REACTIVE PROPERTIES OF CULTURES OF PERIPHERAL BLOOD LYMPHOCYTES FROM RABBITS WITH POSTERIOR HYPOTHALAMIC INJURIES

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Reactive properties of cultures of peripheral blood lymphocytes are unchanged in rabbits with injury to the posterior hypothalamus and a lowered level of production of humoral complement-fixing antibodies. Animals with injury to the posterior hypothalamic nucleus provide a convenient model for differential investigation of specific humoral factors of immunity (antibodies) and cellular factors of immunity in vitro.

Local injury to the posterior hypothalamic nucleus (NHP) affects the viability of skin allografts [14, 15], substantially modifies the response of the cornea to burns, and leads to inhibition of homoral antibody production [9, 11, 12, 16]. It is not known how similar destruction of the hypothalamic structures affects reactivity of the lymphocytes.

In the investigation described below, the reactive properties of cultures of peripheral blood lymphocytes from rabbits with injury to the posterior hypothalamic structures were studied.

EXPERIMENTAL

Blast transformation of stimulated and unstimulated cells (BTSC and BTUC), macrophage transformation of the corresponding cultures (MTSC and MTUC), and the phenomenon of lysis of stimulated lymphocyte cultures (PLSL) [5, 6] were investigated in view of recent evidence showing that these types of response of the cultures to various forms of stimulation are exhibited unequally [1, 3, 4, 8].

Experiments were carried out on rabbits weighing 2-3 kg into which a steel monopolar electrode (200 μ in diameter), insulated throughout its length except at the tip, was inserted stereotaxically under hexobarbital anesthesia. Unilateral electrocoagulation with a direct current of 1 mA for 15 sec was carried out in the region of the posterior hypothalamus. The reference electrode was placed on the rabbit's ear on the same side as the coagulating electrode. Three days after the operation the animal received an intravenous injection of 0.25 ml/kg normal horse serum (NHS), and for the next 25 days the titer of complement-fixing antibodies (CFA) in the blood was investigated and the complement fixation test (CFT) in the cold was performed [10]. The extent of injury to NHP was judged from the results of two tests: changes in CFA production and morphological investigation of the brain of the animals undergoing the operation.*

The reactive properties of the peripheral blood lymphocyte cultures were studied repeatedly: before the operation, on the 2nd-3rd day after the operation but before injection of NHS, and on the 5th-7th and

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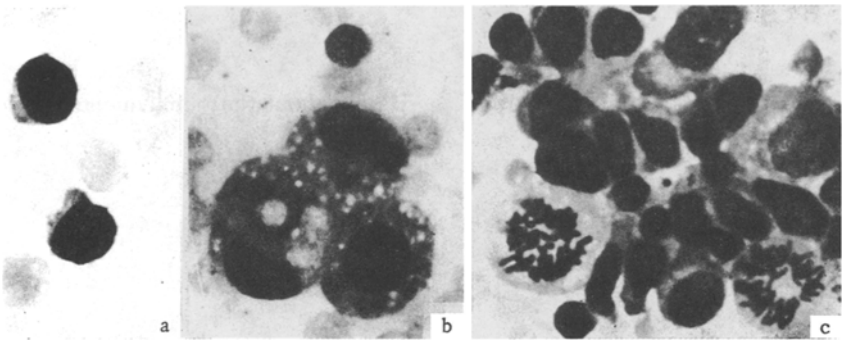

Fig. 1

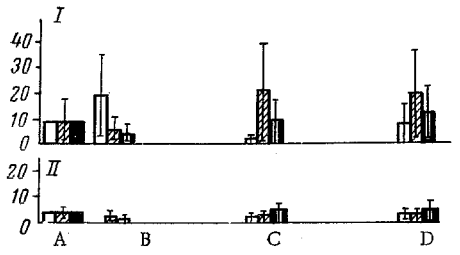

Fig. 2

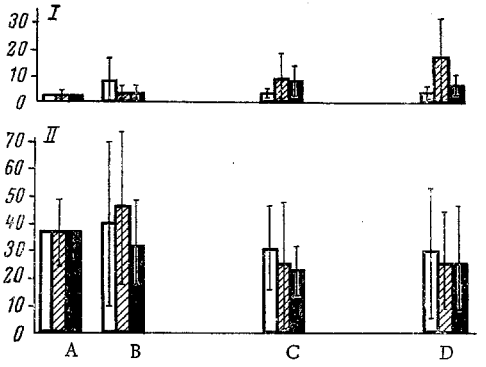

Fig. 3

Fig. 1. Culture of rabbit peripheral blood lymphocytes after cultivation for 96 h. a) Lymphocytes, 1600 ×; b) macrophages in unstimulated culture, 1280 ×; c) cellular adhesion in culture consisting of lymphocytes, transformed lymphoid cells, and blast cells in mitosis, stimulated by phytohemagglutinin M, 1088 ×. Stained with azure II-eosin.

Fig. 2. Reactivity of lymphoid cells in unstimulated culture. I) Macrophage transformation; II) blast transformation. Abscissa – days of observation: A) before operation; B) 2nd-3rd day after operation and before injection of protein; C) 5th-7th day; D) 10th-12th day after immunization. Ordinate – number of cells in percent. Unshaded columns, Group 1; obliquely shaded columns, Group 2; black columns, Group 3.

Fig. 3. Reactivity of lymphoid cells in cultures stimulated by phytohemagglutinin M. Legend as in Fig. 2.

10th-12th days after immunization. The cells were cultivated and counted by the usual methods [2, 7, 17, 19] in Eagle's MEM medium for suspended cells [18] with the addition of 18-20% autologous plasma. Phytohemagglutinin M (Wellcome) was used as the stimulator.

EXPERIMENTAL RESULTS

On the basis of the serological data (CFA production) and the results of the morphological investigation of the brain (localization of the focus of destruction in the hypothalamus) all the animals were divided into three groups: group 1 (six rabbits) included animals in which no CFA could be detected, but the focus of destruction was situated in NHP; group 2 (three rabbits) contained animals in which traces of CFA were found in serum diluted 1:10, and the focus of destruction involved NHP; group 3 (seven rabbits) included animals in which the immunological response to NHS was well marked, i.e., CFA were detected in serum in dilutions of 1:20 or above, and the focus of destruction was located in various structures of the posterior hypothalamus. Bearing in mind that the process of CFA production in the rabbits of group 3 was similar in intensity to that in many of the animals in the preliminary experiments before the operation, and also to results given in the literature [9, 13] it was decided that the rabbits of group 3 could serve as the control for groups 1 and 2.

The culture of peripheral blood lymphocytes from all three groups of animals was viable and most of the cells of the unstimulated cultures on the 4th day consisted of small and medium-sized lymphocytes (Fig. 1a), the number of large lymphocytes not exceeding 1-3% (Fig. 2, II). Only in one rabbit was a high BTUC (up to 25%) observed before the operation and throughout the subsequent period of the investigation. In the animals with undisturbed CFA production (group 3) the value of MTUC remained virtually unchanged on the 5th-7th and 10th-12th days after injection of NHS. The rabbits of group 2 (partial injury to NHP and a decrease in CFA production) gave an increase in MTUC on the 5th-7th and 10th-12th days after immunization. However, because of the small number of observations (three rabbits) it is impossible to determine the significance of this change in MTUC. In the animals of group 1, MTUC was not significantly different from its value in group 3 throughout the experiment (Fig. 2, I; Fig. 1b). In the rabbits of all three groups no significant difference was found in BTSC (Fig. 3, II), while MTSC in the animals of groups 1 and 3 was similar in type at all times of observation (Fig. 3, I). In the rabbits of group 2 an increase in MTSC was observed on the 10th-12th day after immunization, just as was found with MTUC in this group. PLSL was not observed in the cell cultures from any of the groups of animals. The study of peripheral blood lymphocyte cultures from rabbits with injury to NHP thus revealed no significant changes.

The experiments on rabbits with injury to NHP showed that CFA production in response to injection of NHS was virtually absent, but there was a normal response of the peripheral blood lymphocyte cultures with respect both to MTUC and to MTSC and BTSC. Electrolytic injury to the hypothalamic structures produced no marked changes in the reactive properties of lymphocytes of all investigated groups of animals. This investigation suggests that animals with injury to NHP provide a convenient model for differential investigation of specific humoral factors of immunity and its cellular factors.

The question of which link in the cell–antibody chain is broken by injury to NHP, and how NHP exerts its influence on antibody production is extremely important and complicated. The problem must be tackled by parallel experiments on intact animals and in vitro.

It is evident from these investigations that central influences on the morphological manifestations of reactivity of cells of the lymphoid system are not sufficiently intensive or prolonged to enable them to be discovered by analysis of the lymphocyte response in vitro.

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