT with sera obtained before immunization of the animals was negative.

Suspensions of brain tissue, boiled for 3-5 min at 100°C, also were tested as antigens (the considerable thermostability of the agent of scrapie was allowed for). In these tests the bulky residue was removed by centrifugation. The supernatant, not containing protein (as shown by the biuret test), reacted in the CFT almost identically with the heated suspension in the same dilution, and there was no difference between the behavior of the suspensions from the brains of infected and normal mice.

The immunizing power of the suspensions of normal brain and brain infected with virus thus did not in general differ, suggesting that the antisera possessed antitissue activity. Participation of the virus in immunization may be extremely slight and not be detectable in the CFT. For this reason, an attempt was made to absorb the antisera with a residue of the tissue suspension of normal and infected brain and with denatured protein obtained from a mouse brain suspension by precipitation with ethanol. The absorbed serum reacted equally in the CFT with suspensions from brain infected with virus and normal brain.

The CFT with the sera of immunized rats likewise detected no difference in the titers of the antibodies when tested with antigens obtained from the brain tissues of mice infected with scrapie virus and from normal mice. The CFT with the sera of mice with scrapie in an advanced stage of the disease was negative. No neutralizing action of the serum obtained from mice infected with scrapie could be detected. All inoculated mice developed the disease and died or were sacrificed in a severely ill condition.

The neutralization test in vivo did not reveal virus-neutralizing antibodies against the agent of scrapie adapted to mice.

Amyotrophic lateral sclerosis and scrapie are similar in some features. No complement-fixing, agglutinating antibodies have been found in amyotrophic lateral sclerosis [1]; neither have complement-fixing or neutralizing antibodies been found in scrapie [3, 4]. It is possible that antibodies neutralizing the agent of scrapie could be found in the sera of patients with amyotrophic lateral sclerosis.

The test carried out failed to reveal any virus-neutralizing antibodies against scrapie in the sera of patients with amyotrophic lateral sclerosis.

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