DEMYELINATING ACTION OF IMMUNE LYMPHOCYTES IN NERVE TISSUE CULTURES

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The cytotoxic and demyelinating effects of the lymphocytes from guinea pigs sensitized with an oily suspension of Bordetella pertussis cells, whether mixed with brain tissue or not, was demonstrated in cultures of nerve tissue from newborn rats and young rabbits. The immune lymphocytes possess this action during the incubation period and for the next 2 weeks of sensitization irrespective of whether clinical manifestations of encephalomyelitis develop or not, and the effect then diminishes gradually until the 28th-30th day. During interaction between the nerve tissue culture and immune lymphocytes a marked blast-transformation of the latter was observed on the 3rd-4th day of culture. It is postulated that the immune lymphocytes exert their cytotoxic and myelotoxic action by means of local antibody synthesis as a result of specific blast transformation.

Despite all efforts of investigators the type of immunological reaction responsible for the development of experimental allergic encephalomyelitis (EAE) has not yet been established. In the view of the majority allergic encephalomyelitis is an expression of delayed hypersensitivity to nerve tissue [6, 9, 10]. This has been demonstrated by numerous investigations which have shown the specific reactivity of lymphocytes to encephalitogenic proteins [3, 5, 7, 8]. Using a model of whooping cough encephalomyelitis, the role of cells of the lympho-macrophagal series was convincingly demonstrated by the results of work by Kanchurin and Kapitonova [1, 2].

Fig. 1. Explant of cerebellum of newborn rat on collagen. Haloes of migrating cells and axons of neurons can be seen; 5th day of cultivation. Hematoxylin-eosin, 90×.

The object of the investigation described below was to use a culture of mammalian nerve tissue in order to reproduce as closely as possible conditions in vivo when studying the action of immune lymphocytes in whooping cough EAE.

EXPERIMENTAL METHOD

Guinea pigs weighing 250-300 g were divided into 2 groups for immunization. The animals of group 1 received intradermal injections of 0.2 ml of an encephalitogenic emulsion containing homologous brain and spinal cord, in the ratio of 2:1 and Bordetella pertussis cells (2 mg/ml) mixed with Arlacell and petrolatum (1.5:8.5) into the plantar pads. The guinea pigs of group 2 received injections of the same dose of emulsion containing B. pertussis cells in oily suspension without the brain tissue.

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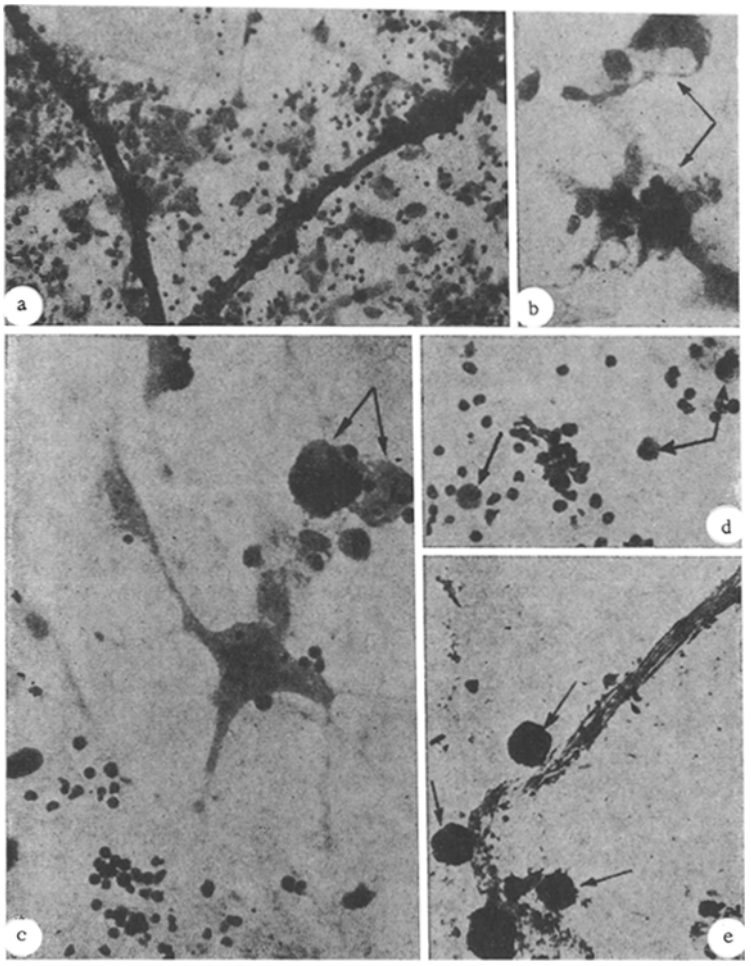

Fig. 2. Demyelinating and cytopathogenic action of immune lymphocytes in nerve tissue culture: a) agglutination of immune lymphocytes in culture of rat cerebellum (contact with lymphocytes for 6 h). Hematoxylin-eosin, 90×; b) changes in culture with disturbance of cell contacts in monolayer under the influence of lymphocytes on 2nd day of interaction during EAE. Hematoxylin-eosin, 200×; c) reduction of processes of oligodendroglial cells on 2nd day of interaction of lymphocytes in culture of rat cerebellum during EAE. Hematoxylin-eosin, 200×; d) blast-transformation of immune lymphocytes in culture of nerve tissue on 3rd day of interaction. Hematoxylin-eosin, 200×; e) demyelination of fiber in culture of rabbit cerebellum; spherical globules of myelin after lysis can be seen. Sudan black, 200×.

The animals were sacrificed on the 5th-30th day. Under aseptic conditions the popliteal, inguinal, and axillary lymph glands were removed and placed in buffer solution (pH 7.2). The tissue of the lymph glands was thoroughly broken up in sterile Petri dishes and the cell suspension was twice washed in cold buffer and centrifuged at 800 rpm for 10 min. The resulting cell residue was resuspended and stained with a mixture of trypan blue and eosin (1 : 1), after which the number of living cells in 1 ml of the suspension was counted.

Newborn rats and young rabbits weighing 1.5-2 kg were killed by exsanguination. Under aseptic conditions the cerebellum of the rabbits was removed through a transoccipital approach and the brain of the rats through a transorbital incision. Pieces of the cerebellum of these animals were placed in sterile Petri dishes with medium No. 199, freed from membranes and blood vessels, and washed twice with the medium. The brain tissue treated in this way was cut into small pieces up to 0.5 mm in size with great care, by means

of a sterile razor blade, avoiding compression and placed 2 or 3 at a time on coverslips coated with collagen [4]. The coverslips with the explants were placed in test tubes and incubated for 12 h at 36°C. The tubes with the cultures were then filled with nutrient medium containing as its basis Eagle's medium with twice the glutamine content, 30% lactalbumin hydrolysate, 20% inactivated calf serum, 800 mg/ml of glucose, and penicillin with streptomycin. The tubes with the cultures were placed in a revolving drum and incubated at 36°C. The medium was changed every 2-3 days.

The culture medium in 20-day cultures of nerve tissue was replaced by a suspension of lymphocytes in 1 ml nutrient medium, after which the cultures were again incubated. The number of lymphocytes in 1 ml medium was about $(8-10) \cdot 10^6$. The first observations under the microscope were made 1 h after addition of the lymphocytes, and the next after 3 and 6 h. Specimens were taken from the cultures after 24, 48, and 72 h and after 6 days, fixed, stained by Nissl's method, with hematoxylin-eosin, and Sudan black and impregnated with silver by the methods of Avtsyn and Gliss.

EXPERIMENTAL RESULTS

On the 4th-5th day of cultivation of the nerve tissue a clearly distinguishable halo of cells forming a monolayer could be seen around the explants, and the first fibers from axons of the nerve cells surviving in the explants were visible (Fig. 1). Starting from the 7th-8th day elongated fusiform cells with a nucleus in the middle of the narrow cytoplasm could be seen in the cultures along the axons. By the 12th day of cultivation myelin formation had begun and glial cells were coiled around the axons, and by the 18th-20th day the cultures were practically completely myelinated.

Altogether 180 specimens from the cultures were investigated and the action of lymphocytes from 50 sensitized and 10 intact animals was studied.

During the first 1-3 h of contact between the culture and immune lymphocytes definite agglutination of the lymphocytes was observed around the glial cells and nerve fibers (Fig. 2a). After 24 h, besides agglutination of the lymphocytes the first signs of alteration were seen in the culture: intermittent swelling of the myelinated fibers and swelling of individual glial cells. On the 2nd day the contacts between the cells in the culture were disturbed and there was a marked decrease in size of the neuroglial processes (Fig. 2b, c). By the end of the 3rd day the picture of the changes showed the addition of gradual lysis of glial cells and myelin sheaths, while among the lymphocyte population cells of larger size with a rim of basophilic cytoplasm around the large nucleus could be seen. In their morphology these cells could be regarded as lymphoblasts and protoplasmic cells (Fig. 2d). On the 4th-6th day of cultivation hardly any glial cells were left in the specimen and only the mesenchymal cells remained intact.

The fibers in the cultures at these times were completely demyelinated and spherical globules of different fibers could be seen around the intact axons. After staining with Sudan black they appeared as black spheres along the demyelinated fibers, and they consisted of fat droplets formed as the result of lysis of the myelin (Fig. 2e).

The cytopathogenic and demyelinating effects observed were most marked in the case of lymphocytes obtained during the incubation period of the disease (on the 5th-7th day of immunization of animals with B. pertussis cells mixed with brain tissue and on the 7th-15th day after immunization with B. pertussis cells only). The immune lymphocytes possessed this property during the next 2 weeks of sensitization regardless of whether clinical features of EAE developed or not, and a tendency for weakening of the property appeared by the 28th-30th day.

The results showed that in both cases of immunization sensitization of the lymphocytes to brain antigen developed in the guinea pigs. It could be concluded from the appearance of blast forms of lymphocytes in the nerve tissue culture that a similar mechanism operates in the living organism. Sensitized lymphocytes, penetrating into the brain through the blood–brain barrier, combine with encephalitogenic proteins by which they are specifically stimulated, so that they proliferate, undergo blast transformation, and liberate antibodies which cause demyelination of the nerve tissue.

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