ROLE OF CELLULAR FACTORS IN PATHOGENESIS OF NERVOUS COMPLICATIONS OF TYPHOID VACCINATION

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Following intradermal injection of typhoid bacteria in Freund's incomplete adjuvant, without the addition of brain tissue, into guinea pigs,the sensitivity of cells of the regional lymph glands and peritoneal exudate to brain antigens was increased even in the early periods, and it remained high throughout the period of observation. Spleen cells exhibited increased resistance to brain antigens.

The Soviet and Western literature contains isolated reports of the encephalitogenic activity of typhoid bacteria [2, 3, 6, 10]. However, injection of typhoid bacteria along with brain tissue, during the reproduction of experimental allergic encephalomyelitis (EAE) is a model which is remote from clinical practice. Experimental data [2, 3] indicating a possible encephalitogenic action of typhoid bacteria when injected without brain tissue are therefore interesting.

The object of this investigation was to study the role of various cell factors (cells of regional lymph glands, peritoneal exudate, and spleen) in the development of EAE caused by injection of typhoid bacteria alone without the addition of brain tissue.

EXPERIMENTAL METHOD

Encephalitogenic properties of typhoid bacteria of strain $Ty_2$4446 were investigated. A culture of the microorganisms, heated for 1.5 h at 56°, was suspended in sterile adjuvant mixture consisting of 8.5 parts mineral oil and 1.5 parts Arlacel A (5 mg bacteria/ml). The adjuvant mixture was injected into the plantar pads of all four limbs in a dose of 0.2 ml per guinea pig. The methods of obtaining suspensions of cells from peritoneal exudate and lymph glands and of preparation of spleen explants were described by the writers previously [4]. Medium No. 199 with antibiotics but without serum was used as culture medium. The antigen was a 20% saline extract (per moist weight) of bovine spinal cord [7] containing 5 mg/ml total nitrogen. As a result of preliminary titration of the spinal cord extract on cells of nonimmunized animals, it was discovered to have a toxic effect in concentrations of 5% and above. For this reason, in all the experiments, concentrations of spinal cord extracts of 1, 0.1, and 0.01% were used, although in the experiments with spleen explants in some cases a 5% concentration of spinal cord extract also was used. To discover whether nonspecific sensitization of the cells could be produced, the following ingredients were used: 20% saline extract of bovine kidney (2.28 mg/ml total nitrogen), concentrated purified diphtheria toxoid, batch 127 (235 1F/ml, degree of purity 1477 1F/1 mg protein nitrogen), produced at the Mechnikov Research Institute of Vaccines and Sera, and a 0.1% heated bovine serum without preservative (batch 230568). Titration of kidney extract on cells of nonimmunized animals showed that in 0.01% concentration it had no toxic action, so that it was used in this concentration. Experiments were carried out on 246 noninbred guinea pigs weighing 320 ±50 g. On the 3rd, 5th, 9th, 12th, 20th, 30th, and 45th days of sensitization, peritoneal exudate, regional lymph glands, and the spleen were removed from the animals under sterile conditions.

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Chambers containing the cells were incubated at 37° for 24 h, after which the results were read. The outlines of the migration zone were traced by means of a drawing apparatus. The area of migration was measured with a planimeter. During analysis of the experiments, the mean area of migration for each dilution of antigen from 4 capillary tubes or explants was considered. All data were calculated by the formula:

$$\frac{\text{mean area of migration with antigen}}{\text{mean area of migration without antigen}} \times 100\%$$

For convenience of graphic presentation of the results, the cytotoxic index was used [8]. All the results were subjected to statistical analysis [5].

EXPERIMENTAL RESULTS

Migration of regional lymph gland cells was inhibited by 1% extract of spinal cord tissue starting from the 3rd day of sensitization. The mean percentage of migration at this time was 79.8% compared with 125.8% migration of lymph gland cells of nonimmune animals. A similar increase in sensitivity of the regional lymph gland cells was also observed on the 5th day after injection of typhoid bacteria. By the 9th day of sensitization, the sensitivity of the lymph gland cells was close to normal, after which it again increased by the 12th day. By the end of the 3rd week the sensitivity of the cells was the same as that in normal animals. On the 30th and 45th days of sensitization, sensitivity increased again, this time to the highest level (migration on the 30th day 66.4%).

Migration of peritoneal exudate cells was inhibited starting from the 3rd day of sensitization by all three concentrations of spinal cord antigen. By the 5th day the inhibition of migration of the cells was statistically significant only under the influence of the lowest (0.01%) concentration of antigen. On the 9th, 12th, and 20th days of sensitization, inhibition of functional activity of peritoneal exudate cells was most marked, as reflected in the considerable inhibition of migration of the cells when incubated with all doses of spinal cord antigen used in the experiment. By the 30th day, only the two lowest (0.1 and 0.01%) concentrations of antigen had an inhibitory effect. By the 45th day of observation the sensitivity of the peritoneal exudate cells to spinal cord antigen had returned to normal.

Changes in the sensitivity of spleen cells to spinal cord antigen were observed on the 3rd day of sensitization. At this time considerable stimulation of the migratory activity of the cells from the explants was observed under the influence of 1% spinal cord extract, and this effect was specific in character because it was not observed under the influence of diphtheria toxoid or of kidney extract and 0.1% bovine serum. In the period from the 5th to the 12th day of sensitization, sensitivity of the spleen cells was indistinguishable from sensitivity of the cells of nonimmunized animals. On the 20th and 30th days, specific stimulation of the migratory activity of the spleen cells was again observed (124.4% compared with a normal value of 103%). A 5% concentration of spinal cord antigen, which had a marked toxic effect on spleen cells of nonimmunized animals (inhibition of migration of 62%) not only had no toxic action on the spleen cells of the immunized animals on the 3rd, 20th, and 30th days, but it revealed their increased resistance to spinal cord antigen. By the 45th day of observation the sensitivity of the spleen cells of the sensitized animals was normal. The absence of inhibition and stimulation of migratory activity of the cells under the influence of bovine kidney extracts, diphtheria toxoid, and bovine serum ruled out any possibility of nonspecific sensitization of the investigated cell systems.

These results indicate that following injection of typhoid bacteria without brain tissue into guinea pigs, a specific increase in the sensitivity of cells of the lymphocyte – macrophage series to brain antigens is observed. These findings can be explained on the basis of the assumption that typhoid bacteria evoke primary injury to nerve tissue with the formation of complex antigens (microoorganisms+nerve tissue). As a result of sensitization of the recipient to the complex antigens, autoallergic chain injuries to nerve tissue develops, in the form of disseminated encephalomyelitis.

These results indicate an active role of the lymphocyte–macrophage system in realization of the encephalitogenic activity of typhoid bacteria. Early sensitization of cells of the regional lymph glands and peritoneal exudate (3rd day) to nerve tissue antigens was found and was specific in character.

Throughout 45 days of observation, the sensitivity of lymph gland cells to spinal cord antigen was increased three times, each time returning to normal. Against the background of these intermittent changes in sensitivity of the lymph gland cells, the sensitivity of the peritoneal exudate cells also changed. This intermittent type of sensitization evidently accounts for the character of the disease, which is marked by an indolent and protracted course [2, 3].

The sensitivity of the spleen cells in these experiments changed only in the direction of increased resistance to spinal cord antigen. Consequently, the function of the spleen as a trap to catch sensitized lymphocytes [9] could take place at these times sufficiently actively. This may account for the long incubation period (up to 30 days) and the benign outcome of the process [3], in agreement with clinical observations. Such a change in the sensitivity of the spleen cells can be explained on the assumption that nerve tissue, a component of the complex antigens formed during the process, also contains neurotoxic substances. The possibility is not ruled out that the increased resistance of the spleen cells is formed in response to this toxic factor.

By the 45th day of sensitization the increased resistance of the spleen cells disappeared, and this was accompanied by a fresh increase in sensitivity of the lymph gland cells. It was against this background that the first clinical signs of damage to the central nervous system appeared.

The results obtained provide experimental proof of the earlier hypothesis [1], and they suggest that complications following typhoid vaccination, such as encephalomyelitis in man, are based on autoallergic pathological changes in the nervous system.

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