IMMUNOHISTOCHEMICAL ANALYSIS OF RAT SPLEEN TISSUE AFTER HYPOTHALAMIC DESTRUCTION

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The role of the nervous system in the response of the whole organism to an antigen is a case when priority belongs to Soviet science. An important contribution to the study of this problem was made by the discovery of modulation of immunological processes through local action directed toward certain hypothalamic structures [2]. For instance, it was shown in experiments with local destruction of hypothalamic formations that the CNS participates in modulation of the humoral and cellular immune response and of allergic reactions [5, 8, 11, 13]. This same model was used to determine the character of the corrective influences of the CNS on particular stages of formation of the immune response, on activity of certain cell populations involved in immunogenesis, such as activity of cells of the mononuclear phagocyte system [4], and on migration of immunocompetent cells from bone marrow into lymphoid organs [3]. Modulation of activity of migration of lymphoid cells in vivo and the associated change in relations between different subpopulations in peripheral lymphoid organs can serve as a basic mechanism of neurohumoral regulation of immunological processes. Despite many investigations, showing that hypothalamic correction of the blood antibody level is possible, we have as yet no clear understanding of the number and distribution of B cells in lymphoid organs when hypothalamic function is disturbed, i.e., of one possible mechanism of realization of regulatory influences on B-cell function. Among the peripheral organs of the lymphoid system the spleen is a special organ because of the presence and properties of its B lymphocytes. Two populations of B cells are distinguished: the first consists of cells carrying IgM and IgD on their surface, and located in the zone of primary follicles of the spleen. These are circulating B lymphocytes. Noncirculating B cells, carrying IgM, are located in the marginal zone of the spleen. B cells present in other peripheral lymphoid organs belong to the first population [10, 12]. The differences between these B-cell populations are revealed after single irradiation of the animal relative to the rate of repopulation of splenic tissue [6, 7].

In the investigation described below the above-mentioned features of distribution of functionally different B cells were used in order to analyze the degree and character of the corrective influences of the hypothalamus on different B-cell populations.

EXPERIMENTAL METHOD

Experiments were carried out on male Wistar rats aged 8-10 months, obtained from the "Rappolovo" Nursery. Bilateral electrolytic destruction of hypothalamic structures was carried out under hexobarbital anesthesia in a stereotaxic apparatus for small laboratory animals (Experimental Workshops, Academy of Medical Sciences of the USSR) using coordinates from the atlas [9] and platinum electrodes, insulated with fluorine plastic, with a tip 0.1 mm in diameter (current 1 mA, duration 10 sec). Seven days after the operation on the rats, the spleen was removed and weighed, fixed in Carnoy's fluid for 18 h at 4°C, dehydrated with ethanol (4 h) and xylol (18 h), and impregnated with Paraplast (2 h at 56°C). By means of a precision microtome, serial tissue sections 4 μ thick were cut, and dewaxed in xylol and alcohol. To demonstrate the localization of the B cells in splenic tissue, an indirect immunoperoxidase method with rabbit antisera to rat IgM and IgD was used [6, 7]. Morphometric analysis of the ratios

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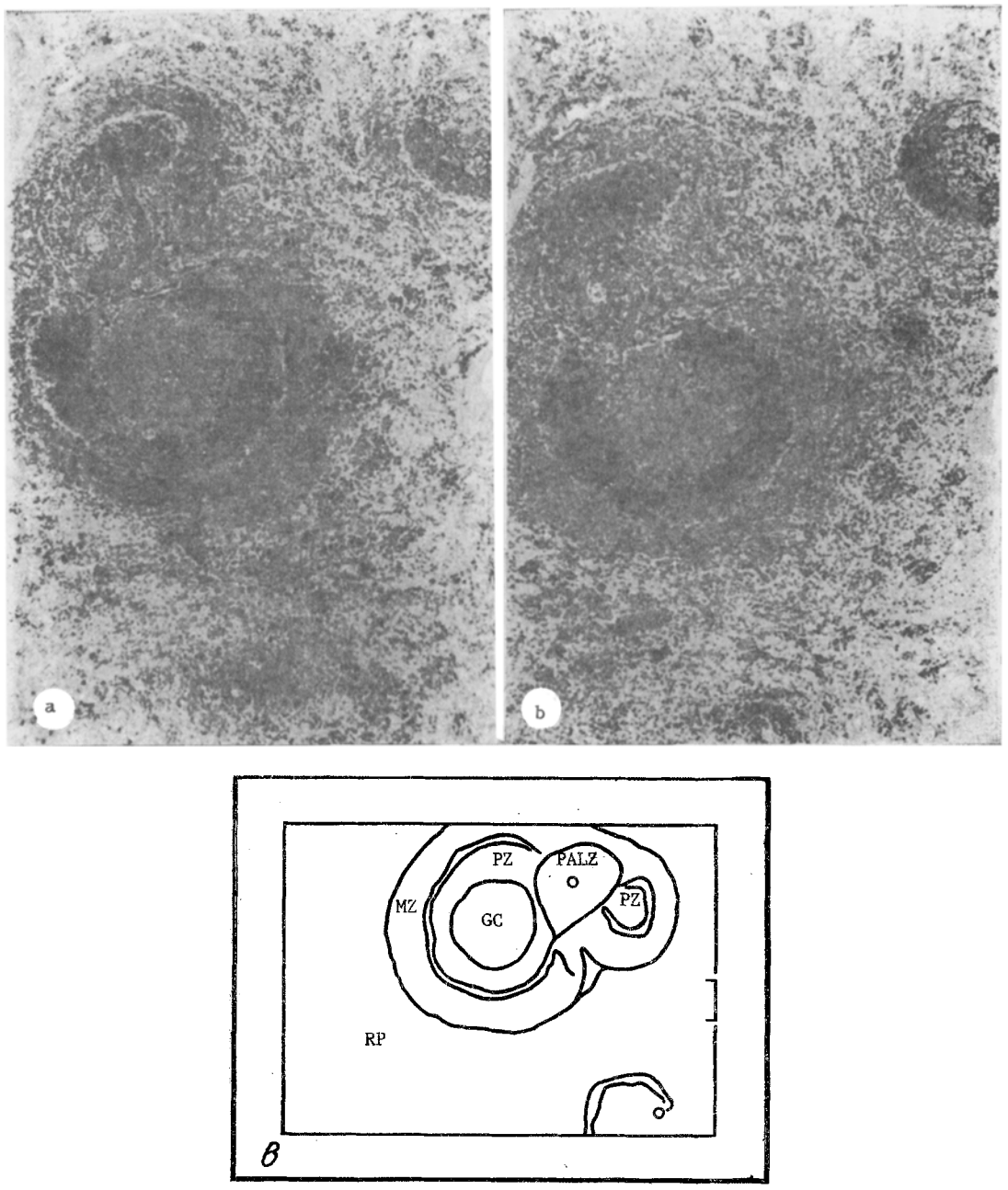

Fig. 1. Splenic tissue sections from Wistar rats, stained to reveal B cells carrying IgM (a) and IgD (b), and diagrammatic representation of their arrangement in a and b (c). PALZ) Para-arteriolar lymphocytic zone; PZ) zone of primary follicles; MZ) marginal zone; GC) germinative center (secondary follicle); RP) red pulp. Marker 100 μ.

of the different zones of splenic tissue was undertaken by the Weibel 2 microscopic system ("Gradicule," England), followed by calculation of the weight of individual tissue components of the spleen [12]. The size and location of the lesion in the hypothalamic structures were determined in serial sections of the rat brain fixed in formalin. Animals with the same type of lesion were combined into one group. The results were subjected to statistical analysis by the Wilcoxon–Mann–Whitney nonparametric test [1].

EXPERIMENTAL RESULTS

Immunoenzyme staining of two neighboring ultrathin sections of splenic tissue, using antibodies to IgM or IgD, revealed B cells carrying one or both of these markers (Fig. 1). This technique enabled zones in the white pulp of the spleen occupied by two different populations

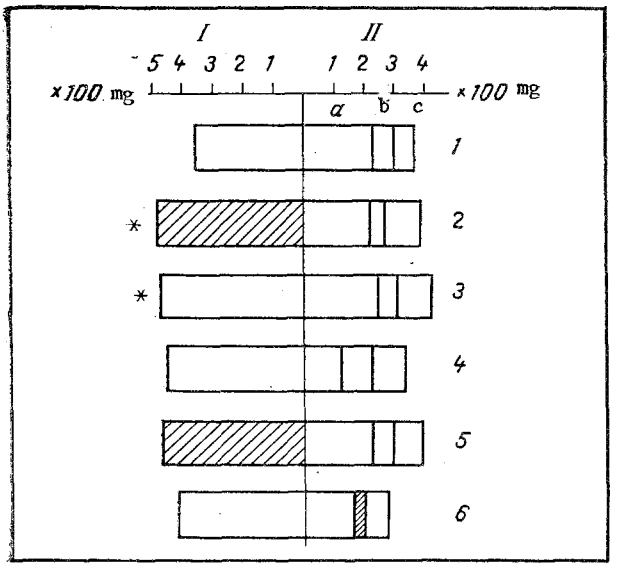

Fig. 2. Changes in weight of spleen and of individual components of splenic tissue in rats after hypothalamic destructive lesions. I) Red pulp; II) white pulp. a) Weight of marginal zone (noncirculating B lymphocytes), b) weight of zone of primary follicles (circulating B lymphocytes), c) weight of para-arteriolar lymphocytic zone (T lymphocytes). Zone of germinative centers did not exceed 1% of weight of spleen and is therefore not indicated. 1) Intact rats, 2) rats undergoing mock operation (anesthesia, trephining of the skull), 3) destructive lesions in the cerebral cortex, 4) destruction of posterior hypothalamic area, 5) destruction of anterior hypothalamic area, 6) destruction of lateral hypothalamic area. Asterisk indicates statistically significant increase in weight of the spleen compared with intact rats; shaded area corresponds to statistically significant increase in weight of red or weight pulp compared with intact rats.

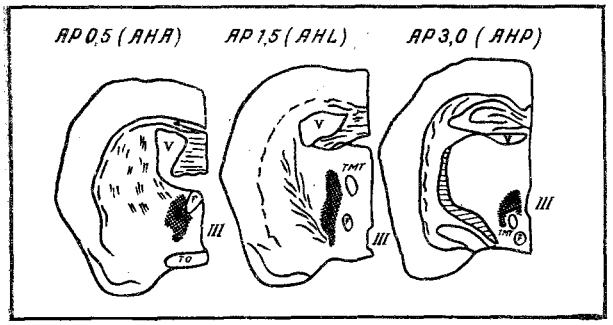

Fig. 3. Location of hypothalamic lesions in rats. Cross-hatching – zone of lesions; black shading – average size of area of tissue destruction in individual animals.

of B cells to be revealed: IgM^+ IgD^+ and IgM^+ IgD^- [6, 7]. It was shown that the first population consists of recirculating cells, and is rapidly renewed after irradiation [6, 7].

The results of morphometric analysis of the splenic tissue from animals with structural damage to the hypothalamus are demonstated in Fig. 1. In animals undergoing a mock operation (anesthesia and trephining of the skull) or with lesions of areas of the cerebral cortex, there was a significant increase in weight of the spleen, mainly on account of an increase in weight of the nonlymphoid part of the spleen – the zone of the red pulp. There was no change in the ratio of the zones in the white pulp of these animals on the 7th day after the operation.

Lesions in the hypothalamus differed in their effect on the morphological characteristics of splenic tissue (Figs. 2 and 3). For instance, with lesions of the posterior hypothal-

amic area no change was found in the weight of the spleen or in the ratio between the zones, i.e., destructive lesions in this area prevent effects induced by the operation. Injury to the anterior hypothalamic area did not prevent an increase in weight of the red pulp characteristic of the group of animals undergoing the mock operation.

The most significant changes were found after local coagulation of the lateral hypothalamic area. In these cases not only was the weight of the spleen and the weight of the red pulp not increased, but there was a significant decrease in weight of the zone of the primary follicles, in which B cells belonging to the population of circulating B cells were distributed.

The investigation thus revealed effects of destructive lesions in the hypothalamus, expressed as changes in the relations between different components of splenic tissue, and that they depend on the location of the hypothalamic lesion. Essentially, in the case of bilateral local destruction of the lateral hypothalamic area, there is a decrease in weight of the B-dependent zone of the spleen in which B lymphocytes of the circulating pool accumulate. These findings are evidence in support of involvement of the CNS in the regulation of B-cell migration in vivo.

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