CHANGES IN LEVELS OF BRAIN ANTIBODIES AND AUTONOMIC NERVOUS SYSTEM MEDIATORS IN THE COURSE OF EXPERIMENTAL ALLERGIC ENCEPHALOMYELITIS

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After sensitization of dogs with homologous brain tissue together with Freund's adjuvant changes were found in the levels of brain antibodies and complement and in the concentrations of adrenalin, noradrenalin, and acetylcholine in the blood. Changes in the function of the sympathoadrenal system and in the levels of the immunologic indices depended on the presence or absence of clinical manifestations of experimental allergic encephalomyelitis.

The mechanism of development of experimental allergic encephalomyelitis (EAE) and the role of serum anti-brain antibodies have not yet been finally settled [2], one of the main obstacles to the recognition of the pathogenetic role of these antibodies being the absence of correlation between their titers and the development of EAE [10].

The levels of brain antibodies and complement during the development of EAE were investigated. Because of the important role of the autonomic nervous system in the formation and course of allergic reactions [1, 4, 5, 7, 8, 11, 13], it was decided to study changes in the concentrations of certain mediators (adrenalin, noradrenalin, and acetylcholine) parallel with the immunologic tests.

EXPERIMENTAL METHOD

Experiments were carried out on 52 noninbred male dogs weighing 10-16 kg. EAE was produced by a single injection of homologous brain tissue together with Freund's adjuvant. The clinical and histological manifestations of EAE in dogs when this method of immunization is used have been described previously [3].

The disease developed in 31 dogs mainly on the 9th-11th day of sensitization; most animals died during the first days of development of clinical symptoms. Depending on the presence or absence of clinical manifestations, two groups of animals were distinguished: 1) dogs with clinical features of EAE, and 2) dogs without clinical features of EAE. Blood samples for testing were taken on the 3rd, 5th, 7th, and 9th days of sensitization and in the period of clinical manifestations of EAE. Catecholamines – adrenalin (A) and noradrenalin (NA) – were estimated by Men'shikov's trihydroxyindole fluorometric method [6], and acetylcholine (AC) by Hestrin's spectrophotometric method [12]. The complementary activity of the serum was determined by titration to 100% hemolysis; brain antibodies were detected by the complement consumption test as described by Chudomiel et al. [9], and their titer was expressed in 100% hemolysis units.

EXPERIMENTAL RESULTS

The titer of complement-fixing brain antibodies in the animals of group 1 in the incubation period was higher than that in the dogs of group 2 (Fig. 1). On the 3rd day of sensitization, for example, a signi-

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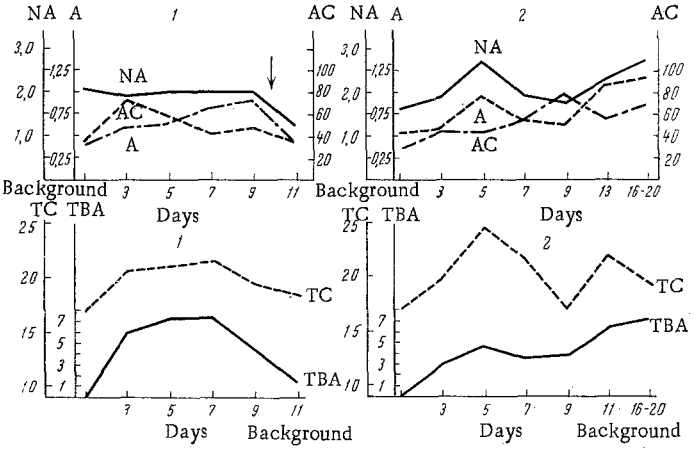

Fig. 1. Content of anti-brain antibodies, complement, catecholamines, and acetylcholine in the blood of dogs with EAE: 1) dogs of group 1 with clinical manifestations of EAE; 2) dogs of group 2 without clinical manifestations of EAE; A) adrenalin concentration (in μg/1000 ml); NA) noradrenalin concentration (in μg/1000 ml); AC) acetylcholine concentration (in μg/ml); TC) titer of complement (in 100% hemolysis units); TBA) titer of brain antibodies (in 100% hemolysis units) from results of complement test. Arrow indicates appearance of clinical symptons of EAE.

ficant difference was found (5.7 ± 0.8 and 3.0 ± 0.55; $P < 0.01$); on the 5th day there was a slight increase in the titer of antibodies (6.6 ± 1.22 and 4.7 ± 1.04; $P > 0.1$). On the 7th day the antibody titer in the animals of group 1 reached its maximum, namely 7.1 ± 0.76, whereas in the animals of group 2 it was only 3.6 ± 0.67 ($P < 0.05$). A few days before manifestations of the condition appeared the antibody titer fell (4.2 ± 0.94; $P < 0.05$ relative to the 7th day), and it reached its minimum in the stage of neurological symptoms (1.2 ± 0.39; $P < 0.001$ relative to the 7th day), while in animals with a severe form of the disease, which usually died during the first day of development of clinical symptoms, usually no antibodies whatsoever could be detected. In the dogs without clinical manifestations, the antibody levels were raised at these times.

The complement level before immunization was virtually the same in the two groups: 16.8 ± 1.8 in group 1 and 17.0 ± 1.3 in group 2. During the incubation period a tendency was observed for the complement titer to rise in all animals: on the 3rd day the complement level in the dogs of group 1 was 20.3 ± 1.7, compared with 20.0 ± 1.2 in group 2; on the 5th day the corresponding values were 21.0 ± 1.3 and 24.6 ± 1.6; on the 7th day 21.5 ± 1.9 and 21.6 ± 1.6. The differences between groups 1 and 2 were not significant, although on the 5th day the increase in the complement titer was greater in animals not subsequently developing the disease. On the 9th day the complement level fell: to 17.2 ± 1.1 ($P < 0.05$) in animals without neurological symptoms and to 18.3 ± 1.14 ($P > 0.05$) in animals developing the disease. Subsequently, in animals not developing the disease there was a tendency for the complement level to rise.

The dynamics of the concentration of mediators of the autonomic nervous system also differed in dogs developing and not developing the disease (Fig. 1).

The initial concentrations of adrenalin did not differ significantly in the two groups: 0.43 ± 0.08 μg/1000 ml in group 1 and 0.53 ± 0.07 μg/1000 ml in group 2 ($P > 0.05$). In the dogs of group 1, the adrenalin level reached its maximum on the 3rd day of sensitization (0.90 ± 0.20 μg/1000 ml), whereas in the dogs of group 2 it was virtually unchanged on the 3rd day (0.59 ± 0.08; $P > 0.05$). On the 5th day, a tendency was observed in group 1 for the adrenalin concentration to fall (0.73 ± 0.13 μg/1000 ml), whereas in group 2 at this period the adrenalin concentration reached its maximum (0.98 ± 0.12 μg/1000 ml; $P < 0.05$). By the 7th day, the adrenalin level in group 1 had fallen to its initial values (0.53 ± 0.05 μg/1000 ml), while in group 2, although it was lower than on the 5th day (0.70 ± 0.09 μg/1000 ml), it was higher than initially ($P < 0.05$). On the day of manifestation of the clinical picture, a tendency was noted for the adrenalin concentration to

rise (0.59 ±0.14 μg/1000 ml; P > 0.05), falling to the background level (0.42 ± 0.11 μg/1000 ml) by the 2nd-3rd day of the disease. In dogs without clinical manifestations of EAE the adrenalin concentration at these times was higher than normal: on the 9th day it was 0.67 ± 0.09 μg/1000 ml (P > 0.05), by the 11th day it had reached 1.11 ± 0.22 μg/1000 ml (P < 0.05), and it remained high until the end of the experiment.

The noradrenalin level in the animals of group 1 showed no significant change in the course of the incubation period. On the day of development of clinical symptoms the noradrenalin level likewise showed no change from its initial value (2.0 ± 0.49 μg/1000 ml, initially 2.11 ± 0.17 μg/1000 ml; P > 0.05), but by the 2nd or 3rd day of the disease it had fallen (1.30 ± 0.15 μg/1000 ml; P < 0.05). In the dogs of group 2 on the 5th day after immunization the noradrenalin concentration reached its maximum (2.77 ± 0.32 μg/1000 ml, initially 1.66 ± 0.24 μg/1000 ml; P < 0.05), after which it returned to its normal level, rising again later during sensitization: on the 13th day, for example, it was 2.35 ± 0.69 μg/1000 ml (P < 0.05), and on the 16th-20th day 2.75 ± 0.38 μg/1000 ml (P < 0.05).

The dynamics of acetylcholine concentration in the animals of both groups showed no substantial differences during the period of sensitization. A marked increase in concentration of this mediator was found on the 7th-9th day (up to 80.85 ± 6.14 μg/ml, initial concentration 32.52 ± 3.84 μg/ml; P < 0.001). On the day of appearance of clinical symptoms of EAE the acetylcholine level was high, falling to the initial level (33.37 ± 4.5 μg/ml; P > 0.05) by the 2nd-3rd day of the disease. In the dogs of group 2 the acetylcholine concentration fell a little in the later stages of sensitization, although its values still remained higher than initially (57.0 ± 5.45 μg/ml; P < 0.05).

Hence, whereas over a period of time significant differences were found in the catecholamine concentrations in animals developing and not developing the disease, the changes in the acetylcholine concentration in all dogs were similar in the incubation period. The general direction of the changes in level of these mediators suggests that in the initial period of sensitization with brain antigen, the tone of the sympathetic nervous system is predominant, while later the tone of the parasympathetic system becomes predominant.

The definite differences in titers of brain antibodies, especially the more marked reaction in the incubation period in animals subsequently developing the disease, indicate an important pathogenetic role of serum antibodies in EAE. From the point of view of the mechanism of development of allergic alteration of nerve tissue, an interesting feature is the decrease in antibody titers at the stage of clinical manifestations, due in all probability to fixation of the antibodies by nerve tissue.

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