SPECIFICALLY REACTIVE CELLS IN EXPERIMENTAL ALLERGIC PERTUSSIS ENCEPHALOMYELITIS

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The dynamics of appearance of cells specifically reactive to brain antigen (SRC) in the regional lymph nodes and peripheral blood was studied in guinea pigs with experimental allergic pertussis encephalomyelitis (EAE). In the middle of the incubation period of EAE (the 6th-7th day) the largest number of SRC was found in the regional lymph nodes, and this was followed by a marked decline until the 9th day of sensitization, whereas the greatest number of SRC in the peripheral blood was observed at this time. In the period of clinically marked EAE (20th day) the number of SRC in the regional lymph nodes was increased, whereas the number in the peripheral blood was reduced. It is concluded that the SRC discovered may belong to the population of T-lymphocytes.

KEY WORDS: allergic encephalomyelitis; specifically reactive cells.

Convincing evidence has now been obtained that experimental allergic encephalomyelitis (EAE) is due to the activity of sensitized lymphocytes [3, 10, 12]. It has been shown, in particular, that T-cells are responsible for the development of EAE and of cellular immunity to the basic protein of myelin in sensitized animals [5, 10, 15].

The dynamics of appearance of specifically reactive lymphocytes in the regional lymph nodes and peripheral blood of guinea pigs during the development of pertussis EAE was studied by the fluorescent antibodies method.

EXPERIMENTAL METHOD

Experiments were carried out on noninbred guinea pigs weighing 250-300 g (132 animals altogether). The animals of group 1 were immunized with 0.2 ml of an encephalitogenic emulsion consisting of two parts of homologous brain homogenates and three parts of an oily mixture (mineral oil and Arlacel in the ratio of 8.5:1.5) with 2.5×10^{10} killed Bordetella pertussis cells of production strains Nos. 305 or 312, inactivated by 0.1% formalin. The dry bacterial mass contained 62.5×10^9 microbial cells/mg. The animals of group 2 were immunized with basic proteins (BP) of human brain myelin in a dose of 500 μg in oily adjuvant containing the same number of bacteria. The myelin BP was prepared by Kies' method [8]. The control group consisted of normal animals.

As the antigens in the experiments in vitro a 20% saline extract of bovine spinal cord and liver and BP of human brain myelin in a dose of 50 μg/ml were used.

A hyperimmune rabbit antibrain serum (HBRS), prepared by the method described previously [1], and serum against BP obtained by Hruby's method [7] were used. Dried luminescent donkey serum against rabbit globulin (LDRS), produced by the N. F. Gamaleya Institute of Epidemiology and Microbiology, Academy of Medical Sciences of the USSR, was used in a dilution of 1:16, after preliminary absorption with guinea pig liver powder for 1 h at room temperature (100 mg powder to 1 ml LDRS).

The popliteal, inguinal, and axillary lymph nodes were removed from animals killed at different times of immunization and placed in Petri dishes with Hanks's solution. The tissue of the lymph nodes was carefully loosened and the resulting cell suspension was washed with phosphate buffer (pH 7.2). Peripheral blood leuko-

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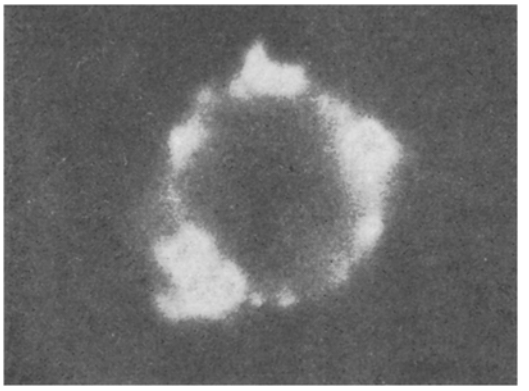

Fig. 1. Specific granular luminescence of peripheral blood lymphocytes from guinea pig with pertussis EAE. 450×.

cytes were obtained as described previously [2]. To isolate a pure suspension of lymphocytes, 4 ml of leukocytic suspension was poured in a layer above 9% dextran-250 in a test tube, after which the tube was centrifuged at 1000 rpm for 5-7 min. The layer of cells formed at the partition boundary was transferred to another tube and washed twice with phosphate buffer. The lymphocytes were resuspended in medium No. 199 with the addition of 20% fresh normal guinea pig serum in a final concentration of 1×10^6 cells/ml.

The reaction was set up as follows. A suspension of lymphocytes from regional lymph nodes or peripheral blood, in a volume of 0.8 ml, was incubated successively for 30 min with 0.2 ml of each of the ingredients, and washed off at each stage for 15 min with phosphate buffer: specific antigen (brain or BP) + specific antisera + LDRS. The cells washed after LDRS were examined in a luminescence microscope, where the number of specifically luminescent lymphocytes in 100 cells was counted.

EXPERIMENTAL RESULTS

Starting from the 14th day, guinea pigs immunized with homogenate of homologous brain tissue or myelin BP in pertussis adjuvant developed the characteristic clinical features of EAE, namely loss of weight, apathy, ataxia, pareses and, in some cases, severe paralysis of the lower limbs. The reactivity of regional lymph node lymphocytes was studied on the 3rd, 6th, 9th, 14th, 20th, and 35th days of sensitization of the animals. In preparations of the suspension of lymph node cells from animals immunized with brain homogenate, after incubation with brain antigen, HBRS, and LDRS, in ultraviolet light the lymphocytes gave characteristic greenish-yellow luminescence at the periphery of the cell, in the form of irregularly distributed small or large luminescent granules (Fig. 1). In cells stained with acridine orange the luminescent lymphocytes could be seen to be of the small and medium-sized types rather than large. The intensity of luminescence varied depending on the experimental conditions. Diffusely stained cells were disregarded.

Table 1 shows that by the 3rd day of immunization the number of specifically reactive cells (SRC) reached $15.6 \pm 1.3\%$, and on the 6th day it was more than twice as high. On the 9th day of immunization a sharp decrease in the number of SRC was observed ($13.0 \times 1.2\%$), followed by another increase toward the 20th day, i.e., during the period of most marked clinical features of the disease.

Investigation of SRC against myelin BP revealed a similar pattern: an increase in the number of SRC in the middle of the incubation period of the disease – on the 6th day, a sharp decrease in their number at the end of the incubation period – on the 9th day, and a further increase in the period of development of clinical manifestations of EAE (Table 1).

The number of SRC in the peripheral blood of animals immunized with brain tissue homogenate in pertussis adjuvant was determined on the 9th and 20th days of sensitization and revealed a different pattern. At the end of the incubation period of EAE (9th day) the number of cells with bright specific luminescence was at a maximum, with a mean value of $22.9 \pm 3.6\%$, whereas in the period of marked clinical features of the disease (on the 20th day of sensitization) the number of SRC was $3.9 \pm 0.85\%$. The percentage of SRC in the peripheral blood and lymph nodes of normal animals did not exceed three.

The significance of the results was determined by means of control experiments: a) incubation of lymphocytes of sensitized animals with LDRS; b) incubation of lymphocytes of sensitized animals with liver antigen,

TABLE 1. Number of SRC in Lymph Nodes of Guinea Pigs with EAE

Day of sensitization	Immunization with brain antigen		Immunization with myelin BP	
	no. of animals	no. of SRC, % (M ± m)	no. of animals	no. of SRC, % (M ± m)
3	8	15,6±1,3	5	3,4±0,4
6	13	35,7±2,1	5	20,0±3,6
9	13	13,0±1,2	5	4,5±0,11
14	14	27,7±3,1	4	15,8±0,98
20	14	33,7±2,8	5	14,0±0,45
35	7	23,4±2,5	—	—

then with HBRS and LDRS; c) incubation of lymphocytes of sensitized animals with brain antigen, followed by HBRS + unlabeled donkey serum against rabbit globulins + LDRS. No luminescence was observed in the first control, whereas in the other two, one or two weakly luminescent cells were sometimes seen.

The investigation thus revealed the dynamics of appearance of specifically reactive lymphocytes in the regional lymph nodes and peripheral blood of guinea pigs during the development of pertussis EAE. The largest number of SRC was observed in the regional lymph nodes in the middle of the incubation period of EAE (the 6th day), it fell sharply toward the end of that period (the 9th day), whereas in the peripheral blood at that time the number of SRC was at its highest. Divergence also was observed during the period of clinical manifestations of the disease – on the 20th day, when the number of SRC in the regional lymph nodes was increased, whereas in the peripheral blood it was reduced. The differences were statistically significant ($P < 0.01$).

It can be tentatively suggested that the SRC discovered in animals immunized with brain antigens could correspond, because of the dynamics of their appearance in the course of EAE, to T-cells, which can recirculate from the regional lymph nodes through the peripheral blood into the tissue of the CNS. A similar regular increase in the number of early (or active) T-cells in the peripheral blood before the beginning of clinical manifestations of EAE in guinea pigs immunized with myelin BP in Freund's complete adjuvant, and their sudden disappearance from the circulation at the height of the clinical symptoms shortly before death of the animals were revealed by the rosette-formation method [13]; the authors cited were able to show subsequently that these cells left the blood stream to enter the brain as the target organ, in which they formed foci of mononuclear infiltration [14]. Lymphocytes of normal guinea pigs are known to have on their surface receptors for myelin BP and to be capable of immune differentiation under the influence of BP bound with syngeneic macrophages [9, 11, 17]. After injection of the encephalitogenic emulsion into animals (except those of lines resistant to EAE) the number of lymphocytes with receptors for myelin BP increased, whereas the participation of antibodies with specificity for BP in the determination of SRC is ruled out [6]. There is another interesting problem. Although under normal conditions T-lymphocytes with receptors for brain antigen (myelin BP) exist in animals, there is also a regulatory mechanism which, in vivo, can prevent immune activation of the animal's own lymphocytes under the influence of brain autoantigens, with subsequent development of autoimmune reactions. Suppressor T-cells, responsible for tolerance to the animal's own antigens, constitute such a regulatory mechanism [3, 4, 10, 12, 16]. It can be tentatively suggested that in this particular experimental model injection of pertussis vaccine into the animals leads to collapse of tolerance, i.e., to inhibition of the function of the suppressor T-cells.

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