ROLE OF PERIPHERAL BLOOD LYMPHOCYTES IN EXPERIMENTAL ALLERGIC (PERTUSSIS) ENCEPHALOMYELITIS

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Peripheral blood lymphocytes of guinea pigs with experimental allergic encephalomyelitis (EAE) induced by a single injection of an oily suspension of Bordetella pertussis cells, when cultured with heterologous brain antigen undergo blast transformation, which is most marked on the 14th-21st day after sensitization. With the development of clinical symptoms of EAE the level of blast transformation of the lymphocytes fell and reached its minimum on the 40th day. It is postulated that this may be connected with the development of transient hypersensitivity and dissemination of sensitized lymphocytes into the CNS, leading to its injury.

KEY WORDS: allergic encephalomyelitis; lymphocytes; blast transformation.

Recent work has shown that some Gram-negative microorganisms, including Bordetella pertussis, have an encephalitogenic action [5, 7]. An important role in the mechanism of the encephalitogenic action of B. pertussis is played by cells of the lymphoid series [6, 8]. The lymphocytes of sensitized animals, under the influence of specific brain antigen in vitro, undergo changes and conversion into cells of the blast type; this process is most marked in the incubation period of experimental allergic encephalomyelitis (EAE) [9, 17]. The peripheral blood lymphocytes of animals with EAE undergo similar changes in vivo during the development of sensitization [14] and, in particular, after the injection of a specific antigen during skin tests [15].

In the present investigation the transforming activity of peripheral blood lymphocytes of guinea pigs sensitized with an oily suspension of B. pertussis cells was studied in vitro.

EXPERIMENTAL METHOD

Experiments were carried out on 80 noninbred guinea pigs weighing 250-300 g. The animals were sensitized with B. pertussis cells (2 mg/ml) suspended in an oily emulsion (8.5 parts of mineral oil + 1.5 parts Arlacel A). Production strains of B. pertussis Nos. 305 and 312, inactivated with 0.1% formalin for 24 h, were used for immunization. The preparations contained 62.5×10^9 bacterial cells/mg dry weight.

At various times after sensitization blood was taken from the animals by cardiac puncture in a volume of 5 ml. A 1.5% solution of the sodium salt of EDTA (chelaton-3) was used as the anticoagulant and mixed with blood in the proportion of 1:2. To remove red blood cells and to obtain a suspension of white blood cells, the blood was mixed with an equal volume of 6% dextran (molecular weight 250,000). After incubation for 20-25 min at 37°C, the plasma rich in white cells was transferred to a sterile tube and centrifuged at 1000 rpm for 5 min.

The cell residue was washed three times with medium No. 199 and resuspended in 4 ml of the same medium containing 20% inactivated calf serum. After determination of the total number of white cells, the

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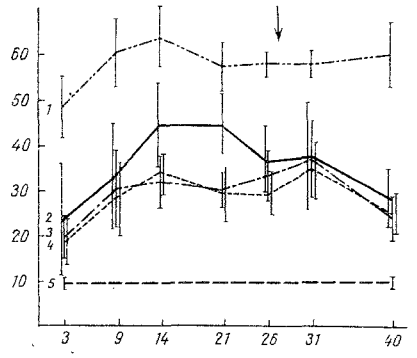

Fig. 1. Transformation of peripheral blood lymphocytes during sensitization to spinal cord antigen: 1) sensitized lymphocytes + PHA; 2) sensitized lymphocytes + spinal cord antigen; 3) sensitized lymphocytes + kidney antigen; 4) sensitized lymphocytes + medium; 5) normal lymphocytes + brain antigen. Arrow marks average time of appearance of clinical features of EAE. Abscissa, days after sensitization of animals; ordinate, percentage of blast and "intermediate" cells.

cell concentration was adjusted to 2 × 10^6 cells/ml and penicillin and streptomycin (100 units/ml of each) were added. The resulting suspension was poured into sterile penicillin flasks in volumes of 1 ml and incubated at 37°C.

Blast transformation of the lymphocytes was investigated in four systems: 1) during culture with a 0.1% nerve tissue antigen prepared from bovine spinal cord by the method of David and Paterson [11]; 2) in the presence of a 0.1% medullary antigen from bovine kidney, prepared by the same method; 3) in the presence of phytohemagglutinin M (PHA) in a dilution of 1 : 100; 4) during culture without the addition of antigens.

The PHA and antigens were added to the flasks immediately before culture. The contents of the flasks were centrifuged at 1000 rpm for 5 min 96 h later, or in the case of the PHA, 72 h later. The supernatant was then poured off and films prepared from the cell suspension. The films were fixed in Nikiforov's fluid for 30 min, stained with azure-eosin, and examined in the light microscope. The reaction was read by counting 1000 cells from each film and, taking advantage of the cytological criteria of blast transformation, the percentage of transformed cells was determined.

EXPERIMENTAL RESULTS

On the average on the 26th-28th day after sensitization with the oily suspension of B. pertussis cells most animals (55%) developed the clinical picture of EAE.

The spontaneous blast-transformation of peripheral lymphocytes of normal guinea pigs, investigated in preliminary experiments, was at the level of 12.4 ± 1%. In stained preparations after culture for 96 h typical small lymphocytes, "intermediate" lymphocytes, and lymphoblasts were observed. The "intermediate" lymphocytes and lymphoblasts were classed as transformed cells.

The reactivity of the peripheral blood lymphocytes of the immunized animals was studied starting from the 3rd day after sensitization. In the presence of heterologous spinal cord antigen in vitro blast transformation was observed with effect from the 3rd day (23.9 ± 5.1%) and reached its maximum on the 14th-21st day (45 ± 3.8 and 44.9 ± 3%, respectively). With the development of the clinical symptoms of EAE the level of transformed lymphocytes fell appreciably, regaining its initial values by the 40th day (Fig. 1).

The parameters of transforming activity of the peripheral lymphocytes grown without antigen and also in the presence of nonspecific kidney antigen did not differ statistically significantly from each other at any period of sensitization (Fig. 1). The small increase in the incubation period of blast transformation in vitro of the sensitized lymphocytes without antigen most probably reflected spontaneous transformation of lymphocytes activated in vivo by the circulating antigen. The reactivity of the sensitized lymphocytes to kidney antigen observed in these experiments may be attributable to the adjuvant action of the B. pertussis cells.

Under the influence of PHA the lymphocytes exhibited the greatest activity, which varied only slightly throughout the period of sensitization.

The results suggest that during immunization of guinea pigs with B. pertussis cells in an oily suspension immunological changes take place as the result of which the lymphocytes become sensitive to spinal cord antigen. This may be connected with a disturbance of the permeability of the blood-brain barrier by endotoxins of B. pertussis [13] and the possible formation of highly active complex (toxin + brain tissue) antigens [1, 4]. The presence of common antigens between B. pertussis and brain tissue likewise cannot be ruled out [3], and this could also lead to the development of sensitization of the animals to brain antigen. The corresponding data from mycobacteria in Freund's adjuvant exist in the literature. When animals were immunized with Freund's complete adjuvant crossed cellular reactivity was obtained between encephalitogenic peptide 1-43 and the mycobacterial protein [10, 12].

During immunization of animals with an oily suspension of B. pertussis cells a dynamic pattern of transformation of peripheral blood lymphocytes was thus revealed, with an increase in transformation activity in the incubation period and a decrease in this activity after the development of the disease. This fact may be connected with the temporary disappearance of cellular hypersensitivity, described as the Jones–Mote phenomenon [16, 18]. The development of cellular hypersensitivity on the first days suggests that the sensitized lymphocytes are disseminated from the regional lymph glands into the peripheral blood, from which they pass through the blood-brain barrier into the tissue of the CNS, which they subsequently damage [2].

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