DEVELOPMENT OF IMMUNOLOGICAL TOLERANCE TO BRAIN ANTIGENS AS A METHOD OF SPECIFIC PREVENTION AND TREATMENT OF EXPERIMENTAL ALLERGIC ENCEPHALOMYELITIS

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Experimental allergic encephalomyelitis was induced in guinea pigs by subcutaneous injections of rabbit brain with an adjuvant of the Freund type. Before or after injection of the encephalitogenic mixture, the animals received repeated injections of rabbit brain antigen without adjuvant but together with amobarbital. If given before sensitization, this treatment in most cases prevented the disease, and if given after sensitization it reduced the mortality.

The mechanisms of transplantation immunity and of autoallergic reactions have much in common. They are based on hypersensitivity of delayed type [1, 13]. Experimental allergic encephalomyelitis (EAE) is a typical autoallergic process. The frequency of production, duration of the course, and outcome of EAE in guinea pigs are dependent on the dose of BCG given as a component of the encephalitogenic mixture (EGM) [2]. The higher the dose of BCG, the more severe the course of the disease and the more likely it is to be fatal. Reports of prevention of EAE by reducing the activity of the immunogenetic system of the guinea pig by various nonspecific procedures have been reported in the literature. These procedures include administration of rabbit antilymphocytic serum (ALS) [14], antirabic γ-globulin, ACTH, and cortisone [5]. Tolerance to EAE has been obtained experimentally by injecting homologous brain tissue of adult animals into newborn rats or guinea pig embryos, followed by their sensitization after birth in the adult state [8, 12]. Cases of prevention of EAE in adults by repeated injections of large doses of antigen of both homologous and heterologous brain, either before sensitization or in the early stages (until the 7th day inclusive) thereafter, have also been described [6, 7, 10, 11]. The authors cited regarded the resistance to EAE as a form of Felton's "immunological paralysis."

In the investigation described below, the possibility of specific prevention and treatment of EAE in the later stages (after 7 days) of the incubation period was studied. Efimov's method [3, 4] was used to produce tolerance.

EXPERIMENTAL METHOD

Experiments were carried out on 108 noninbred guinea pigs weighing 400 ± 30 g, preliminarily quarantined 7-10 days. The EGM consisted of a 50% suspension of brain and spinal cord of adult rabbits with Freund's adjuvant in the ratio 1:1. The EGM was injected subcutaneously in a total dose of 1 ml made up of 5 simultaneous injections each of 0.2 ml of the mixture. Depending on the series of the experiments the dose of BCG was 10 or 5 mg to 1 ml of a mixture of lanoline and mineral oil. Tolerance was produced by repeated injections of supernatant of a rabbit brain homogenate (20 injections, each of 5 ml) in isotonic NaCl solution (1:5), after nonspecific depression of activity of the immunogenetic system by amobarbital (5 mg/100 g body weight, 20 injections). The character of the clinical picture of EAE was recorded:

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TABLE 1. Clinical Features of EAE in Guinea Pigs Receiving Prophylactic and Therapeutic Treatment

Series of experiments, dose of BCG	Group of animals	Character of treatment	Induction of tolerance (amobarbital + homogenate)	Time of injection of EGM	Number of animals	Outcome of experiment				P
						did not develop disease	lethal result	chronic form	recovery	
I, 5 mg	1	Control	–		10	–	9	–	1	–
	2	Preliminary formation of tolerance (prophylactic)	20 injections on alternate days	3 days after end of injections	9	5	2	–	2	< 0.01
	3	Production of tolerance after 11th day of incubation period (therapeutic)	20 daily injections	11 days before beginning injections	10	2	7	–	1	> 0.05
	4	Production of tolerance after development of initial clinical manifestations of the disease	20 daily injections	14 days before beginning injections	20	–	20	–	–	–
II, 2.5 mg	1	Control	–		24	–	22	2	–	–
	2	Prophylactic	13 daily injections	3 days after injection	13	12	1	–	–	< 0.01
	3	Therapeutic	20 daily injections	11 days before beginning injections	22	6	13	1	2	< 0.01

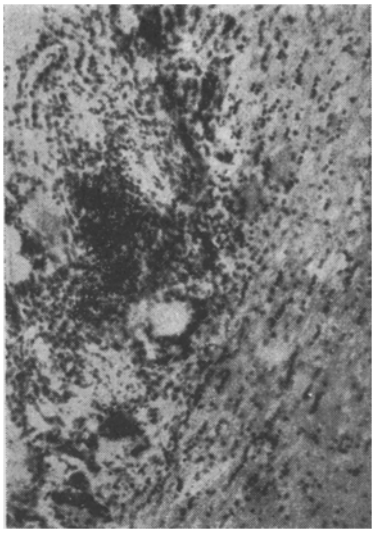

Fig. 1. Perivascular infiltration, predominantly by lymphocytes and histiocytes, in the pia mater. Hematoxylin-eosin, 56 ×.

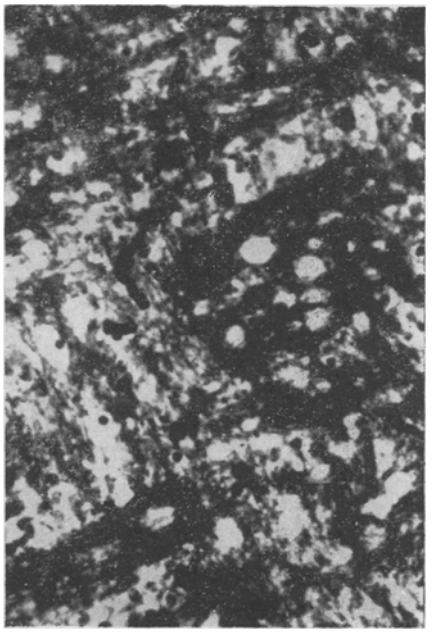

Fig. 2. Focal demyelination of some fibers in the medulla. Spielmeyer, 400 ×.

paralysis and paresis of the hind limbs and of the rectal sphincters. Meanwhile the brain and spinal cord of intact animals and of animals recovering and dying from the disease were examined histologically (stained with hematoxylin-eosin and by Spielmeyer's and Nissl's methods). Altogether material from 46 animals was investigated. Guinea pigs which survived till the end of the period of observation were sacrificed by rapid exsanguination. When the different groups were compared attention was concentrated on the survival rate of the animals, and the differences from the control were subjected to statistical analysis by Fisher's method [9].

EXPERIMENTAL RESULTS

The clinical results are shown in Table 1.

On histological investigation of the brain and spinal cord of 10 guinea pigs dying from EAE in series I (groups 1-4), characteristic changes were observed: massive infiltration around the pial and intracerebral blood vessels mainly by lymphocytes and histiocytes (Fig. 1). Demyelination was observed in the white matter of the spinal cord and in the subependymal zones, where it was due to inflamation and developed around the blood vessels or in the area of nodules of gliosis (Fig. 2). The nerve cells in the cerebral hemispheres, cerebellum, and spinal cord showed swelling, total tigrolysis, and neuronophagy.

In the four animals of this series which recovered after paralysis (total recovery of functions of the limbs and pelvic organs), the changes were mixed inflammatory and proliferative in character, and the degree of demyelination was slight. No gross degenerative changes were seen in the neurons. The time of recovery of the guinea pigs from the moment of appearance of clinical manifestations until their disappearance averaged 45 days.

Investigation of the brain from guinea pigs of this series which did not develop disease (2 from group 2, 2 from group 3) revealed in one case (group 3) the characteristic changes of EAE, although to only a slight degree. The other three guinea pigs showed no signs of EAE. Guinea pigs which recovered or which did not develop the disease were sacrificed on the 125th day after sensitization.

The results of histological investigation of the animals of series II on the whole agreed with those of series I. Three guinea pigs of this series with chronic paresis of the hind limbs were sacrificed on the 145th day after sensitization. They showed the characteristic demyelination and small foci of perivascular infiltration characteristic of EAE. The cells in various parts of the brain were mostly unchanged. Examination of the brain of 12 guinea pigs of this series which did not develop the disease (6 in group 3) showed in 10 cases none of the characteristic changes of EAE, while in 2 cases (group 3) the characteristic changes of this disease were slight.

The results show that if BCG was injected in a dose of 5 and 2.5 mg per animal along with the EGM, all the guinea pigs in the control groups developed the disease and, as a rule, died. The results of the histological investigation confirmed the clinical findings and largely corresponded to the severity of the course of the disease. In groups with prophylactic formation of tolerance, as a rule the guinea pigs remained healthy. In the experiments in which therapeutic treatment was given after the 11th day of the

incubation period, different results were obtained. A positive result to BCG in a dose of 2.5 mg per guinea pig was obtained only in the experiments of series II. The prophylactic effect obtained in these investigations by the use of an original method on the whole is in full agreement with the results published previously [6, 7]. However, the question of whether tolerance (immunological paralysis) can be obtained after sensitization is not yet settled [5, 7]. Positive results were obtained in the present experiments when therapeutic treatment was given after the 11th day of the incubation period. This is the first reported case of positive results having been obtained experimentally at this stage of the disease.

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