EFFECT OF ANTILYMPHOCYTIC SERA ON THE DEVELOPMENT OF EXPERIMENTAL ALLERGIC ENCEPHALOMYELITIS

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Experimental allergic encephalomyelitis (EAE) was induced in guinea pigs by sensitization with myelin of homologous or heterologous (rabbit) brain in Freund's complete adjuvant. In the early stages before or after sensitization the animals were given subcutaneous injections of antilymphocytic serum (ALS), obtained by immunizing rabbits with lymphocytes from guinea pig lymph nodes, daily for 3-6 days. The ALS had a marked inhibitory action on the development of neurological manifestations of EAE and histological changes, namely demyelination and perivascular cell infiltration in the lumbosacral and cervical segments of the spinal cord. ALS was most effective when injected starting from the third to fifth day before sensitization or from the day of induction of EAE. A later start of the injections (from the seventh or 12th day after sensitization) was accompanied by increased morbidity in the groups. A decrease in morbidity correlated with a decrease in the number of positive skin tests to intradermal injection of homologous myelin. The production of complement-fixing antibodies against heterologous myelin was not reduced in the animals receiving ALS. This points to the participation of cellular factors in the development of the pathological process.

KEY WORDS: experimental allergic encephalomyelitis; antilymphocytic serum; immunodepression.

To study the immunological factors concerned in the development of experimental allergic encephalomyelitis (EAE) it is interesting to use immunodepressive agents and, in particular, antilymphocytic sera (ALS). The inhibitory action of ALS on the development of EAE has been demonstrated by several workers [3, 8-10, 13]. However, some problems still remain unsolved, including the mechanisms (cellular or humoral) of the effect of ALS, the existence or otherwise of correlation between the dynamic of these parameters and the development of the disease, and also the nature of the morphological picture in the brain during inhibition of EAE by ALS.

The present investigation was carried out to study these problems.

EXPERIMENTAL METHOD

EAE was produced in adult guinea pigs weighing 350-400 g by inoculation with myelin from homologous or heterologous (rabbit) brain in Freund's complete adjuvant [4]. To obtain ALS, rabbits were immunized intravenously with lymphocytes from guinea pig lymph nodes on the 1st, 2nd, 3rd, 8th, 9th, 10th, 15th, 16th, and 17th days with a suspension containing $3 \cdot 10^8$ cells. Blood was obtained 7 days after the last injection. The sera were inactivated by heating and absorbed with guinea pig red cells until all hemagglutinins had completely disappeared. Activity of the sera was assessed by their ability to inhibit the development of cutaneous reactivity to tuberculin in guinea pigs sensitized 12 days before the skin tests with killed, dried, Mycobacterium tuberculosis cells in a dose of 5 mg per guinea pig. ALS was injected subcutaneously 24 h before the skin tests in a volume of 2 ml. To influence the development of EAE, the ALS was injected subcutaneously in a dose of 1.5 ml at different times relative to the injection of the encephalitogenic mixture (EGM). Antibodies in the sera of the

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TABLE 1. Effect of ALS on Development of EAE in Guinea Pigs

Serum	Scheme of injection of sera (days relative to induction of EAS)	Total dose of sera (in ml)	Number of animals in group		Number of animals with manifestations of EAE	
Antilymphocytic	from −5 to 0	9	7	26	0	1
	" −3 " +2	9	9		0	
	" 0 " +5	9	10		1	
	" +7 " +9	4,5	16	43	8	21
	" +7 " +11	7,5	12		4	
	" +12 " +16	7,5	15		9	
Normal rabbit	from −3 to +2	9	6		5	
	" +7 " +11	7,5	5		4	
No serum injected		—	19		16	

TABLE 2. Comparison of Inhibitory Action of ALS on Development of EAE and Skin Reactions to Myelin of Homologous Brain in Guinea Pigs

Scheme of injection of serum (days relative to injection of encephalitogenic mixture	Daily dose of ALS (in ml)	Total dose of serum (in ml)	Incidence of disease	Skin reactions for myelin (days after induction of EAE)		
				sixth day	tenth day	total
−2, −1, 0	3	9	2/12	0/6	1/6	1/12
	1,5	4,5	5/10	1/5	2/5	3/10
	0,75	2,25	7/10	2/5	3/5	5/10
Control group (no ALS injected)			9/10	3/5	4/5	7/10

Legend. Denominator gives number of animals in group; numerator gives number of animals with manifestations of EAE or with positive skin tests.

TABLE 3. Complement-Fixing Antibodies in Sera of Guinea Pigs Sensitized with Rabbit Brain Myelin

ALS	Number of sera tested				Number of positive reactions	
	total	with antibody titer				
		1:10–1:20	1:40–1:80	1:160–1:320	absolute	%
Injected	79	21	29	9	59	74,7
Not injected	24	11	7	1	19	79,2

immunized rabbits were determined in the complement fixation test in the cold [2], using a saline suspension of rabbit brain myelin as the antigen. To assess cellular reactivity of delayed type, skin tests were studied in response to injection of 1% myelin (0.1 ml) of homologous brain. Tests were regarded as positive if the diameter of the reactive zone was not less than 1 cm 24 h after the test. The lumbosacral and cervical segments of the spinal cord of guinea pigs sensitized with EGM and treated with ALS by effective schemes, or of control animals, were investigated microscopically by the methods of Marchi and Nissl. The brain was not investigated in these experiments, for as was shown previously [7], even in the most severe forms of EAE in guinea pigs, demyelination does not occur in the brain. The animals were killed at times between the 17th and 38th days after inoculation with EGS.

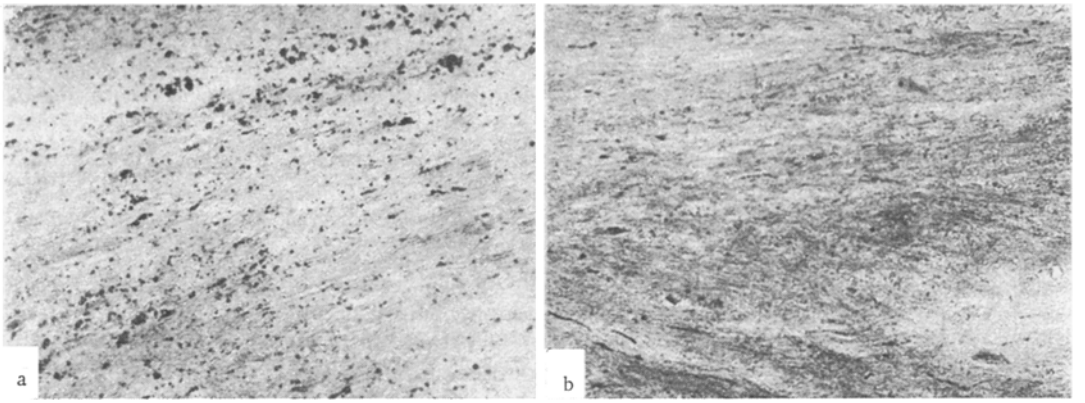

Fig. 1. Effect of ALS on fragmentation of myelin in EAE: a) severe fragmentation of myelin in lumbosacral segments of spinal cord of guinea pig with EAE (21st day after inoculation with myelin of homologous brain); b) absence of fragmentation of myelin in lumbosacral segments of spinal cord of guinea pig receiving ALS (27th day after inoculation with myelin of homologous brain). Impregnation by Marchi's method, 100 ×.

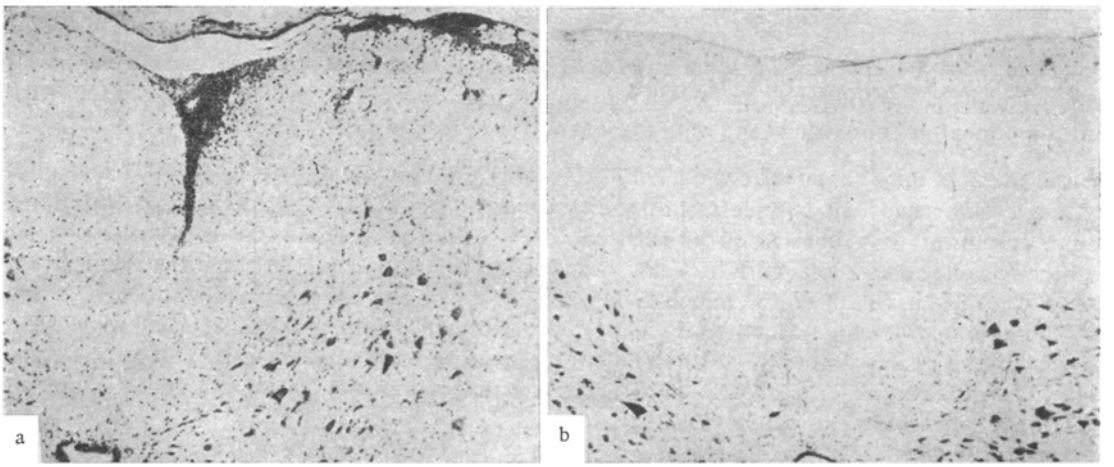

Fig. 2. Effect of ALS on inflammatory infiltration in EAE: a) inflammatory infiltration of leptomeninges in lumbosacral segment of spinal cord of guinea pig with EAE (21st day after inoculation with homologous brain myelin); b) absence of inflammatory infiltration in lumbosacral segments of spinal cord of guinea pig receiving ALS (27th day after inoculation with homologous brain myelin). Stained by Nissl's method, 72 ×.

EXPERIMENTAL RESULTS

As Table 1 shows, of the 26 guinea pigs injected with 1.5 ml ALS starting from the third or fifth day before induction of EAE or from the day of its induction in a total dose of 9 ml, only one developed the disease.

If the serum was injected after the seventh day of the latent period or later, neurological manifestation of EAE were observed in half of the guinea pigs.

As Table 2 shows, injection of ALS in a daily dose of 3 ml for 3 days before inoculation with the antigen protected 10 of the 12 animals against the disease in the absence of skin reactions on the sixth day, whereas on the 10th day a positive skin reaction was observed in only 1 of the 6 guinea pigs. After injection of 1.5 or 0.75 ml serum daily, 5 and 7 guinea pigs respectively of the 10 developed the disease later; the results of the skin test with myelin were positive in 3 of 5 guinea pigs. In the control group 9 to 10 guinea pigs developed the disease and in 7 of them the skin tests were positive.

It is interesting to compare the humoral reaction to sensitizing antigen in the animals of the control group and in guinea pigs treated with ALS. The results of determination of complement-fixing antibodies against myelin at different times after sensitization are shown in Table 3.

Morphological changes in the lumbosacral and, to a rather lesser degree, in the cervical segments of the spinal cord in 16 control animals (either recieving or not receiving normal serum) were manifested chiefly as demyelination, which was most marked along the longitudinal fissure and around the thin-walled blood vessels of the white matter (Fig. 1a). Extensive foci of fragmentation of myelin with the formation of small clumps and grains, intensely impregnated with silver by Marchi's method and appearing black, were observed in the white matter of the spinal cord. In areas of demyelination, marked proliferation and hypertrophy of oligodendrocytes were observed. Inflammatory infiltration was observed in the pia mater, mainly close to the spinal roots, and in the walls of the venules in the white matter; in these cases the endothelial and adventitial cells of the vessels were hypertrophied (Fig. 2). Changes in the spinal roots and ganglia were absent in 10 of the 12 paralyzed animals; in one guinea pig slight fragmentation of myelin was found in one spinal ganglion in the cervical portion of the spinal cord; in one animal demyelination in the lumbosacral portion and infiltration of the pia mater spread also to the spinal root. On the other hand, in only 2 of the 11 animals remaining healthy as a result of the injection of ALS were morphological changes expressed as localized areas of fragmentation of myelin observed in the lumbosacral portion of the spinal cord in regions along the anterior longitudinal fissure and in the white matter of the lateral columns. Meanwhile weak inflammatory infiltration was observed in the wall of some blood vessels. Such changes were absent in the remaining guinea pigs (Figs. 1 and 2).

Injection of ALS thus inhibited the development of EAE in guinea pigs and the maximal inhibitory effect was observed when ALS was injected early (a few days before inoculation with EGS). Inhibition of development of the pathological process in the central nervous system was confirmed by morphological data. Leibowitz et al. [10] found that, under similar experimental conditions, EAE did not develop in guinea pigs receiving ALS both during the first (0-8th) and subsequent (10th-18th) days after sensitization, but on the 54th day inflammatory infiltration of the meninges of the brain and spinal cord was observed. However, Leibowitz gives no information in his paper on demyelination, which is essential evidence of EAE.

As the present experiments showed, inhibition of the development of EAE by ALS correlated with inhibition of allergic skin reactions to myelin in the experimental animals. This correlation confirms the role of cellular factors in the development of the pathological process as a whole, but it is still not enough to be able to regard these factors as the agents directly responsible for demyelination. On the other hand, in animals receiving ALS the production of complement-fixing antibodies was preserved in the absence of development of the disease. Complement-fixing antibodies evidently do not play a direct pathogenetic role in EAE. The question of the effect of ALS on myelinotoxic antibodies, which have been described in EAE [5, 6, 14], still remains unanswered. The different effects of ALS on allergic skin reactions and on complement-fixing antibodies are in agreement with data in the literature [11, 12].

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