ROLE OF CELLULAR FACTORS IN THE PATHOGENESIS OF EXPERIMENTAL ALLERGIC ENCEPHALOMYELITIS

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After subcutaneous injection of guinea pigs with homologous brain tissue together with Freund's adjuvant a change in the sensitivity of lymphocytes and macrophages to brain antigens is observed. At the beginning of the incubation period of the disease the sensitivity of cells of the regional lymph glands is increased. After the middle of the incubation period until death of the animals increased sensitivity of cells of the peritoneal exudate is found. Sensitivity of the spleen cells also changes after the middle of the incubation period but returns to normal with the development of clinical manifestations of the disease.

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According to results obtained by most investigators, experimental allergic encephalomyelitis is based on mechanisms of increased sensitivity of delayed type [10, 12]. Transfer of the disease by cells [1, 3, 9], together with the recently published fact that migration of cells of the peritoneal exudate of guinea pigs with encephalomyelitis is inhibited by the action of brain antigen [6], show conclusively that cells in fact can play an important role in the pathogenesis of this disease. However, the study of changes in cell reactivity during the development of experimental allergic encephalomyelitis is far from complete.

In this investigation we studied the reactivity of cells from lymph glands, peritoneal exudate, and the spleen at various stages of the disease.

EXPERIMENTAL METHOD

Experiments were carried out on 183 noninbred male guinea pigs weighing 250-350 g from which cells of the regional lymph glands, peritoneal exudate, and spleen were taken at various times after sensitization. Homologous nerve tissue was added to the adjuvant mixture (1.5 ml Arlacell A, 8.5 ml mineral oil, 5 mg/ml BCG) in the ratio 2:3. The encephalitogenic mixture was injected into the plantar pads in a dose of 0.2 ml per guinea pig. Control animals received adjuvant without brain tissue and incomplete adjuvant with brain tissue. Cells of the peritoneal exudate were obtained after irritation of the peritoneal cavity with 2% peptone solution 48 h before the experiment [2]. Changes in reactivity of cells of the peritoneal exudate and lymph glands were studied by the method of inhibition of migration activity in capillary tubes [4, 5]. Reactivity of the spleen cells was detected by the plasma culture method [11]. The culture medium consisted of medium No. 199 with antibiotics. The antigen was a 20% extract (wet weight) of bovine spinal cord containing 5.3 mg total nitrogen per ml by the Kjeldahl method [6]. Antigen was used in the experiments in concentrations of 0.01-5%. Since the lymphocytes themselves are unable to migrate in cultures, reactivity of the lymph gland cells was studied by adding 10% of living cells from the regional lymph glands draining the site of injection of the antigen to cells of the peritoneal exudate from nonimmune animals. The area of cell migration was measured with a planimeter [2]. The experimental results were subjected to statistical analysis [8].

EXPERIMENTAL RESULTS

An increase in sensitivity of cells of the regional lymph glands draining the site of injection of the antigen began to appear on the 3rd day after sensitization. This was shown by a marked inhibition of migration of the peritoneal exudate cells of nonimmune animals with 10% of cells from lymph glands of sensitized

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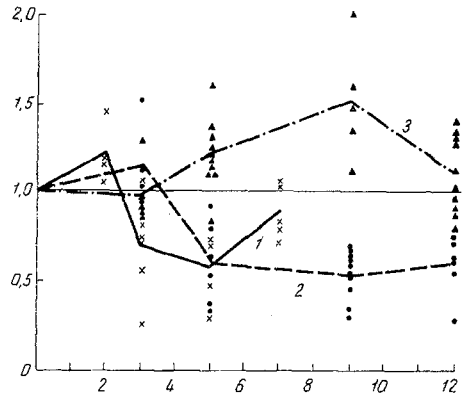

Fig. 1. Sensitization of lymphocytes and macrophages to nerve tissue at various stages of experimental allergic encephalomyelitis. 1) Cells of regional lymph glands; 2) cells of peritoneal exudate; 3) spleen cells. Abscissa, days after sensitization; ordinate, ratio between experimental and normal values of cytotoxic index.

animals after incubation for 18-24 h with brain antigen. Increased sensitivity of the cells persisted until the 5th day, returning to normal by the 7th day of sensitization, i.e., before the development of clinical manifestations. The main control for the experiments was the change in migration of the same peritoneal exudate cells with 10% of lymph gland cells from intact animals.

Migration of peritoneal exudate cells taken from sensitized animals began to be inhibited on the 5th day. Increased sensitivity of these cells was marked not only in the period before development of clinical manifestations, but also at the height of the disease and until death of the animals on the 12th-14th day after sensitization. Similar inhibition of migration was observed under the influence of all doses of antigen used in the experiment.

Experiments to study the reactivity of spleen cells using the method of plasma cultures showed changes in the sensitivity of the cells from the 5th day after sensitization. At this time the area of migration of the spleen cells of sensitized animals was increased by the action of 1% brain antigen compared with the area of migration of cells of nonimmune animals. The same picture was observed on the 9th day of the disease. By the 12th day this type of sensitization was no longer apparent and the reactivity of the spleen cells was indistinguishable from that on intact animals. This shows the need for a more detailed study of the role of the spleen in this method of sensitization. Experiments were carried out with a 5% concentration of antigen which had a toxic action on the spleen cells of nonimmune animals. On the 5th-9th day after sensitization the spleen cells were found to be resistant to this toxic concentration of antigen. In the period of development of clinical manifestations of the disease, the reaction of the spleen cells to 5% brain antigen was no longer different from the reaction of nonimmune animals.

The results thus indicate that in the course of experimental allergic encephalomyelitis a change is first observed in the sensitivity of cells of the regional lymph glands draining the site of injection of antigen. This agrees with results obtained by other workers who found that the cells of the regional lymph glands are the first to be involved in experimental autoimmune processes [7]. The period of increased sensitivity of the lymph gland cells of sensitized animals (3rd-5th day) coincided with the incubation period of the disease. Sensitivity of these cells returns to normal before the development of clinical manifestations.

At the height of modified sensitivity of cells of the regional lymph glands (Fig. 1), sensitivity of the cells of the peritoneal exudate, with its very heterogeneous cell composition, began to increase. According to one hypothesis [4], under the influence of a specific antigen sensitized lymphocytes secrete an agent inhibiting the migratory power of macrophages. These authors consider that the migration power of the macrophages as such is not modified by the action of the antigen, but the macrophages are an indicator of the degree of sensitization of the lymphocytes.

Increased sensitivity of the peritoneal exudate cells was considerable in degree and persisted for a long time from the middle of the incubation period until death of the animals with marked clinical manifestations of experimental allergic encephalomyelitis.

The role of the spleen in connection with the results described above have not yet been finally elucidated. However, the experiments showed that a change in sensitivity of its cells was observed by the 5th day of sensitization. This was shown both by increased reactivity of the spleen cells and by their resistance to brain antigen in the incubation period of the disease. At the height of development of clinical manifestations the reactivity of the spleen cells of the sensitized animals was indistinguishable from the reactivity of the same cells taken from nonimmune animals. The reason for these findings is not yet clear. Perhaps because of the complex antigenic composition of brain tissue, different antigens act on the same cells, thus producing a dynamic pattern of changes in sensitivity. On the other hand, allowance must be made also for the very heterogeneous cell composition of the spleen, when different cells act as targets for antigenic action.

The results obtained indicate a regular pattern of development of increased sensitivity of delayed type during the course of experimental allergic encephalomyelitis. In sensitization by this method a leading role is played by sensitization of cells of the lymph glands draining the site of injection. Later migration of the sensitized cells takes place and this causes injury to the central nervous system in experimental allergic encephalomyelitis.

LITERATURE CITED

1. A. Kh. Kanchurin, The Pathogenesis of Allergic (Demyelinizing) Diseases of the Nervous System [in Russian], Doctorate Dissertation, Moscow (1963).
2. O. V. Protasova, I. M. Pozina, O. S. Sergeeva, et al., Lab. Delo, No. 11, 667 (1966).
3. T. M. Tsaregorodtseva, Vestn. Akad. Med. Nauk SSSR, No. 3, 78 (1964).
4. B. R. Bloom and B. Bennett, Science, 153, 80 (1966).
5. J. R. David, H. S. Lawrence, and L. Thomas, J. Immunol., 93, 264 (1964).
6. J. R. David and P. Y. Paterson, J. Exp. Med., 122, 1161 (1965).
7. J. Freund and M. M. Lipton, J. Immunol., 75, 454 (1955).
8. D. H. Heilman and H. W. Bernton, Am. Rev. Resp. Dis., 84, 862 (1961).
9. P. Y. Paterson, J. Exp. Med., 111, 119 (1960).
10. P. Y. Paterson, Advances in Immunology, 5, 131 (1966).
11. J. Svejcar and J. Johanovsky, Z. Immun.-Forsch., 122, 398 (1961).
12. J. W. Uhr, Physiol. Rev., 46, 359 (1966).