PATHOGENESIS OF EXPERIMENTAL ALLERGIC ENCEPHALOMYELITIS PRODUCED BY SENSITIZATION WITH Bordetella pertussis MIXED WITH BRAIN TISSUE

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UDC 616.832-002-02:616-056.3-092.9]-06

After subcutaneous injection of Bordetella pertussis cells mixed with brain tissue, early sensitization of cells of the regional lymph glands, peritoneal exudate, and spleen is observed. Increased sensitivity of cells of the lymphocyte-macrophage series is most marked in the incubation period of the disease. Sensitivity of the lymph gland cells to brain antigen is restored to normal by the end of the incubation period of the disease, and the sensitivity of exudate and spleen cells by the 21st-30th day after sensitization.

Cases of allergic reactions of the nervous system in response to injection of whooping cough vaccines have been described [8, 10, 18[. The results of investigations showing that Bordetella pertussis, under experimental conditions, like Mycobacterium tuberculosis, possesses adjuvant activity [2, 3, 17, 22].

During recent years increasing importance has been attached to increased sensitivity of delayed type in the pathogenesis of classical experimental allergic encephalomyelitis (EAE) [20, 22, 24, 25].

EXPERIMENTAL METHOD

Experiments were carried out on 240 noninbred guinea pigs weighing 300 g. Production strains Nos. 305 and 312 of B. pertussis, inactivated with 0.1% formalin for 24 h, were used. EAE was induced by Waksman's method [25]. Homologous nerve tissue was mixed in the ratio of 2 : 3 with adjuvant (Arlacel A 1.5 ml, mineral oil 8.5 ml, and B. pertussis cells 125 · 10^9/ml adjuvant). The encephalitogenic mixture was injected into the plantar pad in a dose of 0.2 ml per animals.

At various stages of sensitization of the animals the reactivity of cells from the regional lymph glands, peritoneal exudate, and spleen was investigated. To obtain the peritoneal exudate cells, 48 h before the experiment the animals received an intraperitoneal injection of 10 ml 2% peptone solution. The reactivity of the peritoneal exudate cells was studied by the method of inhibition of cell migration in capillary tubes [12]. The activity of the regional lymph gland cells was investigated by adding 10% of living lymph gland cells of sensitized animals to peritoneal exudate cells of intact animals [11]. The viability of the lymphocytes was determined by staining with trypan blue. To study the reactivity of the spleen cells, the method of plasma cultures was used [23]. Spleen explants measuring 1 mm^3 were prepared and placed in a coagulating mixture (a drop of homologous plasma with a drop of 50% chick embryonic extract).

The culture fluid consisted of medium No. 199 with antibiotics. The antigen was a 20% (initial concentration) saline extract of bovine spinal cord, frozen 3 times [13]. In the experiments in vitro, 0.01, 0.1, and 1% concentrations of brain antigen were used. At certain times of sensitization, additional tests were carried out on the spleen cells with a 5% concentration of brain antigen.

Laboratory of Allergy, I. I. Mechnikov Moscow Research Institute of Vaccines and Sera, Ministry of Health of the USSR. (Presented by Academician of the Academy of Medical Sciences of the USSR A. D. Ado.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 72, No. 9, pp. 73-77, September, 1971. Original article submitted November 23, 1970.

TABLE 1. Change in Migration Activity of Cells of Lymphocyte-macrophage Series at Various Stages of Sensitization of Guinea Pigs with B. pertussis Mixed with Brain Tissue (M±m)

Day of sensitization	Area of migration (in %)									
	lymph gland cells		peritoneal exudate cells				spleen cells			
	number of expts.	dose of antigen (in %)	number of expts.	dose of antigen (in %)			number of expts.	dose of antigen (in %)		
		1,0		1	0,1	0,01		1	0,1	0,01
Control	14	122±4,0	14	135±8,6	131±7,8	136±8,5	14	122±5,1	122±6,2	122±7,2
2nd *P*	6	98±7,8 <0,05		—	—	—	—	—	—	—
3rd *P*	9	88±6,0 <0,01	5	105±6,7 <0,05	102±6,0 <0,05	114±8,7 >0,05	12	92±4,3 <0,001	105±3,1 <0,001	115±5,7 >0,05
5th *P*	6	77±10,0 <0,05	6	55±10,0 <0,001	63±3,8 <0,002	95±7,0 <0,02	8	69±8,3 <0,1	98±10,6 <0,05	100±10,4 <0,05
7th *P*	5	99±10,0 >0,05	16	70±5,5 <0,001	81±5,6 <0,001	92±5,5 <0,001	11	91±9,6 <0,02	99±7,5 <0,05	116±7,5 >0,05
8th *P*	5	117±4,0 >0,05	—	—	—	—	—	—	—	—
9th P	—		6	53±6,6 <0,001	96±7,4 <0,02	122±5,3 >0,05	14	122±10,3 >0,05	120±9,3 >0,05	154±16,8 >0,05
11th P	—		11	87±5,6 <1,001	100±7,6 <0,05	107±6,6 <0,05	15	92±5,6 <0,001	82±4,5 <0,01	96±5,0 <0,01
21st *P*	—		12	99±7,8 <0,01	84±7,4 <0,001	109±7,6 <0,05	16	123±9,0 >0,05	118±8,6 >0,05	116±4,5 >0,05
30th *P*	—		12	118±3,3 <0,05	131±8,2 >0,05	123±9,0 >0,05	—	—	—	—

The cells were incubated at 37°C for 24 h in a special chamber [6]. After incubation, the migration zone was drawn with a drawing apparatus and measured with a planimeter. The ability of the cells to migrate was determined by the formula:

$$\frac{\text{Migration with antigen}}{\text{Migration without antigen}} \times 100 = \text{Migration of cells (in \%)}.$$

The results were subjected to statistical analysis [5].

EXPERIMENTAL RESULTS

Clinical manifestations of EAE appeared in the guinea pigs on the 9th-11th day after sensitization. The disease was manifested as paresis of the muscles of the hind limbs and sphincters, and disturbances of movement coordination.

Preliminary experiments showed that the addition of different concentrations (0.01, 0.1, and 1%) of brain antigen had a stimulating action on migration of the lymph gland, peritoneal exudate, and spleen cells of the nonimmunized (control) animals.

The results of the study of the reactivity of cells belonging to the lymphocyte-macrophage system in EAE induced by injection of B. pertussis cells mixed with brain tissue are given in Table 1 and Fig. 1.

The sensitivity of cells of the lymph glands draining the site of injection of the antigen began to rise on the 2nd day of sensitization. Inhibition of migration was most marked on the 5th day. On the 8th day, i.e., in the period preceding the appearance of clinical signs of the disease, migration of the lymph gland cells returned to normal. The principal control in this series was the reactivity of peritoneal exudate cells from intact animals with 10% living lymph gland cells of the same animals.

Migration of the peritoneal exudate cells was inhibited by brain antigen from the 3rd day after sensitization. Increased sensitivity to brain antigen was most marked in the incubation period, on the 5th-7th day after sensitization. Starting from the 9th-11th day, the sensitivity of the cells was slightly reduced, but it did not return to normal while marked manifestations of the disease persisted until the 30th day after sensitization.

Increased sensitivity of the spleen cells to the action of brain antigen also appeared on the 3rd day after sensitization and was observed until the 7th day of sensitization. On the 9th day, i.e., at the time of appearance of clinical manifestations of the disease, the sensitivity of the spleen cells to brain antigen returned to normal. Additional experiments with a 5% concentration of brain antigen were carried out at the same time, and no stimulating effect of the antigen on migration of the spleen cells of the intact animal was found. On the 9th day of sensitization, 5% brain antigen had a stimulating effect on the migration of these cells. On the 11th day, a further inhibition of migration of the spleen cells by brain antigen was observed. The normal sensitivity of the spleen cells was restored by the 21st-30th day after sensitization. Bovine kidney antigen and bovine serum both had a stimulating action on migration of the tested cells from both intact and immunized animals.

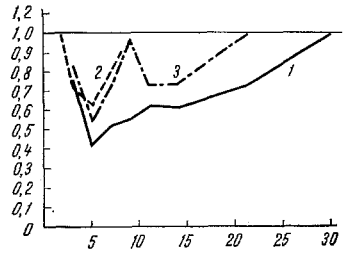

Fig. 1. Sensitization of cells of lymphocyte-macrophage series in nerve tissue at different stages of EAE: 1) peritoneal exudate cells; 2) regional lymph gland cells; 3) spleen cells. Abscissa, day after sensitization; ordinate, ratio between cytotoxic index [20] in experimental series and cytotoxic index under normal conditions.

The results thus indicate that if this method of administration of the encephalitogenic mixture is used, optimal conditions are created for sensitization of regional lymph gland cells. Already sensitized lymphocytes from these glands are rapidly disseminated throughout the body, and this explains the inhibition of migration of the exudate and spleen cells as early as the 3rd day of sensitization. This fact of early "involvement" of cells of the regional lymph glands observed in the present investigations is in agreement with results obtained by other workers [7, 13]. According to the hypotheses of Bartford and Kelly [9], macrophages play the role of indicator of the degree of lymphocyte sensitization.

Sensitized lymphocytes undoubtedly penetrate through the blood-brain barrier into nervous tissue and injure it, thus giving rise to histologically demonstrable vascular lesions characteristic of encephalitis [2, 3].

In classical EAE, inhibition of migration of the regional lymph gland cells [4], peritoneal exudate cells [4, 13, 15, 16], and spleen cells is observed under the influence of brain antigens. However, in EAE induced by injection of homologous brain mixed with B. pertussis cells suspended in an oily mixture, the change in reactivity of cells of the lymphocyte-macrophage series take place earlier – at the beginning of the incubation period of the disease. The regular increase in sensitivity of cells of the lymphocyte-macrophage series of brain antigen in the incubation period of the disease thus is evidence that a leading role in the pathogenesis of EAE induced by injection of homologous brain mixed with B. pertussis cells suspended in an oily mixture is played by allergic reactions of delayed type.

Bearing in mind Kanchurin's [1] hypothesis of the autoallergic nature of postvaccinal encephalomyelitis, the results of the present investigation may be considered to be of value to the investigation of side effects arising in response to injection of various whooping cough vaccines.

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